Topics in Medicinal Chemistry 29

Anil Kumar Saxena *Editor*

Communicable Diseases of the Developing World

Springer

29
Topics in Medicinal Chemistry

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Anil Kumar Saxena
Editor

Communicable Diseases of the Developing World

With contributions by

C.S. Azad · S.S. Bhunia · P. Eleftheriou · M. Ferri · A. Geronikaki · R. Kalyanasundaram · S. Pandey · V. Poroikov · A. Saxena · A.K. Saxena · P.K. Shukla · P. Singh · R.K. Yadav

Springer

Editor
Anil Kumar Saxena
Central Drug Research Institute
Division of Medicinal & Process Chemistry
Lucknow, Uttar Pradesh
India

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Preface

Communicable diseases have always been a major concern to human health, particularly in the developing world where millions of people die every year. This scenario is changing with increased human mobilization and immigration coupled with trading of foodstuffs and biological products and so communicable diseases are no more localized but may affect population worldwide. Furthermore the rapid adaptation by microorganisms is leading to the resurgence of communicable diseases of the past, drug resistance to the existing diseases, and the emergence of new ones. Most of the communicable diseases have not been paid adequate attention by the developed world. These diseases include malaria, tuberculosis, leishmaniasis, filariasis, dengue, HIV/AIDS, diarrhea, cholera, leprosy, buruli ulcer, trachoma, and schistosomiasis. In view of this, a book volume covering tuberculosis, malaria, kinetoplastids, dengue fever, and diarrhea was published in the *Topics in Medicinal Chemistry* series by Prof. Richard L. Elliott. Following this previous publication and considering the recent outbreak of Ebola virus disease, the present volume entitled *Communicable Diseases of the Developing World* was envisaged to cover leftover diseases including a chapter on Ebola. So the important diseases such as HIV/AIDS, filariasis, fungal infections, and neglected virus disease, bacterial diseases including Ebola virus disease have been described in the five chapters of this volume. The first chapter on Ebola virus disease deals with its clinical management and the control measures to be followed along with the current status of drugs and vaccine development for the prevention and cure of this disease. In the second chapter the current state of HIV therapeutics as well as the status of candidate drugs under development along with the future prospects of HIV treatment has been dealt. The third chapter is on the important neglected parasitic disease known as lymphatic filariasis where the targets and strategies for intervention and elimination of this disease along with the existing chemotherapy and newer developments for lymphatic filariasis are described. The fourth chapter is on the antifungal agents, which provides an overview of the past, present, and future prospects of drugs, cationic peptides, and monoclonal antibodies as antifungal agents. The fifth chapter covers neglected

tropical bacterial diseases like leprosy, buruli ulcer, and trachoma along with the chemotherapeutic approaches to their treatment.

It has been an immense pleasure to be the editor of this volume. I gratefully acknowledge all the authors for their contributions and thank them for taking time and care in composing the chapters. I wish that this volume will be useful for researchers working on these diseases and will inspire many more to develop strategies for the discovery and development of new therapies for these diseases.

Lucknow, India Anil Kumar Saxena

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Clinical Management of Ebola Virus Disease: Current and Future Approaches

Aaruni Saxena and Mauricio Ferri

Abstract Ebola virus disease (EVD) is a notoriously dreadful disease. The acute viral syndrome, which has an incubation period ranging from 2 to 21 days, is characterized by fever and diarrhea, along with bleeding diathesis. Mortality rates are high. The natural reservoir is thought to be the fruit bat of the Pteropodidae family. Nonhuman primates, including monkeys, chimpanzees, and gorillas, are primary hosts to the virus. Transmission occurs through direct contact with bodily fluids containing the virus. Currently available laboratory tests include the rapid diagnostic tests ELISA and PCR. A patient's chance of survival depends on multiple factors, such as the initial viral load at the time of exposure, their immune response to the virus, and access to proper care. Currently, there is no specific treatment or cure; however, clinical management mainly consists of supportive measures. Novel drugs and vaccines are undergoing clinical trials to determine their safety and efficacy for use in humans.

Keywords Drugs, Ebola virus disease, Epidemic, Protection, Treatment, Vaccines

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The online version of this chapter has been revised.

A. Saxena
Clinical Praxis, Schulstrasse 36, Hilden 40721, Germany

M. Ferri (✉)
Health Service Research, Rede Metropolitana de Saúde, Sarandí, Brazil
e-mail: mbellerferri@gmail.com

1 Introduction

Ebola virus disease (EVD) first appeared in 1976, simultaneously in the Democratic Republic of Congo (DRC) and Sudan [1]. Ebola virus (EBOV) belongs to the virus family *Filoviridae* [2]. Apart from the Ebola virus, the other main member of the family is the Marburg virus. It was discovered earlier than the Ebola virus, in 1967. The discovery took place after several commercial laboratory workers in Germany were admitted to hospital with an unusual illness. The clinicians observed a different pattern of clinical symptoms in each patient but a similar course of disease. Further investigation revealed the source of infection to be a virus isolated from green monkeys imported from Africa for research purposes. Quarantine procedures were subsequently implemented to halt further transmission, and countries advised to exercise caution when importing monkeys [3].

Ebola is the second known *Filovirus* and can be more lethal than the Marburg virus. It was often reported in cynomolgus monkeys (*Macaca fascicularis*) in the early and late 1990s [4, 5]. Ebola in humans was seen again from 1994 to 1996. This time the infection included the subtype *Zaire ebolavirus* (EBOV-Z) and a new subtype known as *Côte d'Ivoire ebolavirus* (EBOV-CI) or *Taï Forest ebolavirus* [6]. At the time, little was known about the occurrence and transmission of the Ebola virus. Later, three more species were discovered and added to the genus *Ebolavirus*: *Bundibugyo*, *Reston*, and *Sudan*. The *Bundibugyo*, *Zaire*, and *Sudan* viruses have been responsible for outbreaks in Africa; the *Reston* virus is predominantly found in animals [7, 8]. After long-running discussion, the scientific community concluded that fruit bats of the Pteropodidae family may likely be the natural hosts of the Ebola virus. The fruit bat species includes *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata* [9, 10]. All other animals infected by Ebola, especially monkeys, gorillas, and chimpanzees, are accidental hosts [11, 12], although there remains the possibility of other natural hosts. Research efforts have been complicated by the lethal nature of the virus.

2 Epidemiology

2.1 Geographic Spread of Ebola over Time

EVD was first discovered in 1976 when two outbreaks of different strains occurred simultaneously in the Democratic Republic of Congo (DRC, formerly Zaire) and Sudan, infecting 318 (case fatality ratio (CFR): 88%) and 151 people (CFR: 53%), respectively [1]. In 1979, *Sudan* strain reemerged in Nzara, Maridi, the same location as the 1976 Sudan outbreak [13].

Thereafter, sporadic outbreaks due to *Zaire*, *Sudan*, and *Bundibugyo* subtypes occurred in central Africa, namely, Côte d'Ivoire, DRC, Gabon, Republic of the Congo, Sudan, and Uganda. An isolated event was reported in South Africa in 1996 after a medical professional, who had been exposed to the virus while treating Ebola-infected patients in Gabon, traveled to Johannesburg and infected a healthcare worker while seeking care [14]. EBOV was introduced to other parts of the world through exportation of infected monkeys or cynomolgus macaques to Italy and the United States from the Philippines (none of the exposed persons developed symptomatic infection since Reston EBOV is not pathogenic to humans) [15–17]. In England and Russia, accidental needlestick injuries and contamination in laboratory settings were responsible for four infections [18–20]. While there is evidence suggesting that the Ebola virus may have been previously circulating beyond central Africa [21], it was not until 2014 that the virus posed a major threat in a previously unaffected region. The 2014–2016 outbreak, which began in Guéckédou, Guinea, is the largest and most widespread in history, affecting not only Guinea but also neighboring Liberia and Sierra Leone.

As of June 10, 2016, the World Health Organization (WHO) estimated a total of 28,616 confirmed, probable, and suspected cases and 11,310 deaths in the three affected countries. Whereas prior outbreaks occurred in remote areas, mainly in countries that have built capacity to respond to the disease after previous outbreaks, this epidemic illustrated a wider geographic spread for a variety of reasons including, but not limited to, porous borders, modern-day travel, and fragile health systems in three impoverished countries that had never previously experienced an Ebola outbreak. Imported cases were also reported in Mali, Nigeria, and Senegal, as well as the United States and several European countries [22–24].

2.2 Transmission

EVD is considered to be a zoonotic disease. Although live virus has not been isolated from fruit bats, increasing evidence suggests that they are a likely reservoir [9, 10, 25, 26]. Nonhuman primates are unlikely to be reservoirs because of the high mortality in nonhuman primates. Fruit bats either directly transmit the virus to humans through infected saliva or excreta or to nonhuman primates, especially

apes, gorillas, and chimpanzees, which may become infected and serve as intermediate hosts before the virus is passed onto humans through direct contact with blood or bodily fluids. Butchering or hunting of these animals has been associated with Ebola virus infection in humans [27, 28].

Once animal-to-human transmission occurs, human-to-human transmission is possible. Like animal-to-animal and animal-to-human transmission, human-to-human transmission requires direct contact with infected bodily fluids. In humans, viral RNA has been detected as long as 40 days after symptom onset in sweat [28]; 33 days post-symptom onset from vaginal, rectal, and conjunctival swabs [28]; 30 days in urine [28]; 22 days in saliva; and 15 days in breast milk [28]. In addition, viable Ebola virus has been found in semen 82 days after symptom onset [28], while viral RNA has been detected up to 284 days post-symptom onset in semen [28]. Although rare, sexual transmission has been reported [29–32]. Healthcare facility-associated transmission is not uncommon, particularly in the early phase of an outbreak, since the symptoms of EVD mimic many diseases that are endemic to regions where Ebola virus is known to exist, and laboratory testing for EBOV is not routinely performed. Therefore, healthcare workers are often among the first to become infected especially in the absence of personal protective measures [1]. Reuse of contaminated needles has also been associated with nosocomial infections [32]. In community settings, caring for family members at home has been associated with infection, as has funeral attendance. Funeral practices in some cultures involve direct contact with the deceased (e.g., body washing) [33, 34]. The infectious dose of Ebola virus is only 1–10 organisms [35]. The only documented aerosol transmission was in nonhuman primates in a controlled laboratory setting [36].

2.3 Surveillance

A robust surveillance system to rapidly collect and analyze data to understand the epidemiological situation and to inform outbreak response strategies is crucial in containing an EVD outbreak. The major components of surveillance are case investigation, which is prompted after case detection, along with contact tracing to promote the early detection of new cases.

2.4 Case Investigation

During an outbreak, investigation of any individual suspected of having EVD must begin promptly. Case investigation is prompted when the surveillance team is notified of an EVD case. Notification is typically provided through telephone hotline alerts, rumors, and deaths in the community or proactive efforts by the surveillance team to identify new cases (also known as active surveillance). A sick

Table 1 Example of Ebola virus disease case definitions for areas where an outbreak has been declared [37]

Suspected case
Any person, alive or dead, suffering or having suffered from a sudden onset of high fever and having had contact with a suspected, probable, or confirmed Ebola case
OR
Any person with sudden onset of high fever and at least three of the following symptoms:
Headaches
Vomiting
Anorexia/loss of appetite
Diarrhea
Lethargy
Stomach pain
Aching muscles or joints
Difficulty swallowing
Breathing difficulties
Hiccups
OR
Any person with inexplicable bleeding
OR
Any sudden inexplicable death
Probable case
Any deceased suspected case (where it has not been possible to collect specimens for laboratory confirmation) having an epidemiological link with a confirmed Ebola case
OR
Any suspected case evaluated by a clinician
Laboratory-confirmed case
Any suspected or probable case with a positive laboratory result. Laboratory-confirmed cases must test positive for the virus antigen, either by detection of virus RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) or by detection of IgM antibodies directed against Ebola

person may also present at a healthcare facility, and the surveillance team notified to follow up to obtain more information. Case investigation must also be conducted for the contacts of an EVD case who are being monitored for 21 days as soon as they begin to start to show symptoms (21 days is the maximum surveillance incubation period of Ebola virus).

Case investigation consists of an interview with an EVD case or a proxy of a deceased case. It serves several important purposes, including the assessment of clinical signs and symptoms, epidemiological risk factors, and sources of exposure to determine if a person meets the case definition (see Table 1). For patients who do meet the case definition, testing is indicated for laboratory confirmation. A case definition that is used to guide epidemiological investigations may sometimes differ from the one used in clinical settings, since the former serves to maximize sensitivity for surveillance purposes at the expense of specificity while the latter informs clinical decision-making in patient management.

Identification of contacts also occurs during case investigation in order to initiate contact tracing. In the event a healthcare worker becomes an EVD case, a more thorough investigation is needed to understand the occupational conditions under which he or she became infected and to identify any specific exposure that may be unique to healthcare workers that needs to be addressed for prevention and control purposes.

2.5 Contact Tracing

Contact tracing, which occurs after contacts have been identified through case investigation, is the daily monitoring of contacts of an EVD case for 21 days with the purpose of detecting new cases as early as possible so as to allow for isolating them to prevent further transmission to other members of the community. In addition, early detection is intended to promote the early treatment of patients, increasing their likelihood of survival. A contact is defined as anyone who has had any of the following types of exposure [38]:

1. Touched the bodily fluids of the case (saliva, urine, feces, or semen)
2. Had direct physical contact with the body of the case (alive or dead)
3. Received breast milk from the case
4. Attended the funeral of the case
5. Touched or shared the linens, clothes, or dishes/eating utensils of the case
6. Slept, ate, or spent time in the same household or room as the case

2.6 Control Measures

Prevention of person-to-person transmission is the key to containment of an outbreak. Isolation of cases to prevent contact with non-cases is the most obvious strategy to limit transmission [39].

Distribution of personal protective equipment to healthcare workers has been effective in prior outbreaks, along with appropriate infection prevention and control practices in formal care settings hospital-wide and in Ebola treatment units [40]. These practices must also be applied in informal patient care and social settings in the community. Safe burial rituals are effective in reducing transmission [37]. The community's willingness to participate in appropriate control measures is an important factor in outbreak response (see Table 1).

3 Pathology

Ebola virus is a member of *Filovirus* and its genome is similar to *Paramyxoviridae*. The five known strains of the virus are Bundibugyo, Reston, Taï Forest, Sudan, and Zaire. The Zaire subtype is the most pathogenic and lethal [41]. The Ebola virus is an enveloped, non-segmented, negative-sense, and single-stranded RNA virus [42]. It is threadlike in structure with a diameter of 80 nm and has a 19 kb-long genome. It has a leader and trailer region which is responsible for controlling transcription, replication, and packing of the genome in new virions. The seven main genes are presented in the following sequence: 30-leader-NP-VP35-VP40-GP/sGP-VP30-VP24-L-trailer-50 [43]. These seven genes consist of open reading frames responsible for encoding of structural proteins [44]. The structural proteins include the glycoprotein (GP) envelope, nucleoprotein (NP), RNA polymerase (L), matrix protein viral proteins 24 (VP24) and 40 (VP40), and nonstructural viral proteins VP30 and VP35 [45]. VP40 and VP24 are the viral proteins present between the capsid and envelope [46]. The exact mechanism for the entry of the virus and its pathogenicity is still unknown. However, there are recent scientific studies showing some possible mechanisms of virus entry into the host cell.

A study conducted by Carette and coworkers explored infection caused by both Ebola and Marburg viruses resulting in hemorrhagic diathesis accompanied by fever. Their study revealed that entry of the *Filovirus* is mediated by a viral spike in glycoprotein. This glycoprotein acts as an anchor to the cell membrane of the host cell and attaches the viral particle to the cell surface. With the help of additional host factors, fusion between viral and endosomal membranes occurs, resulting in entry of the virus to the cell. Further investigation to explore the critical host factors responsible for facilitation of fusion of viral and endosomal membrane showed the importance of endolysosome cholesterol transporter protein Niemann-Pick type C1 (NPC1). Fibroblast derived from patients suffering from human Niemann-Pick type C1 disease showed resistance to Ebola and Marburg infection [47]. Another important point revealed by studies on the potential entry mechanism of Ebola virus into host cells is the importance of Ebola virus GP. Yonezawa and coworkers showed the use of a virion-based fusion assay by substitution of Ebola virus GP for the human immunodeficiency virus type 1 (HIV-1) envelope. The experiment results proved that entry and fusion induced by the Ebola virus GP occurred at much slower kinetics than with vesicular stomatitis virus G-protein (VSV-G). Furthermore, fusion was blocked by depletion of cholesterol membrane and by inhibition of vesicular acidification with bafilomycin A1. Fusion promotion factors revealed by the experiment were the microtubules. Paclitaxel (Taxol), a microtubule-stabilizing agent, facilitated early fusion of Ebola virus GP pseudotypes but not VSV-G- or HIV-1-enveloped pseudotypes. Similarly, in the presence of a microtubule-disrupting agent like nocodazole (1), the fusion was impaired in the case of Ebola virus GP pseudotypes. Other agents identified for Ebola virus GP-mediated entry and fusion by disrupting the microfilament function

included cytochalasin B (2), cytochalasin D (3), latrunculin A (4), and jasplakinolide (5).

These results suggest that apart from Ebola virus GP, both microtubules and microfilaments play an important role in facilitating virus entry and fusion. It is possible that the microtubules and microfilaments are responsible for transporting the Ebola virions after they anchor on the cell surface to the appropriate acidified vesicular compartment where fusion occurs. Host cells highly sensitive for Ebola virus entry include primary macrophages and target cells, whereas the monocyte shows reduced levels of entry and fusion. Another important fusion promotion factor identified in this study was the presence of tumor necrosis factor alpha (TNF-α), which is released by monocytes/macrophages infected by Ebola virus. The presence of TNF-α enhanced the Ebola virus GP-mediated entry into human umbilical vein endothelial cells in the experiment [48, 49].

The importance of Ebola viral glycoproteins revealed in the scientific studies encouraged researchers to study insights into the type of glycoproteins released by the lethal virus. Wahl-Jensen et al. showed that the mononuclear phagocytic systems in both primates and non-primates are the primary targets for the Ebola virus and are also responsible for the release of inflammatory mediators after infection has occurred. The study revealed that activation of macrophages is not dependent on virus replication and that it is possible that the initial interactions of mononuclear phagocytic cells with glycoproteins released by the virus play a role in the activation of the immune system [50]. After fusion and entry of the Ebola virus into the host cell, it produces four types of soluble glycoproteins. These are nonstructural small glycoprotein (sGP), delta peptide (Δ-peptide), GP1, and GP1,2Δ. The presence of these glycoproteins has been confirmed in blood and other in vitro systems. The glycoprotein gene is responsible for the production of the glycoproteins. The full-length GP1,2 found on the surface of the virus is responsible for receptor binding and fusion with target cells [51].

Later, the full-length GP1,2 gets further processed through proteolysis into GP1 and GP2. Both GP1 and GP2 are disulfide linked and form the mature spike protein. GP1 forms the transmembrane portion of the protein [52–54]. GP1,2Δ is the product of metalloprotease cleavage of GP1,2 [55]. Soluble GP is mostly identical to GP1 but differs in the amino acid present at 69 C-terminal, and it also forms homodimers in antiparallel orientation [56]. Soluble GP synthesis occurs from the unedited GP mRNA that is thought to be to be secreted from the infected host cells [57, 58]. Some studies report that soluble GP binds to neutrophils, thereby paralyzing the inflammatory defense machinery of the host [59]. The study by Wahl-Jensen showed the particular importance of viral glycoprotein GP1,2 among the four soluble glycoproteins. It needs to be present in its rigid form on the surface of the virus, and then it acts as repetitive antigenic stimulus to the macrophages, resulting in their activation (Fig. 1).

Virulent factors currently being considered as potential therapeutic targets include VP35, VP24, and viral selenoproteins, in addition to the aforementioned glycoproteins. VP40 is coded by the third gene of Ebola genome and is thought to help and maintain the structural integrity of the virus. It is present beneath the viral

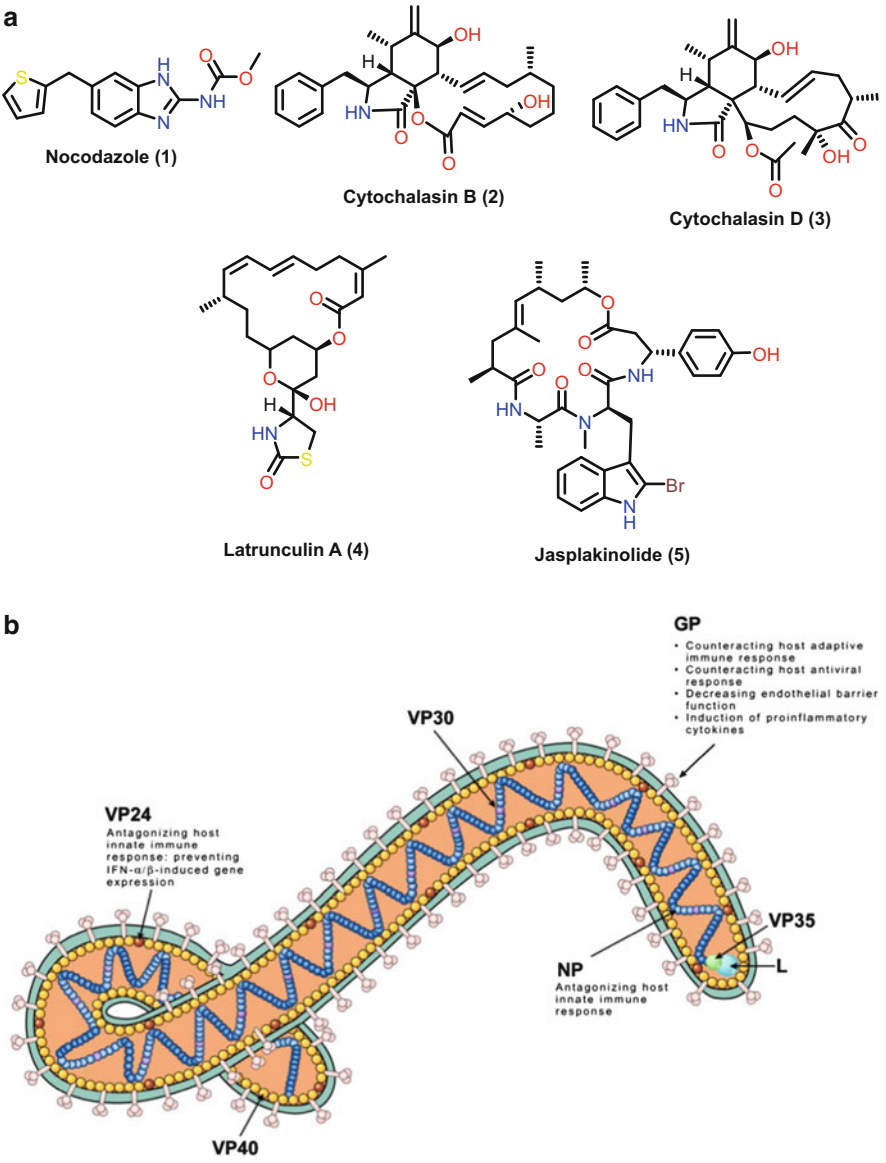


Fig. 1 (**a**) Microtubule-disrupting agent 1–5 and (**b**) determinants of Ebola virus pathogenicity showing the roles of GP, NP, VP24, and VP35 in the pathogenesis of EBOV. Figure (**b**) is used with permission from Wit et al. [49]

envelope and is likely to also mediate the budding of the virus [60]. VP24 is involved in the suppression of the immune system. Zhang et al. did an insight study into the role of VP24 in suppression of the immune system [61] and interferon-γ (IFN-γ) by trapping the karyopherin α-proteins (α1, α5, and α6) in the

cytoplasm of the host cell [59]. VP24 directly binds with STAT1 and thereby interferes in the interferon pathway [61]. VP24 causes inhibition in the signal mechanism responsible for both interferon-α and interferon-β (IFN-α/β). STAT-1 in the host cell plays a key role in the immune signaling and is most often referred to as the common target for the viral machinery [62]. After viral infection, production of IFN-α/β, IFN-γ, tumor necrosis factor α (TNF-α), interleukin-6, and interleukin-10 causes phosphorylation of STAT-1 into P-STAT1. Later, complex formation might occur between P-STAT1 and STAT2 or IRF-9, or P-STAT1 otherwise homodimerizes. These STAT-1-containing complexes are then transported to the nucleus of the host cell by karyopherin α-proteins. These complexes are thought to be involved in the regulation of genes involved in immune response. Due to binding of VP24 directly with STAT-1 and karyopherin-α, the process of immune response is impaired [59]. VP24 also plays an important role in the correct assembly of nucleocapsid. In the absence of VP24, there is a reduction in transcription and translation of VP30 (Fig. 1) [63].

VP35 binds with double-stranded RNA, and it acts as antagonist to several antiviral signaling pathways [64]. Studies conducted at the Basler laboratory showed the importance of VP35 for interferon inhibition [65]. VP35 N-terminus contains an oligomerization domain [66] which is critical for NP-binding, VP40-binding [67] viral polymerase complex formation [68], RNAi silencing suppression [69], and protein kinase (PKR) inhibition [70]. VP35 inhibits PKR by inhibiting the phosphorylation of PKR (Fig. 1) [70].

The most fatal complication of Ebola infection is hemorrhagic diathesis. Computational genomic analysis of the Ebola virus suggests that viral selenoproteins might be responsible for the bleeding complications [71]. It has been well documented that selenium has an effect on the thromboxane and prostacyclin ratio and is involved in enhancing glutathione peroxidase activity and prostacyclin release in the endothelial cells [72]. An increase in synthesis has been noted in selenium supplementation and its deficiency results in low glutathione peroxidase activity [73]. Ramanathan's computational genomic analysis of the Ebola virus showed the presence of UGA-rich PPCRs and potential SECIS elements which might be responsible for rapid depletion of selenium in the host by synthesis of viral selenoproteins.

The mechanism involved in the aforementioned cellular entry has been termed a macropinocytosis-like mechanism [74]. Saeed and coworkers showed that the endocytosis of the virus is facilitated by actin polymerization. They also revealed that the process of macropinocytosis requires the activity of p53-activated kinase 1. Based on these findings, it has been suggested that the macropinocytosis-like pathway is the primary internalization mechanism for Ebola. The process of Ebola virus transcription within the host cell is still unknown. However, the key roles of VP30 and VP24 have been investigated in scientific studies. VP30 is thought to determine the process of transcription and its preinitiation [75, 76], whereas VP24 is involved in the inhibition of transcription and the replication of the Ebola virus genome [77].

Ebola glycoprotein is thought to disrupt cell adhesion. Francica and coworkers showed that the heavily glycosylated domain of the Ebola virus glycoproteins forms a steric shield over proteins at the surface of the host cell. This blocks the detection of the affected surface protein using antibody reagents. It also abrogates cell adhesion and prevents interactions with CD8 T-cells. Another strategy for avoiding CD8 T-cell-mediated killing of Ebola-infected host cells is thought to be the ability of viral glycoprotein to mask MHC-1 [78]. VP40 matrix protein has been documented as a viral protein responsible for budding newly assembled Ebola virus virions [79]. VP40 matrix protein interacts with cellular factors Nedd4 and Tsg101 to mediate the process of budding [80].

To summarize the total patho-mechanism involved in Ebola virus infection, it is important to consider the following points:

1. GP-mediated receptor binding followed by macropinocytosis of the Ebola virion into the endothelial cells.
2. Transport of the macropinosomes containing the Ebola virion into acidic compartments of the host cell where the fusion of the viral and cellular membranes occurs [75].
3. In this process, the infected host cell detaches itself from the neighboring cells and the basement membrane using the mechanism of GP-mediated steric occlusion by viral glycoprotein [78].
4. The newly created particles in the form of detached cells destabilize the vascular systems, resulting in the massive bleeding which is a typical symptom of Ebola patients [81].
5. The immune system is not able to react because of suppression of interferon production by VP35 action on interferon regulatory factor 3 [82].
6. Soluble GP limits the movement of white blood cells and plays an anti-inflammatory role by protecting the endothelial cell barrier function [83].
7. Due to the release of proinflammatory cytokines from the destroyed vascular endothelium, there is an activation of coagulation cascade leading to hypovolemic shock as a result from massive hemorrhage [84].

4 Clinical Presentation and Laboratory Diagnosis

Ebola virus disease (EVD) is the clinical syndrome caused by human infection with the Ebola virus species. The recent adoption of this term, in preference to Ebola hemorrhagic fever, recognizes that only a small proportion of patients experience major bleeding early in the disease course and, predominantly, initial clinical presentation results from substantial volume depletion caused by fever, profuse vomiting, diarrhea, and poor oral intake. In fact, clinical presentation ranges from asymptomatic to severe disease with ensuing shock, hemorrhagic symptoms, multiorgan failure dysfunction, and potentially death. The findings of epidemiological studies on the 2014–2016 West Africa Ebola outbreak corroborate the fact that

hemorrhagic symptoms are late and common only in the most severely ill patients [85–89].

The correct diagnosis and triage of EVD relies on epidemiological, clinical, and laboratory components. Potential patients may present with an abrupt onset of nonspecific flu-like symptoms such as fever, malaise, and headache followed by gastrointestinal symptoms like nausea, vomiting, abdominal pain, and diarrhea [86–91]. The incubation period ranges from 2 to 21 days; individuals are considered at risk if they had direct contact with a person with possible EVD within the last 3 weeks, highlighting the importance of obtaining a detailed epidemiological and travel history during clinical assessment [88, 92–94]. Frontline clinicians face a great challenge when dealing with patients with febrile illness and clinical history compatible with EVD, especially early in an EVD outbreak, when a large list of differential diagnoses with similar presentation in sub-Saharan Africa, combined with unawareness of the local introduction of Ebola virus in humans, usually leads to missed or delayed identification of cases with continued person-to-person transmission [92, 93].

Local health authorities and most international organizations providing care for EVD patients in the 2014–2016 West Africa outbreak relied on case definitions adapted to local context to guide the initial clinical approach, infection prevention and control, and epidemiological efforts [92, 93]. Ebola virus disease has no approved specific treatment, but prompt identification and diagnosis is crucial to initiate resuscitative and supportive measures before the development of shock and multi-organ failure and to institute infection prevention and control procedures halting further transmission [95].

4.1 Clinical Presentation

Outbreaks of different species of Ebola virus may have distinct clinical courses and overall prognoses [89]. For example, the case fatality rate for one Bundibugyo Ebola strain outbreak was approximately 25%, whereas Zaire outbreaks may have up to 90% mortality. The reason for such wide variation is not currently known, but availability of resources, differences in case mix, and high viral loads may all play a role separate to the differences in virus strains [85–88, 91]. Patients with confirmed EVD in the 2014–2016 West Africa outbreak usually presented with abrupt and unspecific signs and symptoms after 3–10 days (incubation period range 2–21 days) of presumed exposure. The most common initial presentations included sudden-onset fever and weakness/fatigue with a combination of the following: chills, maculopapular rash, malaise, headaches, nausea, vomiting, watery diarrhea, and loss of appetite. Other common complaints early in the disease course were retrosternal pain, abdominal pain, hiccups, and sore throat. Poor documentation and considerable variation in clinical presentation resulted in delayed characterization of a typical EVD case [85–87, 91]. Cases at the severe end of the clinical spectrum presented with additional symptoms indicating impairment of immune,

vascular, and coagulation systems. A typical pathway for the severe patients resembled septic shock, with a continuum of dehydration and systemic inflammatory response leading to hypotension, shock, and multi-organ failure, often progressing to death [95].

The erythematous maculopapular rash present in previous outbreaks was less common in West Africa. Minor hemorrhagic manifestations such as petechiae, ecchymoses, mucosal bleeding, pregnancy-related vaginal bleeding, and oozing from vascular access and other venipuncture sites were described in 30–50% of patients. Conversely, major bleeding was rare and usually happened late in the course of the disease in severely ill patients, predicting death. Central nervous system manifestations such as delirium or a meningoencephalitis-type syndrome with decreased level of consciousness, nuchal rigidity, and seizures were frequently found in EVD patients. However, shock and progression to multi-organ failure, markers of severe clinical course, were the most likely causes of decreased level of consciousness in the majority of this population [85–87, 91]. As expected in critically ill patients, some developed complications secondary to the severity of illness or following therapy (i.e., bacterial sepsis, renal failure, or respiratory failure after fluid resuscitation). These patients required intensive life support measures which were not available in Africa [96–98].

Lack of standardized registries, limited clinical documentation, and shortcomings in the working practices of Ebola treatment centers hindered efforts to improve the understanding of the early specific signs and symptoms associated with severe disease. Non-survivors seemed to have early clinical deterioration invariably progressing to shock and multi-organ failure, but it was difficult for healthcare providers to predict this progress at the bedside early in the course of the disease [85–87, 91, 95].

Retrospective studies on previous EVD outbreaks in sub-Saharan Africa have tended to be focused on detailed clinical and epidemiological accounts of severe and fatal cases admitted to healthcare facilities. A few cases of asymptomatic seroconversions and less severe clinical presentations have also been described, suggesting that hospitals and Ebola treatment centers are likely to miss cases at both ends of the severity spectrum [99].

Survivors usually start improving toward the end of the second week of illness and experience a prolonged convalescence period with ongoing and new symptoms and signs specific to the chronic phase of the disease [100–102].

4.2 Laboratory Findings

Laboratory investigations are necessary in EVD patients for four reasons: differential diagnosis from other infectious and noninfectious diseases, confirmation and assessment of severity of EVD, documentation of cure or noninfectiousness of convalescent patients, and assessment of severity or organ dysfunction (i.e., hematology, biochemistry, liver function). Any blood or other bodily fluid from

these patients should be considered highly infectious. Proper risk assessment procedures and infection prevention and control measures must be in place prior to the decision to attempt specimen collection so as to ensure staff safety during collection and handling of the material. EVD confirmation usually requires shipment of the specimen to highly specialized laboratories in reference centers. Detailed guidelines on EVD specimen collection, handling, and shipment are available from the World Health Organization, US Centers for Disease Control and Prevention, and Public Health England and should be followed according to standard operational procedures for each organization or healthcare facility.

An efficient and effective approach to the vast list of differential diagnosis in patients with a clinical presentation compatible with EVD depends on demographic characteristics, specific signs and symptoms, residence location, local endemic disease profile, travel history, risk assessment of potential contacts, vaccination, and comorbidities [93, 94]. In regions where malaria is endemic, frontline healthcare providers should consider rapid diagnostic testing for malaria in the initial assessment of all potential EVD patients that meet case definition criteria. Other differential diagnostic exams should be ordered on an individual basis (i.e., typhoid fever, blood and other fluid cultures, Lassa fever, other viral hemorrhagic fevers, leptospirosis, cholera, pregnancy, etc.) and tailored to the factors outlined above [93].

The vast majority of patients that presented with clinical features that met case definition criteria, even during the 2014–2016 West Africa outbreak, did not have EVD but rather a different diagnosis. This was particularly relevant after the early phase of the outbreak, when surveillance efforts improved and the number of patients on contact tracing efforts increased; also most healthcare facilities were closed or working with reduced capacity during the outbreak, concentrating acute care of all patients in Ebola treatment centers.

Patients that met case definition in the 2014–2016 West Africa outbreak usually had their EVD diagnosis confirmed with a RT-PCR assay. This test detects specific RNA sequences of the Ebola virus within 3 days of symptom initiation. However, in some cases, the rise in blood viral load may take longer to be detectable by the RT-PCR assays utilized in clinical practice. For this reason, it is not recommended to test asymptomatic individuals or patients that do not meet case definition. A negative RT-PCR within 3 days of symptom onset must be repeated in 24–48 h, whereas a negative test after 3 days of symptoms rules out EVD. As an alternative, testing for viral antigens by enzyme-linked immunosorbent assay (ELISA) was widely available and performed in local and field laboratories during previous outbreaks and, depending on the local expertise and availability, could be utilized to confirm EVD. Other assays currently have limited clinical use [85, 86, 91]. The correct clinical interpretation of blood test results may follow local guidelines and require consultation with a specialist in infectious diseases or viral hemorrhagic fevers [92–94]. High viral loads, and consequently low Ct values, may be associated with the severity of clinical presentation, organ dysfunction, and mortality [103].

Discharge criteria for confirmed EVD patients, with minor variations in each facility, typically required a negative result on a repeat RT-PCR 72 h after symptom resolution, i.e., gastrointestinal together with the ability to perform self-care or availability of family support to assist in basic daily activities [89, 104]. The rapidly growing caseload and unprecedented demand for hospital admissions in already precarious healthcare systems led to calls for a discharge criteria that did not require a repeat negative RT-PCR [105].

Assessment of organ or system dysfunction is the third reason to conduct laboratory investigations in EVD patients. In West African Ebola treatment centers, most blood tests were made in adjacent, deployable mobile laboratories operated by international organizations [104]. The primary function of these mobile laboratories was to perform real-time reverse transcriptase-polymerase chain reaction (RT-PCR) testing specific to the Ebola virus; most of these laboratories did not offer other simpler tests for clinical care (i.e., hematology, biochemistry). Lack of availability of clinical tests in local and international laboratories and lack of material and human resources hindered the delivery of supportive care, leading to common abnormalities going unrecognized and untreated in Ebola treatment centers [95, 104].

Laboratory findings in EVD have great variation and overlap during disease progression phases, making it difficult to establish when specific abnormalities should be present. In the 2014–2016 West Africa outbreak, leukopenia, thrombocytopenia, and abnormal transaminases were common in the first 48–72 h after symptom onset. However, transaminase abnormalities and thrombocytopenia usually persisted after the initial period. Renal dysfunction and electrolyte abnormalities (hypomagnesaemia, hypokalemia, and hyponatremia) developed as gastrointestinal symptoms and volume depletion worsened [85–87, 91]. Electrolyte abnormalities may be challenging to manage in clinical settings, in particular with continuing gastrointestinal symptoms and a lack of laboratory resources [95, 106]. When renal failure ensued, hyperkalemia and severe metabolic acidemia were of concern in patients treated in high resources settings. Later in the disease course, coagulation tests are usually abnormal even in the absence of clinical bleeding. Other laboratory findings included severe hypoalbuminemia, elevated amylase, lactate, metabolic acidosis, and worsening of the other parameters described above [85–87, 91].

5 Clinical Management: Current and Future Approaches

5.1 Supportive Care

Current treatment available for EVD mainly includes supportive care including fluid management, antipyretics, analgesics, antiemetics, and blood transfusion for acute bleeding manifestations. A study of 27 patients (median age, 36 years, range

25–75) treated in the United States and Europe showed that early presentation of disease and receipt of supportive care, intravenous fluid resuscitation, careful fluid management and electrolyte replacement to overcome metabolic abnormalities, nutritional support, and critical care might help to reduce mortality in patients with EVD [98].

5.2 Vaccines

The vaccines under development for Ebola can be classified into two main categories, namely, non-replicating and replicating vaccines. The non-replicating vaccine category is subclassified into inactivated vaccines, subunit vaccines, and vector-based vaccines. In the case of vector-based vaccines, the immunogen is expressed from DNA or viral vectors, whereas in subunit vaccines, the immunogen is delivered in the form of viruslike particles or recombinantly expressed purified proteins.

5.2.1 Non-replicating Ebolavirus Vaccines

Inactivated Vaccines

This was the first attempt to develop a vaccine against Ebola. Formalin or heat-inactivated virus preparations were used, but unfortunately no significant immunogenic response was observed in mice and nonhuman primates (NHP) [107–109].

Replicons

In the second major attempt to design a vaccine suitable for Ebola, Venezuelan equine encephalitis virus was used to design the replicon for Ebola by replacing its structural genes with *Zaire* Ebola virus glycoprotein. These replicons were highly protective in mice after two vaccination doses but unfortunately failed to protect nonhuman primates [108].

DNA Vaccines

A three-plasmid DNA vaccine, which encodes the envelope glycoproteins from the *Zaire* and *Sudan* Ebola was evaluated in a randomized, placebo-controlled, double-blinded, dose escalation study by Martin and coworkers. This study was conducted as a phase I clinical trial in healthy adults between 18 and 44 years of age [110, 111]. This vaccine is found to be well tolerated in the healthy individuals and is immunogenic in humans. But the prime/boost dose was found to be

ineffective at initiating immune response after 1 year. The possibility of combining the vaccine with others to increase the efficacy needs further investigation.

Ebola rAd5 Replication Defective Vaccine

This vaccine is genetically engineered to express the genes for EBOV glycoprotein *Zaire* type (GP Zaire) and glycoprotein *Sudan* type (SUDV GP). The phase I trials showed that this vaccine provided 100% protection in *Cynomolgus macaques*, if vaccination was done 28–35 days prior to challenge. Both potent humoral and cell-mediated immune responses were observed [112]. In human trials, 31 healthy adults received vaccine at 2 × 10(9) (*n*¼ 12) or 2 × 10 (10) (*n*¼ 11) viral particles as an intramuscular injection or placebo (*n*¼ 8). Significant antibody titers were seen after 48 weeks of vaccination [113]. The T-cell activation correlated with the dose administered. Further investigations are ongoing to develop the vaccine for commercial use.

Other Adenovirus Vector Vaccines

rAd5 vaccine is undergoing intensive development with the help of complex adenovirus technology to increase the genetic payload capacity of the vector. This new strategy also involves blending the glycoprotein of *Zaire ebolavirus* (Ebola virus, EBOV), *Sudan ebolavirus* (Sudan virus, SUDV), and *Marburg* virus (MARV). A *panfilovirus* vaccine based on the complex adenovirus technology was found to demonstrate 100% protection against two species of Ebola and three species of Marburg virus in nonhuman primates [114]. Although the new adenovirus vector vaccine seems to be promising, preexisting immunity to the Ad5 vector might be problematic in its practical immunogenic utility.

Subunit Vaccines

Subunit vaccines are divided into classical subunit vaccines and viruslike particles. Classical subunit vaccines use purified recombinantly expressed viral proteins. Subunit vaccines have been much developed for cases of Ebola. An attempt was made to use baculovirus-derived glycoprotein to produce immunity against Ebola in guinea pigs. After three immunizations and subcutaneous application of the vaccine into guinea pigs, 50% protection was achieved against Ebola [115].

Another attempt to develop a subunit vaccine included a vaccine in which the ectodomain of *Zaire ebolavirus* glycoprotein was fused to a human Fc fragment in order to achieve purification. This subunit vaccine showed 83% protection in challenged mice after four vaccinations [116]. However, classical subunit vaccines still need further development to be eligible candidates for use in nonhuman primates.

On the other hand, the complex viruslike particle-based vaccines appear to be highly promising. Viruslike particles containing viral protein 40, glycoprotein, and nucleoprotein showed protection against Ebola in rodents [117, 118]. Vaccination efficacy appeared to be dose-dependent in the case of mice [119]. One of the main problems in further development of viruslike particles for use in humans is the difficulty in large-scale production of such particles, which requires sophisticated manufacturing conditions.

Replication-Deficient Ebola Viruses

With the help of reverse genetic engineering, Ebola virus vaccines are under development [120]. rEBOVΔVP30 is under development to provide protection in humans. It has been shown to provide 100% protection in both mice and guinea pigs against a challenge test after two vaccinations [121]. The rEBOVΔVP30 lacks viral protein 30, which is required to produce infectious progeny. This limits the life span of this virus to only one infectious cycle.

5.2.2 Replicating Ebola Viruses Vaccines

Recombinant Vesicular Stomatitis Virus (rVSV)

This is the first replicating Ebola virus vaccine which showed protection in nonhuman primates [122]. There were no signs of disease seen after vaccination. This vaccine was developed by replacing vesicular stomatitis virus glycoprotein with *Zaire ebolavirus* glycoprotein, resulting in rVSV/ΔG/GP virus. This virus can undergo replication, and hence a relatively small dose is required for successful vaccination [123]. VSV did not show any disease signs in nonhuman primates infected with simian-human immunodeficiency virus [124]. The rVSV vaccine showed 100% protection after mucosal immunization through the oral or intranasal route in nonhuman primates [125].

The recombinant VSV has also shown postexposure protection in 24 h post-challenge experiments [126]. If the vaccine is provided within 30–60 min postexposure, then the chances of survival lie between 50 and 83% in nonhuman primates [127]. This makes it a promising candidate for laboratory postexposure prevention. Clinical trials are underway to prove its efficacy and safety in humans. The Guinea ring vaccination cluster-randomized trial (phase III study), conducted between April 1, 2015, and July 20, 2015, to test the effectiveness of an rVSV-vectored vaccine expressing Ebola surface proteins, showed that rVSV-ZEBOV might be highly efficacious and safe in preventing EVD, if it is administered during an Ebola virus disease outbreak via a ring vaccination strategy. As per definition, ring vaccination means vaccination of individuals at high risk of infection [128].

Recombinant Human Parainfluenza Virus Type 3

Human parainfluenza virus type 3 is a respiratory pathogen. It was chosen to develop vaccines against Ebola which can be administered via the respiratory route. To develop this vaccine, a transcription cassette encoding the glycoprotein gene was inserted between the P and M genes of HPIV3. This resulted in the formation of recombinant HPIV3 virus (rHPIV3/GP) which carried *Zaire ebolavirus* glycoprotein on its surface [129]. This vaccine was found to provide 100% protection in guinea pigs and in nonhuman primates without any signs of viremia [130]. The only problem which exists in the use of the vaccine in humans is preexisting immunity to adenovirus.

Rabies Virus

Rabies virus has been also explored to serve as vaccine platform against Ebola viruses. Point mutation in rabies glycoprotein gene was done to reduce the neurovirulence, and *Zaire ebolavirus* glycoprotein was introduced as an additional transcription unit between the N and P genes. The administration of this recombinant vaccine did not produce disease symptoms in mice [130]. A single vaccination showed 100% protection in the mice after single vaccination [131]. There is no data available about the vaccine's efficacy in nonhuman primates.

5.3 Novel Compounds and Drugs

To date, Ebola treatment is more or less confined to symptomatic treatment. However, in the West Africa outbreak, attempts were made to treat infected patients with convalescent serum [18] or equine anti-Ebola immunoglobulin with interferon [132]. Although the recipients of these interventions survived, the mechanism of their action still remains unknown.

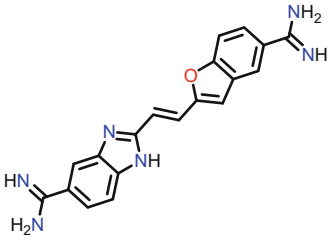
Since last year, there has been rapid development of antiviral compounds which are undergoing tests in rodents and nonhuman primates.

5.3.1 Antiviral Compounds Undergoing Test in Rodents

FGI-103, FGI-104, and FGI-106

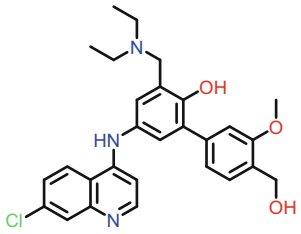
FGI-103 (6) [133] is a low-molecular-weight compound which was discovered during an in vitro screening assay utilizing a variant of *Zaire ebolavirus* that expresses green fluorescent protein. The study conducted by Warren's research

group showed that in vivo administration of FGI-103 as single intraperitoneal dose of 10 mg/kg delivered within 24 h of infection could sufficiently protect mice in the lethal challenge experiment with *Zaire ebolavirus* strain. FGI-103 was found to reduce the burden of virus on kidney, liver, and spleen tissues. The chemical compound FGI-103 is 2-(2-(5-(amino(imino)methyl)-1-benzofuran-2-yl)vinyl)-1*H*-benzimidazole-5-carboximidamide. FGI-103 showed dose-dependent inhibition of not only *Zaire ebolavirus* strain but also *Sudan ebolavirus* and *Marburg* virus.



FGI-103 (6)

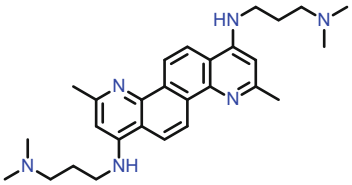
FGI-104 (7) [134] demonstrates inhibition of multiple pathogens (hepatitis C, hepatitis B, HIV) and biothreats (Ebola, cowpox). The chemical name of FGI-104 is 4-[(7-chloroquinolin-4-yl)amino]-2-(diethylaminomethyl)-6-[4-(hydroxymethyl)-3-methoxyphenyl]phenol. FGI-104 showed a dose-dependent inhibition of Ebola virus in the cell-based assays of Ebola hemorrhagic fever virus. FGI treatment in Ebola-infected mice prevented the death of the infected animals.



FGI-104 (7)

FGI-106 (8) is the last of the small molecule therapeutics undergoing testing in rodents. Like FGI-103 and FGI-104, FGI-106 has shown inhibition activity not only against Ebola but also against Rift Valley and dengue fever viruses in cell-based assays. The study conducted by Aman and coworkers showed that a single treatment, administered 1 day after infection, is sufficient to protect animals from a lethal Ebola virus challenge. The rodent model experiment revealed that treatment with 2 μM FGI-106 mediated a 4 log reduction in infectious viral titers relative to matched controls, with an EC90 (effective concentration) for inhibition of

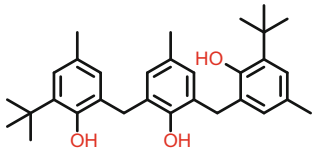
viral killing of host cells estimated to be 0.6 μM [135]. The chemical compound FGI-106 is quino[8,7-h]quinoline-1,7-diamine,N,N_0-bis[3-(dimethylamino)propyl]-3,9-dimethyl-tetrahydrochloride.



FGI-106 (8)

NSC 62914

NSC 62914 (9) was identified during small molecule chemical screening for Ebola virus inhibitors. Panchal and coworkers observed its anti-filovirus activity in cell-based assays. It protected mice in vivo following challenge with Ebola or Marburg virus [136]. In addition, the compound showed inhibition of Rift Valley fever virus, Lassa virus, and Venezuelan equine encephalitis virus. The antiviral action is based on the antioxidant properties of the compound.



NSC62914 (9)

The compound NSC 62914's antioxidant property is due to the presence of the three aryl-OH groups that function by scavenging reactive oxygen species before they can interact with other molecules. This prevents cellular damage to in vivo systems.

However, the compound NSC 62914 evaluation in the mouse model for Ebola infection [137] showed that a treatment dose of 2 mg/kg injection provided protection in 50% of the mice, but treatment with a higher dose of 5 mg/kg injection did not improve the protection due to the toxic side effects of the compound. This has restricted its trials in nonhuman primate models. Nonetheless, the discovery of this compound might help develop further antioxidant compounds against filoviruses.

Small Interfering RNAs (siRNAs)

siRNAs are also called short-interfering RNAs or silencing RNAs. They are a class of double-stranded RNA molecule, 20–25 base pairs in length, with the potential to

interfere in the transcription process. siRNAs are under development, and the investigation phase for their therapeutic use is ongoing because disease is also an outcome of multiple gene activity.

An important example in reference to Ebola is TKM-100802 lipid nanoparticle siRNA, a product of Tekmira. siRNA specifically targets RNA polymerase I protein in the *Zaire ebolavirus*. They work by inhibiting transcription by breaking down mRNA [138]. A study conducted by Geisbert showed that nonhuman primates survived Ebola challenge testing after administration of anti-ZEBOV siRNAs at time intervals of 30 min, day 1, day 3, and day 5 [139]. Another study conducted by Emily and coworkers showed that siRNAs adapted to target the *Makona* outbreak strain of Ebola virus are able to protect 100% of rhesus monkeys against lethal challenge if treatment is initiated within 3 days after exposure. siRNA administration in the postexposure phase also reduced the intensity of disease symptoms in the infected animal [140]. A recent single-arm phase 2 trial showed that Tekmira's siRNA did not improve survival of adults with advanced Ebola virus disease when compared to historic controls. Additional work is required to understand if the results of this trial are generalizable to all Ebola virus disease subpopulations (e.g., less severe cases) and treatment settings [103].

Phosphorodiamidate Morpholino Oligomers (PMOs)

Phosphorodiamidate morpholino oligomers (PMOs) are uncharged nucleic acid-like molecules designed to inactivate the expression of specific genes via antisense-based steric hindrance of mRNA translation. Swenson and coworkers showed that PMO can be a valuable therapeutic agent in treating filovirus infections. They showed that mice treated with a PMO sequence complementary to a region spanning the start codon of VP24 mRNA were protected against lethal Ebola virus challenge [141]. Recent investigations have shown that VP24 is associated with the ribonucleoprotein complex, inhibiting viral RNA replication and transcription [77].

PMOs specifically targeting VP24 and VP35 are also under investigation. Intraperitoneal administration of a VP24-specific PMO has shown to be protective in mice following a lethal EBOV challenge [141].

Enterlein and coworkers showed that conjugation of a VP35-specific PMO with an arginine-rich cell-penetrating peptide improved its efficacy in mice [142]. Another study conducted by Warfield and coworkers showed that PMOs also protected 75% of rhesus macaques from lethal EBOV infection [143].

The in vivo evaluations of the PMOs have shown its effect as a prophylactic treatment in mice, but its potential in a postexposure scenario needs to be studied.

Monoclonal Neutralizing Antibodies (NABs)

Antiviral antibodies play a critical role in protection against infection or disease. The role of antibodies in treatment of lethal EBOV infection has been long under

investigation. It has included transferring serum or immune globulin from an immune to a naive individual either before or immediately after exposure to the lethal virus. Parren and coworkers investigated the pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody called KZ52 [144]. Administration of KZ52 before or up to 1 h after challenge resulted in dose-dependent protection by antibodies in guinea pigs. The results revealed that KZ52 was more effective as pre-exposure prophylaxis but could be also considered as a postexposure prophylactic candidate, if administered within 1 h of exposure to the virus. Monoclonal antibody KZ52 provided protection against Ebola virus by reducing plasma viremia. However, further studies in nonhuman primate models are required to further develop KZ52 use in humans.

Triple Monoclonal Antibody Cocktail (ZMapp™)

Monoclonal antibody (mAb) cocktails have also been considered as potential candidates for the postexposure treatment of Ebola. MB-003 and ZMAb have been extensively evaluated for their efficacy in both in vitro and in vivo studies.

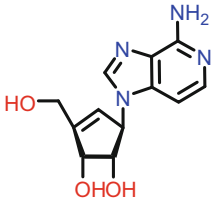
ZMapp™ is a combination of the two monoclonal antibodies. However, the epitope information and mechanism of action are unknown for most of the monoclonal antibodies. Computational biological studies conducted by Murin and coworkers showed that there are sites of vulnerability on the Ebola virus glycoprotein which might be used as future target for the development of monoclonal antibodies [145]. Another study in nonhuman primates conducted by Qiu and coworkers showed that the ZMapp™ monoclonal antibodies could reverse the elevated liver enzymes, mucosal hemorrhages, and generalized petechial caused by Ebola virus in nonhuman primates [146]. ZMapp™ was given to seven patients with EVD in 2014. Five of these patients survived, leading to the recognition of ZMapp™ as a potential candidate to be developed at rapid pace against Ebola [147].

ZMapp™ composition consists of three monoclonal antibodies: c13C6, c2G4, and c4G7 [146]. The three monoclonal antibodies bind to the mucin containing Ebola glycoprotein. The epitope region GP1/GP2 is responsible for binding c2G4 and c4G7, whereas c13C6 binds to glycan Cap/sGP [145]. The binding sites on the virus are considered to be the vulnerability regions on the surface of the virus. Ongoing clinical trials will determine the potential side effects and contraindication for the treatment. In the 2014–2016 West Africa outbreak, 25 patients received ZMapp™, of which only 2 died. However, further trials are required since those 25 patients received intensive supportive care along with ZMapp™ [97].

S-Adenosyl-L-Homocysteine Hydrolase Inhibitors

S-adenosyl-L-homocysteine hydrolase is an enzyme of the activated methyl cycle, required for the reversible hydration of *S*-adenosyl-L-homocysteine into adenosine

and homocysteine. It is a ubiquitous enzyme which binds and requires NAD+ as a cofactor. Blockage of this cellular enzyme can block replication of both RNA and DNA viruses. A study conducted by Bray and coworkers showed that 3-deazaneplanocin A, an *S*-adenosyl-L-homocysteine hydrolase inhibitor, prevented illness and death in mice infected with a mouse-adapted variant of *Zaire* Ebola *Zaire* strain [148].



3- Deazaneplanocin A (10)

In the study, it was observed that 3-deazaneplanocin A (10) appears to reverse interferon-α suppression induced by Ebola virus in host cells and restricts viral dissemination. However, further development studies are still needed to identify other adenosine analogues which can also produce similar effects in Ebola virus-infected primates.

5.3.2 Antiviral Compounds Currently Tested in Nonhuman Primate Models of Ebola Infection

Recombinant Human-Activated Protein C (rhAPC)

Later complications of Ebola virus infection involve hypotension, coagulation disorders, and an impaired immune response that resembles sepsis. Hensley and coworkers tested the effectiveness of rhAPC in 14 rhesus macaques challenged with a uniformly lethal dose of *Zaire* Ebola virus (ZEBOV). 11 monkeys out of 14 were treated by IV infusion with rhAPC within 30–60 min after challenge, and treatment was continued for a period of 7 days. It was observed that 2 out of the 11 rhAPC-treated monkeys survived [149].

The ZEBOV-infected monkeys that showed response to rhAPC infusion had low viral loads, reduced activation of coagulation, and attenuation of the systemic inflammatory response. However, the investigators could not identify the reason that the remaining nine macaques showed no response to rhAPC. rhAPC is currently licensed for use in the treatment of sepsis. It has been shown to have antithrombotic properties related to inactivation of factors Va and VIIa [150] and inhibition of plasminogen activator inhibitor 1 [151]. The use of rhAPC in

combination with other antiviral approaches might be helpful to improve survival rates during an Ebola outbreak; however, further investigation is still required in this field to obtain clear evidence.

Recombinant Nematode Anticoagulant Protein C2 (rNAPC2)

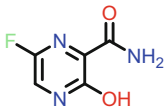
It is well known that individuals infected with the Ebola virus develop coagulation abnormalities. The coagulation disorders have been observed in primates [152]. This activation of coagulation disorder was the reason for the administration of rNAPC2, a potent inhibitor of tissue factor-initiated blood coagulation, to the macaques either 10 min (n¼ 6) or 24 h (n¼ 3) after a high-dose lethal injection of Ebola virus. In this experiment, it was observed that survival time was increased by approximately 33% [153] in each treatment group. It was found that rNAPC2 attenuated the proinflammatory response in the host cell.

5.3.3 Other Drugs with Antiviral Activity Against Ebola

Favipiravir

Favipiravir (11), also known as pyrazinecarboxamide derivative T-705, was first produced by Toyama Chemical (Japan) as a potent inhibitor of influenza virus replication [154]. The drug is under the final phase of clinical development for the treatment of flu [155]. It has been observed that favipiravir is converted by host enzymes into T-705-ribofuranosyl-50-triphosphate and presumably acts as a nucleotide analogue that selectively inhibits the viral RNA-dependent RNA polymerase. This prevents virus replication [156]. T-705 has not only shown antiviral activity against influenza virus but also against other negative-strand RNA viruses such as arenavirus and bunyavirus [157]. In light of these reports, Ostereich and coworkers tested the compound's antiviral activity against EBOV, a negative-strand RNA virus. It was observed that T-705 suppressed the replication of *Zaire* EBOV in cell culture by 4 log units and it has an IC90 (inhibitory concentration) of 110 μM. In the mouse experiment, it was observed that if T-705 is administered at day 6 postinfection, then it induces rapid virus clearance and reduces the biochemical parameters of the disease [158]. However, further studies in the nonhuman primate model are required to assess the antiviral activity of favipiravir against Ebola.

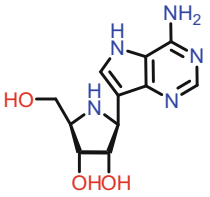
A recent nonrandomized trial study of 126 patients, who were included between December 17, 2014, and April 8, 2015, during 2014–2016 Ebola outbreak, showed that favipiravir monotherapy requires further study in patients with medium to high viremia. It was not found to be a promising candidate for treatment of very high viremia. Out of the 111 patients who could be analyzed out of the 126, 60 patients died and 51 patients survived [159].



Favipiravir (11)

BCX4430

BCX4430 (12) is an adenosine analogue antiviral drug [160], developed specifically against hepatitis C by BioCryst Pharmaceuticals. BCX4430 inhibits viral RNA polymerase activity indirectly through non-obligate RNA chain termination. Apart from filovirus, BCX4430 has also shown antiviral properties against *Togaviridae*, *Bunyaviridae*, *Arenaviridae*, *Paramyxoviridae*, *Orthomyxoviridae*, *Picornaviridae*, and *Flaviviridae* [161].

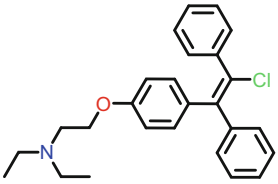


BCX4430 (12)

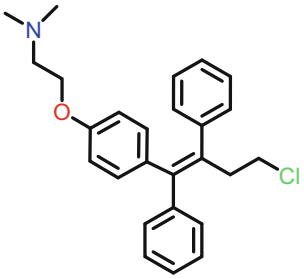
Selective Estrogen Modulator

In 2014, the US Food and Drug Administration (FDA) performed an in vitro screen of US approved drugs which could show antiviral activity against *Zaire ebolavirus* (EBOV-Z). The study, conducted by Johansen and coworkers, showed that clomiphene (13) and toremifene (14) can act as potent inhibitors of EBOV infection. It was observed that clomiphene and toremifene inhibits EBOV infection in both in vitro and in vivo models. The ELISAs showed that the compounds inhibited viral infection of all *Zaire ebolavirus*, *Sudan ebolavirus*, *Marburg* virus, and *Ravn* virus. The PCR confirmed this result for both the compounds. In murine EBOV infection models, clomiphene and toremifene were administered for 6 consecutive days after target virus exposure. The treatment of infected mice showed significant survival benefits. Later, the mechanism underlying the antiviral property of selective estrogen modulators was investigated, and it was found that anti- EBOV activity occurred even in the absence of detectable estrogen receptors. This suggests that the response is an outcome of an off-target effect in which the compounds interfere with a step late in viral entry and likely interfere with fusion of the virus [162].

Currently, the selective estrogen modulators are being considered for use as medical countermeasures alone or in combination with antiviral drugs against Ebola.



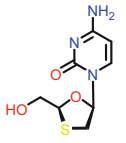
Clomiphene (13)



Toremifene (14)

Lamivudine

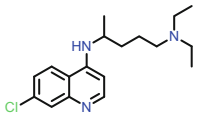
Lamivudine (15) is a well-known, antiretroviral drug approved for the treatment of HIV and hepatitis B virus. Its mechanism of action includes inhibition of HIV reverse transcription by DNA chain termination. Structurally it is a cytosine analogue, and it also inhibits the RNA and DNA polymerase activity of reverse transcriptase [163]. To date, there are no in vivo or in vitro studies evaluating lamivudine antiviral activity against Ebola virus.



Lamivudine (15)

Chloroquine

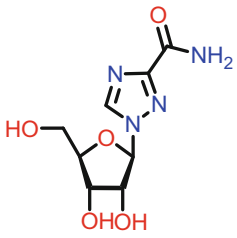
Chloroquine (16) is a well-known antimalarial drug. In the FDA screening program, chloroquine was tested for its antiviral activity against Ebola virus. It was observed that chloroquine possessed antiviral activity against Ebola in both in vitro and in vivo studies. 90 mg/kg dose of chloroquine, if administered twice a day, resulted in survival rates of 90 and 80% in mouse Ebola virus models [164]. However, chloroquine has not shown survival benefits in human studies against dengue virus and chikungunya virus infection [165]. This raises doubts on its effectiveness against Ebola virus in humans.



Chloroquine (16)

Ribavirin

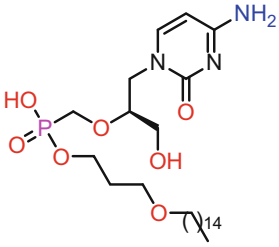
Ribavirin (17) is a well-known broad-spectrum antiviral drug. It has shown both prophylactic and therapeutic efficacy against arenavirus infection in guinea pigs and monkeys. The compound reduced mortality in humans suffering from Lassa fever. Unfortunately, it is not effective in animal models of filoviral and flaviviral infection [166].



Ribavirin (17)

Brincidofovir

Brincidofovir (18) is hexadecylpropyl ester of cidofovir, which can be administered orally and has less nephrotoxicity than cidofovir. It is regarded as a prodrug form of cidofovir. It has been developed by Chimerix, who have also conducted clinical trials into its efficacy against viral diseases. It was initially developed against DNA virus types, but it showed inhibition activity against Ebola virus in in vitro tests. However, there was no evidence of efficacy in small animal models or nonhuman primates [167].



Brincidofovir (18)

6 Conclusion

There has been a substantial progress in the effort to control EVD. During the course of the 2014–2016 outbreak, the research and development of both vaccines and drugs against EVD have ignited opportunities that were not previously foreseen. In addition, it must not be forgotten that control and prevention should take a holistic approach. Personal protective equipment and primary prevention measures remain the first line of defense against the deadly virus. Supportive care should continue to be encouraged. The considerable efforts of participating organizations have enhanced knowledge and awareness about EVD worldwide. It remains hopeful that an effective vaccine or drug will be available for safe administration in humans against the Ebola virus in the near future.

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References

1. WHO (1978) Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. Bull World Health Organ 56:247–270
2. Martini GA, Siegert R (1971) Marburg virus disease. Springer, Berlin
3. Demarcus TA, Tripple MA, Ostrowski SR (1999) US policies for disease control among imported nonhuman primates. J Infect Dis 179(Suppl 1):282–283
4. Jahrling PB, Geisbert TW, Dalgard DW (1990) Preliminary report: isolation of Ebola virus from monkeys imported to USA. Lancet 335:502–505
5. Peters CJ, Jahrling PB, et al (1991) Filoviruses. Emerging viruses. Oxford University Press, New York
6. Le Guenno B, Formenty P, Wyers M, et al (1995) Isolation and partial characterization of a new strain of Ebola virus. Lancet 345:1271–1274
7. Junfa Y, Zhang Y, Jialu L, et al (2012) Serological evidence of ebolavirus infection in bats, China. J Virol 9:236
8. Barrette RW, Metwally SA, Rowland JM, et al (2009) Discovery of swine as a host for the Reston ebolavirus. Science 325:204–206
9. Leroy EM, Kumulungi B, Pourrut X, et al (2005) Fruit bats as reservoirs of Ebola virus. Nature 438:575–576
10. Gonzalez JP, Pourrut X, Leroy E (2007) Ebolavirus and other filovirus. Curr Top Microbiol Immunol 315:363–387
11. Walsh PD, Abernethy KA, Magdalena B, et al (2003) Catastrophic ape decline in western equatorial Africa. Nature 422:611–614
12. Vogel G (2003) Conservation biology can great apes be saved from Ebola? Science 300(5626):1645
13. Baron RC, Zubeir OA (1983) Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. Bull World Health Organ 61:997–1003
14. Richards GA, Murphy S, Jobson R, et al (2000) Unexpected Ebola virus in a tertiary setting: clinical and epidemiologic aspects. Crit Care Med 28:240–244

15. Miranda ME, White ME, Dayrit MM, et al (1991) Seroepidemiological study of filovirus related to Ebola in the Philippines. Lancet 337:425–426
16. CDC (1990) Update: filovirus infection in animal handlers. MMWR Morb Mortal Wkly Rep 39:221
17. Miranda ME, Ksiazek TG, Retuya TJ, et al (1999) Epidemiology of Ebola (subtype Reston) virus in the Philippines, 1996. J Infect Dis 179(Suppl 1):115–119
18. Emond RT, Evans B, Bowen ET, et al (1977) A case of Ebola virus infection. Br Med J 2:541–544
19. Akinfeyeva LA, Vasilyevich IV, Ginko ZI, et al (2005) A case of Ebola hemorrhagic fever. Infektsionnye Bolezni 3:85–88
20. Borisevich IV, Markin VA, Firsova IV, et al (2006) Hemorrhagic (Marburg, Ebola, Lassa, and Bolivian) fevers: epidemiology, clinical pictures, and treatment. Vopr Virusol 51:8–16
21. Schoepp RJ, Rossi CA, Khan SH, et al (2014) Undiagnosed acute viral febrile illnesses, Sierra Leone. Emerg Infect Dis 20:1176–1182
22. Shuaib F, Gunnala R, Musa EO, et al (2014) Ebola virus disease outbreak – Nigeria, July-September 2014. MMWR Morb Mortal Wkly Rep 63:867–872
23. WHO (2014) Disease outbreak news: Ebola virus disease – Mali. http://www.who.int/csr/don/31-october-2014-ebola/en/. Retrieved 28 June 2015
24. WHO (2014) Disease outbreak news: Ebola virus disease update – Senegal. http://www.who.int/csr/don/2014_08_30_ebola/en/. Retrieved 28 June 2015
25. Georges AJ, Leroy EM, Renaut AA, et al (1999) Ebola hemorrhagic fever outbreaks in Gabon, 1994–1997: epidemiologic and health control issues. J Infect Dis 179(Suppl 1):65–75
26. Pourrut X, Kumulungui B, Wittmann T, et al. (2005) The natural history of Ebola virus in Africa. Microbes Infect 7:1005–1014
27. Leroy EM, Epelboin A, Mondonge V, et al (2009) Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. Vector Borne Zoonotic Dis 9:723–728
28. Rodriguez LL, De Roo A, Guimard Y, et al (1999) Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis 179(Suppl 1):170–176
29. Christie A, Davies-wayne GJ, Cordier LT, et al (2015) Possible sexual transmission of Ebola virus – Liberia, 2015. MMWR Morb Mortal Wkly Rep 64:479–481
30. Rowe AK, Bertolli J, Khan AS, et al (1999) Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidemies a Kikwit. J Infect Dis 179(Suppl 1):28–35
31. Mupapa K, Massamba M, Kibadi K, et al (1999) Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. J Infect Dis 179(Suppl 1):18–23
32. WHO (1978) Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ 56:271–293
33. Bausch DG, Towner JS, Dowell SF, et al (2007) Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J Infect Dis 196(Suppl 2):142–147
34. Sagripanti JL, Rom AM, Holland LE (2010) Persistence in darkness of virulent alphaviruses, Ebola virus, and Lassa virus deposited on solid surfaces. Arch Virol 155:2035–2039
35. Franz DR, Jahrling PB, Friedlander AM, et al (1997) Clinical recognition and management of patients exposed to biological warfare agents. JAMA 278:399–411
36. Johnson E, Jaax N, White J, et al (1995) Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. Int J Exp Pathol 76:227–236
37. Lamunu M, Lutwama JJ, Kamugisha J, et al (2004) Containing a haemorrhagic fever epidemic: the Ebola experience in Uganda (October 2000-January 2001). Int J Infect Dis 8: 27–37

38. WHO (2015) Emergency guideline: implementation and management of contact tracing for Ebola virus disease. http://www.who.int/csr/resources/publications/ebola/contact-tracing/en/. Retrieved 31 Jan 2016
39. WHO (2001) Outbreak of Ebola haemorrhagic fever, Uganda, August 2000–January 2001. Wkly Epidemiol Rec 76:41–46
40. Muyembe-tamfum JJ, Kipasa M, Kiyungu C, et al (1999) Ebola outbreak in Kikwit, Democratic Republic of the Congo: discovery and control measures. J Infect Dis 179:259–262
41. Kuhn JH, Becker S, Ebihara H, et al (2010) Proposal for a revised taxonomy of the family Filoviridae: classification, names of taxa and viruses, and virus abbreviations. Arch Virol 55: 2083–2103
42. Muhlberger E (2007) Filovirus replication and transcription. Futur Virol 2:205–215
43. Crary SM, Towner JS, Honig JE, et al (2003) Analysis of the role of predicted RNA secondary structures in Ebola virus replication. Virology 306:210–218
44. Sanchez AA, Khan S, Zaki SR, et al (2001) Filoviridae: Marburg and Ebola viruses. Lippincott Williams & Wilkins, Philadelphia
45. Nanbo A, Watanabe S, Halfmann P, et al (2013) The spatio-temporal distribution dynamics of Ebola virus proteins and RNA in infected cells. Sci Rep 3:1206
46. Feldmann H, Klenk HD, Sanchez A (1993) Molecular biology and evolution of filoviruses. Arch Virol Suppl 7:81–100
47. Carette JE, Raaben M, Wong AC (2011) Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature 477:340–343
48. Yonezawa A, Cavrois M, Greene WC (2005) Studies of Ebola virus glycoprotein-mediated entry and fusion by using pseudotyped human immunodeficiency virus type 1 virions: involvement of cytoskeletal proteins and enhancement by tumor necrosis factor alpha. J Virol 79:918–926
49. Wit E, Feldmann H, Munster VJ (2011) Tackling Ebola: new insights into prophylactic and therapeutic intervention strategies. Genome Med 3(1):5
50. Wahl-Jensen V, Kurz SK, Hazelton PR, et al (2005) Role of Ebola virus secreted glycoproteins and virus-like particles in activation of human macrophages. J Virol 79:2413–2419
51. Feldmann HV, Volchkiva AV, Stroeher U, et al (2001) Biosynthesis and role of filoviral glycoprotein. J Gen Virol 82:2839–2848
52. Sanchez A, Yang ZY, Nabel GJ, et al (1998) Biochemical analysis of the secreted and virion glycoproteins of Ebola virus. J Virol 72:6442–6447
53. Volchkov VE, Volchkova VA, Slenczka W, et al (1998) Release of viral glycoproteins during Ebola virus infection. Virology 245:110–119
54. Yang Z, Delgado R, Todd RF, et al. (1998) Distinct cellular interactions of secreted and transmembrane Ebola virus glycoproteins. Science 279:1034–1037
55. Dolnik O, Volchkova V, Garten W, et al (2004) Ectodomain shedding of the glycoprotein GP of Ebola virus. EMBO J 23:2175–2184
56. Volchova VA, Klenk HD, Volchkov VE (1999) Delta-peptide is the carboxy-terminal cleavage fragment of the nonstructural small glycoprotein sGP of Ebola virus. Virology 265:164–171
57. Sanchez A, Trappier SG, Mahy BW, et al (1996) The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. Proc Natl Acad Sci U S A 93:3602–3607
58. Volchkov VE, Becker S, Volchkova VA, et al (1995) GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. Virology 214:421–430
59. Reid SP, Leung LW, Hartman AL, et al (2006) Ebola virus VP24 binds karyopherin alpha1 and blocks STAT1 nuclear accumulation. J Virol 80:5156–5167
60. Jasenosky LD, Neumann G, Lukashevich I, et al (2001) Ebola virus VP40-induced particle formation and association with the lipid bilayer. J Virol 75:5205–5214
61. Zhang AP, Abelson DM, Bornholdt ZA, et al (2012) The ebolavirus VP24 interferon antagonist: know your enemy. Virulence 3:440–445

62. Najjar I, Fagard R (2010) STAT1 and pathogens, not a friendly relationship. Biochimie 92:425–444
63. Hoenen T, Groseth A, Kolesnikova L, et al (2006) Infection of naive target cells with virus-like particles: implications for the function of Ebola virus VP24. J Virol 80:7260–7264
64. Leung DW, Prins KC, Basler CF, et al (2010) Ebolavirus VP35 is a multifunctional virulence factor. Virulence 1:526–531
65. Basler CF, Wang X, Muhlberger E, et al (2000) The Ebola virus VP35 protein functions as a type I IFN antagonist. Proc Natl Acad Sci U S A 97:12289–12294
66. Reid SP, Cardenas WB, Basler CF (2005) Homo-oligomerization facilitates the interferon-antagonist activity of the ebolavirus VP35 protein. Virology 341:179–189
67. Johnson RF, Mccarthy SE, Godleweski PJ, et al (2006) Ebola virus VP35–VP40 interaction is sufficient for packaging 3E-5E minigenome RNA into virus-like particles. J Virol 80: 5135–5144
68. Muhlberger E, Lotfering B, Klenk HD, et al (1998) Three of the four nucleocapsid proteins of Marburg virus, NP, VP35, and L, are sufficient to mediate replication and transcription of Marburg virus-specific monocistronic minigenomes. J Virol 72:8756–8764
69. Haasnoot J, De Vries W, Geutjes EJ, et al (2007) The Ebola virus VP35 protein is a suppressor of RNA silencing. PLoS Pathog 3:e86
70. Feng Z, Cerveny M, Yan Z, et al (2007) The VP35 protein of Ebola virus inhibits the antiviral effect mediated by double-stranded RNA-dependent protein kinase PKR. J Virol 81:182–192
71. Ramanathan CS, Taylor EW (1997) Computational genomic analysis of hemorrhagic fever viruses. Viral selenoproteins as a potential factor in pathogenesis. Biol Trace Elem Res 56: 93–106
72. Ricetti MM, Guidi GC, Perona G, et al (1994) Selenium enhances glutathione peroxidase activity and prostacyclin release in cultured human endothelial cells. Concurrent effects on mRNA levels. Biol Trace Elem Res 46:113–123
73. Schiavon R, Freeman GE, Guidi GC, et al (1984) Selenium enhances prostacyclin production by cultured endothelial cells: possible explanation for increased bleeding times in volunteers taking selenium as a dietary supplement. Thromb Res 34:389–396
74. Saeed MF, Kolokoltsov AA, Albrecht T, et al (2010) Cellular entry of ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes. PLoS Pathog 6:e1001110
75. Weik M, Modrof J, Klenk HD, et al (2002) Ebola virus VP30-mediated transcription is regulated by RNA secondary structure formation. J Virol 76:8532–8539
76. Martinez MJ, Biedenkopf N, Volchkova V, et al (2008) Role of Ebola virus VP30 in transcription reinitiation. J Virol 82:12569–12573
77. Watanabe S, Noda T, Halfmann P, et al (2007) Ebola virus (EBOV) VP24 inhibits transcription and replication of the EBOV genome. J Infect Dis 196(Suppl 2):S284–S290
78. Francica JR, Varela-Rohena A, Medvec A, et al (2010) Steric shielding of surface epitopes and impaired immune recognition induced by the ebola virus glycoprotein. PLoS Pathog 6: e1001098
79. Noda T, Sagara H, Suzuki E, et al (2002) Ebola virus VP40 drives the formation of virus-like filamentous particles along with GP. J Virol 76:4855–4865
80. Timminis J, Schoehn G, Ricard-blum S, et al. (2003) Ebola virus matrix protein VP40 interaction with human cellular factors Tsg101 and Nedd4. J Mol Biol 326:493–502
81. Bavari S, Bocio CM, Wiegand E, et al (2002) Lipid raft microdomains: a gateway for compartmentalized trafficking of Ebola and Marburg viruses. J Exp Med 195:593–602
82. Basler CF, Mikulasova A, Martinez-sobrido L, et al (2003) The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3. J Virol 77:7945–7956
83. Wahl-Jensen VM, Afanasieva TA, Seebach J, et al (2005) Effects of Ebola virus glycoproteins on endothelial cell activation and barrier function. J Virol 79:10442–10450

84. Geisbert TW, Hensley LE, Larsen T, et al (2003) Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. Am J Pathol 163:2347–2370
85. Bah EI, Lamah MC, Fletcher T, et al (2015) Clinical presentation of patients with Ebola virus disease in Conakry, Guinea. N Engl J Med 372:40–47
86. Chertow DS, Kleine C, Edwards JK, et al (2014) Ebola virus disease in West Africa – clinical manifestations and management. N Engl J Med 371:2054–2057
87. Qin E, Zhao M, Wang Y, et al (2015) Clinical features of patients with Ebola virus disease in Sierra Leone. Clin Infect Dis 7:29
88. Feldmann H, Geisbert TW (2011) Ebola haemorrhagic fever. Lancet 377:849–862
89. Kortepeter MG, Bausch DG, Bray M (2011) Basic clinical and laboratory features of filoviral hemorrhagic fever. J Infect Dis 204(Suppl 3):810–816
90. Maganga GD, Kapethshi J, Berthet N (2014) Ebola virus disease in the Democratic Republic of Congo. N Engl J Med 371:2083–2091
91. Schieffelin JS, Shaffer JG, Goba A (2014) Clinical illness and outcomes in patients with Ebola in Sierra Leone. N Engl J Med 371:2092–2100
92. CDC (2015) Ebola virus disease: case definition for Ebola virus disease. http://www.cdc.gov/vhf/ebola/healthcare-us/evaluating-patients/case-definition.html
93. WHO (2014) Clinical management of patients with viral haemorrhagic fever: a pocket guide for the front-line health worker. Interim emergency guidance – generic draft for West African adaptation. WHO, Geneva
94. WHO (2014) Case definition recommendations for Ebola or Marburg virus disease. Accessed 11 May 2015
95. Fowler RA, Fletcher T, Fischer WA, et al (2014) Caring for critically ill patients with ebola virus disease. Perspectives from West Africa. Am J Respir Crit Care Med 190:733–737
96. Wolf T, Kann G, Becker S, et al (2015) Severe Ebola virus disease with vascular leakage and multiorgan failure: treatment of a patient in intensive care. Lancet 385:1428–1435
97. Lyon GM, Mehta AK, Varkey JB, et al (2014) Clinical care of two patients with Ebola virus disease in the United States. N Engl J Med 371:2402–2409
98. Uyeki TM et al (2016) Clinical management of Ebola virus disease in the United States and Europe. N Engl J Med 374:636–646
99. Leroy EM, Baize S, Volchkov VE, et al (2000) Human asymptomatic Ebola infection and strong inflammatory response. Lancet 355:2210–2215
100. Clark DV, Kibuuka H, Millard M, et al (2015) Long-term sequelae after Ebola virus disease in Bundibugyo, Uganda: a retrospective cohort study. Lancet Infect Dis 15:905–912
101. Kraft CS, Hewlett AL, Koepsell S, et al (2015) The use of TKM-100802 and convalescent plasma in 2 patients with Ebola virus disease in the United States. Clin Infect Dis. doi:10.1093/cid/civ334
102. Varkey JB, Shantha JG, Crozier I (2015) Persistence of Ebola virus in ocular fluid during convalescence. N Engl J Med 372:2423–2427
103. Dunning J, Sahr F, Rojek A, Gannon F, Carson G, Idriss B, et al (2016) Experimental treatment of Ebola virus disease with TKM-130803: a single-arm phase 2 clinical trial. PLoS Med 13(4):e1001997. doi:10.1371/journal.pmed.1001997
104. Chua AC, Cunningham J, Moussy F, et al (2015) The case for improved diagnostic tools to control Ebola virus disease in West Africa and how to get there. PLoS Negl Trop Dis 9: e0003734
105. O'Dempsky T, Khan SH, Bausch DG (2015) Rethinking the discharge policy for Ebola convalescents in an accelerating epidemic. Am J Trop Med Hyg 92:238–239
106. WHO Ebola Response Team (2014) Ebola virus disease in West Africa – the first 9 months of the epidemic and forward projections. N Engl J Med 371:1481–1495
107. Rao M, Bray M, Alving CR (2002) Induction of immune responses in mice and monkeys to Ebola virus after immunization with liposome-encapsulated irradiated Ebola virus: protection in mice requires CD4(+) T cells. J Virol 76:9176–9185

108. Geisbert TW, Pushko P, Anderson K (2002) Evaluation in nonhuman primates of vaccines against Ebola virus. Emerg Infect Dis 8:503–507
109. Jahrling PB, Geisbert J, Swearengen JR (1996) Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses. Arch Virol Suppl 11: 135–140
110. Martin JE, Sullivan NJ, Enama ME (2006) A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol 13:1267–1277
111. Jeffs LB, Palmer LR, Ambegia EG (2005) A scalable, extrusion-free method for efficient liposomal encapsulation of plasmid DNA. Pharm Res 22:362–372
112. Sullivan NJ, Sanchez A, Rollin PE (2000) Development of a preventive vaccine for Ebola virus infection in primates. Nature 408:605–609
113. Ledgerwood JE, Costner P, Desai N (2010) A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine 29:304–313
114. Swenson DL, Wang D, Luo M (2008) Vaccine to confer to nonhuman primates complete protection against multi-strain Ebola and Marburg virus infections. Clin Vaccine Immunol 15:460–467
115. Mellquist-Riemenschneider JL, Garrison AR, Geisbert AR (2003) Comparison of the protective efficacy of DNA and baculovirus-derived protein vaccines for EBOLA virus in guinea pigs. Virus Res 92:187–193
116. Konduru K, Bradfute SB, Jacques J (2011) Ebola virus glycoprotein Fc fusion protein confers protection against lethal challenge in vaccinated mice. Vaccine 29:2968–2977
117. Swenson DL, Warfield KL, Wenson DL (2005) Virus-like particles exhibit potential as a pan-filovirus vaccine for both Ebola and Marburg viral infections. Vaccine 23:3033–3042
118. Warfield KL, Bosio CM, Welcher BC (2003) Ebola virus-like particles protect from lethal Ebola virus infection. Proc Natl Acad Sci U S A 100:15889–15894
119. Warfield KL, Posten NA, Swenson DL, et al (2007) Filovirus-like particles produced in insect cells: immunogenicity and protection in rodents. J Infect Dis 196(Suppl 2):S421–S429
120. Hoenen T, Groseth A, De Kok-Mercado F, et al (2011) Mini-genomes, transcription and replication competent virus-like particles and beyond: reverse genetics systems for filoviruses and other negative stranded hemorrhagic fever viruses. Antivir Res 91:195–208
121. Halfmann P, Ebihara H, Marzi A (2009) Replication-deficient ebolavirus as a vaccine candidate. J Virol 83:3810–3815
122. Jones SM, Feldmann H, Stroher U (2005) Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. Nat Med 11:786–790
123. Jones SM, Stroher U, Fernando L (2007) Assessment of a vesicular stomatitis virus-based vaccine by use of the mouse model of Ebola virus hemorrhagic fever. J Infect Dis 196 (Suppl 2):S404–S412
124. Geisbert TW, Daddario-Dicaprio KM, Lewis MG (2008) Vesicular stomatitis virus-based ebola vaccine is well-tolerated and protects immunocompromised nonhuman primates. PLoS Pathog 4:e1000225
125. Qiu X, Fernando L, Alimonti J (2009) Mucosal immunization of cynomolgus macaques with the VSVDeltaG/ZEBOVGP vaccine stimulates strong ebola GP-specific immune responses. PLoS One 4:e5547
126. Tsuda Y, Safronetz D, Brown K, et al (2011) Protective efficacy of a bivalent recombinant vesicular stomatitis virus vaccine in the Syrian hamster model of lethal Ebola virus infection. J Infect Dis 204(Suppl 3):S1090–S1097
127. Feldmann H, Jones SM, Daddario-Dicaprio KM (2007) Effective post-exposure treatment of Ebola infection. PLoS Pathog 3:e2
128. Henao-Restrepo AM et al (2015) Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomized trail. Lancet 386:857–866

129. Bukreyev A, Yang L, Zaki SR (2006) A single intranasal inoculation with a paramyxovirus-vectored vaccine protects guinea pigs against a lethal-dose Ebola virus challenge. J Virol 80: 2267–2279
130. Bukreyev A, Rollin PE, Tate MK (2007) Successful topical respiratory tract immunization of primates against Ebola virus. J Virol 81:6379–6388
131. Blaney JE, Wirblich C, Papaneri AB (2011) Inactivated or live-attenuated bivalent vaccines that confer protection against rabies and Ebola viruses. J Virol 85:10605–10616
132. Kudoyarova-Zubavichene NM, Sergeyev NN, Chepurnov AA (1999) Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. J Infect Dis 179(Suppl 1):S218–S223
133. Warren TK, Warfield KL, Wells J (2010) Antiviral activity of a small-molecule inhibitor of filovirus infection. Antimicrob Agents Chemother 54:2152–2159
134. Kinch MS, Yunus AS, Lear C (2009) FGI-104: a broad-spectrum small molecule inhibitor of viral infection. Am J Transl Res 1:87–98
135. Aman MJ, Kinch MS, Warsfield K, et al (2009) Development of a broad-spectrum antiviral with activity against Ebola virus. Antivir Res 83:245–251
136. Panchal RG, Reid SP, Tran JP, et al (2012) Identification of an antioxidant small-molecule with broad-spectrum antiviral activity. Antivir Res 93:23–29
137. Bray M, Davis K, Geisbert T (1998) A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. J Infect Dis 178:651–661
138. Agrawal N, Dasaradhi PV, Mohmmed A, et al (2003) RNA interference: biology, mechanism, and applications. Microbiol Mol Biol Rev 67:657–685
139. Geisbert TW, Lee AC, Robbins M, et al (2010) Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study. Lancet 375:1896–1905
140. Thi EP, Mire CE, Lee AC, et al (2015) Lipid nanoparticle siRNA treatment of Ebola-virus-Makona-infected nonhuman primates. Nature 521:362–365
141. Swenson DL, Warfield KL, Warren TK, et al (2009) Chemical modifications of antisense morpholino oligomers enhance their efficacy against Ebola virus infection. Antimicrob Agents Chemother 53:2089–2099
142. Enterlein S, Warfield KL, Swenson DL (2006) VP35 knockdown inhibits Ebola virus amplification and protects against lethal infection in mice. Antimicrob Agents Chemother 50: 984–993
143. Warfield KL, Swenson DL, Olinger GG (2006) Gene-specific countermeasures against Ebola virus based on antisense phosphorodiamidate morpholino oligomers. PLoS Pathog 2: e1
144. Parren PW, Geisbert TW, Maruyama T, et al (2002) Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. J Virol 76:6408–6412
145. Murin CD, Fusco ML, Bornholdt ZA, et al (2014) Structures of protective antibodies reveal sites of vulnerability on Ebola virus. Proc Natl Acad Sci U S A 111:17182–17187
146. Qiu X, Wong G, Audet J, et al (2014) Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature 514:47–53
147. Mccarthy M (2014) US signs contract with ZMapp maker to accelerate development of the Ebola drug. BMJ 349:g5488
148. Bray M, Driscoll J, Huggins JW (2000) Treatment of lethal Ebola virus infection in mice with a single dose of an S-adenosyl-L-homocysteine hydrolase inhibitor. Antivir Res 45:135–147
149. Hensley LE, Stevens EL, Yan SB, et al (2007) Recombinant human activated protein C for the postexposure treatment of Ebola hemorrhagic fever. J Infect Dis 196(Suppl 2):S390–S399
150. Marlar RA, Kleiss AJ, Griffin JH (1981) Human protein C: inactivation of factors V and VIII in plasma by the activated molecule. Ann N Y Acad Sci 370:303–310

151. Sakata Y, Curriden S, Lawrence D, et al (1985) Activated protein C stimulates the fibrinolytic activity of cultured endothelial cells and decreases antiactivator activity. Proc Natl Acad Sci U S A 82:1121–1125
152. Geisbert TW, Young HA, Jahrling PB, et al (2003) Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. J Infect Dis 188:1618–1629
153. Geisbert TW, Hensley LE, Jahrling PB, et al (2003) Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. Lancet 362: 1953–1958
154. Furuta Y, Takahashi K, Fukuda Y, et al (2002) In vitro and in vivo activities of anti-influenza virus compound T-705. Antimicrob Agents Chemother 46:977–981
155. Furuta Y, Gowen BB, Takahashi K (2013) Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antivir Res 100:446–454
156. Baranovich T, Wong SS, Armstrong J, et al (2013) T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. J Virol 87:3741–3751
157. Gowen BB, Wong MH, Jung KH, et al (2007) In vitro and in vivo activities of T-705 against arenavirus and bunyavirus infections. Antimicrob Agents Chemother 51:3168–3176
158. Ostereich L, Ludtke A, Wurr S, et al (2014) Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. Antivir Res 105:17–21
159. Sissoko D et al (2016) Experimental treatment with Favipiravir for Ebola virus disease (the JIKI trail): a historically controlled, single-arm proof-of-concept trail in Guinea. PLoS Med 13(3):e1001967. doi:10.1371/journal.pmed.1001967
160. Kamat SS, Burgos ES, Raushel FM (2013) Potent inhibition of the C-P lyase nucleosidase PhnI by Immucillin-A triphosphate. Biochemistry 52:7366–7368
161. Warren TK, Wells J, Panchal RG, et al (2014) Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. Nature 508:402–405
162. Johansen LM, Brannan JM, Delos SE, et al (2013) FDA-approved selective estrogen receptor modulators inhibit Ebola virus infection. Sci Transl Med 5:190ra79
163. Bishop BM (2015) Potential and emerging treatment options for Ebola virus disease. Ann Pharmacother 49:196–206
164. Madrid PB, Chopra S, Manger ID, et al (2013) A systematic screen of FDA-approved drugs for inhibitors of biological threat agents. PLoS One 8:e60579
165. De Lamballerie X, Boisson V, Reynier JC, et al (2008) On chikungunya acute infection and chloroquine treatment. Vector Borne Zoonotic Dis 8:837–839
166. Huggins JW (1989) Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. Rev Infect Dis 11(Suppl 4):S750–S761
167. Mendoza EJ et al (2016) Progression of Ebola therapeutics during the 2014-2015 outbreak. Trends Mol Med 22:164–173

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Anti-HIV Agents: Current Status and Recent Trends

Athina Geronikaki, Phaedra Eleftheriou, and Vladimir Poroikov

Abstract Human immunodeficiency virus is responsible for acquired immunodeficiency syndrome (AIDS), an infectious disease that consists a serious concern worldwide for more than three decades. By the end of 2013 UNAIDS estimated that there were 35 million (range 33.2–37.2 million) adults and children living with HIV/AIDS worldwide. Despite the introduction of highly active antiretroviral therapy (HAART), the need for new anti-HIV agents is extremely high because the existing medicines do not provide the complete curation and exhibit serious side effects, and their application leads to the appearance of resistant strains. This chapter explores the medicinal chemistry efforts that gave rise to currently launched drugs as well as investigational anti-HIV agents. Currently used and studied molecular targets of antiretrovirals and the main classes of HIV-1 inhibitors are presented. Among the future prospects, we discuss the efforts directed to overcome the latent HIV infection, utilization of natural products as potential anti-HIV agents, recent trends on development of biologics as potential anti-HIV medicines, and application of computer-aided methods in the discovery of new anti-HIV drugs.

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A. Geronikaki (✉)
Department of Medicinal Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece
e-mail: geronik@pharm.auth.gr

P. Eleftheriou
Department of Medical Laboratory Studies, School of Health and Medical Care, Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece

V. Poroikov
Institute of Biomedical Chemistry, Moscow, Russia

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Contents

1 Introduction

Human immunodeficiency virus (HIV) is the cause of acquired immunodeficiency syndrome (AIDS), an infectious disease that consists a serious concern worldwide for more than three decades. Although the first recognized cases of AIDS were referred in the USA in 1981, it is now believed that the first incident occurred much earlier, in 1959 or even 1930, as it was proved by the detection of HIV virus in blood and tissue samples of humans who had died in that period [1–3]. According to the origin of most of these samples, the place of first known infections is Central or West Africa. So, although AIDS now concerns all countries of the world, it originally occurred in tropical areas.

HIV is a lentivirus of the larger group of retroviruses and has significant similarity with the simian immunodeficiency virus (SIV) that affects monkeys [4]. Because of the great resemblance between certain strains of SIV virus and the HIV-1 or HIV-2 types, it is now considered that HIV is a descendant of SIV [4–7].

Precaution measures reduced the number of new infections referred each year, worldwide, from about 3.4 million in 2001 to about 2.4 million in 2012 [8]. By the end of 2013 UNAIDS estimated that there were 35 million (range 33.2–37.2

million) adults and children living with HIV/AIDS worldwide. Despite the introduction of highly active antiretroviral therapy (HAART), the number of people living with AIDS remains high, with a slight, constant increase leading to about 32 million patients in 2012 from about 29 million patients in 2001 [8]. This increase may reflect the higher survival of infected people as more potent therapeutic approaches are developed. Unfortunately, existing therapeutic agents can only diminish the viral load but fail to eliminate the virus completely. The need for long-time treatment of infected patients facilitates the development of resistant strains of the virus while also underlines the requirement of low side effect therapies. Consequently, although many anti-HIV drugs are already in the market [9–12], research for the development of novel effective drugs with better efficacy, less side effects, and effective against the resistant strains continues [13–23].

2 The Biology of HIV

2.1 Structure and Organization

HIV belongs to the family of Retroviridae, subfamily of Lentivirinae [24–26]. It is a virus with a long incubation period, capable of infecting nondividing cells.

Following the typical pattern of retroviruses, the HIV genome consists of two copies of a single-stranded, positive-sense ribonucleic acid (RNA) of about 9.7 kilobases (Fig. 1). Each RNA molecule contains nine genes that code for the fourteen proteins of the virus (Fig. 2). The RNA is protected in a bullet-shaped capsid formed by about 2,000 molecules of the viral protein p24. The viral capsid is

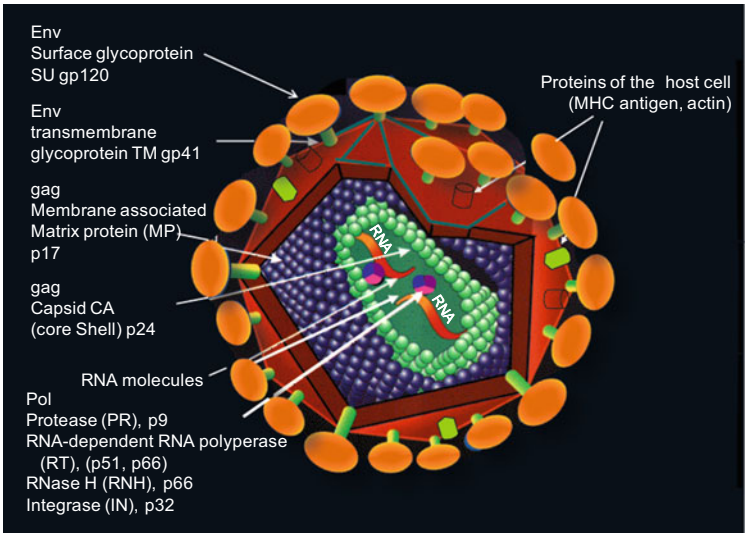


Fig. 1 The structure of HIV virus

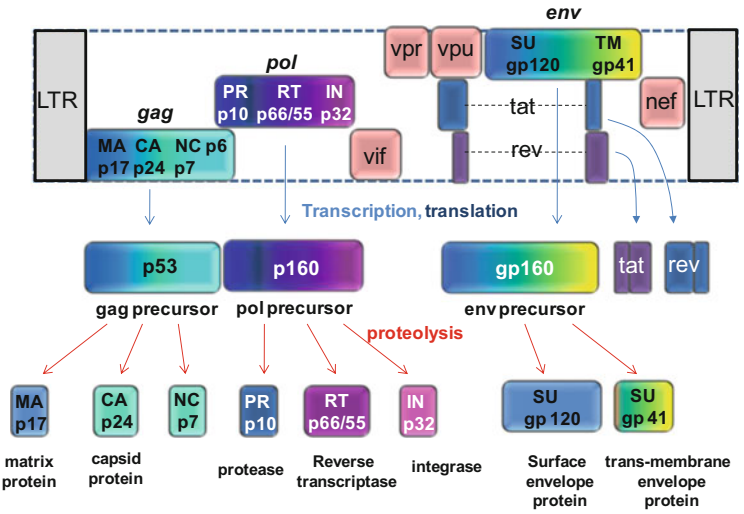


Fig. 2 Structure of HIV genome, transcription, translation and proteolysis products

surrounded by molecules of the matrix protein p17, also known as membrane-associated (MA) viral protein. The outer layer of the virus consists of a lipid bilayer, which has been extracted by the host cell during budding of the newly formed virus. This membrane constitutes the viral envelope. The viral envelope carries a number of proteins with both virus and host-cell origin. The host-cell major histocompatibility complex (MHC) proteins and actin remain embedded within the viral envelope. The envelope consists of the viral transmembrane protein gp41 which forms non-covalent complex with the viral outer membrane glycoprotein gp120. Protein gp120 may separate from the envelope and can be detected in the serum or within the lymphatic tissue of HIV-infected patients. The envelope protein is the most variable component of HIV. It is structurally divided into highly variable (V) and more constant (C) regions. The variability of V regions seems to be related with envelope functionality and may affect co-receptor use. Three reading frames coexist, permitting existence and expression of overlapping gene-coding regions (Fig. 2).

2.2 *HIV Life Cycle*

HIV cannot replicate outside human cells. The HIV replication cycle can be summarized in six steps: (1) binding and entry, (2) uncoating, (3) synthesis of viral DNA, (4) integration of viral DNA in host DNA, (5) virus protein synthesis and assembly, and (6) budding (Fig. 3).

The proteins essential for virus recognition and entry into target cells are the heterodimer proteins gp120 and gp41, present on the viral envelope. The gp41 subunit contains a hydrophobic moiety at its amino terminus, which has an important role in fusion of the viral and cellular membranes [27]. HIV gp120 binds to

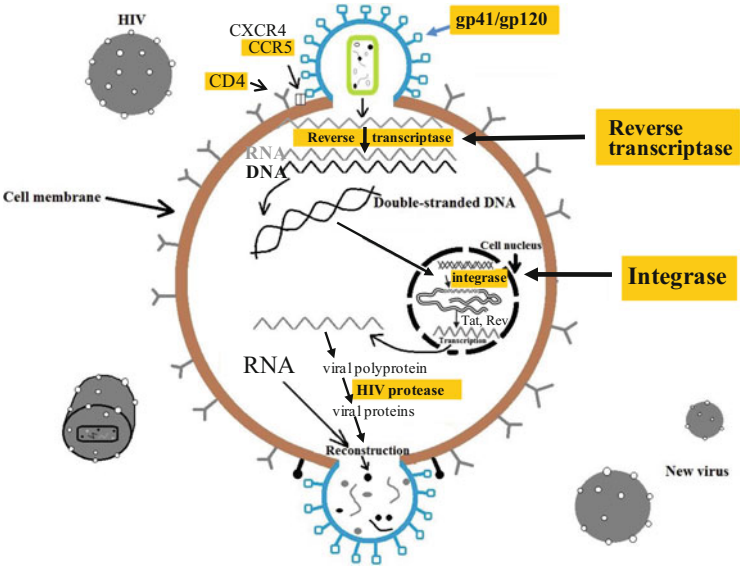


Fig. 3 HIV life cycle. The main drug targets are indicated in *yellow*

CD4a, a glycoprotein present on the cell surface of about 60 % of the circulating T lymphocytes, on the T-cell precursors within the bone marrow and thymus and on monocytes/macrophages, eosinophils, dendritic cells, and microglial cells of the central nervous system. During T-cell recognition of a foreign antigen, the CD4 molecule functions as a co-receptor of the major histocompatibility complex class II [28]. A second co-receptor is also required for viral entry. Such co-receptors may be the CC chemokine receptor 5 (CCR5), the CXC chemokine receptor 4 (CXCR4), and other proteins of the class of seven-transmembrane region receptors [29–34]. After binding of the viral gp120 protein to the CD4 receptor and to the co-receptor, a conformational change in the gp41 protein leads to the insertion of the N-terminal hydrophobic part of the protein into the host-cell membrane [35]. This insertion results in the fusion of the viral and host-cell membranes and to the subsequent entry of the viral contents into the host-cell cytoplasm.

Following membrane fusion, the virus loses the capsid, liberating the viral RNA (uncoating). At the next stage, the viral RNA is used as a template for the synthesis of the proviral DNA by the action of reverse transcriptase (RT) which contains three active sites: a reverse transcriptase, an RNase H, and a DNA polymerase active site. As a first step, RT begins the reverse transcription of viral RNA, through its RNA-dependent DNA polymerase (reverse transcriptase) activity. This leads to the production of a RNA/DNA hybrid double helix. At a second step, RT hydrolyzes the RNA strand of the hybrid, via the RNase activity of the enzyme. At a third step, the DNA-dependent DNA polymerase active site of the enzyme synthesizes the complementary DNA strand to form a double helix DNA molecule. The dsDNA molecule is then integrated within the genome of the host cell by the integrase. This enzyme cleaves nucleotides of each 3′ ends of each strand of the double helix DNA

producing two sticky ends and catalyzes its integration into the host genome. Since the expression of viral proteins require the activation of target cells, monocytes/macrophages, microglial cells, and infected quiescent CD4+ T cells contain integrated provirus genome and represent long-living cellular reservoirs of HIV [36].

Upon cell activation, transcription of the integrated proviral DNA occurs. The three reading frames enable the expression of the 14 viral proteins, although the genetic information for their synthesis is overlapping each other. The first proteins synthesized are the regulatory HIV-1 proteins Tat and Rev. Tat binds to the transactivation response (TAR site) element at the beginning of the HIV-1 RNA and stimulates the transcription and the formation of longer RNA transcripts. On the other hand, Rev induces the transcription of longer RNA transcripts and the expression of structural and enzyme genes and inhibits the production of regulatory proteins. The viral mRNA migrates into the cytoplasm where proteins are synthesized. During the translation process, large precursor protein molecules are produced which are then cleaved by the HIV-1 protease to produce the functional viral proteins. So, the precursor gp160 protein, derived from the env gene, is hydrolyzed into the gp120 and gp41 envelope proteins. The Gag and Pol proteins are also derived from large precursor molecules, from which the HIV protease cleaves the p24, p17, p9, and p7 gag final products and the viral protease, reverse transcriptase, and integrase, which are the Pol final products, respectively.

The formation of the new viral particles is a stepwise process: two viral RNA strands associate together with viral enzymes, and core proteins assemble over them forming the virus capsid. The capsid then migrates toward the cell surface. During the budding process, the viral envelope lipid membrane is formed by extracting phospholipids and cholesterol from the host cell.

2.3 HIV Types

Based on genome sequence, two types of HIV virus are distinguished: HIV-1 and HIV-2. Both types can cause AIDS, although they have differences in pathogenesis. HIV-2 is less virulent than HIV-1, and HIV infection takes longer to progress to AIDS. However, HIV-2 more frequently attacks the central nervous system [37].

3 The Main Classes of Anti-HIV Drugs

The efforts for the development of effective anti-HIV drugs have been focused on several target molecules of viral or host-cell origin. The launched anti-HIV agents belong to two main categories: viral enzyme inhibitors and fusion/entry inhibitors.

3.1 Viral Enzyme Inhibitors

The HIV enzymes were among the first drug targets. The first drug belonged to the family of nucleoside analogs of reverse transcriptase (RT) inhibitors (NRTIs). Zidovudine (Retrovir) was approved in 1987. Drugs of this class are mimicking the dNTPs, the natural substrates of the enzyme, thus inhibiting reverse transcription or viral RNA to DNA. The first molecule of a second family of RT inhibitors is the non-nucleoside reverse transcriptase inhibitors (NNRTIs). The first drug of this class nevirapine (Viramune) was approved in 1996. This kind of inhibitors acts by binding to allosteric site of the enzyme. The first HIV-1 protease-inhibitor saquinavir mesylate (Invirase) was approved in 1995, while the first inhibitor of HIV-1 integrase raltegravir was approved only in 2007 (Table 1).

3.2 Fusion or Entry Inhibitors

Drugs that prevent entering of the virus to the host cells are known as fusion or entry inhibitors. This kind of inhibitors may interact either with the viral transmembrane envelope protein gp120 or gp41, which has an essential role in viral entrance into the host cell, or bind to certain molecules of the host-cell surface that act as co-receptors (Fig. 4, Table 1).

4 Current State of Anti-HIV Therapy and Recent Studies

4.1 HIV-1 Reverse Transcriptase (RT) Inhibitors

HIV-1 reverse transcriptase inhibitors inhibit the viral enzyme, which catalyze the reverse transcription of viral RNA to DNA.

The active form of the enzyme is a heterodimer composed of two subunits, p66 and p51. The p51 subunit has identical sequence with part of the p66 subunit but a different 3D structure. So, while p51 has a structural function, the p66 subunit contains the catalytic sites of the enzyme, a polymerase active site, and an RNase H active site [38]. Three distinct enzymatic activities were found in RT: (a) an RNA-dependent DNA polymerase activity where the synthesis of the negative strand of the proviral DNA takes place, (b) an RNase H activity which is responsible for the degradation of the RNA portion of the RNA/DNA hybrid, and (c) a DNA-dependent DNA polymerase activity that catalyzes the synthesis of the positive DNA strand. The RNase H activity is also involved in the removal of the tRNA primer that is used to initiate synthesis of the first strand [39, 40]. After synthesis of the first DNA strand, the genomic retroviral RNA template is cleaved into multiple fragments, one of which, a 19-base RNA primer with a purine-rich sequence, is used by the reverse transcriptase as a primer [41].

Table 1 Categories of anti-HIV drugs

Drug target		Antiretroviral drug class	Approved and experimental drugs		First approved	Mechanism of action
			Nature	Name		
Viral enzymes	*Viral reverse transcriptase (HIV-1 RT)*	*Nucleoside/nucleotide reverse transcriptase inhibitors* (NRTIs, nucleoside analogs, nukes)	Small organic molecule	Abacavir, emtricitabine, tenofovir, zidovudine, lamivudine, stavudine	1987	NRTIS mimic natural dNTPs and inhibit reverse transcription of viral RNA to DNA
		Non-nucleoside reverse transcriptase inhibitors (NNRTIs, non-nucleosides, non-nukes)	Small organic molecule	Efavirenz, etravirine, nevirapine	1996	NNRTIs inhibit viral reverse transcriptase protein by binding to an allosteric center of the enzyme
		Nucleotide-competing RT inhibitors (NcRTIs)	Small organic molecule	INDOPY-1, DAVPs	–	Non-nucleotide RT inhibitors (NNRTIs) which exhibit competitive mode of inhibitory action against dNTPs
	Viral protease	*Protease inhibitors* (PIs)	Small organic molecule	Ritonavir, nelfinavir, amprenavir, lopinavir, atazanavir, tipranavir, darunavir	1995	PIs inhibit viral protease, involved in maturation of viral enzymes
	Viral integrase	*Integrase inhibitors*	Small organic molecule	Raltegravir, elvitegravir, dolutegravir	2007	Integrase inhibitors interfere with the integrase enzyme, which HIV needs to insert its material into human cells
Viral transmembrane envelop protein pg41		*Fusion inhibitors*	Oligopeptide	Enfuvirtide	2003	Fusion or entry inhibitors prevent HIV from binding to or entering human immune cells
Host-cell secondary co-receptor CC chemokine receptor 5 (CCR5)		*Entry inhibitors*	Small organic molecule	Maraviroc	2007	
Host-cell CD4		*Entry inhibitors*	Humanized antibody	Ibalizumab	2014	

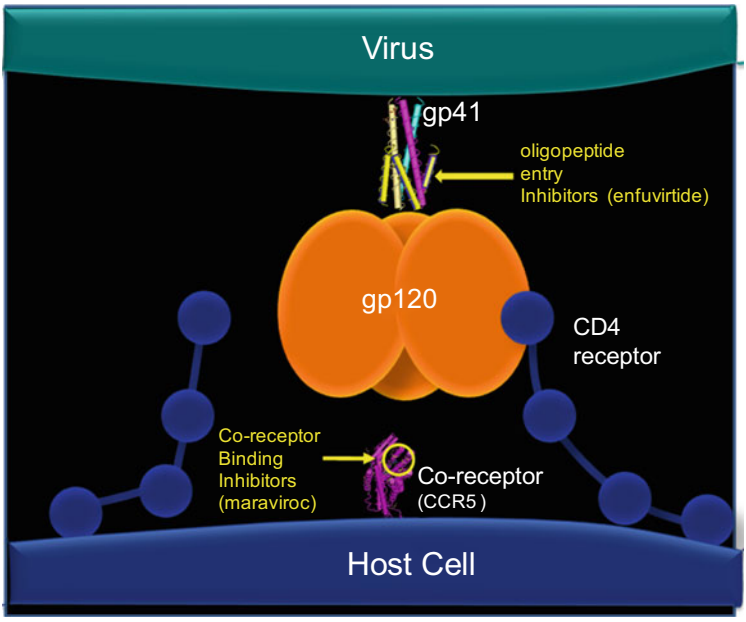


Fig. 4 HIV virus binding to the host cell

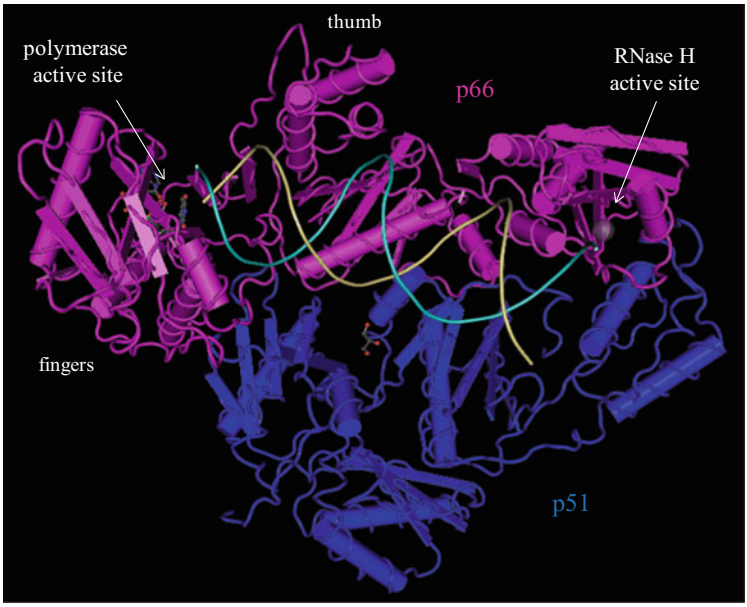


Fig. 5 HIV-1 reverse transcriptase structure from (PDB ID: 3KLF) [44, 45]

The structure of HIV-1 RT is shown in Fig. 5 [42, 43]. The N-terminal portion of the p66 subunit attains a structure that resembles an open right hand containing three domains, known as: fingers, palm, and thumb [44, 45]. Polymerase active site is placed in this domain, while RNase H active site is located in the C-terminal part of RT.

The approved RT inhibitors belong to two families: the nucleoside/nucleotide RT inhibitors (NRTIs) and the non-nucleoside RT inhibitors (NNRTIs). A novel group of inhibitors characterized as nucleotide-competitive RT inhibitors (NcRTIs) also exist.

4.1.1 Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)

The nucleoside reverse transcriptase inhibitors (NRTIs) were among the first medicines approved for anti-HIV treatment. Abacavir (Ziagen), emtricitabine (Emtriva), tenofovir (Viread), zidovudine (Retrovir), lamivudine (Epivir), and stavudine (Zerit) belong in this category.

The NRTIs are prodrugs that are structurally similar to the endogenous deoxynucleosides, with structural substitutions of the 3′ OH group of deoxyribose. After insertion into host target cells, NRTIs are phosphorylated by kinases of the host cell to form their active triphosphate derivatives (ddNTPs). Since NRTIs lack a 3′ hydroxyl group on their ribose or ribose mimic moiety, the synthesis of the DNA strand is terminated after incorporation of nucleotide mimic-drug derivative in the newly synthesized DNA strand. In practice, the drugs' triphosphates inhibit HIV RNA reverse transcription through two mechanisms [46–49]. Firstly, their phosphorylated and non-phosphorylated forms act as competitive inhibitors of the enzyme against dNTPs, while at a second phase they stop DNA elongation after incorporation.

Representative structures of NRTIs are shown in Fig. 6.

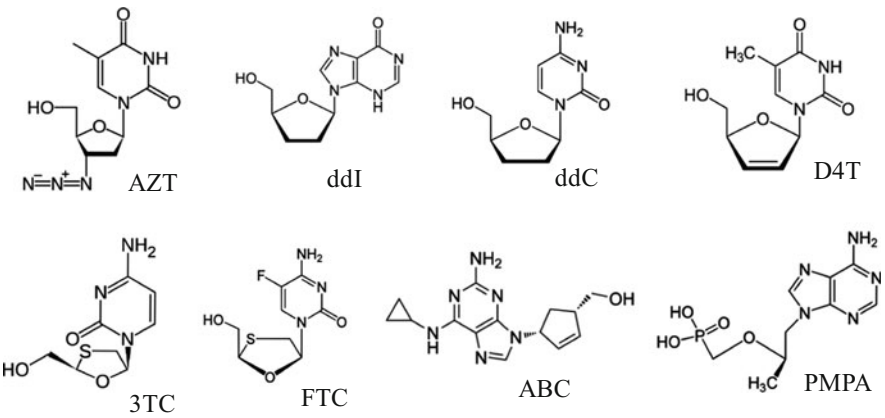


Fig. 6 FDA-approved NRTIs. *AZT* zidovudine, *ddI* didanosine, *ddC* zalcitabine, *D4T* stavudine, *3TC* lamivudine, *FTC* emtricitabine, *ABC* abacavir sulfate, *PMPA* tenofovir disoproxil fumarate

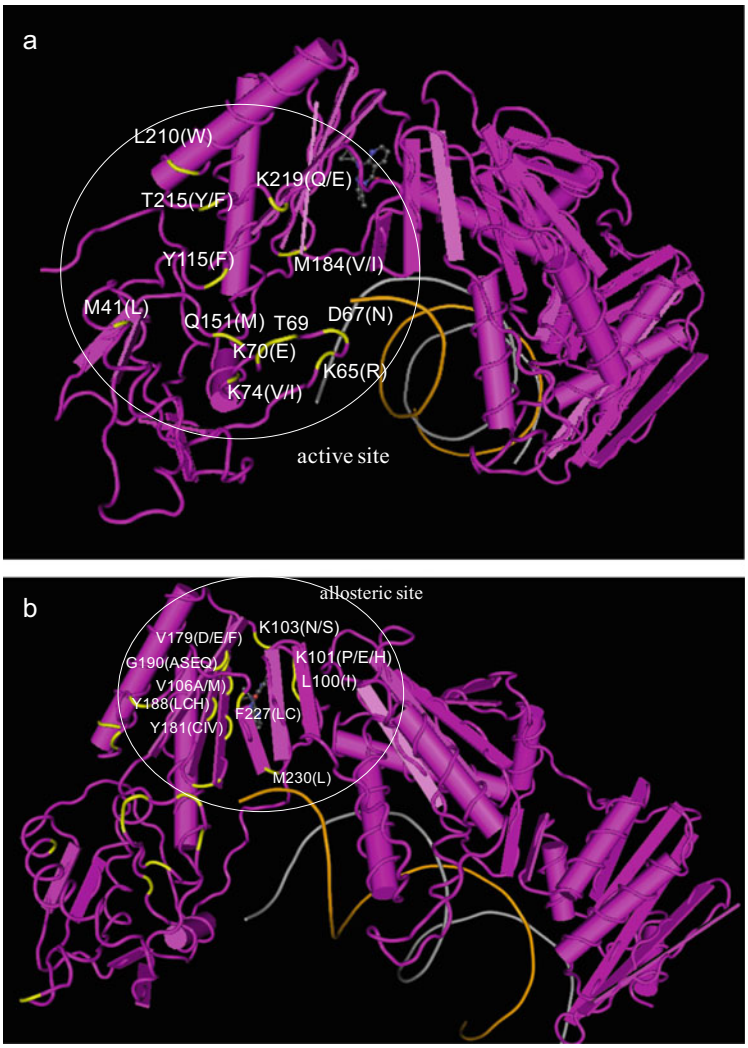


Fig. 7 (**a**) Active and (**b**) allosteric site of HIV-1 RT. Amino acid residues involved in interaction with the inhibitors and subject to mutations are indicated with *yellow color* on HIV-1 reverse transcriptase complex with the NNRTI, nevirapine (PDB ID: 3V81). Amino acid residues present at the same position in resistant strains are shown in *brackets* [44, 45]

This kind of medicines is not associated with the high rate of resistance development. However, a few strains resistant to NRTIs have been developed. The active site of reverse transcriptase is shown in Fig. 7a. The amino acid residues surrounding the active center, which are mutated in resistant strains, are presented in the picture. Amino acid residues located in the same position of resistant strains are shown in brackets.

Undesired Side Effects of NRTIs

NRTIs are often related with undesired side effects, mainly derived from the NRTI-induced inhibition of the mitochondrial DNA polymerase gamma [50]. Among the disorders associated with long-term use of NRTIs are hematologic disorders, peripheral neuropathies, myopathy, and cardiotoxic and hepatotoxic effects [51]. Increased levels of lactate in blood and lactic acidosis are observed because of the toxic effect [52, 53]. NRTIs have also been associated with peripheral lipodystrophy [54–56].

4.1.2 Non-nucleoside RT Inhibitors (NNRTIs)

As crystallographic studies have revealed, NNRTIs bind to an allosteric center that is located near the RNA-dependent polymerase active site of the enzyme on subunit p66 (Fig. 7b) [57–61]. The allosteric center of the enzyme is formed by a hydrophobic cleft surrounded by the aromatic amino acid residues Tyr181, Tyr188, Phe227, Trp229, and Tyr318 and the hydrophobic amino acid residues Pro95, Leu100, Val106, Val108, Val179, Leu234, and Pro236 [62]. Apart from hydrophobic and aromatic π–π interactions, which are essential for RT-inhibitor complex stabilization, hydrogen bonds with Lys101 or Lys103 are formed in many cases. This may be the reason for the >50 % of mutations of Lys103 in resistant strains. Crystallographic studies of the first-generation NNRTIs indicated that a butterfly conformation (Fig. 8) of the molecules favored binding. The ability to adapt this conformation was considered as mandatory for effective compounds [62, 65]. However, inhibitors with different conformations, such as the 4-dihydroquinoxalin-2(1*H*)-thione derivative, HBY097, were also found to interact with the active site [66]. Moreover, flexible molecules, capable of acquiring multiple conformations, like etravirine, were found to present inhibition activity against more mutated strains [67]. The allosteric center is practically absent in RT enzyme and is created after interaction with the inhibitor [68].

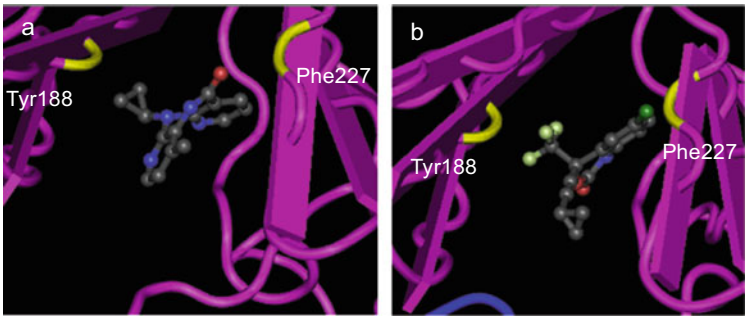


Fig. 8 3D structure of (**a**) nevirapine (PDB ID: 1S1X) and (**b**) efavirenz (PDB ID: 1FK9) in complex with RT [45, 63, 64]

All approved and most of the investigated NNRTIs exhibit a noncompetitive mode of action. However, for a few NNRTIs different modes of actions have been described. The bis(heteroaryl)piperazine inhibitor (BHAP), U-90152E, acts as a mixed inhibitor with respect to the template: primer and dNTP and for both the RNA- and DNA-directed DNA polymerase activities of the enzyme [69] while chloroxoquinolinic ribonucleoside, 6-chloro-1,4-dihydro-4-oxo-1-(beta-D-ribofuranosyl) quinoline-3-carboxylic acid, was found to inhibit RT with an uncompetitive mode of action with respect to dTTP and a noncompetitive mode of action with respect to RNA: primer template [70]. (4/6-Halogen/MeO/EtO-substituted benzo[d]thiazol-2-yl) thiazolidin-4-one derivatives were found to act as uncompetitive inhibitors or competitive inhibitors against dNTPs depending on the substitution [71].

Commercially available NNRTIs are compounds bearing a variety of heterocyclic rings such as benzoxazin-2-one (efavirenz), dipyrido[1,4]diazepine-6-one (nevirapine), pyrimidine (etravirine) [72], piperazine, and indolyl (delavirdine) moieties [73, 74]. Reverse transcriptase inhibition potency differs among the inhibitors. Apart from molecules having received FDA acceptance (Fig. 9), many compounds have been found to exhibit RT inhibitory action [66, 68, 75–79] such as benzothiazine dioxides [80], N1,N3-disubstituted uracils [81], 6-arylmethyl-substituted S-DABOs [82], indolyl aryl sulfones [83], 2-adamantyl-substituted

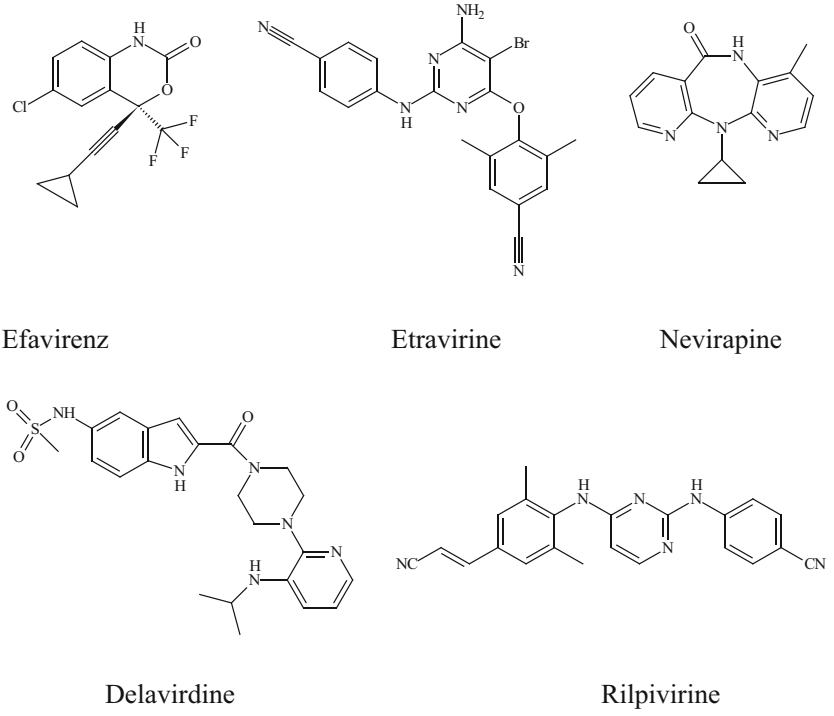
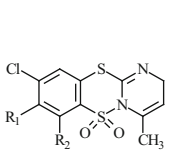
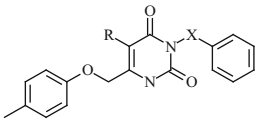


Fig. 9 FDA-approved NNRTIs

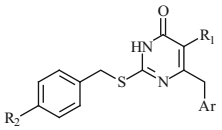


Benzothiazine dioxides

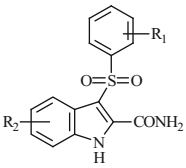


R = CH_3, H; X = CH_2, $(CH_2)_2$, $CH(CH_3)$

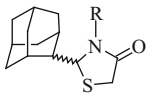
N1, N3-Disubstituted uracils



6-Arylmethyl-substituted S-DABOs



Indolyl Aryl Sulfones



2-Adamantyl-substituted thiazolidin-4-ones

Fig. 10 Structure of compounds with the RT inhibitory activity

thiazolidin-4-ones [84], lectins [85], and many others [10, 86–93]. Representative structures of these inhibitors are shown in Fig. 10.

Though all approved NNRTI have different chemical structures, all of them contact the same site in the RT structure. Therefore, a mutation providing resistance to one NNRTI also provides resistance to all other NNRTIs ("cross resistance") [94–96]. Amino acid residues of the allosteric site, which are subject to mutations, are indicated in Fig. 7b. Amino acid residues located in the same position of resistant strains are shown in brackets.

Undesired Side Effects of NNRTIs

Non-nucleoside RT inhibitors do not present the same side effects as NRTIs but are related with high frequencies of resistance development. Undesired side effects are also associated with the use of NNRTIs, adding the goal of finding lower toxicity agents among the research targets [97, 98]. NNRTIs may also cause rash, Stevens–Johnson syndrome and toxic epidermal necrolysis. More specifically, efavirenz is associated with symptoms of the central nervous system disorders and fatigue and may also affect liver function and induce hyperlipidemia. Etravirine and nevirapine have been also related to liver disorders.

4.1.3 Nucleotide-Competing RT Inhibitors (NcRTIs)

Non-nucleotide/non-nucleoside RT inhibitors (NNRTIs) which do not incorporate into the newly synthesized DNA strand but exhibit competitive mode of inhibitory action against dNTPs belong to a different category and have been proposed to be

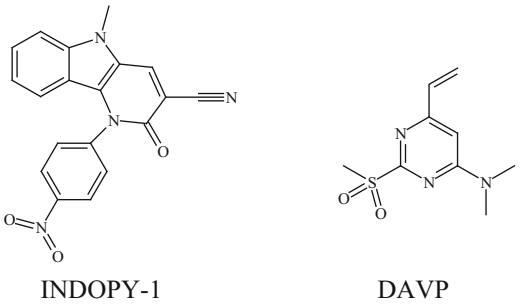


Fig. 11 Representative structures of NcRTIs: 5-methyl-1-(4-nitrophenyl)-2-oxo-2,5-dihydro-1*H*-pyrido[3,2-b]indole-3-carbonitrile (INDOPY-1 *left*) and 6-ethenyl-*N*,*N*-dimethyl-*N*,*N*-dimethyl-2-(methylsulfonyl)-4-pyrimidamine (DAVP *right*)

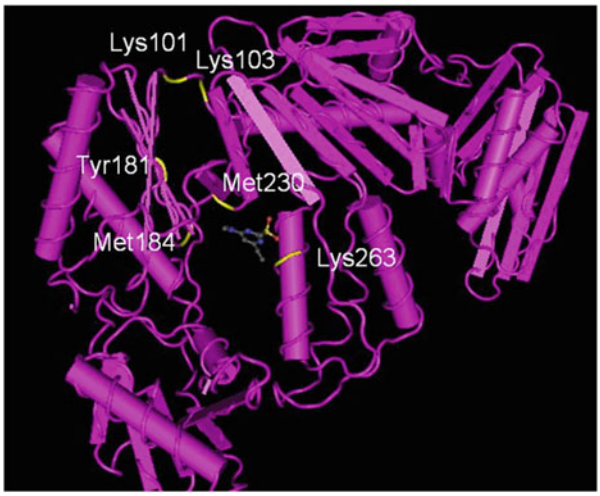


Fig. 12 HIV-1 reverse transcriptase complex with the NcRTI, 4-dimethylamino-6-vinylpyrimidine (PDB ID: 3ISN) [104]

called as nucleotide-competing RT inhibitors (NcRTIs) [99]. Among these inhibitors, 5-methyl-1-(4-nitrophenyl)-2-oxo-2,5-dihydro-1*H* pyrido[3,2-b]indole-3-carbonitrile (INDOPY-1) (Fig. 11) inhibits RT with a competitive [100] or mixed-type [101] mode with respect to dNTPs and seems to interact with the amino acid residues involved in dNTPs associations such as Met184 and Tyr115. 4-Dimethylamino-6-vinylpyrimidines (DAVPs) also compete with the incoming dNTP [102, 103]. They bind to an RT site distinct from the NNRTI-binding pocket and close to the RT polymerase catalytic site [104]. This site comprises from the amino acid residues Met230, Gly231, Gly262, Lys263, Trp266, Met184, and Asp186 (Fig. 12).

4.2 HIV-1 Integrase Inhibitors

HIV integrase is a promising drug target for HIV treatment because of its central role in the HIV life cycle and the absence of analog enzymes in human organism. Integrase is a 32 kDa protein that acts as a tetramer (Fig. 13) [105].

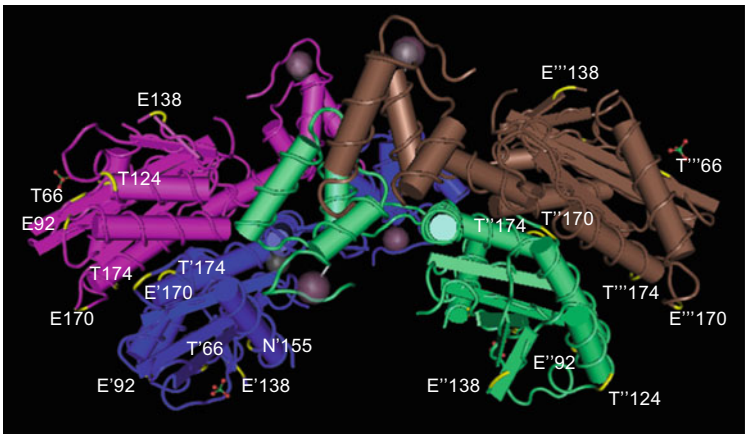


Fig. 13 3D structure model of HIV-1 integrase (PDB ID: 1K6Y) [105]

Like all retroviral integrases, the HIV integrase contains three domains: an N-terminal – Zinc-binding domain, consisted by three helices – a catalytic domain, and a C-terminal DNA-binding domain surrounded by the amino acid residues Thr66 and Glu92 [106]. Retroviral IN catalyzes: (a) a process called 3′-end processing, in which two or three nucleotides are removed from one or both 3′ ends of the viral DNA to expose the invariant CA dinucleotides at both 3′-ends of the viral DNA, and (b) the strand-transfer reaction, in which the 3′ ends of the viral DNA are covalently ligated to the host chromosomal DNA. Both reactions are catalyzed by the same active site. Several host-cell proteins bind to HIV integrase, facilitating its action. Human chromatin-associated protein LEDGE is one of them. LEDGE interacts with HIV integrase at the area of amino acid residues Thr124 and Glu170 (Fig. 14) [107].

Investigation for the finding of integrase inhibitors led to the development of inhibitors [108–130] that bind to either the catalytic site [109] or an allosteric site (Fig. 15) [110]. Allosteric inhibitors, such as the (2*S*)-2-*tert*-butoxy-2-[4-(4-chlorophenyl)-6-(3,4-dimethylphenyl)-2,5-dimethyl-3-pyridyl]acetic acid, LF8, are found to occupy the LEDGE interaction site (Fig. 15b). However, most of the inhibitors found and the FDA-approved integrase inhibitors are molecules that bind to the active site of the enzyme.

The first integrase inhibitor received FDA approval in October 2007 for the treatment of HIV-1 as part of combination antiretroviral therapy. This first approved drug of this category was raltegravir (RAL) (Fig. 16). Two other integrase inhibitors have been approved for the treatment of HIV till now: elvitegravir (ELV) and dolutegravir (DTG).

Raltegravir is a 1-*N*-alkyl-5-hydroxypyrimidinone [127]. It is a structural analogue of the diketo acid class of inhibitors [114, 127]. It possesses metal-chelating functions and can interact with the divalent metals Mg^{2+} or Mn^{2+} within the active site of HIV-1 integrase (Fig. 17) [128].

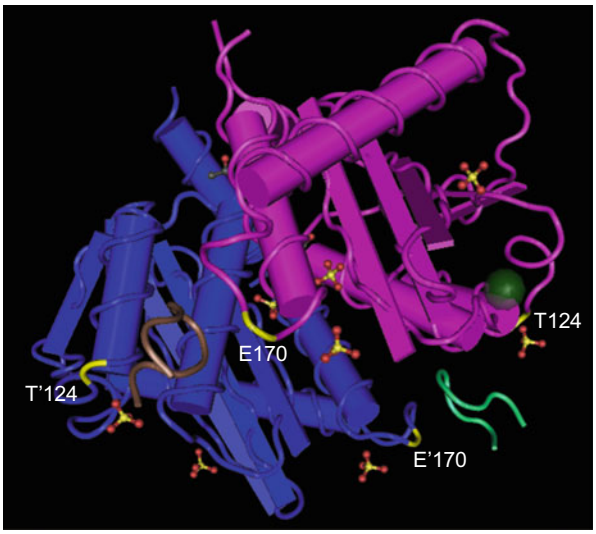


Fig. 14 HIV-1 integrase complex with the peptide LEDGE (*brown* and *green chains*). PDB ID: 3AVA [107]

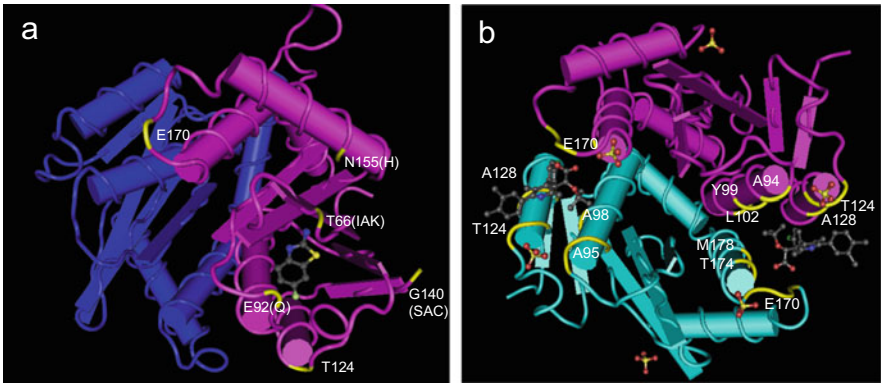
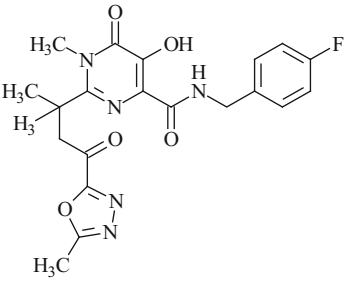


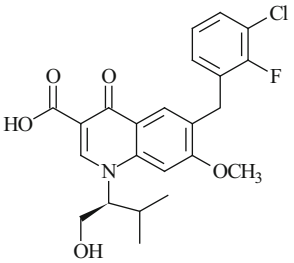
Fig. 15 (**a**) HIV-1 integrase catalytic core complex with 2-amino-6-fluorobenzothiazole (PDB ID: 3VQ9). Amino acid residues at the same position of the resistant strains are shown in *brackets* [82]. (**b**) HIV-1 complex with the allosteric inhibitor (2*S*)-2-*tert*-butoxy-2-[4-(4-chlorophenyl)-6-(3,4-dimethylphenyl)-2,5-dimethyl-3-pyridyl]acetic acid (PDB ID: 4O0J) [110]

Raltegravir has an IC_{50} value of approximately 10 nM and is active on many different HIV-1 and HIV-2 virus strains.

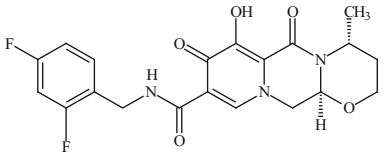
Elvitegravir is a dihydroquinoline carboxylic acid compound that also posses the β-hydroxyketone structural motif [129]. This drug is active against HIV-1 and HIV-2, with an IC_{90} of 1.2 nM in peripheral blood mononuclear cells (PBMCs), and an IC_{50} of 0.2 nM. Elvitegravir was licensed by Tokyo Tobacco in 2008 and was approved by FDA in the USA in 2012.



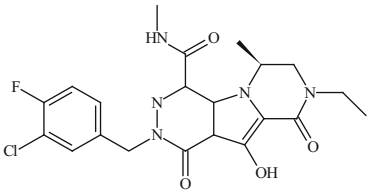
Raltegravir



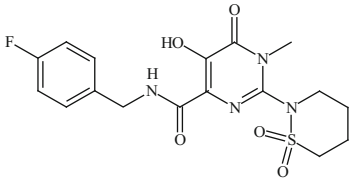
Elvitegravir



Dolutegravir



MK-2048



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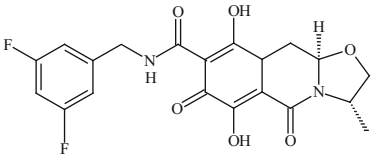
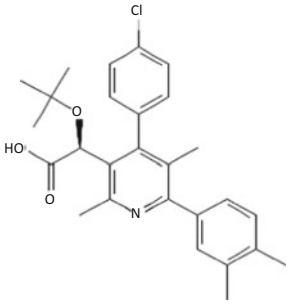


Cabotegravir



LF8

Fig. 16 Integrase inhibitors (INIs)

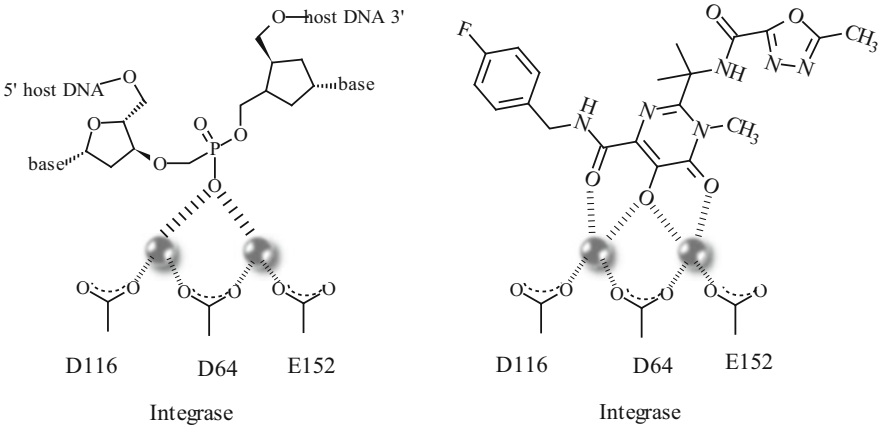


Fig. 17 Probable mechanism of action of integrase inhibitors bound to the active site of the enzyme [105]

Dolutegravir [130] was approved for the treatment of HIV in 2013 in the USA and Canada and in 2014 in Europe.

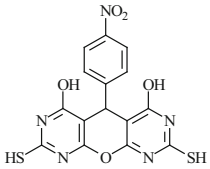
MK-2048 [126] belongs to the second generation of integrase inhibitors that are currently under development. MK-2048 is more potent than raltegravir, and it is being investigated for use as part of preexposure prophylaxis (PrEP).

Despite the achievements in the design of effective integrase inhibitors, the development of resistant strains is still an essential limitation to use these drugs for antiretroviral therapy. Resistance to both raltegravir and elvitegravir as well as to dolutegravir has been observed [112, 121, 126–128]. Since all approved INIs practically have the same mode of action, through binding at the catalytic site of the enzyme of INSTIs, a number of virus strains appeared with mutations that enable resistance to two or three approved integrase inhibitors. Therefore, the design and discovery of other classes of integrase inhibitors with a mechanism of action distinct from that of INSTIs still represents a highly attractive antiretroviral strategy.

There are many reports in the literature regarding HIV-1 IN inhibitors tested in vitro [129–131], but most of them did not exhibit antiviral activity in cell culture or have not appropriate selectivity indices. Furthermore, even if most of them show antiviral effect, it is not clear if the integration step is really targeted.

Pannecouque et al. [132] studied 5-(4-substituted-phenyl)-5-*H*-pyrano[2,3-d:-6,5-d]dipyrimidines (PDPs) as inhibitors of viral integration in cell culture. They found that compound V-165 or 5-(4-nitrophenyl)-2,8-dithiol-4,6-dihydroxy-5*H*-pyrano[2, 3-d:-6,5-d′]dipyrimidine was not only exhibiting an EC50 of 8.9 μM but was also active against HIV-1 (NL4.3 and L1), HIV-1 (NDK, NL4.3, and L1) strains, HIV-2 (ROD and EHO), and SIV (MAC251), at EC_{50} values in range 3.7–30 μM. Based on the obtained results, the authors concluded that V-165

could be a lead compound for further synthesis and development of novel HIV drugs for combination therapy.



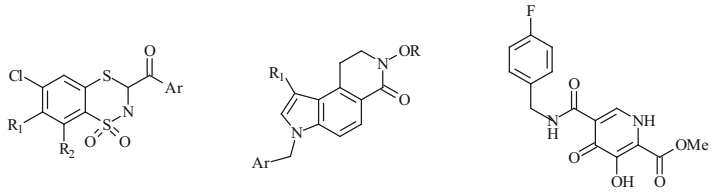
V-165

The search for new integrase inhibitors is continued. In 2009 Brzozowski et al. [133] reported the synthesis and biological evaluation of a series of novel 3-aroyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazines (Fig. 18). All the compounds **1**–**3** inhibited IN-mediated strand-transfer reaction with IC_{50} values ranging from 3 to 30 μM. The studies on mutants revealed that the Y99S mutant in general was about fivefold more resistant than the H114A. This implies that the tested compounds most likely bind to these novel sites.

Johnson et al. [134] reported the synthesis of novel tricyclic *N*-hydroxy-dihydronaphthyridinones (2) as potent, orally bioavailable HIV-1 integrase inhibitors. The evaluation of integrase inhibitory activity showed that IC_{50} of *N*-hydroxy-dihydronaphthyridinones is in range between 2.9 and 250 nM. It was found that antiviral activity in cell assays is comparable to the currently marketed HIV-1 integrase inhibitor raltegravir (EC_{50} 10 nM).

Kawasuji et al. [135] reported their studies on new carbamoyl pyridines (3) for their chelating properties in order to design new compounds with improved pharmacokinetic (PK) and resistance profile. Thus, the designed compounds with carbamoyl pyridone nucleus appeared to be good inhibitors in enzymatic as well as in antiviral assays with IC_{50} values in nanomolar range. Furthermore, these compounds being administered to rats, dogs, and monkeys exhibited good PK profile.

Tsiang et al. [136] aimed to test *tert*-butoxy-(4-phenyl-quinolin-3-yl)-acetic acids (Fig. 19), which were shown to be analogues to LEDGINs in order to



1 2 3

Fig. 18 Structures of 3-aroyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazines (*1*), tricyclic *N*-hydroxy-dihydronaphthyridinones (*2*), and carbamoyl pyridines (*3*)

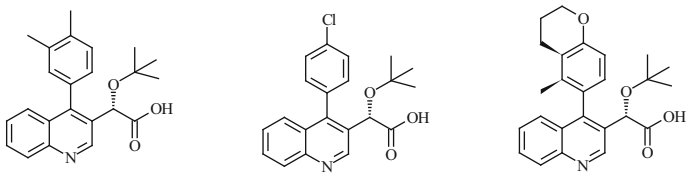


Fig. 19 Structure of *tert*-butoxy-(4-phenyl-quinolin-3-yl)-acetic acids

explicate their mechanism of action. This study revealed that tBPQAs appear to be potent inhibitors of HIV-1 replication with EC_{50} values of 10–20 nM. For this study a variety of infected cells including primary PBMC were used. Regarding mechanism of tBPQAs' action, authors showed that these compounds could be inhibitors of HIV-1 integration through binding to the IN dimer interface. It was also shown that they could be dual inhibitors, since they are responsible for loss of flexibility of IN dimer, which did not allow correct assembly of viral DNA-IN complex. On the other hand, it inhibits the interaction of IN with LEDGF.

4.2.1 Side Effects of Integrase Inhibitors

There are some common side effects reported in the literature such us creatinine kinase elevations, myopathy, and some others [120, 121]. Thus, common side effects of elvitegravir include diarrhea, while common side effects of dolutegravir include insomnia and headache. Serious side effects also include allergic reactions and abnormal liver function in patients who were simultaneously infected with hepatitis B or C.

4.3 HIV-1 Protease Inhibitors

HIV protease is an aspartate protease. Although similar to other aspartate proteases of human organism, its action in HIV replication is essential and cannot be replaced by proteases of the host cell [114, 115, 137, 138]. HIV protease consists of two identical protein subunits. The active site of the enzyme is placed between the two subunits at the area of amino acid residues Asp25, Thr26, and Gly27 of the first and Asp25′, Thr26′, and Gly27′ of the second subunit (Fig. 20). Peptide-like compounds have been used since the 1990s as HIV protease inhibitors, exhibiting competitive inhibition activity. These drugs inhibit proteolytic cleavage of HIV Gag, Pol, and Env polyproteins to the active proteins of the virus [140, 141].

All the commercial HIV protease inhibitors (Table 2) consist of a central core of hydroxyethylene scaffold with the exception of tipranavir, whose central core is a coumarin [142–145] (Fig. 21).

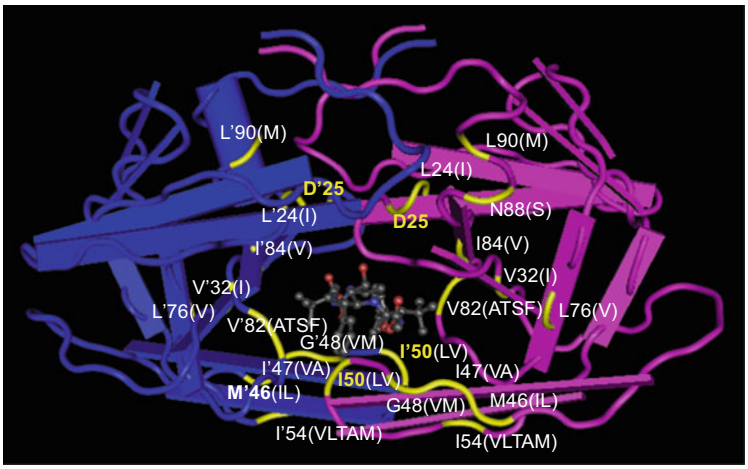


Fig. 20 3D structure of HIV-1 protease complex with fosamprenavir (PDB ID:3S85) *Yellow lines* represent amino acid residues involved in interactions with inhibitors or amino acid residues which are mutated at resistant strains of the virus. The amino acid residues present at the same position of mutated strains are shown in *brackets* [139]

Table 2 HIV-1 protease inhibitors approved by FDA

Drug	Nature	Date approved by FDA
Saquinavir	Peptidomimetic	1995
Ritonavir	Peptidomimetic	1996
Nelfinavir	Nonpeptidic	1997
Amprenavir	Nonpeptide	1999
Lopinavir	Peptidomimetic	2000
Fosamprenavir	Phosphoester prodrug	2003
Atazanavir	Azapeptide	2003
Tipranavir	Nonpeptide	2005
Darunavir	Nonpeptide	2006

Hydrogen bonds between hydroxyl groups of the inhibitor and the carboxylic acids of Asp25 and Asp25′ of the enzyme are involved in complex stabilization. Hydrogen bonds are also formed between a water molecule, which is linked to Ile50 and Ile50′ and carbonyl groups of the peptidomimetic inhibitors. Interactions with Ile50 residues of the enzyme are present in case of the non-peptidyl inhibitors as well. In general, the ability to form hydrogen bonds is essential for complex stabilization. In enzyme interaction with its natural substrate, four hydrophobic amino acid residues of the substrate are placed in four hydrophobic pockets of the enzyme. The existence of hydrophobic moieties capable to interact with these pockets in the molecule of inhibitors increases the potency of the inhibitor (Fig. 20).

Even though ritonavir was developed as HIV protease inhibitor, it is mostly used as a booster of other protease inhibitors. More specifically, because of its structural similarity with the known CYP3A inhibitors (Fig. 22), ritonavir acts as an inhibitor

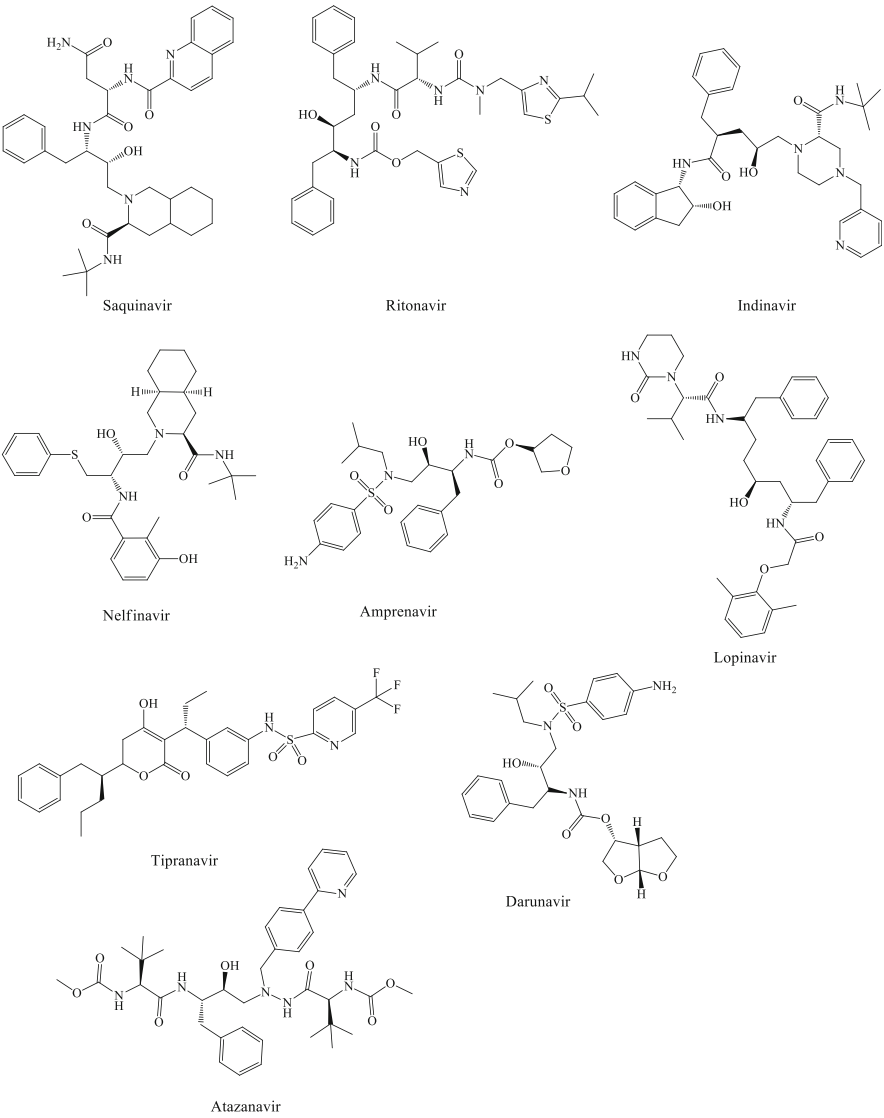


Fig. 21 Structures of the approved HIV protease inhibitors

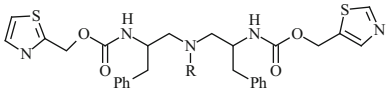


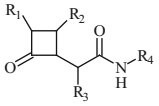
Fig. 22 Structure of CYP3A inhibitor

of cytochrome P450 3A4 (CYP3A4) that normally metabolizes protease inhibitors in the liver. Low doses of ritonavir can be used to enhance the other protease inhibitors [146].

Unfortunately, ritonavir and other protease inhibitors are associated with several side effects as well as development of HIV-resistant strains. The changes in amino acid residues in resistant strains are shown in Fig. 20 in brackets. Moreover, strains resistant to one PI may show resistance to other protease inhibitors as well (cross resistance). Thus, the development of novel inhibitors, with less adverse effects and ability to act on resistant mutants, is of major interest, and various groups have reported on their progress in this area [147].

Sperka et al. [148] based on the previous publications [149, 150] reported the synthesis of β-lactam derivatives and their evaluation as uncompetitive PIs. Authors [148] used a colorimetric microtiter plate method [151] to screen a 126-member combinatorial monocyclic beta-lactam library [152] for inhibition of the enzyme. It was found that several of the compounds exhibited more than 60 % inhibition that was proved for some compounds by HPLC method under the same assay conditions. The Ki values for the compounds were determined by HPLC method [153].

It should be mentioned that the type of inhibition depends on the conditions in which assay was performed. Thus, under low ionic strength conditions, the type of inhibition appeared to be uncompetitive, while at high ionic strength which is optimal for HIV protease [154, 155], the type of inhibition was mixed.



Beta-lactam derivatives

Analyzing these results, the authors believed that the inhibitor might interact with the closed flap region of the enzyme–substrate complex [148]. On the other hand, change in the type of inhibition at higher ionic strength may be due to the more favorable binding of these compounds to the active site of the enzyme through hydrophobic contact with the appropriate side chains [148]. The same was observed for peptidomimetic inhibitors, which bind much more strongly toward the active site of the enzyme in high ionic strength [145].

Cigler et al. [156] reported the potent, specific, and selective inhibition of HIV PR by parental and substituted metallacarboranes, namely, cobalt bis (1,2-dicarbollides). They provided evidence for the mechanism of action of these compounds, showed their antiviral activity in tissue cultures, analyzed their binding toward the enzyme by X-ray crystallography, and showed the potential of this class of compounds to become a novel pharmacophore for enzyme inhibition. Authors identified 12-vertex metallacarborane clusters as suitable hydrophobic, stable, and nontoxic structural analogues of aromatic compounds. These compounds showed good antiviral activity with IC_{50} values ranged from 0.13 to 1.4 μM.

Ghosh and Anderson [157] in their review presented the design of novel HIV-1 protease inhibitors with heterocyclic core scaffolds that have been reported in the recent years (2005–2010). They pointed out on the role that heterocycles play as scaffold and bioisosteres in HIV protease-inhibitor drug development. Some of them are shown in Fig. 23.

Wang et al. [158] reported the evaluation of anti-HIV activity of mangiferin. According to the authors, mangiferin (Fig. 24) can inhibit HIV-1IIIB-induced syncytium formation at noncytotoxic concentrations, with a 50 % effective concentration (EC_{50}) at 16.9 μM and a therapeutic index (TI) above 140. It should be mentioned that inhibitory activity of this compound was dose dependent. Furthermore, it showed activity against (NNRTIs) resistant strain HIV-1A17 with EC_{50} 22.75 μM.

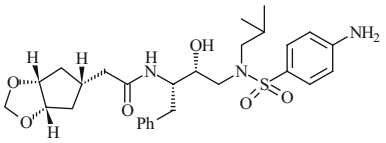
Jonckers et al. [159] reported the discovery of a novel class of benzoxazole and benzothiazole amides that were designed to have no other primary activity than CYP3A4 inhibition together with an acceptable toxicity/side effect profile.

A diverse set of benzoxazole and benzothiazole amide derivatives was obtained using a convenient synthesis (Scheme 1) and evaluated for their anti-HIV activity against wild-type HIV-1 which was studied on acutely infected lymphoblastic cell line (MT4-LTR EGTP) using gene assay [160]. None of the compounds showed significant activity with $EC_{50} > 10$ μM in each case.

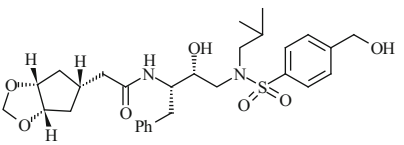
Compounds were also tested for the CYP3A4 inhibition in vitro using a human liver microsome (HLM)-based assay in which conversion of midazolam to 10-OH-midazolam was measured (by LC/MS) in the presence and absence of the inhibitor. Fortunately, all compounds exhibited very good inhibitory activity with IC_{50} values in range from 0.022 to 2.7 μM. Analysis of the results revealed some structure–activity relationships. Thus, it was observed that overall, having a 5-thiazolyl fragment (R1) present in the molecule resulted, in most cases, in potent CYP3A4 inhibition, with the 3-pyridyl and 5-benzo[1,3]dioxolyl fragment as good alternatives. On the contrary, the 4-pyridyl group is clearly unfavorable as a tenfold loss in inhibitory potency was observed. It could be mentioned that the authors identified a novel class of CYP3A4-inhibiting benzoxazole and benzothiazole amides that are devoid of HIV protease-inhibiting activity following a key "sulfonamide-to-amide" switch.

4.3.1 Side Effects of Protease Inhibitors

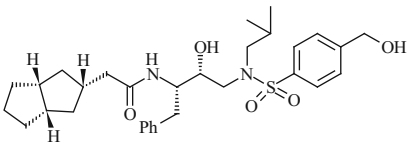
Even protease inhibitors play an important role in antiretroviral therapy and have dramatically improved the life expectancy of HIV-infected individuals; they are also associated with abnormalities in glucose/lipid metabolism and body fat distribution. There is no clear picture regarding the pathogenesis of protease-inhibitor-associated metabolic and body fat changes and their potential treatment; thus, further studies are required. Many protease inhibitors have been accused for gastrointestinal disorders, increasing of bleeding, insulin resistance, hyperglycemia, and hyperlipidemia and have also been associated with increased incidents of coronary artery disease and lipodystrophy [161–165].



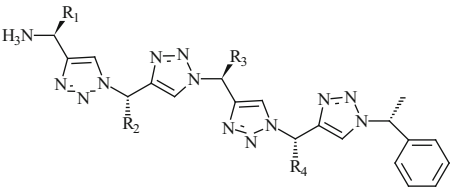
Ki = 0.14 nM
EC_{50} = 8 nM

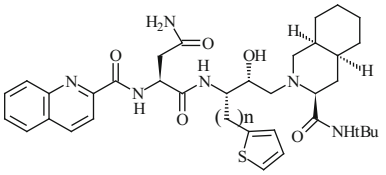


(GRL-06579A)
Ki = 4.5 pM
EC_{50} = 1.8 nM

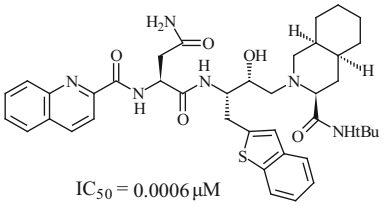


Ki = 5.3 nM
EC_{50} > 1000 nM

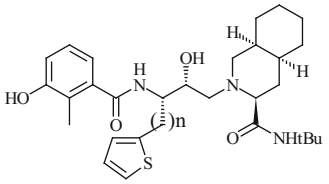




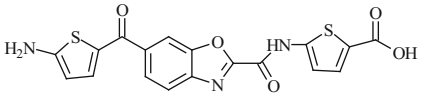
n = 0; IC_{50} = 5 μM
n = 1; IC_{50} = 0.02 μM



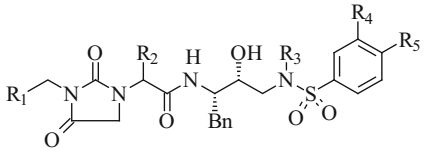
IC_{50} = 0.0006 μM



n = 0; IC_{50} = 30 μM
n = 1; IC_{50} = 0.003 μM



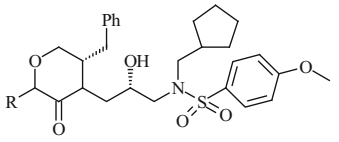
Ki = 1.6 μM, IC_{50} = 17 μM



Imidazoline-2,4-dione

R_1 = 2-MOM-4-thz
R_2 = 2-(S)-butyl
R_3 = 2-Propyl
R_4 = OMe
R_5 = H

EC_{50}wt = 1nM
EC_{5017} = 5 nM
AUC = 7μgh/ml



Morpholinone PIs

R = (*R*) allyl, Ki = 2 nM
R = spyro-cyclohexyl, Ki - 2 nM

Fig. 23 PIs with the heterocyclic scaffolds

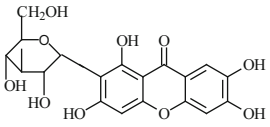
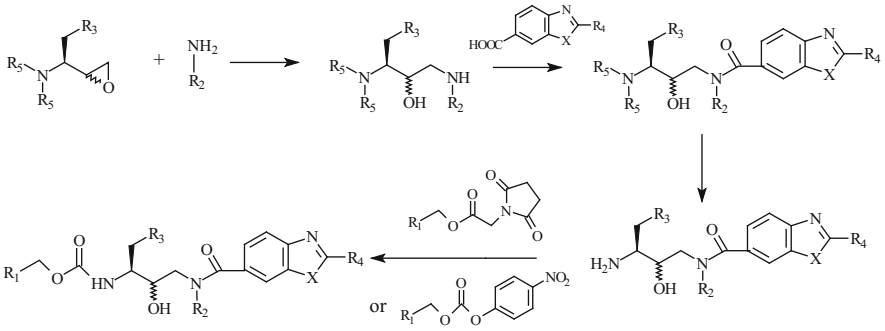


Fig. 24 Structure of mangiferin



Scheme 1 Synthesis of benzoxazole and benzothiazole amide derivatives as potential anti-HIV agents

Moreover, there is a link between HIV-PI usage and increased ROS production as shown in the literature established by human [166]-, animal [167–170]-, and cell-based studies [171–175], which include numerous cell and tissues.

4.4 HIV Fusion Inhibitors

The drugs of this class are responsible for binding, fusion, and entry of HIV virions into a human cell. They attached themselves to proteins of the surface of HIV virion or to proteins of the surface of CD4 cells. The US Food and Drug Administration (FDA) approved only two entry inhibitors.

The first one is Fuzeon (enfuvirtide) (Fig. 25), which is an oligopeptide, approved in March 2003, that targets the gp41 protein on HIV's surface [176–178]. Apart from enfuvirtide, several other oligopeptides have been found to exhibit inhibitory action targeting at the same viral protein (Fig. 26).

The second category of entry inhibitors includes maraviroc, which acts as a negative allosteric modulator of the CCR5 co-receptor (Fig. 27). This drug avoids the association of HIV protein gp120 to the CCR5, thus blocking the entry of the virus into the host cell. However, HIV can also use the other co-receptors such as the CXCR4.

In a way similar to other drugs targeting to viral proteins, strains resistant to enfuvirtide have also been developed. The most common mutations leading to resistance involve the amino acid residues 36–45 of gp41 such as Gly361→Asp,

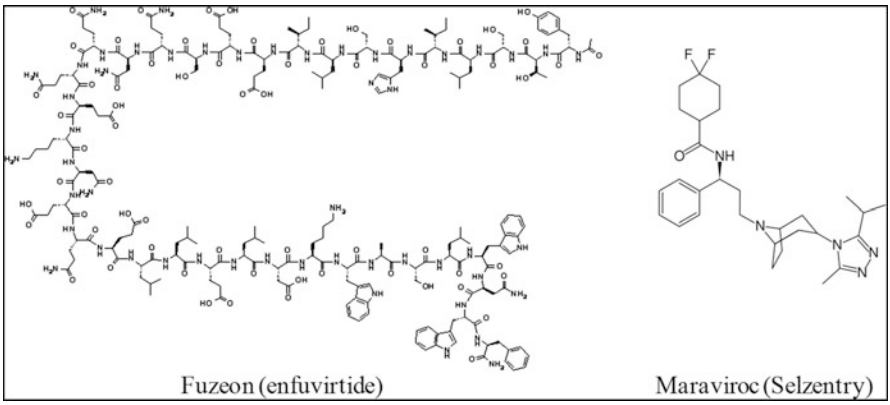


Fig. 25 FDA-approved HIV entry inhibitors

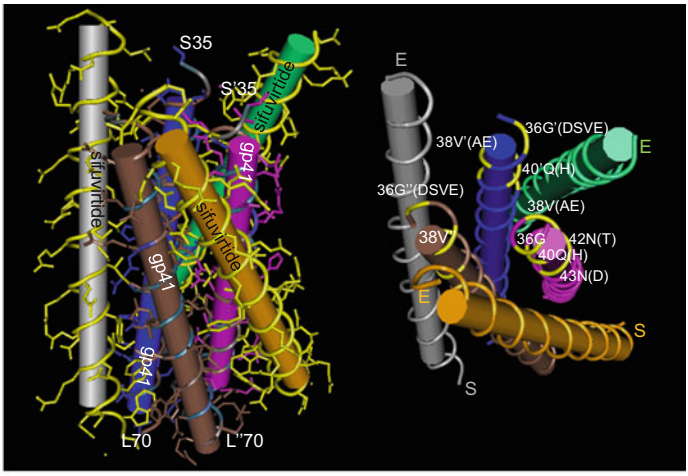


Fig. 26 HIV-1 envelop glycoprotein gp41 complex with the fusion oligopeptide inhibitor sifuvirtide (PDB ID: 3VIE) [186]. Amino acid residues mutated at strains resistant to enfuvirtide are shown with *yellow lines* at the *right structure*. The residues at the same position of resistant strains are shown in *brackets*

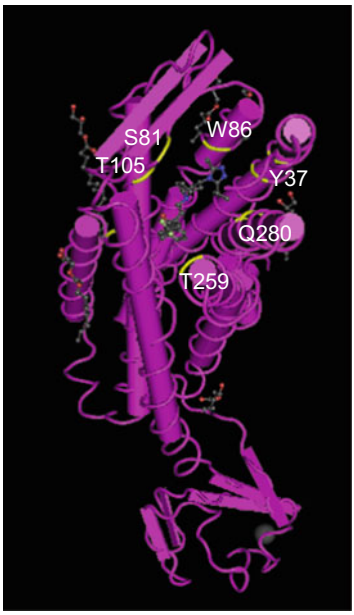


Fig. 27 CCR5 co-receptor complex with maraviroc (PDB ID: 4MBS) [179]

Ser, Val, or Glu; Val38→Ala, Glu, or Met; Gln40→His; Asn42→Thr; and Asn43→Asp [180–185] (Fig. 26).

Experimental drugs include Schering-Plough's CCR5-blocking entry inhibitor vicriviroc, Progenics's CCR5-blocking monoclonal antibody PRO 140, and Tanox's TNX-355, a drug that targets the CD4 protein on CD4 cells.

Since only two drugs were approved by FDA as fusion inhibitors and taking into account that this is an important target in the battle against HIV, scientific community continued the search for new potent fusion inhibitors. Jiang et al. [187] based on their previous works [188, 189] on the synthesis of *N*-(4-carboxy-3-hydroxy) phenyl-2,5-dimethylpyrrole (1) and *N*-(3-carboxy-4-chloro)phenylpyrrole (2) as well as series of 2-aryl-5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl) furans (3a-o) reported the synthesis of new 5-((arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl) *phenyl*)thiazolidin-4-ones (12a-o), modifying chemical structures of previous compounds (3a-o). The modifications are deleting of the CH_2CH_2 side-chain linker and also in some cases changing the carboxyl group for a tetrazolyl unit and/or the furan ring for pyrrole (Fig. 28).

These modifications resulted in improved activity almost for all compounds. It should be mentioned that two of them, 12-l and 12-m (X-tetrazolyl, Y=O, R=Cl, H and R_1=H, F, respectively), showed inhibitory activity against HIV-1IIIB at low nanomolar level (EC_{50} 0.018 ± 0.002 and 0.014 ± 0.005, respectively) and selectivity indexes (SI values) of >2,000. Furthermore, analysis of structure–activity relationships showed that furan derivates were more potent than the pyrroles (12f-i) against HIV-1 IIIB infection (about 40-fold), indicating the favorable role of oxygen at position Y.

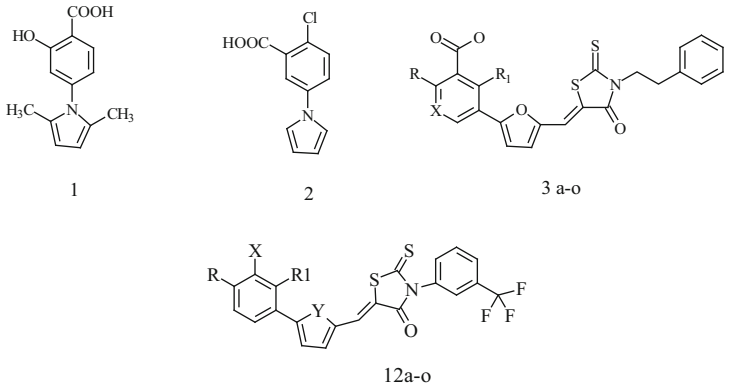


Fig. 28 Fusion inhibitors of HIV

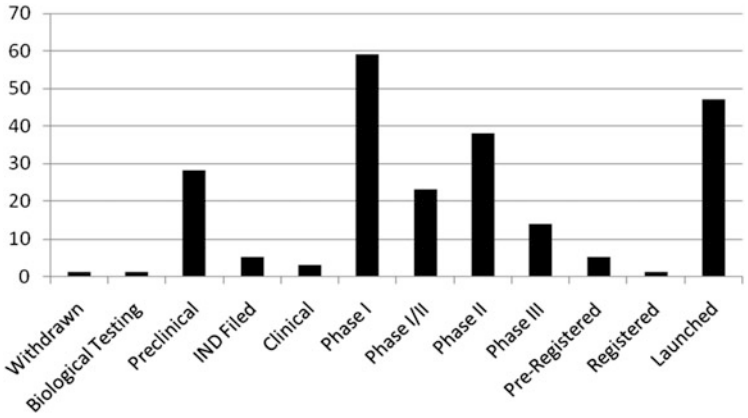


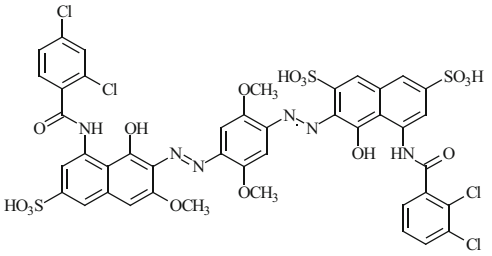
Fig. 29 The number of current anti-HIV/AIDS agents at different stages of research and development

Also it was found that tetrazole group in position X is more preferable than COOH group. However, molecular docking studies of active compound with COOH group and with tetrazole revealed that both of them docked in the hydrophobic cavity almost in the same way [164, 187].

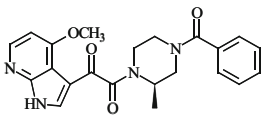
Even gp120 protein was recognized as drug target [190, 191] until recently, effective, potent and selective small molecules that act on gp120 were not discovered.

Dezube et al. [192] reported, a bis(disulfonaphthelene) derivative (FP-21399) (Fig. 29) as anti-HIV agent. Unfortunately, despite that it was introduced to phase I clinical studies, its profile in reducing viral load was not good. However, these attempts were continued and led to a molecule 4-methoxy-7-azaindole derivative (BMS-378806), which inhibits infection by HIV-1 strains at nanomolar level

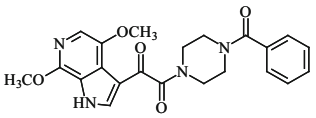
[190]. Although the clinical development of this molecule was terminated, a second-generation analogue BMS-488043 replaced the BMS-378806 which showed promising oral bioavailability and safety profile.



FP-21399



BMS-37806



BMS-488043

4.4.1 Side Effects of Fusion Inhibitors

Among the minor adverse effects of fusion inhibitors are pain, erythema, nodules, or cysts at the site of injection. Other adverse effects may include headache, dizziness, pain or tenderness around the eyes, cough and shortness of breath, loss of appetite and weight loss, and pain in the arms, legs, hands, or feet. Severe adverse effects may include allergic reactions fever, vomiting, kidney problems, low blood pressure, and paralysis.

4.5 Novel Drug Targets: TAR, Tat-Binding Drugs

It is known that the regulatory proteins Tat and Rev are important for HIV replication. The protein Tat (trans-activator of transcription) binds to trans-activator responsive region (TAR) of HIV RNA, stimulating the transcription [170, 193]. An arginine-rich area of Tat recognizes the base sequence and the conformation of TAR RNA. Two kinds of inhibitors targeted the Tat–TAR interaction. The first binds directly to TAR RNA, while, the second binds to the Tat protein. Both of them block the formation of Tat–TAR complex [194, 195].

According to Aboul-Ela et al. [196], small molecules may be able to lock the RNA structure into a conformation that does not allow binding of the Tat protein. The antibiotics neamin and neomycin and their derivatives are representatives of Tat–TAR interaction blockers and may also prevent secondary infections in HIV patients [197, 198].

Furthermore, purine nucleoside analogs such as 5,6-dichloro-1-b-D-ribofuranosylbenzimidazole [199] and carbocyclic adenosine analogs [200, 201] inhibit the Tat action.

On the other hand, Rev protein, which recognizes the Rev-response element (RRE) [202], could be also a potential target for anti-HIV therapy.

5 HAART and Combined Formulations

Currently, the most effective treatment of HIV/AIDS patients is highly active antiretroviral therapy (HAART), which results in sustained reductions in viral load and increases in CD4 cell counts [203, 204].

It includes three or more anti-HIV drugs in combination. First-line regimens at the current time consist of two nucleoside or nucleotide reverse transcriptase inhibitors with a non-nucleoside reverse transcriptase inhibitor, protease inhibitor, or integrase inhibitor [205]. However, most patients continued to have low levels of HIV-1 detectable in the blood using assays that can measure as little as one copy per mL [206].

Initially, several antiretroviral drugs were combined as separate dosage forms; later, to reduce the pill burden and increase patient compliance, such medicines were developed and launched as the combined formulations. Such combination products are listed below.

Drug	Mechanism of action	Company	Year
Lamivudine/zidovudine (Combivir)	Prodrug: active metabolites of lamivudine are lamivudine triphosphate (3TC-TP) and zidovudine triphosphate (ZDV-TP); both are RT inhibitors. 3TC-TP is also a weak inhibitor of the cellular DNA polymerases alpha, beta, and gamma, while ZDV-TP is a weak inhibitor of only the alpha and gamma subtypes	GlaxoSmithKline	1997
Lamivudine/zidovudine/ abacavir sulfate (Trizivir)	Triple synthetic nucleoside analogue combination therapy. Prodrugs, active metabolites of lamivudine, zidovudine, and abacavir sulfate are 3TC-TP, ZDV-TP, and carbovir triphosphate (CBV-TP), respectively. 3TC-TP	GlaxoSmithKline	2000

(continued)

Drug	Mechanism of action	Company	Year
	and CBV-TP are also weak inhibitors of the cellular DNA polymerases alpha, beta, and gamma, while ZDV-TP is a weak inhibitor of only the alpha and gamma subtypes		
Lopinavir/ritonavir (Kaletra)	Lopinavir is an inhibitor of the HIV protease; ritonavir inhibits the CYP3A-mediated metabolism of lopinavir that increases plasma levels of lopinavir	AbbVie	2000
Abacavir sulfate/lamivudine (Epzicom)	Combination product containing two synthetic nucleoside analogs acting as RT inhibitors	GlaxoSmithKline	2004
Tenofovir disoproxil fumarate/emtricitabine (Truvada)	Combination of two nucleoside reverse transcriptase inhibitors (NRTIs). Tenofovir diphosphate is also weak inhibitor of mammalian DNA polymerases alpha, beta, and mitochondrial DNA polymerase gamma, while emtricitabine is a weak inhibitor of mammalian DNA polymerase alpha, beta, epsilon, and mitochondrial DNA polymerase gamma	Gilead	2004
Tenofovir disoproxil fumarate/emtricitabine/efavirenz (Atripla)	Atripla(TM) is a combination of Bristol-Myers Squibb's non-nucleoside reverse transcriptase inhibitor (NNRTI), Sustiva (R) (efavirenz), and Gilead Science's Truvada(TM), itself a combination of two nucleoside transcriptase inhibitors (NRTI): emtricitabine and tenofovir disoproxil fumarate	Bristol-Myers Squibb/Gilead	2006
Tenofovir disoproxil fumarate/emtricitabine/rilpivirine hydrochloride (Complera)	Combination of Truvada (R) (tenofovir disoproxil fumarate/emtricitabine) and TMC-278 (rilpivirine hydrochloride), non-nucleoside reverse transcriptase inhibitors	Gilead	2011
Elvitegravir/GS-9350/Truvada (Stribild)	Combination of elvitegravir, GS-9350, tenofovir disoproxil fumarate, and emtricitabine, which jointly act as RT inhibitors, integrase (IN) inhibitors, DNA polymerase inhibitors, and cytochrome P450 CYP3A4 inhibitors	Gilead	2012

(continued)

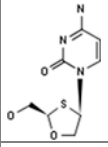
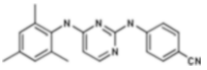
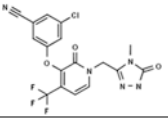
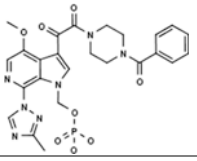
Drug	Mechanism of action	Company	Year
Darunavir/cobicistat (Prezcobix)	Combination of darunavir, a HIV protease inhibitor, and cobicistat, a cytochrome P450 CYP3A4 inhibitor	Janssen	2014
Dolutegravir/abacavir/ lamivudine (Triumeq)	Combination of RT inhibitors and HIV IN inhibitors	ViiV Healthcare	2014
Atazanavir sulfate/cobicistat (Evotaz)	Combination of HIV protease inhibitors and cytochrome P450 CYP3A4 and CYP2D6 inhibitors	Bristol-Myers Squibb	2015

6 Current Anti-HIV/AIDS Agent Pipeline

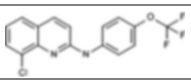
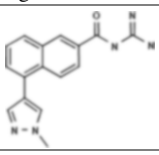
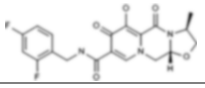
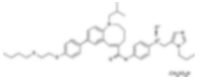
Despite the availability of HAART therapy, the further research and development of new anti-HIV agents is needed due to the non-sufficient efficacy of the existing drugs as well as because of severe side effects and arising resistance to the present therapy. Representative examples of novel small molecule drugs and biologics under development are listed below.

Name	Structural formulae	Mechanism of action	Company	Stage
Raltegravir potassium/ lamivudine	–	RT inhibitors/IN inhibitors	Merck & Co.	Registered
Elvitegravir/ cobicistat/ tenofovir alafenamide/ emtricitabine	–	RT inhibitors/IN inhibitors/ CYP3A4 inhibitors	Gilead	Preregistered
Emtricitabine/ rilpivirine hydrochloride/ tenofovir alafenamide fumarate	–	RT inhibitors	Gilead	Preregistered
Emtricitabine/ tenofovir alafenamide fumarate	–	RT inhibitors	Gilead	Preregistered
HIV-1 immunogen (Remune)	–	HIV vaccine candidate	Immune Response BioPharma	Preregistered
Darunavir/ cobicistat/	–	RT inhibitors/ protease	Gilead	Phase III

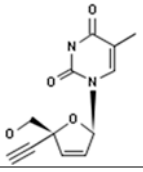
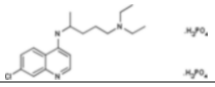
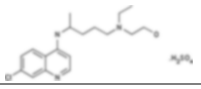
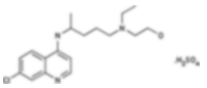
(continued)

Name	Structural formulae	Mechanism of action	Company	Stage
emtricitabine/ tenofovir alafenamide fumarate		inhibitors/ CYP3A4 inhibitors		
Dolutegravir/ rilpivirine	–	RT inhibitors/IN inhibitors/	ViiV Healthcare/ Janssen	Phase III
AIDSVAX gp120 B/E	–	Bivalent vaccine candidate	Walter Reed Army Institute	Phase III
ALVAC E120TMG	–		Walter Reed Army Institute/ Sanofi Pasteur	Phase III
Albuvirtide	Polypeptide	HIV fusion inhibitors	Frontier Biotechnologies	Phase III
Apricitabine		RT inhibitors	Avexa	Phase III
Dapivirine		RT inhibitors	International Partnership Microbicides	Phase III
Doravirine		RT inhibitors/IN inhibitors/ CYP3A4 inhibitors	Merck & Co.	Phase III
Fostemsavir		HIV attachment inhibitors/ CYP3A4 inhibitors	Bristol-Myers Squibb	Phase III
MK-1439A	–	RT inhibitors/IN inhibitors/DNA polymerase inhibitors/ CYP3A4 inhibitors	Merck & Co.	Phase III
PRO-140	Humanized monoclonal IgG4 kappa antibody	Anti-CD195 (CCR5)/signal transduction modulators/viral entry inhibitors	CytoDyn	Phase III
S-247303	–	IN inhibitors	ViiV Healthcare	Phase III
Tubercin T-5	–	Carbohydrate complex, a mixture of low molecular-	Artec	Phase III

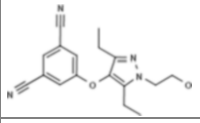
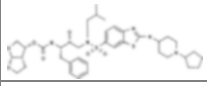
(continued)

Name	Structural formulae	Mechanism of action	Company	Stage
		weight polysaccharides with arabinomannan structure extracted from *Mycobacterium tuberculosis*		
Alpha1-Antitrypsin (human)	Biological source-derived proteins		Grifols	Phase II/III
4E10/2F5/2G12	Combination of the anti-HIV-1 human monoclonal antibodies 4E10, 2F5, and 2G12	Viral entry inhibitors	Polymun	Phase II
ABX-464		HIV replication inhibitors	Abivax	Phase II
AGS-004	AIDS vaccine consisting of dendritic cells electroporated with autologous amplified HIV-1 gag, nef, rev, and vpr RNA antigens and CD40 ligand RNA		Argos Therapeutics	Phase II
BIT-225		Nucleocapsid p7 protein (NCp7) zinc finger inhibitors	Biotron Ltd.	Phase II
BMS-955176	–		Bristol-Myers Squibb	Phase II
C7-DHAdC	Oral prodrug of KP-1212-triphosphate, the active RT metabolite and substrate		Koronis	Phase II
Cabotegravir		IN inhibitors	ViiV Healthcare	Phase II
Cenicriviroc mesylate		HIV attachment inhibitors/chemokine CCR5 antagonists/chemokine CCR2B receptor ligands/signal transduction modulators	Tobira Therapeutics	Phase II

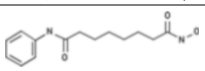
(continued)

Name	Structural formulae	Mechanism of action	Company	Stage
Censavudine		RT inhibitors	Oncolys	Phase II
Chloroquine phosphate		Apoptosis inducers	NIAID	Phase II
FIT-06	AIDS vaccine consisting of a DNA plasmid expressing HIV-1 B-clade nef, rev, tat, gag, pol, env, and CTL epitopes		FIT Biotech	Phase II
GS-9883	–	IN inhibitors	Gilead	Phase II
GTU-MultiHIV multiclade	DNA-based HIV vaccine candidate		FIT Biotech	Phase II
HIV-LIPO-5	AIDS vaccine candidate that contains five lipopeptides from gag, nef, and pol corresponding to more than 50 epitopes		ANRS	Phase II
Hydroxychloroquine sulfate		Autophagy inhibitors	Medical Research Council (MRC)	Phase II
IR-103		TLR9 receptor agonists/signal transduction modulators	Immune Response BioPharma	Phase II
ITV-1	AIDS vaccine consisting of an inactivated purified extract of porcine pepsin recognizing HIV gp41 and gp120 proteins		Immunotech Laboratories	Phase II
Ibalizumab	Immunoglobulin G4, anti-(human CD4 (antigen)) (human–mouse monoclonal 5A8 gamma4-chain), disulfide with human–mouse monoclonal 5A8 kappa-chain, dimer	HIV attachment inhibitors/anti-CD4	TaiMed Biologics	Phase II

(continued)

Name	Structural formulae	Mechanism of action	Company	Stage
LC-002	DNA vaccine		Genetic Immunity	Phase II
Lersivirine		RT inhibitors	ViiV Healthcare	Phase II
Lexgenleucel-T	Lentiviral vector expressing an anti-sense sequence targeted to the HIV-1 envelope (env) gene	env expression inhibitors	VIRxSYS	Phase II
MVA-62B (GOVX-B11)	Modified vaccinia Ankara vector containing HIV-1 gag, pr, rt, and env genes from clade B	Recombinant vector vaccines	GeoVax Labs/ NIAID	Phase II
PF-232798	–	HIV attachment inhibitors/chemokine CCR5 antagonists/signal transduction modulators	ViiV Healthcare	Phase II
Rintatolimod	5′-Inosinic acid homopolymer, complex with 5′-cytidylic acid polymer with 5-′-uridylic acid (1:1)	TLR3 receptor agonists/signal transduction modulators	HemispheRx	Phase II
SB-728-T	Autologous CD4+ cells genetically modified at the CCR5 gene by zinc finger nucleases	CCR5 expression inhibitors	Sangamo	Phase II
Sevelamer carbonate	Epichlorohydrin-cross-linked polyallylamine carbonate		NIAID	Phase II
Sifuvirtide	Polypeptide	HIV fusion inhibitors	FusoGen Pharmaceuticals	Phase II
TMC-310911		HIV protease inhibitors	Janssen R&D Ireland	Phase II
UB-421	Anti-CD4 monoclonal antibody	Anti-CD4	United Biomedical	Phase II
VAC-3S	Peptide vaccines		InnaVirVax	Phase II
VM-1500		RT inhibitors	Viriom	Phase II
VRC-HIVADV014-00-VP	The vaccine candidate is composed of four adenoviral vectors		NIAID	Phase II

(continued)

Name	Structural formulae	Mechanism of action	Company	Stage
	(in a 3:1:1:1 ratio) that encode the HIV-1 Gag/Pol polyprotein from clade B and HIV-1 Env glycoproteins from clades A, B, and C			
VRC-HIVDNA016-00-VP	Multivalent HIV-1 DNA vaccine		NIAID	Phase II
Vacc-4x	HIV vaccine consisting of four water-soluble synthetic HIV-1 core protein (p24)-like modified consensus peptides (Vac-10, −11, −12 and −13)		Bionor Pharma	Phase II
Vorinostat		Histone deacetylase 1 (HDAC1) inhibitors/apoptosis inducers/ histone deacetylase 2 (HDAC2) inhibitors/histone deacetylase 3 (HDAC3) inhibitors/histone deacetylase 6 (HDAC6) inhibitors	Merck & Co.	Phase II
pGA2/JS7 (GOVX-B11)	DNA plasmid containing gag, pro, RT, env, tat, rev, and vpu genes from HIV-1 clade B		GeoVax Labs/ NIAID	Phase II
rTat (IIIB-BH-10)	Recombinant HIV-1 (HTLV-IIIB strain, clone BH-10) Tat protein-based vaccine		Istituto Superiore di Sanita	Phase II
791760	HIV clade B′/C DNA vaccine		Chinese Center Disease Control Prevent	Phase II

According to the data presented in Thomson Reuters Integrity database (http://integrity.thomson-pharma.com), there are about 100 other anti-HIV/AIDS agents at earlier stages of clinical trials or in preclinical studies. The distribution of all anti-HIV/AIDS agents versus different stages of R & D is given in Fig. 29.

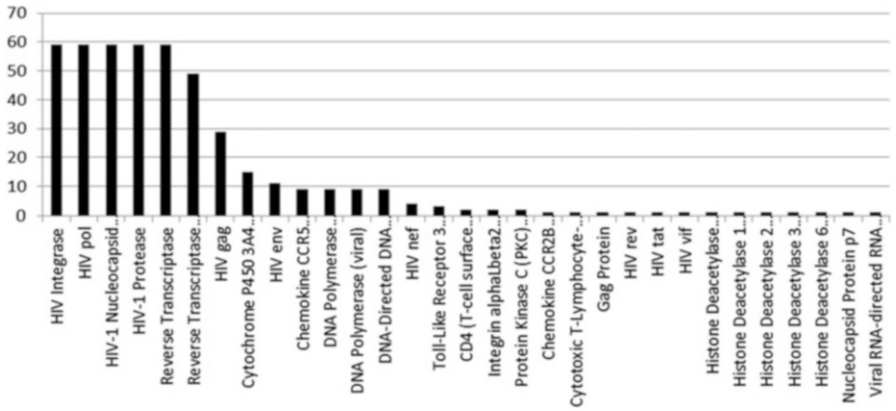


Fig. 30 The number of anti-HIV/AIDS agent with different targets

As one may see from the data presented in Fig. 29, only one anti-HIV agent was already withdrawn from the market. It was amprenavir, HIV-1 protease inhibitor, that was launched in 1999 under a collaboration agreement between GlaxoSmithKline, Kissei Pharmaceutical, and Vertex for the oral treatment of HIV infection in combination with other antiretrovirals in children 4 years of age and older and in adults. Amprenavir works by binding to the active site of HIV-1 protease, thereby, preventing the processing of viral gag and gag–pol polyprotein precursors. This results in the formation of immature and noninfectious virions. In 2005, Kissei Pharmaceutical issued a decision to voluntarily withdraw the marketing authorization for the product in Japan. Marketing authorization in the EU was withdrawn in 2010.

Distribution of all anti-HIV/AIDS agents versus different molecular targets is given in Fig. 30. As one may see from this data, the most popular targets now are: HIV integrase = HIV pol = HIV-1 nucleocapsid protein p7 = HIV-1 protease > reverse transcriptase > HIV gag > HIV env > chemokine CCR5 receptor, etc. Fifteen compounds are CYP3A4 inhibitors, which act in combination with anti-HIV agents increasing their concentration in plasma.

7 The Future Trends

As one may see from the presented above overview of the current status of the anti-HIV agents development, nowadays antiretroviral therapy is able to decrease significantly the mortality of HIV-infected people in industrially developed countries. However, the existing antiretroviral therapy is still too expensive for patients living in low-income and middle-income countries [207, 208]. Moreover, the available antiretroviral drugs do not lead to complete curation of HIV infection, cause severe adverse effect, and lead to the appearance of resistant strains. Thus, the

discovery of the novel more safety and efficacious anti-HIV medicines still remains the essential challenge.

7.1 Attempts to Overcome the Latent HIV Infection

One of the problems that prevent the complete curation of HIV-1 infection is the persistence of a viral reservoir that harbors integrated provirus within host-cellular DNA. This latent infection is unaffected by antiretroviral therapy and unseen by the immune system. To solve this problem, the mechanisms of latent infection and the sources of viral reservoirs are studied in detail now [207, 209]. Recent achievements in understanding of the latent reservoirs and new approaches to eradicate established HIV-1 infection and avoid the burden of lifelong ART are reviewed in several publications [209, 210]. In particular, one established mechanism of the patent HIV infection is associated with the repression of chromatin on the HIV-1 promoter. Histone deacetylation is a key modification connected with transcriptional repression of the HIV-1 promoter, and inhibition of histone deacetylase (HDAC) enzymes reactivates the latent HIV-1. Therapeutic potential in reactivating the latent HIV-1 by different HDAC inhibitors is discussed [211–214].

7.2 Natural Products as Potential Anti-HIV Agents

Natural products are known as the primary source of over 50 % of currently existing drugs [215]. It was demonstrated that they provide higher chemical diversity in comparison with the libraries of organic synthetic molecules [216]. Due to that, screening of plant extracts and other libraries of natural products is widely used for discovery of new anti-HIV agents [216–233] (http://www.ibscreen.com). We predicted the biological activities associated with the molecular mechanisms of anti-HIV action using computer program PASS (Prediction of Activity Spectra for Substances) [234] (http://www.way2drug.com/passonline) for the library of over 56,000 natural compounds, their analogs, and derivatives provided by InterBioScreen Ltd. (http://www.ibscreen.com). As one may see from the Fig. 31, this library is rather promising for screening of novel anti-HIV agents.

7.3 Biologicals as Potential Anti-HIV Agents

In addition to the small molecules of natural or synthetic origin, which are presently studied as novel anti-HIV agents, the large number of biological products is investigated. According to the Thomson Reuters Integrity (http://integrity.thomson-pharma.com), over 100 biologicals are studied as anti-HIV medicines. Their

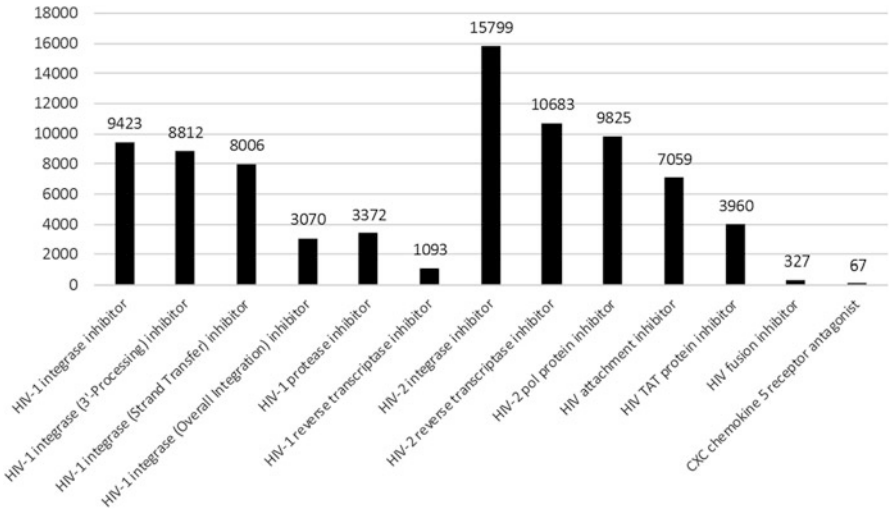


Fig. 31 Anti-HIV mechanisms of action predicted by PASS of InterBioScreen library of natural compounds (cutoff Pa>Pi, where Pa is the probability to be active, Pi is the probability to be inactive)

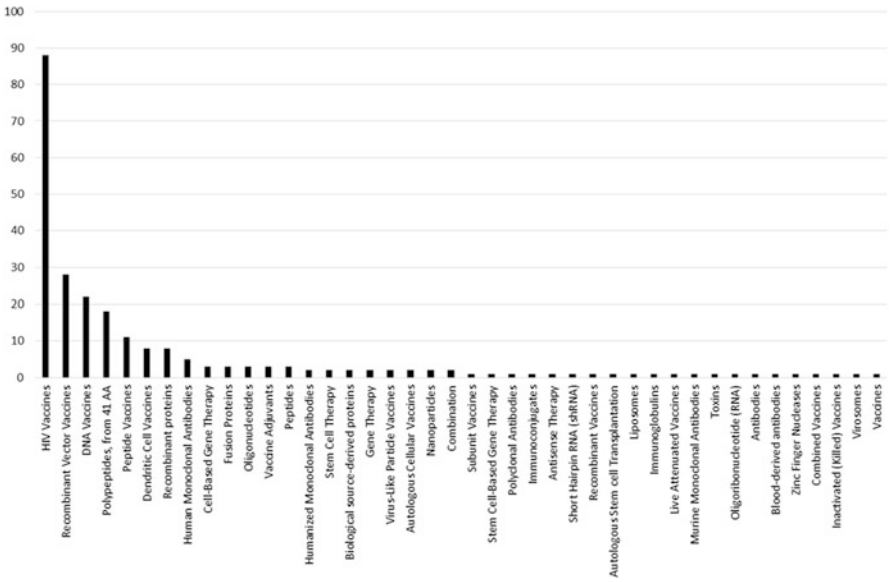


Fig. 32 Products categories of biologics under R & D as anti-HIV medicines

distribution by product categories is shown in Fig. 32. As one may see from the data presented here, the most actively studied are HIV vaccines > recombinant vector vaccines > DNA vaccines > polypeptides, from 41 AA > peptide vaccines > dendritic cell vaccines, etc. Also, among the different studied approaches to anti-

HIV therapy, one finds the gene therapy, antisense therapy, short hairpin RNA (shRNA), and oligoribonucleotide (RNA). Gene-based therapies that utilize RNA interference (RNAi) to silence the expression of viral or host mRNA targets that are required for HIV-1 infection and/or replication are reviewed recently [225].

It was shown that HIV-1 infectivity is influenced by the host-cellular miRNAs, and current results in the field of miRNA and HIV-1 interplay were recently discussed [226]. Didigu C and Doms R. considered the effects of gene therapy targeting HIV entry and impacts of allogeneic stem cell transplantation in the development of strategies to cure HIV infection [208]. Also, DNA aptamers to the HIV-1 reverse transcriptase are studied as potential therapeutic agents for treatment of HIV/AIDS [227].

However, till now the only biological product is launched as a remedy for anti-HIV therapy. This is the immune globulin intravenous (IGIV-C) developed as an immunomodulator and launched by Bayer for treatment of HIV infections in 2004 and approved in the USA for the treatment of primary immunodeficiency diseases by Grifols in 2010 (http://integrity.thomson-pharma.com).

7.4 Computer-Aided Drug Discovery of New Anti-HIV Agents

In recent years, computer-aided drug design methods are widely used in research and development of novel pharmacological agents for finding and optimizing hits and lead compounds [228, 229]. The basic computational methods include molecular docking, pharmacophore search, and (Q)SAR modeling. Since the field of anti-HIV drug discovery is extensively studied, both target-based and ligand-based drug design methods could be applied. Some examples of fresh works in this direction are given below.

Recently, novel HIV-1 protease inhibitors were identified by virtual screening using a complementary set of computational methods [230]. The potential HIV-1 protease inhibitors were searched in the National Cancer Institute (NCI) database, which contains 260,000 structures of organic compounds. Six molecules were selected based on computational prediction, and two of them (NSC111887 and NSC121217) showed inhibitory potency against HIV-1 protease in vitro, with IC_{50} values of 62 and 162 μM, respectively. The authors concluded that these compounds could be used for the further optimization as HIV-1 protease inhibitors.

Extract of *Caesalpinia sappan* L. was found to exhibit HIV-1 integrase inhibiting activity [231]. Nine compounds were extracted from the heartwoods and roots of *C. sappan* L. The most potent compounds against HIV-1 IN were sappanchalcone and protosappanin A with IC_{50} values 2.3 and 12.6 μM, respectively. Using molecular docking, the authors determined that these compounds presumably bind to the amino acid residues Gln148 and Thr66 in the core domain of HIV-1 integrase.

A few derivatives of N-substituted benzyl/phenyl-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2(4*H*)-yl)acetamides were found to exhibit the anti-HIV activity with $IC_{50} < 20$ μM [232]. Then, using molecular docking to the RT-bound nevirapine X-ray data, the authors determined that the presumable molecular mechanism of these compounds is binding in the NNRTI pocket of the HIV-1 reverse transcriptase.

Potential HIV-1 reverse transcriptase inhibitors were designed in silico as *N*-heteroaryl compounds bearing pyrimidine and benzimidazole moieties [233]. The designed compounds were synthesized and tested in cell assays using laboratory-adapted strains HIV-1IIIB (X4, subtype B) and HIV-1Ada5 (R5, subtype B) and the primary isolates HIV-1UG070 (X4, subtype D) and HIV-1VB59 (R5, subtype C). It was shown that the compounds were active at IC_{50} 1.4 μM with the selectivity index ranged from 1.29 to 38.39.

Structural details regarding the interactions between the inhibitors and CXCR4 were determined using holographic QSAR, docking, and molecular dynamics studies [235]. It was found that the binding is affected by two crucial residues Asp97 and Glu288. Structure–activity relationships were analyzed, and the obtained results will be useful for rational design of novel CXCR4 inhibitors.

Molecular modeling and site-directed mutagenesis studies on the RNase H domain demonstrated different binding poses for ester and acid diketo acids. It was shown that they interact with residues (Arg448, Asn474, Gln475, Tyr501, and Arg557) involved not in the catalytic motif but in highly conserved portions of the RNase H primer grip motif [236]. Therefore, the authors showed that RNase H inhibition by diketo acids is not only due to their chelating properties but also to the specific interactions with highly conserved amino acid residues in the RNase H domain. This finding provides important insights for the rational design of novel RNase H inhibitors.

To overcome the resistance to the available anti-HIV agents, rational design of inhibitors with dual mechanisms of action was performed [237]. Inhibitors of both HIV-1 reverse transcriptase (RT) DNA polymerase (DP) and ribonuclease H (RNase H) were discovered among the small library of 1,3-diarylpropenones, which exhibited dual inhibition properties in the low-micromolar range.

More information about the multi-targeted antiretroviral agents may be found in the paper [238].

Examples of application of in silico methods to the design and discovery of novel anti-HIV agents presented above clearly demonstrated that both target-based and ligand-based methods are useful for optimization of synthesis and biological testing of hits and lead compounds. Earlier [239], using the information from the NCI database about compounds tested in anti-HIV assays, we demonstrated that based on predictions of the computer program PASS [234], it is possible to reduce the number of experiments up to 17 times.

More information about applications of computational methods to the discovery and optimization of novel anti-HIV agents may be found in the papers [240–244]. Detailed consideration of the dynamics of HIV-1 reverse transcriptase complexes with different ligands and with a number of mutations allowed to reveal a

novel mechanism for drug resistance to non-nucleoside RT inhibitors [245]. - Computer-aided design of protein–protein interaction inhibitors as agents for potential agents for anti-HIV therapy is described in the paper [19].

Some latest algorithmic and methodological developments for application of docking to design of novel pharmacological agents were recently published [246]. An effective strategy was proposed using three orthogonal metrics for assessment and validation: pose reproduction over a large database of protein–ligand complexes, cross docking to 24 drug-target protein families, and database enrichment using large active and decoy datasets for five proteins including HIV-1 protease.

Since in the past few years the data on structures and biological activity of known anti-HIV agents are collected and presented by several publicly available resources (PubChem (http://pubchem.ncbi.nlm.nih.gov), ChEMBL (http://www.ebi.ac.uk/chembl), ChemSpider (http://www.chemspider.com), DrugBank (http://www.drugbank.ca), etc.), this stimulates the creating of numerous (Q)SAR models and their application to design and discovery of novel candidates for HIV/AIDS treatment. As was recently shown [247], this data could not be used for this purpose "as they are"; instead, the experts' estimates and careful prefiltering of the available data are necessary to obtain the (Q)SAR models with reasonable accuracy and predictivity.

Moreover, despite the development and application of powerful computational drug design methods, the essential role of researchers' intuition and serendipity in finding of efficacious antiretroviral drugs is emphasized [248, 249].

References

1. Zhu T, Korber BT, Nahmias AJ, Hooper E, Sharp PM, Ho DD (1998) An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. Nature 391 (6667):594–597
2. Worobey M, Gemmel M, Teuwen DE, Haselkorn T, Kunstman K, Bunce M, Muyembe JJ, Kabongo JM, Kalengayi RM, Van Marck E, Gilbert MT, Wolinsky SM (2008) Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. Nature 455(7213):661–664
3. Frøland SS, Jenum P, Lindboe CF, Wefring KW, Linnestad PJ, Böhmer T (1988) HIV-1 infection in Norwegian family before 1970. Lancet 1(8598):1344–1345
4. Worobey M, Telfer P, Souquière S, Hunter M, Coleman CA, Metzger MJ, Reed P, Makuwa M, Hearn G, Honarvar S, Roques P, Apetrei C, Kazanji M, Marx PA (2010) Island biogeography reveals the deep history of SIV. Science 329(5998):1487
5. Bailes E, Gao F, Bibollet-Ruche F, Courgnaud V, Peeters M, Marx PA, Hahn BH, Sharp PM (2003) Hybrid origin of SIV in chimpanzees. Science 300(5626):1713
6. Gao G, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, Cummins LB, Arthur LO, Peeters M, Shaw GM, Sharp PM, Hahn BH (1999) Origin of HIV-1 in the chimpanzee Pan troglodytes. Nature 397:436–444
7. Lemey P, Pybus OG, Wang B, Saksena NK, Salemi M, Vandamme AM (2003) Tracing the origin and history of the HIV-2 epidemic. Proc Natl Acad Sci 100:6588–6592
8. Global report of the Joint United Nations Program for AIDS (UNAIDS) (2013) http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Global_Report_2013_en_1.pdf

9. De Clercq E (2013) The nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, and protease inhibitors in the treatment of HIV infections (AIDS). Adv Pharmacol 67:317–358
10. Flexner C, Saag M (2013) The antiretroviral drug pipeline: prospects and implications for future treatment research. Curr Opin HIV AIDS 8(6):572–578
11. McGowan I (2014) An overview of antiretroviral pre-exposure prophylaxis of HIV infection. Am J Reprod Immunol 71(6):624–630
12. Assaes CP, Sáez-Cirión A (2014) HIV cure research: advances and prospects. Virology 454–455:340–352
13. De Clercq E (2013) A cutting-edge view on the current state of antiviral drug development. Med Res Rev 33(6):1249–1277
14. Maga G, Veljkovic N, Crespan E, Spadari S, Prljic J, Perovic V, Glisic S, Veljkovic V (2013) New in silico and conventional in vitro approaches to advance HIV drug discovery and design. Expert Opin Drug Discovery 8(1):83–92
15. Métifiot M, Marchand C, Pommier Y (2013) HIV integrase inhibitors: 20-year landmark and challenges. Adv Pharmacol 67:75–105
16. Yu F, Lu L, Du L, Zhu X, Debnath AK, Jiang S (2013) Approaches for identification of HIV-1 entry inhibitors targeting gp41 pocket. Viruses 5(1):127–149
17. Lagunin AA, Filimonov DA, Gloriozova TA, Tarasova OA, Zakharov AV, Guasch L, Nicklaus MC, Poroikov VV (2013) Virtual screening for potential substances for the prophylaxis of HIV infection in libraries of commercially available organic compounds. Pharm Chem J 47(7):343–360
18. Lange JM, Ananworanich J (2014) The discovery and development of antiretroviral agents. Antivir Ther 19(Suppl 3):5–14
19. Veselovsky AV, Zharkova MS, Poroikov VV, Nicklaus MC (2014) Computer-aided design and discovery of protein-protein interaction inhibitors as agents for anti-HIV therapy. SAR QSAR Environ Res 25(6):457–471
20. Di Santo R (2014) Inhibiting the HIV integration process: past, present, and the future. J Med Chem 57(3):539–566. Erratum in: J Med Chem. 2014 Jul 24; 57(14):6273
21. Tintori C, Brai A, Fallacara AL, Fazi R, Schenone S, Botta M (2014) Protein-protein interactions and human cellular cofactors as new targets for HIV therapy. Curr Opin Pharmacol 18:1–8
22. Han YS, Xiao WL, Xu H, Kramer VG, Quan Y, Mesplède T, Oliveira M, Colby-Germinario SP, Sun HD, Wainberg MA (2015) Identification of a dibenzocyclooctadiene lignan as a HIV-1 non-nucleoside reverse transcriptase inhibitor. Antivir Chem Chemother 24(1):28–38
23. Patel RV, Park SW (2015) Pyrroloaryls and pyrroloheteroaryls: inhibitors of the HIV fusion/attachment, reverse transcriptase and integrase. Bioorg Med Chem pii: S0968-0896(15)00510-6. doi:10.1016/j.bmc.2015.06.016. [Epub ahead of print] Review. PubMed
24. Chiu IM, Yaniv A, Dahlberg JE, Gazit A, Skuntz SF, Tronick SR, Aaronson SA (1985) Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. Nature 317(6035):366–3688
25. Wain-Hobson S, Alizon M, Montagnier L (1985) Relationship of AIDS to other retroviruses. Nature 313(6005):743
26. Vogt PK (1997) Historical introduction to the general properties of retroviruses. In: Coffin JM, Hughes SH, Varmus HE (eds) Retroviruses. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 1–27
27. Weiss RA (1993) Cellular receptors and viral glycoproteins involved in retrovirus entry. In: Levy JA (ed) The retroviridae, vol 2. Plenum, New York, pp 1–108
28. Miceli MC, Parnes JR (1993) Role of CD4 and CD8 in T cell activation and differentiation. Adv Immunol 53:59–122
29. Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, Wu L, Mackay CR, LaRosa G, Newman W, Gerard N, Gerard C, Sodroski J (1996) The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 85(7):1135–1148

30. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR (1996) Identification of a major co-receptor for primary isolates of HIV-1. Nature 381 (6584):661–6666
31. Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, Parmentier M, Collman RG, Doms RW (1996) A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 85(7):1149–1158
32. Hoffman TL, Stephens EB, Narayan O, Doms RW (1998) HIV type I envelope determinants for use of the CCR2b, CCR3, STRL33, and APJ coreceptors. Proc Natl Acad Sci U S A 95 (19):11360–11365
33. Scholten DJ, Canals M, Maussang D, Roumen L, Smit MJ, Wijtmans M, de Graaf C, Vischer HF, Leurs R (2012) Pharmacological modulation of chemokine receptor function. Br J Pharmacol 165(6):1617–1643
34. Zhang L, He T, Huang Y, Chen Z, Guo Y, Wu S, Kunstman KJ, Brown RC, Phair JP, Neumann AU, Ho DD, Wolinsky SM (1998) Chemokine co-receptor usage by diverse primary isolates of human immunodeficiency virus type 1. J Virol 72(11):9307–9312
35. Eckert DM, Kim PS (2001) Mechanisms of viral membrane fusion and its inhibition. Annu Rev Biochem 70:777–810
36. Chun TW, Carruth L, Finzi D, Shen X, Di Giuseppe JA, Taylor H, Hermankova M, Chadwick K, Margolick J, Quinn TC, Kuo YH, Brookmeyer R, Zeiger MA, Barditch-Crovo P, Siliciano RF (1997) Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 387:183–188
37. Whittle H, Morris J, Todd J, Corrah T, Sabally S, Bangali J, Ngom PT, Rolfe M, Wilkins A (1994) HIV-2-infected patients survive longer than HIV-1-infected patients. AIDS 8:1617–1620
38. Divita G, Rittinger K, Geourjon C, Deleage G, Goody RS (1995) Dimerization kinetics of HIV-1 and HIV-2 reverse transcriptase: a two step process. J Mol Biol 245:508–521
39. Barat C, Lullien V, Schatz O, Keith G, Nugeyre MT, Gruninger-Leitch F, Barre-Sinoussi F, Grice L, Darlix JL (1989) HIV-1 reverse transcriptase specifically interacts with the anticodon domain of its cognate primer tRNA. EMBO J 8(32):3279–3285
40. Sarih-Cottin L, Bordier B, Musier-Forsyth K, Andreola M-L, Barr PJ, Litvak S (1992) Preferential interaction of HIV RT with two regions of primer $tRNA^{Lys3}$ as evidenced by footprinting studies and inhibition with synthetic oligoribonudeotides. J Mol Biol 226:1–6
41. Litvak LE, Andderola M-L, Nevinsky GA, Sarih-Cofttin L, Litvax S (1994) The reverse transcriptase of HIV-1: from enzyrnology to therapeutic intervention Laboratoire de Replication et Expression des Genomes eucaryotes et Retroviraux, institut Biochimie Cellulaire, CNRS5 33077 Bordeaux cedex, France, vol 8. pp 497–502.8
42. Tu X, Das K, Han Q, Bauman JD, Clark AD, Hou X, Frenkel YV, Gaffney BL, Jones RA, Boyer PL, Hughes SH, Sarafianos SG, Arnold E (2010) Structural basis of HIV-1 resistance to AZT by excision. Nat Struct Mol Biol 17:1202
43. Madej T, Lanczycki CJ, Zhang D, Thiessen PA, Geer RC, Marchler-Bauer A, Bryant SH (2014) MMDB and VAST+: tracking structural similarities between macromolecular complexes. Nucleic Acids Res 42(Database issue):D297–D303
44. Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA (1992) Crystal structure at 3.5 A resolution of HIV-1 RT complexed with an inhibitor. Science 256:1783–1790
45. Huang H, Chopra R, Verdine GL, Harrison SC (1998) Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. Science 28:1669–1675
46. Arts EJ, Wainberg MA (1996) Mechanisms of nucleoside analog antiviral activity and resistance during human immunodeficiency virus reverse transcription. Antimicrob Agents Chemother 40:527–540
47. Squires KE (2001) An introduction to nucleoside and nucleotide analogues. Antivir Ther 6 (Suppl 3):1–14

48. Prasad VR, Goff SP (1990) Structure-function studies of HIV reverse transcriptase. Ann N Y Acad Sci 616:11–21
49. St Clair MH, Richards CA, Spector T et al (1987) 3′-Azido-3′-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. Antimicrob Agents Chemother 31:1972–1977
50. Lewis W, Gonzalez B, Chomyn A, Papoian T (1992) Zidovudine induces molecular, biochemical, and ultrastructural changes in rat skeletal muscle mitochondria. J Clin Invest 89:1354–1360
51. Lewis W, Dalakas MC (1995) Mitochondrial toxicity of antiviral drugs. Nat Med 1:417–422
52. Schambelan M, Benson CA, Carr A, Currier JS, Dube P, Gerber JG, Grinspoon SK, Saag MS (2002) Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. J Acquir Immune Defic Syndr 31:257–275
53. Falco V, Rodriguez D, Ribera E, Martinez E, Miro JM, Domingo P, Diazaraque R, Jose RA, Gonzalez-Garcia JJ, Montero F, Sanchezl L, Pathissa A (2002) Severe nucleoside-associated lactic acidosis in human immunodeficiency virus-infected patients: report of 12 cases and review of the literature. Clin Infect Dis 34:838–846
54. Miller KD, Cameron M, Wood LV, Dalakas MC, Kovacs JA (2000) Lactic acidosis and hepatic steatosis associated with use of stavudine: report of four cases. Ann Intern Med 133:192–196
55. Bissuel F, Bruneel F, Habersetzer F et al (1994) Fulminant hepatitis with severe lactate acidosis in HIV-infected patients on didanosine therapy. J Intern Med 235:367–371
56. Chattha G, Arieff AI, Cummings C, Tierney LM Jr (1993) Lactic acidosis complicating the acquired immunodeficiency syndrome. Ann Intern Med 118:37–39
57. Smerdon SJ, Jager J, Wang J, Kohlstaedt LA, Chirino AJ, Friedman JM, Rice PA, Steitz TA (1994) Structure of the binding site for nonnucleoside inhibitors of the reverse transcriptase of human immunodeficiency virus type 1. Proc Natl Acad Sci U S A 91(9):3911–3915
58. Pitta E, Crespan E, Geronikaki A, Maga G, Samuele A (2010) Novel thiazolidinone derivatives with an uncommon mechanism of inhibition towards HIV-1 reverse transcriptase. Lett Drug Des Discovery 7(4):228–234
59. Das K, Lewi PJ, Hughes SH, Arnold E (2005) Crystallography and the design of anti-AIDS drugs: conformational flexibility and positional adaptability are important in the design of non-nucleoside HIV-1 reverse transcriptase inhibitors. Prog Biophys Mol Biol 88:209–231
60. Hsiou Y, Das K, Ding J, Clark AD Jr, Kleim JP, Rosner M, Winkler I, Riess G, Hughes SH, Arnold E (1998) Structures of Tyr188Leu mutant and wild-type HIV-1 reverse transcriptase complexed with the non-nucleoside inhibitor HBY 097: inhibitor flexibility is a useful design feature for reducing drug resistance. J Mol Biol 284(2):313–323
61. Das K, Clark AD Jr, Lewi PJ, Heeres J, De Jonge MR, Koymans LM, Vinkers HM, Daeyaert F, Ludovici DW, Kukla MJ, De Corte B, Kavash RW, Ho CY, Ye H, Lichtenstein MA, Andries K, Pauwels R, De Bethune MP, Boyer PL, Clark P, Hughes SH, Janssen PA, Arnold E (2004) Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related nonnucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug resistant HIV-1 variants. J Med Chem 47(10):2550–2560
62. Janssen PA, Lewi PJ, Arnold E, Daeyaert F, de Jonge M, Heeres J, Koymans L, Vinkers M, Guillemont J, Pasquier E, Kukla M, Ludovici D, Andries K, de Bethune MP, Pauwels R, Das K, Clark AD Jr, Frenkel YV, Hughes SH, Medaer B, De Knaep F, Bohets H, De Clerck F, Lampo A, Williams P, Stoffels P (2005) In search of a novel anti-HIV drug: multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1E)-2-cyanoethenyl]-2,6-dimethylphenyl]

amino]-2- pyrimidinyl]-amino]benzonitrile (R278474, rilpivirine). J Med Chem 48 (6):1901–1909
63. Ren J, Milton J, Weaver KL, Short SA, Stuart DI, Stammers DK (2000) Structural basis for the resilience of efavirenz (DMP-266) to drug resistance mutations in HIV-1 reverse transcriptase. Struct Fold Des 8:1089
64. Ren J, Nichols CE, Chamberlain PP, Weaver KL, Short SA, Stammers DK (2004) Crystal structures of HIV-1 reverse transcriptases mutated at codons 100, 106 and 108 and mechanisms of resistance to non-nucleoside inhibitors. J Mol Biol 336:569–579
65. Monforte AM, Logoteta P, Ferro S, De Luca L, Iraci N, Maga G, Clercq ED, Pannecouque C, Chimirri A (2009) Design, synthesis, and structure-activity relationships of 1,3-dihydrobenzimidazol-2-one analogues as anti-HIV agents. Bioorg Med Chem 17(16):5962–5967
66. Pauwels R, Andries K, Debyser Z, Van Daele P, Schols D, Stoffels P, De Vreese K, Woestenborghs R, Vandamme AM, Janssen CG (1993) Potent and highly selective human immunodeficiency virus type 1 (HIV-1) inhibition by a series of alpha-anilinophenylacetamide derivatives targeted at HIV-1 reverse transcriptase. Proc Natl Acad Sci U S A 90(5):1711–1715
67. Balzarini J, Orzeszko-Krzesińska B, Maurin JK, Orzeszko A (2009) Synthesis and anti-HIV studies of 2-and 3-adamantyl-substituted thiazolidin-4-ones. Eur J Med Chem 44:303–311
68. Pauwels R, Andries K, Desmyter J, Schols D, Kukla MJ, Breslin HJ, Raeymaeckers A, Van Gelder J, Woestenborghs R, Heykants J, Schellekens K, Janssen M, De Clerq E, Janssen PAJ (1990) Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. Nature 343(6257):470–474
69. Althaus IW, Chou JJ, Gonzales AJ, Deibel MR, Chou KC, Kezdy FJ, Romero DL, Thomas RC, Aristoff PA, Tarpley WG et al (1994) Kinetic studies with the non-nucleoside human immunodeficiency virus type-1 reverse transcriptase inhibitor U-90152E. Biochem Pharmacol 47(11):2017–2028
70. Souza TM, Rodrigues DQ, Ferreira VF, Marques IP, da Costa Santos F, Cunha AC, de Souza MC, de Palmer Paixao Frugulhetti IC, Bou-Habib DC, Fontes CF (2009) Characterization of HIV-1 enzyme reverse transcriptase inhibition by the compound 6-chloro-1,4-dihydro-4-oxo-1-(beta-D-ribofuranosyl) quinoline-3- carboxylic acid through kinetic and in silico studies. Curr HIV Res 7(3):327–335
71. Pitta E, Geronikaki A, Surmava S, Eleftheriou P, Mehta V, Van der Eicken E (2013) Synthesis and HIV-1 RT inhibitory action of novel (4/6-substituted benzo[d]thiazol -2-yl) thiazolidin-4-ones. Divergence from the noncompetitive mechanism. J Enzyme Inhib Med Chem 28(1):113–122
72. Andries K, Azijn H, Thielemans T, Ludovici D, Kukla M, Heeres J, Janssen P, De Corte B, Vingerhoets J, Pauwels R, de Bethune MP (2004) TMC125, a novel next-generation nonnucleoside reverse transcriptase inhibitor active against nonnucleoside reverse transcriptase inhibitor-resistant human immunodeficiency virus type 1. Antimicrob Agents Chemother 48(12):4680–4686
73. Zhan P, Liu X, Li Z, Fang Z, Li Z, Wang D, Pannecouque C, De Clercq E (2008) Novel 1,2,3-thiadiazole derivatives as HIV-1 NNRTIs with improved potency: synthesis and preliminary SAR studies. Acta Pharm 57:379–393
74. Rao A, Balzarini J, Carbone A, Chimirri A, De Clercq E, Monforte AM, Monforte P, Pannecouque C, Zappala M (2004) 2-(2,6-Dihalophenyl)-3-(pyrimidin-2-yl)-1,3-thiazolidin-4-ones as non-nucleoside HIV-1 reverse transcriptase inhibitors. Antiviral Res 63:79–84
75. Debyser Z, Pauwels R, Andries K, Desmyter J, Kukla M, Janssen PA, De Clercq E (1991) An antiviral target on reverse transcriptase of human immunodeficiency virus type 1 revealed by tetrahydroimidazo-[4,5,1-jk][1,4]benzodiazepin-2 (1H)-one and -thione derivatives. Proc Natl Acad Sci U S A 88(4):1451–1455
76. Goldman ME, Nunberg JH, O'Brien JA, Quintero JC, Schleif WA, Freund KF, Gaul SL, Saari WS, Wai JS, Hoffman JM et al (1991) Pyridinone derivatives: specific human

immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. Proc Natl Acad Sci U S A 88(15):6863–6867
77. Miyasaka T, Tanaka H, Baba M, Hayakawa H, Walker RT, Balzarini J, De Clercq E (1989) A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. J Med Chem 32(12):2507–2509
78. Baba M, Tanaka H, De Clercq E, Pauwels R, Balzarini J, Schols D, Nakashima H, Perno CF, Walker RT, Miyasaka T (1989) Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. Biochem Biophys Res Commun 165 (3):1375–1381
79. Rawal RK, Tripathi R, Kulkarni S, Paranjape R, Katti SB, Pannecouque C, De Clercq E (2008) 2-(2,6-Dihalo-phenyl)-3-heteroaryl-2-ylmethyl-1, 3-thiazolidin-4-ones: anti-HIV agents. Chem Biol Drug Des 72(2):147–154
80. Brzozowski Z, Saczewski F, Neamati N (2006) Synthesis, antitumor and anti-HIV activities of benzodithiazine-dioxides. Bioorg Med Chem 14:2985–2993
81. Novikov MS, Valuev-Elliston VT, Babkov DA, Paramonova MP, Ivanov AV, Gavryushov SA, Khandazhinskaya AL, Kochetkov SN, Pannecouque C, Andrei G, Snoeck R, Balzarini J, Seley-Radtke KL (2013) N1, N3-disubstituted uracils as nonnucleoside inhibitors of HIV-1 reverse transcriptase. Bioorg Med Chem 21:1150–1158
82. Wang Y-P, Chen F-E, De Clercq E, Balzarini J, Pannecouque C (2009) Synthesis and in vitro anti-HIV evaluation of a new series of 6-arylmethyl-substituted S-DABOs as potential non-nucleoside HIV-1 reverse transcriptase inhibitors. Eur J Med Chem 41:1016–1023
83. La Regina G, Coluccia A, Piscitelli F, Bergamini A, Sinistro A, Cavazza A, Maga J, Samuele A, Zanoli S, Novellino E, Artico M, Silvestri R (2007) Indolyl aryl sulfones as HIV-1 non-nucleoside reverse transcriptase inhibitors: role of two halogen atoms at the indole ring in developing new analogues with improved antiviral activity. J Med Chem 50:5034–5038
84. Balzarini J, Orzeszko B, Maurin JK, Orzeszko A (2007) Synthesis and anti-HIV studies of 2-adamantyl-substituted thiazolidin-4-ones. Eur J Med Chem 42:993–1003
85. Akkouh O, Tzi Bun N, Singh SS, Yin C, Dan X, Chan YC, Pan W, Cheung RCF (2015) Lectins with anti-HIV activity: a review. Molecules 20:648–668
86. Famiglini V, Coluccia A, Brancale A, Pelliccia S, La Regina G, Silvestri R (2013) Arylsulfone-based HIV-1 non-nucleoside reverse transcriptase inhibitors. Future Med Chem 5(18):2141–2156
87. De Clercq E (2013) Dancing with chemical formulae of antivirals: a personal account. Biochem Pharmacol 86(6):711–725
88. Veljkovic N, Glisic S, Prljic J, Perovic V, Veljkovic V (2013) Simple and general criterion for "*in silico*" screening of candidate HIV drugs. Curr Pharm Biotechnol 14(5):561–569
89. Li D, Zhan P, Liu H, Pannecouque C, Balzarini J, De Clercq E, Liu X (2013) Synthesis and biological evaluation of pyridazine derivatives as novel HIV-1 NNRTIs. Bioorg Med Chem 21:2128–2134
90. La Regina G, Coluccia A, Brancale A, Piscitelli F, Gatti V, Maga G, Samuele A, Pannecouque C, Schols D, Balzarini J, Novellino E, Silvestri R (2011) Indolylarylsulfones as HIV-1 non-nucleoside reverse transcriptase inhibitors: new cyclic substituents at indole-2-carboxamide. J Med Chem 54:1587–1598
91. La Regina G, Coluccia A, Brancale A, Piscitelli F, Famiglini V, Cosconati S, Maga G, Samuele A, Gonzalez E, Clotet B, Schols D, Esté JA, Novellino E, Silvestri R (2012) New nitrogen containing substituents at the indole-2-carboxamide yield high potent and broad spectrum indolylarylsulfone HIV-1 non-nucleoside reverse transcriptase inhibitors. J Med Chem 55:6634–6638
92. Rotili D, Samuele A, Tarantino D, Ragno R, Musmuca I, Ballante F, Botta G, Morera L, Pierini M, Cirilli R, Nawrozkij MB, Gonzalez E, Clotet B, Artico M, Esté JA, Maga G, Mai A (2012) 2-(Alkyl/aryl)amino-6-benzylpyrimidin-4(3H)-ones as inhibitors of wild-type and mutant HIV-1: enantioselectivity studies. J Med Chem 55:3558–3562

93. Rawal RK, Tripathi R, Katti SB, Pannecouque C, De Clercq E (2008) Design and synthesis of 2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4-ones as anti-HIV agents. Eur J Med Chem 43:2800–2806
94. Ravichandran S, Veerasamy R, Raman S, Krishnan PN, Agrawal RK (2008) An overview on HIV-1 reverse transcriptase inhibitors. Dig J Nanomater Biostruct 3(4):171–187
95. Paolucci S, Baldanti F, Tinelli M et al (2002) Q145M, a novel HIV-1 reverse transcriptase mutation conferring resistance to nucleoside and nonnucleoside reverse transcriptase inhibitors. Antivir Ther 7(2):S35
96. Johnson VA, Brun-Vezinet F, Clotet B, Conway B, D'Acquila RT, Demeter LM, Kuritzkes DR, Pillay D, Shapiro JM, Telenta A, Richman DD (2004) Update of the drug resistance mutations in HIV-1: 2004. Top HIV Med 12(4):119–124
97. Mbuagbaw LC, Irlam JH, Spaulding A, Rutherford GW, Siegfried N (2010) Efavirenz or nevirapine in three-drug combination therapy with two nucleoside-reverse transcriptase inhibitors for initial treatment of HIV infection in antiretroviral-naive individuals. Cochrane Database Syst Rev 8(12):CD004246
98. Neukam K, Mira JA, Ruiz-Morales J, Rivero A, Collado A, Torres-Cornejo A, Merino D, de Los Santos-Gil I, Macias J, Gonzalez-Serrano M, Camacho A, Parra-Garcia G, Pineda JA, On behalf of the SEGURIDAD HEPATICA Study Team of the Grupo HEPAVIR de la Sociedad Andaluza de Enfermedades Infecciosas (SAEI) (2011) Liver toxicity associated with antiretroviral therapy including efavirenz or ritonavir-boosted protease inhibitors in a cohort of HIV/hepatitis C virus co-infected patients. J Antimicrob Chemother 66(11):2605–2614
99. Esposito F, Corona A, Tramontan E (2012) HIV-1 reverse transcriptase still remains a new drug target: structure, function, classical inhibitors, and new inhibitors with innovative mechanisms of actions. Mol Biol Int 2012:586401
100. Jochmans D, Deval J, Kesteleyn B, Van Marck H, Bettens E, De Baere I, Dehertogh P, Ivens T, Van Ginderen M, Van Schoubroeck B, Ethesami M, Wigerinck P, Gotte M, Hertogs K, Hertogs K (2006) Indolopyridones inhibit human immunodeficiency virus reverse transcriptase with a novel mechanism of action. J Virol 80(24):12283–12292
101. Zhang Z, Walker M, Xu W, Shim JH, Giradet J-L, Hamatake RK, Hong Z (2006) Novel nonnucleoside inhibitors that select nucleoside inhibitor resistance mutations in human immunodeficiency virus type 1 reverse transcriptase. Antimicrob Agents Chemother 50 (8):2772–2781
102. Maga G, Radi M, Zanoli S, Manetti F, Cancio R, Hübscher U, Spadari S, Falciani C, Terrazas M, Vilarrasa J, Botta M (2007) Discovery of non-nucleoside inhibitors of HIV-1 reverse transcriptase competing with the nucleotide substrate. Angew Chem 16 (11):1810–1813
103. Radi M, Falciani C, Contemori L, Petricci E, Maga G, Samuele A, Zanoli S, Terrazas M, Castria M, Togninelli A, Este JA, Clotet-Codina I, Armand-Ugon M, Botta M (2008) A multidisciplinary approach for the identification of novel HIV-1 non-nucleoside reverse transcriptase inhibitors: S-DABOCs and DAVPs. ChemMedChem 3(4):573–593
104. Freisz S, Bec G, Radi M, Wolff P, Crespan E, Angeli L, Dumas P, Maga G, Botta M, Ennifar E (2010) Crystal structure of HIV-1 reverse transcriptase bound to a non-nucleoside inhibitor with a novel mechanism of action. Angew Chem Int Ed Engl 49:1805–1808
105. Wang JY, Ling H, Yang W, Craigie R (2001) Structure of a two-domain fragment of hiv-1 integrase: implications for domain organization in the intact protein. EMBO J 20:7333–7343
106. Lodi PJ, Ernst JA, Kuszewski J, Hickman AB, Engelman A, Craigie R, Clore GM, Gronenborn AM (1995) Solution structure of the DNA binding domain of HIV-1 integrase. Biochemistry 34(31):9826–9833
107. Rhodes DI, Peat TS, Vandegraaff N, Jeevarajah D, Newman J, Martyn J, Coates JA, Ede NJ, Rea P, Deadman JJ (2011) Crystal structures of novel allosteric peptide inhibitors of HIV integrase identify new interactions at the LEDGF binding site. Chembiochem 12 (15):2311–2315

108. Sharma A, Slaughter A, Jena N, Feng L, Kessl JJ, Fadel HJ, Malani N, Male F, Wu L, Poeschla E, Bushman FD, Fuchs JR, Kvaratskhelia M (2014) A new class of multimerization selective inhibitors of HIV-1 integrase. Plos Pathog 10(5):e1004171
109. Wielens J, Headey SJ, Rhodes DI, Mulder RJ, Dolezal O, Deadman JJ, Newman J, Chalmers DK, Parker MW, Peat TS, Scanlon MJ (2013) Parallel screening of low molecular weight fragment libraries: do differences in methodology affect hit identification? J Biomol Screen 18:147–159
110. Hazuda DJ, Felock P, Witmer M, Wolfe A, Stillmock K, Grobler JA, Espeseth A, Gabryelski L, Schleif W, Blau C, Miller MD (2000) Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. Science 287(5453):646–650
111. Pommier Y, Johnson AA, Marchand C (2005) Integrase inhibitors to treat HIV/AIDS. Nat Rev Drug Discov 4(3):236–248
112. Wai JS, Egbertson MS, Payne LS, Fisher TE, Embrey MW, Tran LO, Melamed JY, Langford HM, Guare JP Jr, Zhuang L, Grey VE, Vacca JP, Holloway MK, Naylor-Olsen AM, Hazuda DJ, Felock PJ, Wolfe AL, Stillmock KA, Schleif WA, Gabryelski LJ, Young SD (2000) 4-Aryl-2,4-dioxobutanoic acid inhibitors of HIV-1 integrase and viral replication in cells. J Med Chem 43(26):4923–4926
113. Grobler JA, Stillmock K, Hu B, Witmer M, Felock P, Espeseth AS, Wolfe A, Egbertson M, Bourgeois M, Melamed J, Way JS, Young S, Vacca J, Hazuda DJ (2002) Diketo acid inhibitor mechanism and HIV-1 integrase: implications for metal binding in the active site of phosphotransferase enzymes. Proc Natl Acad Sci 99(10):6661–6666
114. Marchand C, Johnson AA, Karki RG, Pais GC, Zhang X, Cowansage K, Patel TA, Nicklaus M, Burke TR Jr, Pommier Y (2003) Metal-dependent inhibition of HIV-1 integrase by beta-diketo acids and resistance of the soluble double-mutant (F185K/C280S). Mol Pharmacol 64(3):600–609
115. Hazuda DJ, Young SD, Guare JP, Anthony NJ, Gomez RP, Wai JS, Vacca JP, Handt L, Motzel SL, Klein HJ, Dornadula G, Danovich RM, Witmer MV, Wilson KA, Tussey L, Schleif WA, Gabryelski LS, Jin L, Miller MD, Casimiro DR, Emini EA, Shiver JW (2004) Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. Science 305(5683):528–532
116. Asante-Appiah E, Skalka AM (1999) HIV-1 integrase: structural organization, conformational changes, and catalysis. Adv Virus Res 52:351–369
117. Esposito D, Craigie R (1999) HIV integrase structure and function. Adv Virus Res 52:319–333
118. Summa V, Petrocchi A, Matassa VG, Gardelli C, Muraglia E, Rowley M, Paz OG, Laufer R, Monteagudo E, Pace P (2006) 4,5-Dihydroxypyrimidine carboxamides and N-alkyl-5-hydroxypyrimidinone carboxamides are potent, selective HIV integrase inhibitors with good pharmacokinetic profiles in preclinical species. J Med Chem 49(23):6646–6649
119. Savarino A (2006) A historical sketch of the discovery and development of HIV-1 integrase inhibitors. Expert Opin Investig Drugs 15(12):1507–1522
120. Iwamoto M, Wenning LA, Petry AS, Laethem M, De Smet M, Kost JT, Merschman SA, Strohmaier KM, Ramael S, Lasseter KC, Stone JA, Gottesdiener KM, Wagner JA (2008) Safety, tolerability, and pharmacokinetics of raltegravir after single and multiple doses in healthy subjects. Clin Pharmacol Ther 83(2):293–299
121. DeJesus E, Berger D, Markowitz M, Cohen C, Hawkins T, Ruane P, Elion R, Farthing C, Zhong L, Cheng AK, McColl D, Kearney BP (2006) Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. J Acquir Immune Defic Syndr 43(1):1–5
122. Temesgen Z, Siraj DS (2008) Raltegravir: first in class HIV integrase inhibitor. Ther Clin Risk Manag 4(2):493–500
123. Mouscadet J-F, Tchertanov L (2009) Raltegravir: molecular basis of its mechanism of action. Eur J Med Res 14(Suppl III):5–16

124. Shimura KL, Kodama EN (2009) Elvitegravir: a new HIV integrase inhibitor. Antivir Chem Chemother 20(2):79–85
125. Fantauzzi A, Turriziani O, Mezzaroma I (2013) Potential benefit of dolutegravir once daily: efficacy and safety. HIV AIDS (Auckl) 5:29–40
126. Malet I, Delelis O, Valantin M-A, Montes B, Soulie C, Wirden M, Tchertanov L, Peytavin G, Reynes J, Mouscadet J-F, Katlama C, Calvez V, Marcelin A-G (2008) Mutations associated with failure of raltegravir treatment affect integrase sensitivity to the inhibitor in vitro. Antimicrob Agents Chemother 52(4):1351–1358
127. Métifiot M, Marchand C, Maddali K, Pommier Y (2010) Resistance to integrase inhibitors. Viruses 2(7):1347–1366
128. Kobayashi M, Yoshinaga T, Seki T, Wakasa-Morimoto C, Brown KW, Ferris R, Foster SA, Hazen RJ, Miki S, Suyama-Kagitani A, Kawauchi-Miki S, Taishi T, Kawasuji T, Johns BA, Underwood MR, Garvey EP, Sato A, Fujiwara T (2011) In Vitro antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. Antimicrob Agents Chemother 55(2):813–821
129. Pommier Y, Marchand C, Neamati N (2000) Retroviral inhibition of HIV-1 vector integrase inhibitors year 2000: update and perspectives. Antiviral Res 47:139–148
130. Neamati N, Marchand C, Pommier Y (2000) HIV-1 integrase inhibitors: past, present, and future. Adv Pharmacol 49:147–165
131. Young SD (2001) Inhibition of HIV-1 integrase by small molecules: the potential for a new class of AIDS chemotherapeutics. Curr Opin Drug Discov Devel 4:402–410
132. Pannecouque C, Pluymers W, Van Maele B, Tetz V, Cherepanov P, De Clercq E, Debyser Z (2002) New class of HIV integrase inhibitors that block viral replication in cell culture. Curr Biol 12:1169–1177
133. Brzozowski Z, Saczewski F, Sławiński J, Sanchez T, Neamati N (2009) Synthesis and anti-HIV-1 integrase activities of 3-aroyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazines. Eur J Med Chem 44:190–196
134. Johnson TW, Tanis SP, Butler SL, Dalvie D, DeLisle DM, Dress KR, Flahive EJ, Hu Q, Kuehler JE, Kuki A, Liu W, McClellan GA, Peng Q, Plewe MB, Richardson PF, Smith GL, Solowiej J, Tran KT, Yu HWX, Zhang J, Zhu H (2011) Design and synthesis of novel N-hydroxy-dihydronaphthyridinones as potent and orally bioavailable HIV-1 integrase inhibitors. J Med Chem 54:3393–3417
135. Kawasuji T, Johns BA, Yoshida H, Taishi T, Taoda Y, Murai H, Kiyama R, Fuji M, Yoshinaga T, Seki T, Kobayashi M, Sato A, Fujiwara T (2012) Carbamoyl pyridone HIV-1 integrase inhibitors. 1. Molecular design and establishment of an advanced two-metal binding pharmacophore. J Med Chem 55(20):8735–8744
136. Tsiang M, Jones GS, Niedziela-Majka A, Kan E, Lansdon EB, Huang W, Hung M, Samuel D, Novikov N, Xu Y, Mitchell M, Guo H, Babaoglu K, Liu X, Geleziunas R, Sakowicz R (2012) New class of HIV-1 integrase (in) inhibitors with a dual mode of action. J Biol Chem 287:21189–21203
137. Seelmeier S, Schmidt H, Turk V, von der Helm K (1988) Human immunodeficiency virus has an aspartic-type protease that can be inhibited by pepstatin A. Proc Natl Acad Sci U S A 85:6612–6616
138. Kohl NE, Emini EA, Schleif WA, Davis LJ, Heimbach JC, Dixon RA, Scolnik EM, Sigal IS (1988) Active human immunodeficiency virus protease is required for viral infectivity. Proc Natl Acad Sci U S A 85:4686–4690
139. Degoey DA, Grampovnik DJ, Flentge CA, Flosi WJ, Chen HJ, Yeung CM, Randolph JT, Klein LL, Dekhtyar T, Colletti L, Marsh KC, Stoll V, Mamo M, Morfitt DC, Nguyen B, Schmidt JM, Swanson SJ, Mo H, Kati WM, Molla A, Kempf DJ (2009) 2-pyridyl p1-′-substituted symmetry-based human immunodeficiency virus protease inhibitors (A-792611 and a-790742) with potential for convenient dosing and reduced side effects. J Med Chem 52:2571–2586

140. McQuade TJ, Tomasselli AG, Liu L, Karacostas B, Moss B, Sawyer TK, Heinrikson RL, Tarpley WG (1990) A synthetic HIV protease inhibitor with antiviral activity arrests HIV-like particle maturation. Science 247:454–4566
141. Tang J, Lin Y, Co E, Hartsuck J, Lin X (1992) Understanding protease: can it be translated into effective therapy against AIDS. Scand J Clin Lab Invest 52(Suppl 210):127–135
142. Brik A, Wong CH (2003) HIV-1 protease: mechanism and drug discovery. Org Biomol Chem 1(1):5–14
143. Kurup A, Mekapati SB, Garg R, Hansch C (2003) HIV-1 protease inhibitors: a comparative QSAR analysis. Curr Med Chem 10:1679–1688
144. Perez MAS, Fernandes PA, Ramos MJ (2007) Drug design: new inhibitors for HIV-1 protease based on Nelfinavir as lead. J Mol Graph Model 26:634–642
145. Larder BA et al (2000) Tipranavir inhibits broadly protease inhibitor-resistant HIV-1 clinical samples. AIDS 14:1943–1948
146. Zeldin RK, Petruschke RA (2004) Pharmacological and therapeutic properties of ritonavir-boosted protease inhibitor therapy in HIV-infected patients. J Antimicrob Chemother 53 (1):4–9
147. Flentge CA, Randolph JT, Huang PP, Klein LL, Marsh KC, Harlan JE, Kempf DJ (2009) Synthesis and evaluation of inhibitors of cytochrome P450 3A (CYP3A) for pharmacokinetic enhancement of drugs. Bioorg Med Chem Lett 19:5444–5448
148. Sperka T, Pitlik J, Bagossia P, Tozser J (2005) Beta-lactam compounds as apparently uncompetitive inhibitors of HIV-1 protease. Bioorg Med Chem Lett 15:3086–3090
149. Bisacchi GS, Slusarchyk VA, Bolton SA, Hartl KS, Jacobs G, Mathur A, Meng W, Ogletree ML, Pi Z, Sutton JC, Treuner U, Zahle R, Zhao G, Seiler SM (2004) Synthesis of potent and highly selective nonguanidine azetidinone inhibitors of human tryptase. Bioorg Med Chem Lett 14:2227–2231
150. Sutton JC, Bolton SA, Davis ME, Hartl KS, Jacobson B, Mathur A, Ogletree ML, Slusarchyk WA, Zahler SSM, Bisacchi GS (2004) Solid-phase synthesis and SAR of 4-carboxy-2-azetidinone mechanism-based tryptase inhibitors. Bioorg Med Chem Lett 14:2233–2239
151. Stebbins J, Beboucl C (1997) A microtiter colorimetric assay for the HIV-1 protease. Anal Biochem 248(2):246–250
152. Pitlik J, Townsend CA (1997) Solution-phase synthesis of a combinatorial monocyclic β-lactam library: potential protease inhibitors. Bioorg Med Chem Lett 7:3129–3133
153. Tözsér J, Gustchina A, Weber IT, Blaha I, Wondrak EM, Oroszlan S (1991) Studies on the role of the S4 substrate binding site of HIV proteinases. FEBS Lett 279(2):356–360
154. Wondrak EM, Louis JM, Oroszlan S (1991) purification of HIV-1 wild-type protease and characterization of proteolytically inactive HIV-1 protease mutants by pepstatin A affinity chromatography. FEBS Lett 280:347–350
155. Bagossi P, János Kádas J, Gabriella Miklóssy G, Boross P, Weber IT, Tözsér J (2004) Development of a microtiter plate fluorescent assay for inhibition studies on the HTLV-1 and HIV-1 proteinases. J Virol Methods 119:87–93
156. Cígler P, Kožíšek M, Řezáčová P, Brynda J, Otwinowski Z, Pokorná J, Plešek J, Grüner B, Dolečková-Marešová L, Máša M, Sedláček J, Bodem J, Kräusslich H-G, Král V, Konvalinka J (2005) From nonpeptide toward noncarbon protease inhibitors: metallacarboranes as specific and potent inhibitors of HIV protease. Proc Natl Acad Sci U S A 102(43):15394–15399
157. Ghosh AK, Anderson DD (2011) Tetrahydrofuran, tetrahydropyran, triazoles and related heterocyclic derivatives as HIV protease inhibitors. Future Med Chem 3(9):1181–1197
158. Wang RR, Gao Y-D, Ma C-H, Zhang X-J, Huang C-G, Huang J-F, Zheng Y-T (2011) Mangiferin, an anti-HIV-1 agent targeting protease and effective against resistant strains. Molecules 16:4264–4277
159. Jonckers THM, Rouan M-C, Hachı G, Schepens W, Hallenberger S, Baumeister J, Sasaki JC (2012) Benzoxazole and benzothiazole amides as novel pharmacokinetic enhancers of HIV protease inhibitors. Bioorg Med Chem Lett 22:4998–5002

160. Surleraux DLNG, Tahri A, Verschueren WG, Pille GME, de Kock HA, Jonckers THM, Peeters A, De Meyer S, Azijn H, Pauwels R, de Bethune M-P, King NM, Prabu-Jeyabalan M, Schiffer CA, Wigerinck PBTP (2005) Discovery and selection of TMC114, a next generation HIV-1 protease inhibitor. J Med Chem 48:1813–1822
161. Manchanda T, Schiedel D, Fischer D, Dekaban GA, Rieder MJ (2002) Adverse drug reactions to protease inhibitors. Can J Clin Pharmacol 9(3):137–146, Fall
162. Hui DY (2003) Effects of HIV protease inhibitor therapy on lipid metabolism. Prog Lipid Res 42(2):81–92
163. Friis-Moller N, Weber R, Reiss P, Thiıbaut R, Kirk O, Monforte AD, Pradier C, Morfeldt L, Mateu S, Law M, El-Sadr W, DeWit S, Sabin CA, Phillips AN, Lundgren JD (2003) Cardiovascular disease risk factors in HIV patients—association with antiretroviral therapy: results from the DAD study. AIDS 17:1179–1193
164. Friis-Moller N, Reiss P, Sabin CA, Weber R, Monforte AD, El-Sadr W, Thiıbaut R, DeWit S, Kirk O, Fontas E, Law MG, Phillips A, Lundgren JD (2007) Class of antiretroviral drugs and the risk of myocardial infarction. N Engl J Med 356:1723–1735
165. Smith C, Sabin CA, Lundgren JD, Thiebaut R, Weber R, Law RM, Monforte AD, Kirk O, Friis-Moller N, Phillips A, Reiss P, El Sadr W, Pradier C, Worm SW (2010) Factors associated with specific causes of death amongst HIV-positive individuals in the DAD study. AIDS 24:1537–1548
166. Zaera M, Miro O, Pedrol E, Soler A, Picon M, Cardellach F, Casademont J, Nunes V (2001) Mitochondrial involvement in antiretroviral therapy-related lipodystrophy. AIDS 15:1643–1651
167. Zhang S, Carper MJ, Lei X, Cade WT, Yarashesk KE, Ramanadham S (2009) Protease inhibitors used in the treatment of HIV+ induce beta-cell apoptosis via the mitochondrial pathway and compromise insulin secretion. Am J Physiol Endocrinol Metab 296:E925–E935
168. Lagathu C, Eustace B, Prot M, Frantz D, Gu Y, Bastard J-P, Maachi M, Azoulay S, Briggs M, Caron M, Capeau J (2007) Some HIV antiretrovirals increase oxidative stress and alter chemokine, cytokine or adiponectin production in human adipocytes and macrophages. Antivir Ther 12:489–500
169. Chandra S, Mondal D, Agrawal KS (2009) HIV-1 protease inhibitor induced oxidative stress suppresses glucose stimulated insulin release: protection with thymoquinone. Exp Biol Med 234:442–453
170. Touzet O, Philips A (2010) Resveratrol protects against protease inhibitor-induced reactive oxygen species production, reticulum stress and lipid raft perturbation. AIDS 24:1437–1447
171. Ben-Romano R, Rudich A, Etzion S, Potashnik R, Kagan E, Greenbaum U, Bashan N (2006) Nelfinavir induces adipocyte insulin resistance through the induction of oxidative stress: differential protective effect of antioxidant agents. Antivir Ther 11:1051–1060
172. Wang X, Chai H, Lin PH, Yao Q, Chen C (2009) Roles and mechanisms of human immunodeficiency virus protease inhibitor ritonavir and other anti-human immunodeficiency virus drugs in endothelial dysfunction of porcine pulmonary arteries and human pulmonary artery endothelial cells. Am J Pathol 174:771–781
173. Wang X, Mu H, Chai H, Liao D, Yao Q, Chen C (2007) Human immunodeficiency virus protease inhibitor ritonavir inhibits cholesterol efflux from human macrophage-derived foam cells. Am J Pathol 171:304–314
174. Conklin BS, Fu W, Lin PH, Lumsden AB, Yao Q, Chen C (2004) HIV protease inhibitor ritonavir decreases endothelium-dependent vasorelaxation and increases superoxide in porcine arteries. Cardiovasc Res 63:168–175
175. Chai H, Yang H, Yan S, Li M, Lin PH, Lumsden AB, Yao Q, Chen C (2005) Effects of HIV protease inhibitors on vasomotor function and superoxide anion production in porcine coronary arteries. J Acquir Immune Defic Syndr 40:12–19
176. Kilby JM, Hopkins S, Venetta TM, DiMassimo B, Cloud GA, Lee JY, Alldredge L, Hunter E, Lambert D, Bolognesi D, Matthews T, Johnson MR, Nowak MA, Shaw GM, Saag MS (1998)

Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. Nat Med 4(11):1302–1307
177. Robertson D (2003) US FDA approves new class of HIV therapeutics. Nat Biotechnol 21 (5):470–471
178. Este JA, Telenti A (2007) HIV entry inhibitors. Lancet 370(9581):81–88
179. Tan Q, Zhu Y, Li J, Chen Z, Han GW, Kufareva I, Li T, Ma L, Fenalti G, Li J, Zhang W, Xie X, Yang H, Jiang H, Cherezov V, Liu H, Stevens RC, Zhao Q, Wu B (2013) Structure of the CCR5 chemokine receptor-HIV entry inhibitor maraviroc complex. Science 341:1387–1390
180. Marcial M, Lu J, Deeks SG, Ziermann R, Kuritzkes DR (2006) Performance of human immunodeficiency virus type 1 gp41 assays for detecting enfuvirtide (T-20) resistance mutations. J Clin Microbiol 44(9):3384–3387
181. Rimsky LT, Shugars DC, Matthews TJ (1998) Determinants of human immunodeficiency virus type 1 resistance to gp41-derived inhibitory peptides. J Virol 72:986–993
182. Sista PR, Melby T, Davison D, Jin L, Mosier S, Mink M, Nelson EL, DeMasi R, Cammack N, Salgo MP, Matthews TJ, Greenberg ML (2004) Characterization of determinants of genotypic and phenotypic resistance to enfuvirtide in baseline and on-treatment HIV-1 isolates. AIDS 18:1787–1794
183. Marcelin AG, Reynes J, Yerly S, Ktorza N, Segondy M, Piot JC, Delfraissy JF, Kaiser L, Perrin L, Katlama C, Calvez V (2004) Characterization of genotypic determinants in HR-1 and HR-2 gp41 domains in individuals with persistent HIV viraemia under *T-20*. AIDS 18:1340–1342
184. Mink M, Mosier SM, Janumpalli S, Davison D, Jin L, Melby T, Sista P, Erickson J, Lambert D, Stanfield-Oakley SA, Salgo M, Cammack N, Matthews T, Greenberg ML (2005) Impact of human immunodeficiency virus type 1 gp41 amino acid substitutions selected during enfuvirtide treatment on gp41 binding and antiviral potency of enfuvirtide in vitro. J Virol 79:12447–12454
185. Xu L, Pozniak A, Wildfire A, Stanfield-Oakley SA, Mosier SM, Ratcliffe D, Workman J, Joall A, Myers R, Smit E, Cane PA, Greenberg ML, Pillay D (2005) Emergence and evolution of enfuvirtide resistance following long-term therapy involves heptad repeat 2 mutations within gp41. Antimicrob Agents Chemother 49:1113–1119
186. Yao X, Chong H, Zhang C, Waltersperger S, Wang M, Cui S, He Y (2012) Broad antiviral activity and crystal structure of HIV-1 fusion inhibitor sifuvirtide. J Biol Chem 287:6788–6796
187. Jiang S, Tala SR, Lu H, Abo-Dya NE, Avan I, Gyanda K, Lu L, Katritzky AR, Debnath AK (2011) Design, synthesis, and biological activity of novel 5-((arylfuran/1H-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones as HIV-1 fusion inhibitors targeting gp41. J Med Chem 54:572–579
188. Jiang S, Lu H, Liu S, Zhao Q, He Y, Debnath AK (2004) N-substituted pyrrole derivatives as novel human immunodeficiency virus type 1 entry inhibitors that interfere with the gp41 six helix bundle formation and block virus fusion. Antimicrob Agents Chemother 48:4349–4359
189. Katritzky AR, Tala SR, Lu H, Vakulenko AV, Chen Q-Y, Sivapackiam J, Pandya K, Jiang S, Debnath AK (2009) Design, synthesis, and structure-activity relationship of a novel series of 2-aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl) furans as HIV-1 entry inhibitors. J Med Chem 52:7631–7639
190. Teixeira C, Gomes JRB, Gomes P, Maurel F (2011) Viral surface glycoproteins, gp120 and gp41, as potential drug targets against HIV-1: brief overview one quarter of a century past the approval of zidovudine, the first anti-retroviral drug. Eur J Med Chem 46(4):979–992
191. Acharya P, Lusvarghi S, Bewley CA, Kwong PD (2015) HIV-1 gp120 as a therapeutic target: navigating a moving labyrinth. Expert Opin Ther Targets 19(6):765–783
192. Dezube BJ, Dahl TA, Wong TK, Chapman B, Ono M, Yamaguchi N, Gillies SD, Chen LB, Crumpacker CS (2000) A fusion inhibitor (FP-21399) for the treatment of human immunodeficiency virus infection: a phase I study. J Infect Dis 182:607–610

193. Hermann H, Westhof E (1998) RNA as a drug target: chemical, modeling, and evolutionary tools. Curr Opin Biotechnol 9:66–73
194. Yang M (2005) Discoveries of Tat-Tar interaction inhibitors for HIV-1. Curr Drug Targets Infect Disord 5(4):433–444
195. Gait MJ, Karn J (1993) RNA recognition by the human immunodeficiency virus Tat and Rev proteins. Trends Biochem Sci 18:255–259
196. AbouI-Ela F, Karn J, Varani G (1995) The structure of the human immunodeficiency virus type-1 TAR RNA reveals principles of RNA recognition by Tat protein. J Mol Biol 253:313–332
197. O'Brien WA, Sumner-Smith M, Mao SH, Sadeghi S, Zhao JQ, Chen IS (1996) Anti-human immunodeficiency virus type 1 activity of an oligocationic compound mediated via gp120 V3 interactions. J Virol 70:2825–2831
198. Hamasaki K, Ueno A (2001) Aminoglycoside antibiotics, neamine and its derivatives as potent inhibitors for the RNA-protein interactions derived from HIV-1 activators. Bioorg Med Chem Lett 11:591–594
199. Marciniak RA, Sharp PA (1991) HIV-1 Tat protein promotes formation of more-processive elongation complexes. EMBO J 10:4189–4196
200. Daelemans D, Este JA, Witvrouw M et al (1997) S-adenosylhomocysteine hydrolase inhibitors interfere with the replication of human immunodeficiency virus type 1 through inhibition of the LTR transactivation. Mol Pharmacol 52:1157–1163
201. De Clercq E (1998) Carbocyclic adenosine analogues as S-adenosylhomocysteine hydrolase inhibitors and antiviral agents: recent advances. Nucleosides Nucleotides 17:625–634
202. Ratmeyer L, Zapp ML, Green MR, Vinayak R, Kumar A, Boykin DW, Wilson WD (1996) Inhibition of HIM-1 Rev-RRE interaction by diphenylfuran derivatives. Biochemistry 35:13689–13696
203. Kaufmann GR, Cooper DA (2000) Antiretroviral therapy of HIV-1 infection: established treatment strategies and new therapeutic options. Curr Opin Microbiol 3(5):508–514
204. Richman DD (2001) HIV chemotherapy. Nature 410(6831):995–1001
205. Lipshultz SE, Miller TL, Wilkinson JD, Scott GB, Somarriba G, Cochran TR, Fisher SD (2013) Cardiac effects in perinatally HIV-infected and HIV-exposed but uninfected children and adolescents: a view from the United States of America. J Int AIDS Soc 16(1):18597
206. Maldarelli F, Palmer S, King MS, Wiegand A, Polis MA, Mican J, Kovacs JA, Davey RT, Rock-Kress D, Dewar R, Liu S, Metcalf JA, Rehm C, Brun SC, Hanna GJ, Kempf DJ, Coffin JM, Mellors JW (2007) ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. PLoS Pathog 3(4):e46
207. Maartens G, Celum C, Lewin SR (2014) HIV infection: epidemiology, pathogenesis, treatment, and prevention. Lancet 384(9939):258–271
208. Didigu C, Doms R (2014) Gene therapy targeting HIV entry. Viruses 6(3):1395–1409
209. Archin NM, Sung JM, Garrido C, Soriano-Sarabia N, Margolis DM (2014) Eradicating HIV-1 infection: seeking to clear a persistent pathogen. Nat Rev Microbiol 12(11):750–764
210. Siliciano JD, Siliciano RF (2014) Recent developments in the search for a cure for HIV-1 infection: targeting the latent reservoir for HIV-1. J Allergy Clin Immunol 134(1):12–19
211. Manson McManamy ME, Hakre S, Verdin EM, Margolis DM (2014) Therapy for latent HIV-1 infection: the role of histone deacetylase inhibitors. Antivir Chem Chemother 23 (4):145–149
212. Bullen CK, Laird GM, Durand CM, Siliciano JD, Siliciano RF (2014) New *ex vivo* approaches distinguish effective and ineffective single agents for reversing HIV-1 latency *in vivo*. Nat Med 20(4):425–429
213. Archin NM, Bateson R, Tripathy MK, Crooks AM, Yang KH, Dahl NP, Kearney MF, Anderson EM, Coffin JM, Strain MC, Richman DD, Robertson KR, Kashuba AD, Bosch RJ, Hazuda DJ, Kuruc JD, Eron JJ, Margolis D (2014) HIV-1 expression within resting CD4+ T cells after multiple doses of vorinostat. J Infect Dis 210(5):728–735

214. Sogaard OS, Graversen ME, Leth S et al (2014) The HDAC inhibitor romidepsin is safe and effectively reverses HIV-1 latency *in vivo* as measured by standard clinical assays. In: 20th international AIDS conference, Melbourne, Abst TUAA0106LB, 20–25 July 2014
215. Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75(3):311–335
216. Kumari A, Baskaran P, Van Staden J (2015) Enhanced HIV-1 reverse transcriptase inhibitory and antibacterial properties in callus of Catha edulis Forsk. Phytother Res 29(6):840–843
217. Xu L, Grandi N, Del Vecchio C, Mandas D, Corona A, Piano D, Esposito F, Parolin C, Tramontano E (2015) From the traditional Chinese medicine plant Schisandra chinensis new scaffolds effective on HIV-1 reverse transcriptase resistant to non-nucleoside inhibitors. J Microbiol 53(4):288–293
218. Huang SZ, Zhang X, Ma QY, Peng H, Zheng YT, Hu JM, Dai HF, Zhou J, Zhao YX (2014) Anti-HIV-1 tigliane diterpenoids from Excoecaria acertiflia Didr. Fitoterapia 95:34–41
219. Ellithey MS, Lall N, Hussein AA, Meyer D (2014) Cytotoxic and HIV-1 enzyme inhibitory activities of Red Sea marine organisms. BMC Complement Altern Med 14:77
220. Helfer M, Koppensteiner H, Schneider M, Rebensburg S, Forcisi S, Müller C, Schmitt-Kopplin P, Schindler M, Brack-Werner R (2014) The root extract of the medicinal plant *Pelargonium sidoides* is a potent HIV-1 attachment inhibitor. PLoS One 9(1):e87487
221. Suedee A, Tewtrakul S, Panichayupakaranant P (2013) Anti-HIV-1 integrase compound from *Pometia pinnata* leaves. Pharm Biol 51(10):1256–1261
222. Nutan, Modi M, Dezzutti CS, Kulshreshtha S, Rawat AK, Srivastava SK, Malhotra S, Verma A, Ranga U, Gupta SK (2013) Extracts from *Acacia catechu* suppress HIV-1 replication by inhibiting the activities of the viral protease and Tat. Virol J 10:309
223. Leteane MM, Ngwenya BN, Muzila M, Namushe A, Mwinga J, Musonda R, Moyo S, Mengestu YB, Abegaz BM, Andrae-Marobela K (2012) Old plants newly discovered: *Cassia sieberiana* D.C. and *Cassia abbreviata* Oliv. Oliv. root extracts inhibit *in vitro* HIV-1c replication in peripheral blood mononuclear cells (PBMCs) by different modes of action. J Ethnopharmacol 141(1):48–56
224. Park IW, Han C, Song X, Green LA, Wang T, Liu Y, Cen C, Song X, Yang B, Chen G, He JJ (2009) Inhibition of HIV-1 entry by extracts derived from traditional Chinese medicinal herbal plants. BMC Complement Altern Med 9:29
225. Bobbin ML, Burnett JC, Rossi JJ (2015) RNA interference approaches for treatment of HIV-1 infection. Genome Med 7(1):50
226. Swaminathan G, Navas-Martín S, Martín-García J (2014) MicroRNAs and HIV-1 infection: antiviral activities and beyond. J Mol Biol 426(6):1178–1197
227. Lai YT, DeStefano JJ (2012) DNA aptamers to human immunodeficiency virus reverse transcriptase selected by a primer-free SELEX method: characterization and comparison with other aptamers. Nucleic Acid Ther 22(3):162–176
228. Jorgensen WL (2004) The many roles of computation in drug discovery. Science 303 (5665):1813–1818
229. Geppert H, Vogt M, Bajorath J (2010) Current trends in ligand-based virtual screening: molecular representations, data mining methods, new application areas, and performance evaluation. J Chem Inf Model 50:205–216
230. Wei Y, Li J, Chen Z, Wang F, Huang W, Hong Z, Lin J (2015) Multistage virtual screening and identification of novel HIV-1 protease inhibitors by integrating SVM, shape, pharmacophore and docking methods. Eur J Med Chem 101:409–418
231. Tewtrakul S, Chaniad P, Pianwanit S, Karalai C, Ponglimanont C, Yodsaoue O (2015) Anti-HIV-1 integrase activity and molecular docking study of compounds from *Caesalpinia sappan* L. Phytother Res 29(5):724–729
232. Ahmad M, Aslam S, Rizvi SU, Muddassar M, Ashfaq UA, Montero C, Ollinger O, Detorio M, Gardiner JM, Schinazi RF (2015) Molecular docking and antiviral activity of N-substituted benzyl/phenyl-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2 (4H)-yl)acetamides. Bioorg Med Chem Lett 25(6):1348–1351

233. Singh A, Yadav D, Yadav M, Dhamanage A, Kulkarni S, Singh RK (2015) Molecular modeling, synthesis and biological evaluation of N-heteroaryl compounds as reverse transcriptase inhibitors against HIV-1. Chem Biol Drug Des 85(3):336–347
234. Filimonov DA, Lagunin AA, Gloriozova TA, Rudik AV, Druzhilovskii DS, Pogodin PV, Poroikov VV (2014) Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chem Heterocycl Compd 50(3):444–457
235. Zhang C, Du C, Feng Z, Zhu J, Li Y (2015) Hologram quantitative structure activity relationship, docking, and molecular dynamics studies of inhibitors for CXCR4. Chem Biol Drug Des 85(2):119–136
236. Corona A, Di Leva FS, Thierry S, Pescatori L, Cuzzucoli Crucitti G, Subra F, Delelis O, Esposito F, Rigogliuso G, Costi R, Cosconati S, Novellino E, Di Santo R, Tramontano E (2014) Identification of highly conserved residues involved in inhibition of HIV-1 RNase H function by diketo acid derivatives. Antimicrob Agents Chemother 58(10):6101–6110
237. Meleddu R, Cannas V, Distinto S, Sarais G, Del Vecchio C, Esposito F, Bianco G, Corona A, Cottiglia F, Alcaro S, Parolin C, Artese A, Scalise D, Fresta M, Arridu A, Ortuso F, Maccioni E, Tramontano E (2014) Design, synthesis, and biological evaluation of 1,3-diarylpropenones as dual inhibitors of HIV-1 reverse transcriptase. ChemMedChem 9 (8):1869–1879
238. Song Y, Zhan P, Li X, Rai D, De Clercq E, Liu X (2013) Multivalent agents: a novel concept and preliminary practice in Anti-HIV drug discovery. Curr Med Chem 20(6):815–832
239. Poroikov VV, Filimonov DA, Ihlenfeldt W-D, Gloriozova TA, Lagunin AA, Borodina YV, Stepanchikova AV, Nicklaus MC (2003) PASS biological activity spectrum predictions in the enhanced open NCI database browser. J Chem Inf Comput Sci 43(1):228–236
240. Liao C, Nicklaus MC (2010) Computer tools in the discovery of HIV-1 integrase inhibitors. Future Med Chem 7:1123–1140
241. Alcaro S, Artese A, Ceccherini-Silberstein F, Chiarella V, Dimonte S, Ortuso F, Perno CF (2010) Computational analysis of Human Immunodeficiency Virus (HIV) Type-1 reverse transcriptase crystallographic models based on significant conserved residues found in Highly Active Antiretroviral Therapy (HAART)-treated patients. Curr Med Chem 17 (4):290–308
242. Kirchmair J, Distinto S, Liedl KR, Markt P, Rollinger JM, Schuster D, Spitzer GM, Wolber G (2011) Development of anti-viral agents using molecular modeling and virtual screening techniques. Infect Disord Drug Targets 11(1):64–93
243. Rawal RK, Murugesan V, Katti SB (2012) Structure-activity relationship studies on clinically relevant HIV-1 NNRTIs. Curr Med Chem 19(31):5364–5380
244. Hao GF, Yang SG, Yang GF (2014) Structure-based design of conformationally flexible reverse transcriptase inhibitors to combat resistant HIV. Curr Pharm Des 20(5):725–739
245. Seckler JM, Leioatts N, Miao H, Grossfield A (2013) The interplay of structure and dynamics: insights from a survey of HIV-1 reverse transcriptase crystal structures. Proteins 81 (10):1792–1801
246. Allen WJ, Balius TE, Mukherjee S, Brozell SR, Moustakas DT, Lang PT, Case DA, Kuntz ID, Rizzo RC (2015) DOCK 6: impact of new features and current docking performance. J Comput Chem 36(15):1132–1156
247. Tarasova OA, Urusova AF, Filimonov DA, Nicklaus MC, Zakharov AV, Poroikov VV (2015) QSAR modeling using large-scale databases: case study for HIV-1 reverse transcriptase inhibitors. J Chem Inf Model. doi:10.1021/acs.jcim.5b00019. First published online June 5, 2015
248. De Clercq E (2015) Curious discoveries in antiviral drug development: the role of serendipity. Med Res Rev 35(4):698–719
249. Filimonov DA, Lagunin AA, Gloriozova TA, Gawande D, Goel R, Poroikov VV (2014) Libraries of natural and synthetic compounds as sources of novel drug-candidates. In: Chemistry of heterocyclic compounds. Modern trends, vol 1. ICSPF, Moscow. pp 464–471 (Rus)

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Lymphatic Filariasis: Current Status of Elimination Using Chemotherapy and the Need for a Vaccine

Ramaswamy Kalyanasundaram

Abstract During the last one decade, the whole world witnessed one of the most coordinated efforts toward global elimination of lymphatic filariasis (LF), a neglected tropical parasitic infection that affects 120 million people living in 72 different tropical countries. The approach was to use annual mass drug administration (MDA) using a combination of two chemotherapeutic agents to clear circulating parasites (microfilaria) in infected individuals living in various endemic regions of the world. This approach substantially decreased the incidence of infection in almost all the countries where the program was initiated. However, the biggest challenge now is to sustain the success and attain prophylaxis. This can be achieved only by newer chemotherapeutic agents against adult worms and an effective vaccine that can prevent future infections. This chapter summarizes the current status of LF elimination and the need for a more stringent and sustainable approach to control LF infection in endemic regions.

Keywords *Brugia malayi*, Endemic normal, Hot spots, Immune responses, Mass drug administration, Prophylactic chemotherapy, Protection

Contents

R. Kalyanasundaram (✉)
Department of Biomedical Sciences, University of Illinois, College of Medicine, 1601 Parkview Avenue, Rockford, IL 61107, USA
e-mail: ramswamy@uic.edu

1 Introduction

Lymphatic filariasis (LF) is a mosquito-transmitted tropical nematode infection that affects several millions of people living in 72 different countries in the world that are endemic for this infection. The adult parasites reside in the lymphatic circulation and produce larval stages called microfilariae that appear in the peripheral blood circulation, which are then picked up by the mosquitoes during their blood meal. The parasites undergo some development within the mosquitoes, and the infection gets transmitted when the infected mosquitoes feed on a new subject. Thus, microfilaria is the stage that is involved in the transmission of the disease and is the target of nearly all current elimination strategies [1]. Chronic infections with LF are characterized by lymphedema of the limbs, genital manifestations (hydrocele, chylocele, swelling of the scrotum and penis), and recurrent acute attacks, which are extremely painful accompanied by fever [2, 3]. According to the WHO, LF is the second leading cause of physical disability in the world [4]. Chronic LF infections are difficult to treat often requiring surgical intervention. Therefore, it is important to diagnose the infection early on so that appropriate treatment can be instituted. However, acute infections with LF often go undiagnosed because majority of the patients do not show any clinical signs. These asymptomatic carriers are thus a major source of disease transmission in a community. Thus, asymptomatic carriers are the target for elimination of LF from a community [5].

Attempts to accurately diagnose asymptomatic carriers in an endemic population have been a challenging and daunting task because of patient noncompliance. Although control program targeted at the detection of infected individuals through night blood screening followed by selective treatment with antifilarial drugs is an excellent approach that has been practiced for several years, unfortunately the process is highly labor intensive, costly, and difficult to sustain without an effective vector control approach. Mass drug administration (MDA) of a combination of the antifilarial drugs, diethylcarbamazine (DEC), and albendazole or ivermectin (Fig. 1

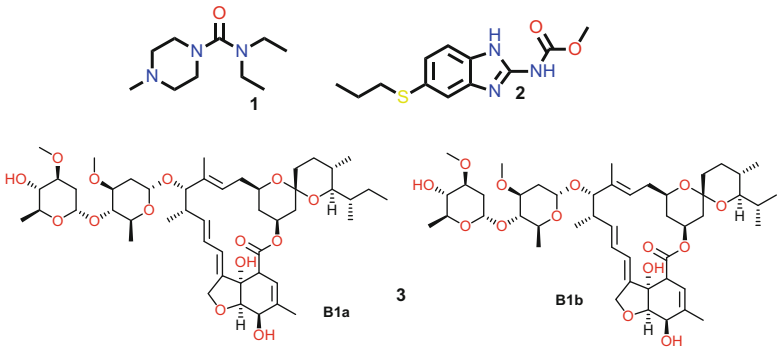

Fig. 1 Structure of DEC (**1**), albendazole (**2**), and ivermectin (**3**)

shows structure of these drugs) was successfully used in the past one decade as a control strategy to interrupt the transmission of LF in the endemic areas [6].

Despite the success, there are few drawbacks to the MDA approach, which include subject noncompliance in some endemic regions [7, 8] and concerns over the development of drug resistance parasites, although none has been reported to date. Nevertheless, the incidence of LF has significantly reduced in majority of the places where the annual MDA was implemented [9]. Focal areas of transmission, called "hot spots," continue to remain in some areas due to noncompliance to MDA [10–12]. For total elimination to be effective, it is important that these "hot spots" be identified and cordoned off to implement more focused and targeted elimination approach. In order to identify the "hot spots," there is an immediate need for a more reliable diagnostic test that can be used quickly in a larger population [13, 14]. Similarly there is a need for a better and sustainable approach for the control of LF in a community. MDA alone will not be effective as an eradication strategy against LF because antifilarial drugs are only effective as a treatment for existing infection. For the eradication of the disease, there is a need to build herd immunity in the endemic areas so reemergence of any residual infection can be prevented [15]. This can only be achieved by vaccinating the endemic population against LF. There are several recent advances in the development of an effective prophylactic vaccine against LF [16]. Thus, MDA combined with a prophylactic vaccination and vector control approach needs to be implemented if "hot spots" are to be eliminated from the endemic regions. Before the momentum gained by MDA campaign is reduced, it is important to capitalize the progress achieved so far in several endemic regions by planning toward eradication strategies. This chapter will review the current approaches in the elimination of LF and detail some of the major achievements in this area including the development of prophylactic vaccines.

2 LF and the Spectrum of Clinical Diseases

The main causative agents of LF are three nematode parasites: *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*. Infections in the human occur when infective larvae (L3) of the parasite are placed in the skin bite wound by infected mosquitoes during their blood meal. The larvae migrate into the lymphatic system to develop into adult males and female parasites. In Bancroftian filariasis, generally the parasite tends to localize to the scrotal lymphatics, in the lymph vessels and lymph nodes draining the lower and upper limbs. In brugian filariasis, the parasites are usually seen in the inguinal and axillary lymphatics and nodes [17]. Individuals infected with LF are presented with at least four different major clinical conditions: (1) asymptomatic carriers, (2) acute adenolymphangitis (ADL), (3) chronic irreversible lymphedema, and (4) tropical pulmonary eosinophilia.

Asymptomatic individuals carry microfilariae in their peripheral circulation. Depending upon the species of the parasite, the microfilariae may appear in the peripheral circulation in the evening when the mosquitoes are highly active. This ensures disease transmission. Depending on the number of adult female parasites and their fecundity, these individuals may show as high as 5000–10,000 Mf per ml of blood. In heavy infections, the Mf may get trapped in the tissues resulting in the formation of acute and/or chronic inflammatory granulomas, splenomegaly, and damage to the kidneys.

Acute adenolymphangitis (ADL) lymphedema, elephantiasis, and hydrocoele are the most common chronic complications of LF [17–21]. Chyluria does occur in few patients but it is relatively infrequent. Prevention and treatment of ADL is very important because distress caused by ADL can lead to severe economic loss and deterioration of quality of life of the patient. Paracetamol is used in the treatment of milder cases, but severe attacks of ADL are treated with oral or parenteral administration of antibiotics like penicillin, ampicillin, or co-trimoxazole [21]. ADL could develop into severe lymphoedema if not properly cared for and can be prevented by following the "foot-care program" which involves washing of the affected area with soap and water, clipping the nails at intervals and keeping them clean, raising the affected limb to reduce the swelling, using antibiotic ointments to treat local injuries, and using antifungal ointment on the feet daily [22]. Secondary bacterial infections are prevented by using antibiotics along with close adherence to hygiene practices [21, 22].

Chronic irreversible lymphedema is the most disabling disease. Patients with enlarged limbs or dependent regions with accompanying pain become severely disabled and unable to perform their normal day to day functions. They find it difficult to find employment, they become socially isolated, and with severe stigma they are a huge burden to the family because of the healthcare cost and no job. In addition to morbidity management and disability prevention, these patients will need significant psychological and socioeconomic support [19–24].

Tropical pulmonary eosinophilia (TPE) is a severe allergic syndrome associated with LF, where patients show wheezing, fever, and high eosinophilia in the blood

(as high as 3×10^6 eosinophils per ml of blood) and bronchoalveolar lavage [25, 26]. TPE reactions largely resemble type I, type III, and type IV hypersensitivity reactions depending on the severity of the condition. In all TPE cases reported, eosinophils are the major effector cells identified. TPE patients also show high titer of serum IgE and IgG antibodies against filarial antigens. It is believed that these antibodies amplify the allergic responses. Patients with TPE may show acute eosinophilic alveolitis and severe histiocytic infiltration depending on the severity of the condition. Pulmonary function tests may show mixed restrictive and obstructive abnormality with a reduction in diffusion capacity. Majority of the TPE cases are misdiagnosed as allergic conditions and are often treated with steroids. Given the parasite etiology of TPE, the use of DEC as a treatment is warranted to reduce the clinical symptoms of TPE. Despite treatment with DEC, about 20% of patients may relapse. Thus, there is a clear need for a diagnostic marker that can be used to differentially diagnose TPE from other pulmonary eosinophilic conditions [26].

3 Targets for Intervention and Elimination of LF

Elimination of LF in a community requires total interruption of the life cycle of the parasite which will prevent further transmission of the disease. Currently this is achieved by chemotherapy and vector control [9]. Adult parasites in the lymphatics produce microfilariae that appear in the peripheral circulation which are then picked up by the mosquitoes for disease transmission. There are at least three highly effective chemotherapeutic drugs (DEC, albendazole, and ivermectin) available to clear the microfilariae from the circulation. If given at an appropriate interval, these drugs are highly effective in prolonged clearing of microfilariae and thus interrupting transmission of the disease to mosquitoes. Thus, chemotherapy is an effective strategy to interrupt transmission of LF in a community [27–29]. In fact, mass administration of DEC was successfully tried in a population in the states of Kerala and Puducherry in India during 1990–1992 resulting in transmission interruption of LF in these regions [30]. Based on the success achieved at Cherthala and Puducherry, the 50th World Health Assembly passed a resolution in 1997 to eliminate LF by 2020 using the mass drug administration (MDA) approach [31].

4 Current Elimination Strategies for LF

4.1 Prophylactic Chemotherapy Using Mass Drug Administration

It is recognized as early as 1996 that the elimination of LF is feasible [32]. Clinical treatment for LF is based on the classification of diseases, whether it is asymptomatic, acute adenolymphangitis, or chronic irreversible lymphedema. Asymptomatic infection can be treated with a combination of oral medication DEC (6 mg/kg) and albendazole (400 mg/kg) in an outpatient setting [33]. In Africa, where onchocerciasis is prevalent, a combination of albendazole (400 mg/kg) and ivermectin (150–200 mg/kg) is used. Acute adenolymphangitis (ADL) occurs when large amounts of adult worms infest the lymph nodes. A course of DEC combined with albendazole or with ivermectin and analgesics is shown to be effective [34]. Antihistaminic and steroids are given to reduce swelling and hypersensitivity. Often antibiotics are used to treat any secondary infections that develop in the patient [35]. There is no radical cure once lymphoedema is established in the patient. Currently available treatments can only prevent further progression of the swellings. Alternative medicine may help reduce the swelling. There is a report that prolonged treatment with oral or topical coumarin or flavonoids can shrink the lymphedema [36]. Nevertheless, DEC, albendazole, and ivermectin are widely used in the control of LF.

4.2 Diethylcarbamazine Citrate

DEC (*N*,*N*-diethyl-4-methyl-1-piperazine carboxamide dihydrogen), a synthetic derivative of piperazine (Fig. 1), is a white crystalline odorless bitter salt used in the treatment of LF primarily as its citrate derivative. Since its discovery as an anthelmintic, DEC remains the drug of choice for LF and is used as an antifilarial drug since 1947. DEC has no known toxicity when given to uninfected individuals [37].

DEC is soluble in water and is available as a white crystalline tablet for treating LF. DEC has a melting point at 141°C and decomposition of DEC by heat emits toxic nitrogen oxides. The salt is very stable under high environmental temperatures and humidity. DEC is readily absorbed from the gastrointestinal tract and peal plasma level occurs in 1–2 h. The drug is metabolized in the liver and the metabolite is excreted in the urine.

The mechanism of action of DEC is not fully characterized, and some of the earlier studies showed that DEC inhibits arachidonic acid metabolism in microfilaria. This makes the microfilaria more susceptible to immune attack by the host. Thus, the lethal effects of DEC on microfilaria are thought to be not direct. A single annual dose of 6 mg/kg of DEC significantly lowers microfilariae count in the blood

[38]. However, its effect against adult worms is seen only in few patients due to the lack of sensitivity of the worms to DEC. Thus, DEC is not an adulticidal drug and it only clears microfilariae from infected patients. Therefore, when DEC is used as a mass drug treatment, there is a need to repeat the DEC treatment for many years. The average reproductive life span of adult female worms is approximately 30 years. Thus, there is a need to repeat DEC treatment as long as the adult female worms are reproductively active. This will ensure killing all microfilariae and possibly interrupt transmission of LF [39]. DEC is also used in the treatment of tropical eosinophilia syndrome; however, it needs to be given for 3–4 weeks. DEC-fortified cooking salt are distributed in several endemic regions as a way of mass distribution [40, 41].

DEC treatments are often followed by complications such as encephalitis and retinal hemorrhage [42–44]. Some patients develop fever, malaise, and headache following treatment with DEC. Some of these symptoms can be minimized by reducing the doses of DEC.

4.3 Ivermectin

Ivermectin, another drug used in the mass treatment of LF, is a macrocyclic lactone that is a semisynthetic derivative of avermectin (Fig. 1) produced by the fungal organism *Streptomyces avermitilis*. The 2015 Nobel Prize in Physiology or Medicine was awarded to Dr. William Campbell, emeritus research fellow at Drew University in Madison, New Jersey, in the USA, and Satoshi Omura, professor emeritus at Kitasato University in Japan, for their discovery of avermectin. Ivermectin has broad-spectrum antiparasitic effect. In the treatment of LF, a single dose of 200–400 ug/kg of ivermectin was highly effective against microfilariae and the immature stages of the parasite including embryos in the uterus of the adult female worms [45]. Nevertheless, ivermectin has no proven action against the adult parasites or in tropical eosinophilia [46].

The mechanism of action of ivermectin involves the interference of glutamate-gated chloride channel that is important for the neural and neuromuscular transmission in the parasite. When given at the recommended dose, ivermectin is well tolerated. Some of the reported side effects following ivermectin treatment in individuals with higher microfilariae counts include fever, myalgia, headache, sore throat, and cough. Ivermectin therapy occasionally causes self-limiting serum aminotransferase elevations along with liver injury.

4.4 Albendazole

Albendazole (methyl 5-(propylthio)-2-benzimidazolecarbamate), a benzimidazole derivative (Fig. 1), is also widely used as a chemopreventive agent against LF in the

MDA. Albendazole has broad-spectrum activity against a wide variety of gastrointestinal helminth parasites. Albendazole is thought to have killing effect on adult lymphatic filarial worms when used at a dose of 400 mg twice daily for 2 weeks. The death of the adult worm may induce severe scrotal reactions in infected patients [47].

A major advantage of using albendazole as an antifilarial agent is that it is also highly effective against other gastrointestinal nematode parasitic infections that are co-endemic in regions where LF is endemic [48]. Albendazole and diethylcarbamazine (DEC) are currently used in combination as an MDA in all parts of the world except Africa [49–52]. This is because DEC cannot be used in areas where LF and onchocerciasis are co-endemic. Onchocerciasis affects the eye, and the inflammation associated with the death of juvenile parasites in the eye following MDA treatment can cause blindness in addition to encephalopathy and kidney damage [53, 54].

Albendazole is metabolized in the liver to albendazole sulfoxide, which is the active form that is effective against parasites. After a single oral dose, albendazole is poorly absorbed through the gastrointestinal tract. However, high-fat diet was shown to increase albendazole absorption resulting in peak plasma concentrations of albendazole. In the blood, albendazole sulfoxide is 70% protein bound and has a half-life of approximately 8–12 h. Majority of the albendazole sulfoxide is secreted in the bile and urine. Hepatic disease or biliary obstruction can affect the clearance of albendazole sulfoxide. The mechanism of action of albendazole involves inhibition of the polymerization of tubulin specifically in the intestinal cells of the parasite. Albendazole has no effect on the host cell tubulin. Polymerization of tubulin is an important event in the formation of microtubules, and microtubule formation is critical for the uptake of glucose by the parasite. Albendazole-induced interference in the formation of microtubules is irreversible. Thus, resultant disruption in the absorption of glucose leads to diminished metabolism and energy production in the parasite resulting ultimately in the death and disintegration of the parasite. Several reports suggest that both human and animal parasites can develop resistance to albendazole. Resistance occurs when albendazole loses its ability to bind to tubulin, usually as a result of mutation in the tubulin gene and/or conformational changes in the tubulin protein of the parasites [55].

All three antifilarial drugs needed for MDA was supplied free of cost by GlaxoSmithKline, Merck & Co. Inc. and Eisai Inc. After 10 years of MDA coverage, the incidence of LF infection has drastically reduced in several parts of the world [3]. This is a great achievement. Nonetheless, certain focal areas of transmission – “hot spots” – continue to remain due to noncompliance to MDA [11, 12, 56]. MDA has little or no effect on the adult parasites, which can produce microfilariae when the effect of the drug is waned [57, 58]. The presence of ample mosquito vectors in the endemic regions can amplify the focal infection resulting in the reemergence of the disease. Therefore, there is a need to develop newer drugs that are effective against the adult worms.

4.5 Surgical Treatment

Surgical options like lymph nodo-venous shunts, omentoplasty, excisional surgery and skin grafting, elevation of the affected limb and compression bandages, regular light massage of the limb, compression of the affected limb using single or multicell jackets, and heat therapy using wet heat or hot oven all have been tried with varying success [23, 34]. Large hydroceles and scrotal elephantiasis can be excised by surgery, but multiple procedures and skin grafting are required in elephantiasis to correct the gross limbs.

Lymphatic drainage could be improved by reconstructive surgery involving lymphatic-venous anastomoses but the long-term benefit is still unclear. Drainage of hydrocele may give some immediate relief. Hydrocelectomies was also found to be helpful in some cases [18–20, 59].

4.6 Vector Control

Patrick Manson was the first to demonstrate that mosquitoes act as vectors for *W. bancrofti* [60]. Since then several species of mosquitoes largely belonging to the four genera (*Anopheles*, *Culex*, *Aedes*, and *Mansonia*) were shown to act as vectors for LF. Over the years several estimates were made as to the number of infective bites required to produce a patent infection in the human. For *Culex quinquefasciatus* mosquito, it is estimated that about 15,500 infective bites are required [61]. For *Anopheles* and *Aedes*, an estimated 2700–100,000 infective bites are needed for a patent infection. The distribution, ecology, biology, and transmission potential of each of the vectors differ greatly. In Southeast Asia, *Aedes* is the main transmitting vector for LF, and in Africa, *Anopheles* is the primary vector. *Aedes* mosquitoes can transmit diurnal subperiodic form of *W. bancrofti* (microfilariae are found in the peripheral blood during day time and night time). Similarly, *Aedes* can breed in any small puddle of stagnant water. Generally, *Anopheles* mosquitoes are active at dusk and dawn. Several species of *Anopheles* mosquitoes can transmit the LF infection in Africa and Southeast Asia. Many of these mosquitoes transmit other diseases as well. For example, *Anopheles* can transmit malaria and *Aedes* can transmit dengue. Thus, LF is often co-endemic with other disease conditions. Thus, an integrated vector control strategy can have significant impact on LF transmission as well. In fact, vector control was used as a primary approach for the control of filariasis initially by spraying indoor with dichlorodiphenyltrichloroethane (DDT) which successfully eliminated *Anopheles*-transmitted filariasis from Solomon Islands and Togo [62].

4.7 Morbidity Management

Major public health problem associated with LF is long-term disability and disfigurement due to lymphedema in the extremities and development of hydrocele [4]. The edematous limb in early ADL stages of the infection has a high risk of injury and possible secondary infection complicating the condition [63]. Therefore, one of the primary necessities will be to educate the patient to use simple hygiene measures, such as basic skin care, to prevent the progression of the edema. The affected parts should be washed with soap and warm clean water twice a day and mopped dry carefully. Topical antibiotic treatment such as penicillin over the affected skin in addition to the cleaning can help prevent secondary infections [21]. Regular mild exercise and keeping the limb elevated while lying down can facilitate lymph flow. Patients should wear comfortable footwear and keep their toe nails and skin between toes clean all the time. Scarification or if any open wound occurs on the skin, they should be treated immediately with antibiotic ointments. Hydrocele condition needs surgical intervention. In additional to the morbidity management, it is important that the patient receives psychological and socioeconomic support to reduce the stigma associated with the disease.

5 Developing Newer Drugs for LF Elimination

As mentioned above current strategy uses the combination of three drugs: DEC, albendazole, and ivermectin. These drugs are highly effective in clearing the parasites in the blood circulation, which includes L3 and microfilariae. However, none of these treatments reliably kills all adult worms in the human host. Therefore, much research effort has gone into identifying drugs and drug targets that are aimed at clearing the adult worms (macrofilaricides) along with the larval forms. Most successful among these are the anti-*Wolbachia* treatment strategies. A number of antibiotics doxycycline, tetracycline, rifampicin, azithromycin, and chloramphenicol (Fig. 2) were tested against *B. malayi* in vitro and were shown to have direct effect on the release of microfilariae from adult female worms [64–66]. At higher concentrations, these antibiotics can even kill the adult worms. Antibiotics such as penicillin, aminoglycosides, erythromycin, and ciprofloxacin (Fig. 2) have no effect against LF. However, corallopyronin A (Fig. 3), a natural antibiotic originally isolated from the myxobacterial strain *Corallococcus coralloides* c127 (DSM 2550), is shown to have significant effect against *Wolbachia* [67]. Additional studies showed that tetracycline, rifampicin, and azithromycin can clear *Wolbachia* from adult worms in physiological concentrations, and this was associated with the damage of the developing embryos [65]. Tetracycline treatment also decreases the expression of genes that are involved in the cuticle biosynthesis and energy metabolism of LF. Among the antibiotics that are effective, doxycycline

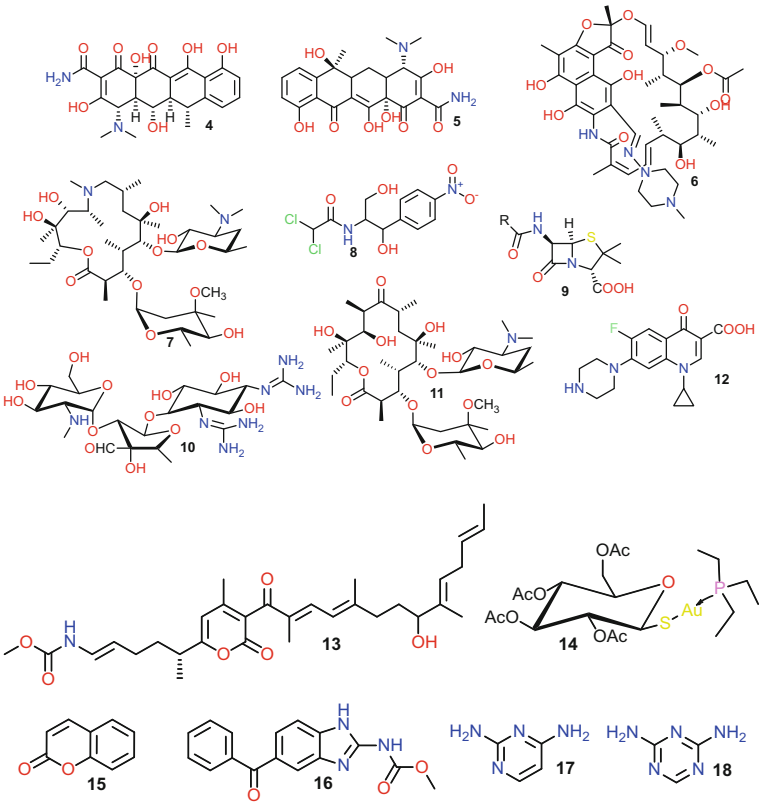

Fig. 2 Structure of doxycycline (**4**), tetracycline (**5**), rifampicin (**6**), azithromycin (**7**), chloramphenicol (**8**), penicillin (**9**), aminoglycosides (**10**), erythromycin (**11**), ciprofloxacin (**12**), corallopyronin A (**13**), auranofin (**14**), coumarin (5, 6 benzo-alpha-pyrone) (**15**), mebendazole (**16**), 2,4-diaminopyrimidine (**17**), and 2,4-diamino-*s*-triazine (**18**)

(C22H24N2O8), a synthetic derivative of tetracycline, has the most potent effect against LF [68].

Doxycycline is an inhibitor of matrix metalloproteases in LF. Few reports show that doxycycline treatment can ameliorate the lymphedema stage in *W. bancrofti* infections [69].

There was also significant decrease in the severity of lymphoedema and hydrocoeles in LF patients following doxycycline therapy. Several studies suggest that doxycycline at a dose of 200 mg daily for 3–8 weeks has no side effects and is more efficient than DEC and albendazole in interfering with the development, embryogenesis, fertility, and viability of filarial worms [70]. Doxycycline is mainly useful in areas where *W. bancrofti* and *Loa loa* is co-endemic. One of the major drawbacks of doxycycline treatment is that it is not suitable for mass treatment due to the possibility of developing drug resistance.

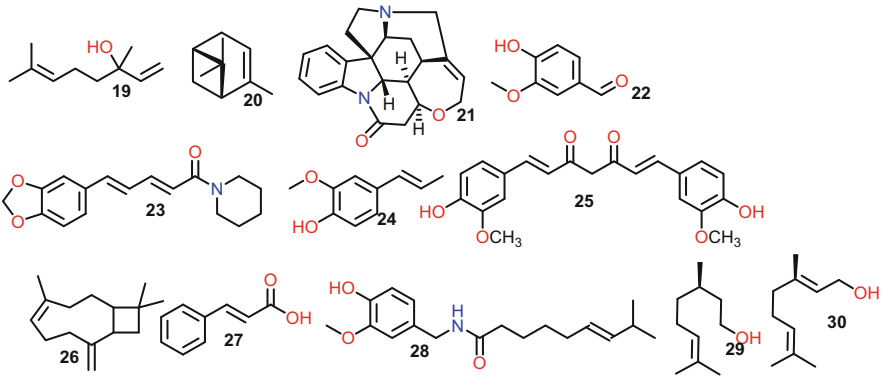

Fig. 3 Structure of GST inhibitory compounds tested against LF: linalool (**19**), alpha-pinene (**20**), strychnine (**21**), vanillin (**22**), piperine (**23**), isoeugenol (**24**), curcumin (**25**), beta-caryophyllene (**26**), cinnamic acid (**27**), capsaicin (**28**), citronellol (**29**), and geraniol (**30**)

Recently, several studies have also attempted to repurpose drugs that are used for other conditions and have already gone through the regulatory approvals. For example, auranofin (Fig. 2), a gold-containing drug used for rheumatoid arthritis, was found to be highly effective in killing both *Brugia* spp. and *Onchocerca* spp. adult worms in vitro in nanomolar range [71].

Some of the other drugs tested or under development against chronic LF infections include: coumarin (5,6 benzo-alpha-pyrone) (Fig. 2) that can reduce the swelling and bursting pain, secondary fungal infections, lymphangitis, and lymphadenitis that accompany LF infection. Unfortunately many of these drugs have severe side effects that can lead to hepatotoxicity [72, 73]. Mebendazole (Fig. 2), a broad-spectrum anthelmintic, has been tried with only partial success against LF [74]. The potential of developing inhibitors of parasite trehalose-6-phosphate phosphatase and 2,4-diaminopyrimidine and 2,4-diamino-*s*-triazine (Fig. 2) derivatives has been tried as a target for LF therapy [75, 76].

Herbal medicines are also being used by about 80% of the world's population in the developing countries and are recognized as a holistic approach toward health. In general most herbal medicines are safe with lesser side effects. Extensive search for herbal medicines against LF resulted in several herbal products that showed both macrofilaricidal and microfilaricidal properties. Some of these include the ethanolic and aqueous extracts of *Azadirachta indica* [77]; crude extracts of *Xylocarpus granatum*, *Tinospora crispa*, and *Andrographis* [88]; aqueous extracts of *Butea monosperma* L.; ethanolic extracts of *Vitex negundo* L. and *Aegle marmelos*; and ethanolic extracts of *nirgundi* roots, *bael* leaves, and *Cardiospermum halicacabum* [78–80]. Similarly, the leaf extracts of *Hibiscus sabdariffa* showed significant effect on both *B. malayi* adult worms and microfilariae. In jirds the leaf extract killed 57% of adult worms and sterilized 64% of female worms. A combination of herbal extracts from *Semen Arecae*, *Rhizoma Alismatis*, *Fructus Forsythiae*, *Spica Prunellae*, *Rhizoma Atractylodis*, *Fructus Chaenomelis Semen Torreya*, *Herba*

Polygoni Avicularis, and *Folium Pyrrosiae* is being developed as a drug against filariasis. Flavonoids (naringenin, hesperetin, flavone, rutin, chrysin, and naringin) and polyphenolic herbal extracts (*Aegle marmelos*, *Vitex negundo*, *Azadirachta indica*) combined with DEC have significant both macrofilaricidal and microfilaricidal properties in vitro [81]. Similarly several GST inhibitory compounds such as linalool, alpha-pinene, strychnine, vanillin, piperine, isoeugenol, curcumin, beta-caryophyllene, cinnamic acid, capsaicin, citronellol, and geraniol (Fig. 3) are being developed as a novel drug therapy for LF [82]. Despite all these drug developments, there are no comprehensive information on the costs and cost-effectiveness of interventions for LF prevention [83, 84].

6 Drug Discovery Pipeline for LF

Newer approaches to drug discovery against LF have begun to look at repurposing already approved drugs from human pharmacopeia. Initial screening of approved drugs focused on targeting the endosymbiont *Wolbachia*. These studies showed that tetracycline, fluoroquinolone, and rifamycin classes of antibiotics are promising as potential antifilarial drugs against adult filarial worms. A second pipeline of drugs being developed against LF uses testing of antiparasitic agents that were identified from veterinary practice and animal health drug discovery efforts. Exploitation of surrogate drug discovery models from veterinary practice is not new. In fact several antiparasitic drugs used in human medicine were first developed for veterinary practice. The third pipelines of drug discovery approach rely on high-throughput screening of FDA-approved pharmacological compounds against select molecular targets. Newer research approaches focus on identifying potential molecular targets in LF for chemotherapy. For example, if the target is a key enzyme critical for the survival of the parasite in the host, such throughput screening can identify molecules that can neutralize the activity of the enzyme. Currently there are several chemotherapeutic drugs that are highly effective against the larval stages of LF. There is an immediate need for drugs that are effective against the adult parasites. If adult parasites are not cleared from the host, it will be very difficult to eliminate LF from endemic region. Clearing microfilariae from infected individuals will only temporarily interrupt transmission. For long-term transmission interruption and elimination, it is critical that the adult worms are removed from infected individuals.

7 Developing Chemotherapy Against the Endosymbiont, *Wolbachia*

Nearly all of the filarial parasites including lymphatic filarial worms have the alphaproteobacteria *Wolbachia* which resides in these worms as an endosymbiont [85]. Transmission of these bacteria occurs vertically through female worms. The relationship between *Wolbachia* and the filarial worms are "mutualism" in that the *Wolbachia* bacteria are essential for the survival and reproduction of filarial worms. Removal of *Wolbachia* with antibiotics can significantly affect the survival of the filarial worms [86]. Preliminary annotation of the genome of *Wolbachia* from *Brugia malayi* is now complete [87] and is helping in identifying targets for chemotherapy and vaccination against *Wolbachia*. Approximately 15% of the genome has repeated elements like transposons and viruses. The *Wolbachia* genome also has a high number of duplicated genes compared to other bacteria. The association of *Wolbachia* spp. with filarial worms led to the speculation that endotoxins and bacterial products released from *Wolbachia* might play a central role in the pathogenesis of LF [88].

8 Public Health Education

Public health education plays a major role in any control programs. Education will remove some of the myths associated with the disease and clear any doubts the patients may have regarding the disease. These educations should start as early as in the lower grade school. In addition discussions about the disease and latest advances about the disease in the community forum on a regular basis can immensely help the effort toward control and elimination of LF. Studies show that public health education improved patient compliance to MDA, improved self-hygiene, and promoted clearing of mosquito breeding grounds from the surrounding environment in a community. Thus, public health education should be an integral part of any LF elimination strategies. The use of focus groups was used as a strategy to improve the quality of life with great success in a study conducted at Bangladesh for people living with LF [89]. Dietary changes consisting of a low-fat, high-protein diets supplemented with medium-chain triglycerides were shown to be beneficial.

9 Vaccine Development Against LF

9.1 Natural Immunity and the Need for a Vaccine Against LF

Natural immunity to LF occurs in the endemic regions. Subjects living in the same household or in the neighborhood same as an LF-infected person are at great risk of getting the infection because they can be repeatedly bitten by the infected mosquito. If some of the infective larvae end up developing into adult worms in the naive nonimmune individual, infection will ensue, thus perpetuating the transmission of the disease. However, in some of the individuals, the infective larvae after entering in the body probably die, and over time these individuals develop immunity to the infection. These individuals carry high titer of antibodies against the parasite in their circulation and show no signs of LF infection. These individuals are called endemic normal (EN) subjects [90]. Repeated exposure to infective larvae in the endemic regions is believed to boost the immunity of EN subjects against LF infection. Experimental studies in rodents show that repeated infections with *B. malayi* L3 as trickle infections can induce resistance against challenge infections [91]. Thus, EN is a heterogeneous group of individuals who are truly free of infections with adult worms but show high titer of antibodies against the infective larvae. Studies have confirmed that naturally immune EN human subjects have higher titer of IgG antibodies against infective larval antigens in their sera [92, 93]. These antibodies were shown to be lethal for infective larvae when tested in vitro using an antibody-dependent cellular cytotoxicity assay [94–96]. When infective larvae were incubated with sera from EN subjects and buffy coat cells from healthy individuals, several cells were found adhered to the larvae (Fig. 4), causing damage to the larval surface resulting in death of the larvae [97, 98].

IgG and IgM antibodies in the sera appeared to be primarily attaching to the larval surface in these assays. Depletion of IgG bodies completely abolished the ability of the EN sera to kill infective larvae in the ADCC assay suggesting an

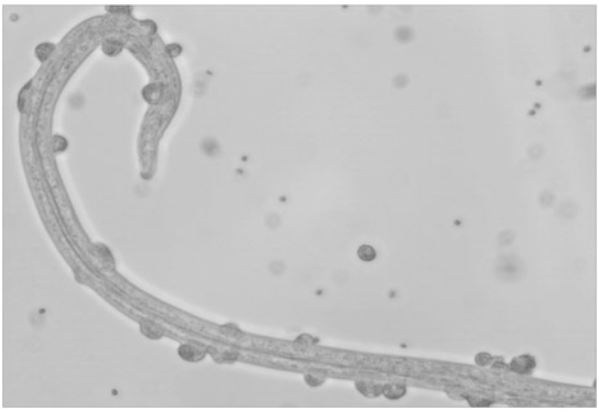

Fig. 4 ADCC assay using sera from an endemic normal (EN) subject. Several cells (mainly macrophages) were found adhered to the *B. malayi* L3 larva resulting in larval death. When EN sera depleted of IgG antibodies were used in the assay, no cells adhered to the larva, and there was no larval death suggesting that IgG antibodies are critical for the ADCC function

important role for IgG antibodies in the protection against LF. Among the buffy coat cells, macrophages that have FcγR1 were shown to be the major cells that were involved in the killing of the parasite [99]. Thus, the presence of protective antibodies is used as a criterion to distinguish EN from asymptomatic microfilaria carriers (MF). In contrast, asymptomatic microfilaraemic individuals have high titer of IgG4 antibodies, and patients with chronic disease have high levels of IgE antibodies against adult worm soluble antigens [100]. Thus, there is a clear dichotomy in the antibody responses between naturally immune EN subjects and patients with acute or chronic infections. In fact, this difference is exploited in many studies to understand the host protective responses to LF in the human and in the development of a vaccine. Mothers who have developed immunity against filariasis can transfer IgG antibodies to fetus via the transplacental route [101]. This is critical for the initial resistance and subsequent immune responses against the parasite in infants. All these reports suggest that development of a vaccine against LF in the human is possible.

Analysis of the response of peripheral blood mononuclear cells (PBMC) of EN subjects to infective larval antigens shows that antigen-responding cells are present in the peripheral circulation of EN subjects [102]. These cells proliferate in response to the antigens and secrete predominantly IFN-γ and IL-4 [94]. A balanced Th1/Th2 response against key antigens from the infective larvae appears to be critical for the protective immune responses in EN subjects. In sharp contrast, the PBMC of asymptomatic microfilaraemic (MF) individuals either fails to respond to the parasite antigens or predominantly secretes IL-10. Thus, a clear dichotomy exists in the cellular responses between EN and MF individuals as well [16]. The presence of protective immunity has been demonstrated in the human [103]. This suggested that development of a vaccine is possible against human LF.

As mentioned above, individuals living in the endemic region can develop natural immunity to LF. However, at this time, we do not know what percentage of individuals actually develop natural immunity and how long the immunity lasts. There is also a need to assess if there is really a need to develop immunity against LF for elimination, especially if MDA by itself can lead the way to eliminate LF from the endemic regions. After about 10 rounds of MDA campaign, incidence of LF has substantially reduced in several parts of the world [28]. Despite the significant achievements, there are several reports of noncompliance to MDA. This has led to the persistence of LF infection in some areas within the endemic regions. These areas are the “hot spots” where the infection still persists and implement targeted control approaches. Once the incidence level is brought to negligible levels, the next step will be to start planning for the eradication of lymphatic filariasis. Some of the countries were able to eradicate lymphatic filariasis; however, the rapidly increasing vector population, temperate climatic conditions, and poor hygienic conditions in some of the affected regions are a big challenge for achieving eradication of lymphatic filariasis. Therefore, there is a

need to compliment vector control measures along with MDA [104]. Similarly, a vaccination strategy against lymphatic filariasis combined with targeted MDA and vector control is probably the most sustainable approach to achieve eradication of lymphatic filariasis.

9.2 Current Status of Vaccine Development Against LF

Morris et al. [105] has extensively reviewed the history of vaccine development against LF in experimental animals. Initial attempts to identify potential vaccine candidates largely relied on characterizing soluble whole worm antigens or excretory secretory antigens using immune sera from experimental animals [106]. Wong et al. [107] demonstrated that partial protection can be achieved when macaques were immunized with excretory secretory products of the infective larvae. Completion of the genome of *B. malayi*; identification of the transcriptomes [108], secretome [109], and inflammatory/immuno-proteome [110]; and availability of cDNA libraries of different stages of the parasite helped in the genome-wide screening for the identification of vaccine candidates against LF [111].

In our laboratory we expressed the cDNA library of the infective stages of *B. malayi* and *W. bancrofti* on the surface of T7 bacteriophage [112]. We then screened these phage expression libraries using several rounds of sequential iterative panning with sera samples from non-endemic normal (NEN, these are individuals never exposed to the infection and have no antibodies to the parasites), asymptomatic carriers (MF) and chronic patients (CP) and finally with sera from the naturally immune EN individuals that contain protective antibodies. We identified several antigens that were subsequently cloned, and recombinant proteins were then evaluated for their vaccine potential in rodent models [16, 113–118]. Antigens that are uniquely recognized by the antibodies in the sera of EN subjects that are specific to the parasite with no homology to human protein sequences were selected for final vaccine development.

Among the several antigens identified and characterized, abundant larval transcript (ALT-2) is the major antigen recognized by all sera samples from EN subjects [119] and was subsequently shown by several laboratories as a major vaccine candidate for LF. Other important antigens identified include thioredoxin peroxidase (TPX-2) [120], glutathione-*S*-transferases (GST) [121], heat shock protein (HSP12.6) [116], vespid venom allergen homologue (VAL-1) [115, 122], tetraspanin LEL (TSP) [117], myosin heavy chain [123], *Bm*T5 [124], *Bm* transglutaminase [125], *Bm* paramomycin [126], *Bm*SPN2 [127], *Bm* AFII [128], *Bm* cysteine protease inhibitor (*Bm*CPI-2M) [95], *Bm* transglutaminase [125], *Bm*-trehalose-6-phosphate phosphatase (*Bm*TPP) [129], *Wolbachia* translation initiation factor-1 (*Wol* Tl IF-1) [130], and cofactor-independent phosphoglycerate mutase (*Bm*iPGM) [131].

Lymphatic filarial parasites are multicellular parasites that use redundant mechanisms for their survival in the host. Thus, targeting one molecule for vaccine development may not be as effective as targeting multiple antigens. Therefore, one of the best approaches in the vaccine development against helminth parasites is to combine two or more key vaccine antigens from different life cycle stages of the parasite and develop those as a multivalent vaccine to achieve maximum protection. Our laboratory originally showed that combining two antigens as bivalent vaccine was better than single monovalent vaccines. Since ALT-2 remains the leading vaccine candidate for LF, combinations of ALT-2 with other potential antigens were tested by us and others [91, 97, 118, 119, 122, 125, 132, 133]. For example, we constructed three different bivalent fusion proteins containing ALT-2 such as HA (HSP+ALT-2), TA (TSP+ALT-2), and VA (VAL-1+ALT-2) [122]. Compared to ALT-2 alone that gave approximately 72% protection, the bivalent constructs HA, TA, and VA gave 90%, 82%, and 80% protein, respectively, in mouse model. These are significant improvement over the monovalent ALT-2 vaccine [94]. Other laboratories also showed similar synergistic enhancement of protection by combining two antigens as multivalent vaccines [120, 122, 125]. The combination of antigens gave over 70% reduction in adult worm establishment following challenge infection in jirds compared to vaccination with individual antigens separately [134]. Another approach was to combine the vaccine antigens together as cocktail vaccines. In this approach, equal amounts of two antigens such as thioredoxin (*Wb*TRX) and thioredoxin peroxidase (*Wb*TPX) were combined as a vaccination dose [135]. This approach reduced the worm establishment by 71% in *Mastomys coucha*. Each of the vaccines as monovalent gave only 57% and 62%, respectively. Similarly, a chimera of TRX and VAH was highly immunogenic in mice [120].

Given the success of a bivalent vaccine, recently we prepared a trivalent fusion protein vaccine consisting of HSP, ALT-2, and TSP. This trivalent vaccine (HAT) gave close to sterile immunity (>94%) against a challenge infection with *B. malayi* L3 in mice and jirds [94, 97, 113]. When tested in nonhuman primates (*Macaca mulatta*), HAT vaccination was highly immunogenic, safe, and conferred about 50% protection compared to controls [16]. Vaccinated macaques did not develop the characteristic lymphatic pathology (Fig. 5) suggesting that HAT is an excellent and safe vaccine for LF.

Significant advances have been made in the last one decade to identify and characterize potential vaccine antigens against lymphatic filariasis. Despite the lack of suitable experimental small animal models, several research laboratories have developed highly effective vaccine candidates by combining two or more antigens as cocktail vaccines, multiepitope vaccine, and/or multivalent vaccines. Several of these vaccine candidates have great potential to be developed as a vaccine against human lymphatic filariasis. There is significant hope that a prophylactic vaccine will be available soon for the control of LF in the endemic regions of the world.

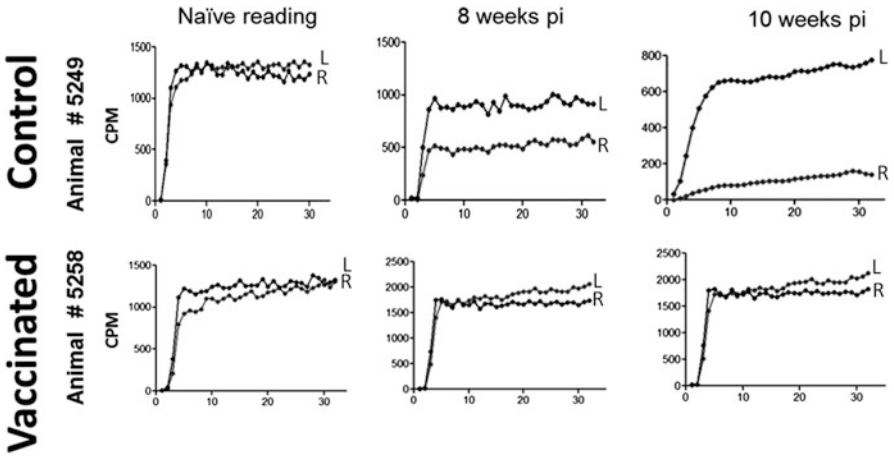

Fig. 5 Macaques were vaccinated three times at 4 weeks interval with alum adjuvanted *Bm*HAT (vaccinated) or alum alone (control). Four weeks after the last immunization, all animals were challenged on the right leg (R) with 500 *B. malayi* L3. To determine the level of damage to lymphatic vessels, we injected 200 μl of ^{99m}TC sulfide colloid on week 0 (before challenger), week 8, and week 10 after challenge between the webbing of each foot. Progression of the isotope from the injection site to the popliteal and inguinal nodes was monitored, and images were acquired every minute for up to 90 min. Results show that there was significant lymphatic damage in the right leg (where challenge infection was given) of control animals, whereas no damage was apparent in the right leg of vaccinated animals. $n = 5$ [16]

10 Future Prospects of Elimination of LF

The MDA approach although highly ambitious has interrupted the transmission of LF in several communities in the endemic regions [136, 137]. Published reports show that the incidence of LF has significantly decreased in communities where MDA coverage is over 75%. The MDA grassroots campaign, the community-wide education on LF by public health workers, various vector control approaches, and the successful morbidity management initiatives all have raised significant public awareness of the disease in the endemic communities. Therefore, even low-level transmission in these regions can be controlled if properly managed. However, in areas where there is significant noncompliance to MDA, the disease transmission continues [58, 84, 138–140]. These LF "hot spot" areas within an endemic region need to be identified and mapped using more reliable mass diagnostic tools that need to be developed. Recent evidences show that a combination of ivermectin and albendazole as MDA is also effective against soil-transmitted helminths, especially hookworm. Therefore, continuing the MDA with albendazole can be beneficial to eliminate some of the gastrointestinal parasites. Since DEC cannot be used in regions of Africa where onchocerciasis is a problem, the combination of ivermectin and albendazole will remain as the frontline chemopreventive approach to eliminate LF, *Onchocerca*, and gastrointestinal parasitic infections.

11 LF Elimination Is Not Close to the Endgame Yet

Over the past 10 years, the MDA approach was used to eliminate LF in several endemic regions. In places where MDA coverage was excellent, the LF incidence dropped significantly. In these regions unfortunately LF is not totally eliminated. The infection is present in low levels. Recently, we conducted a survey in several villages in India to assess the transmission and incidence of LF in communities that had high prevalence of LF and received at least 8 annual rounds of DEC treatment. Alarmingly, we found 5% of new cases of LF in individuals who were previously not reported to be positive for the infection. Since we identified new microfilariae-positive individuals that were not reported previously, we collected mosquitoes from that region using a CDC mosquito mini trap. To our surprise, mosquitoes from four out of seven villages were positive for LF by PCR analysis (unpublished data). These findings clearly show that LF disease is reemerging in select rural areas in India despite 8 years of MDA approach. The transmission assessment (TSA) survey has not begun in many parts of the world where 8–10 rounds of MDA have been completed. We will have to wait and see if our finding was an aberrant one or the situation is same in other parts of the world as well. Few recent reports suggest that this may be the case in other endemic regions also [136–140]. Drugs can clear the infection from infected individuals and transiently reduce the incidence of the infection. Since in most cases the adult worms are not cleared, they can start producing the microfilariae soon after the effect of drugs is waned. The situation becomes more complicated when the infected individuals fail to continue taking the medication due to noncompliance. Thus, although MDA is an excellent approach to eliminate LF, it is not sustainable. Mainly because there is a need to continue MDA every year or biannually to clear the circulating parasites from the blood of infected individual until all the adult parasites naturally die in the infected individuals, which could very well be over 30 years. Another major hurdle in the MDA approach is that elimination of LF from an individual with MDA is no guarantee that the person will not get a new infection, because MDA treatment will not confer protection against future infections. In the villages where we found new cases of LF, all the newly infected individuals claimed that they took DEC every year but still became infected. Therefore, to establish a more sustainable elimination approach, there is a need to build herd immunity. Natural infections or drug-attenuated infections in some cases can induce natural immunity. At least from our survey, there appears to be very few individuals who have developed natural immunity to LF in areas where ten rounds of MDA was given. Herd immunity can be built only by prophylactic vaccination. Thus, a sustainable LF elimination approach should include a prophylactic vaccination, chemotherapy of infected individuals in the "hot spot," and an effective vector control measure which will ensure prevention of any newer infection plus removal of any residual infections in a community [13].

12 Concluding Remarks

The Global Program for Elimination of LF has made significant strides toward eliminating LF using mass drug administration and morbidity management in the endemic regions of the world. Nevertheless, few pockets of residual infections still remain in majority of these endemic regions largely due to noncompliance to MDA. These residual infections can be a nidus and risk for reemergence of LF, thus potentially losing the momentum already gained by MDA. Significant progress has also been achieved in the last two decades in understanding the protective immune responses to LF in the human and experimental animals. All the studies to date confirm the notion that development of an effective prophylactic vaccine is possible against LF. Completion of the genome of *B. malayi* has significantly advanced our understanding of the secretome and proteome of the parasite. This has immensely helped the vaccine development field against LF. Despite the limitations in the availability of a true small experimental animal model, several vaccine candidate antigens have been identified and characterized. Combination of two or more antigens as a multivalent vaccine gave significantly better results than monovalent vaccines. Initial trials using a multivalent vaccine in nonhuman primates are highly encouraging. Prophylactic vaccine combined with MDA is critically needed to contain focal infections in "hot spot" areas. It is needless to stress that prophylactic vaccination is the most sustainable strategy for elimination and possibly eradication of infectious agents from a larger geographical area. There is significant hope that LF will be eliminated and eradicated in the future from the endemic regions.

References

1. Hotez PJ, Bottazzi ME, Strych U et al (2015) Neglected tropical diseases among the Association of Southeast Asian Nations (ASEAN): overview and update. PLoS Negl Trop Dis 9:e0003575
2. Anon (2008) Conclusions of the meeting of the Technical Advisory Group on the Global Elimination of Lymphatic Filariasis, November 2007. Wkly Epidemiol Rec 83:341–347
3. WHO (2012) Lymphatic filariasis. Fact Sheet 102
4. Zeldenryk LM, Gray M, Speare R et al (2011) The emerging story of disability associated with lymphatic filariasis: a critical review. PLoS Negl Trop Dis 5:e1366
5. Ottesen EA (2000) The global programme to eliminate lymphatic filariasis. Trop Med Int Health 5:591–594
6. Hoti SL, Pani SP, Vanamail P et al (2010) Effect of a single dose of diethylcarbamazine, albendazole or both on the clearance of *Wuchereria bancrofti* microfilariae and antigenaemia among microfilaria carriers: a randomized trial. Natl Med J India 23:72–76
7. Nujum ZT, Remadevi S, Nirmala C et al (2012) Factors determining noncompliance to mass drug administration for lymphatic filariasis elimination. Trop Parasitol 2:109–115
8. Alexander ND (2015) Are we nearly there yet? Coverage and compliance of mass drug administration for lymphatic filariasis elimination. Trans R Soc Trop Med Hyg 109:173–174
9. Rebollo MP, Bockarie MJ (2014) Shrinking the lymphatic filariasis map: update on diagnostic tools for mapping and transmission monitoring. Parasitology 141:1912–1917

10. Sunish IP, Munirathinam A, Kalimuthu M et al (2014) Persistence of lymphatic filarial infection in the pediatric population of rural community, after six rounds of annual mass drug administrations. J Trop Pediatr 60:245–248
11. Sinha N, Mallik S, Mallik S et al (2012) Coverage and compliance of mass drug administration in lymphatic filariasis: a comparative analysis in a district of West Bengal, India. Glob J Med Public Health 1:3–10
12. Hussain MA, Sitha AK, Swain S et al (2014) Mass drug administration for lymphatic filariasis elimination in a coastal state of India: a study on barriers to coverage and compliance. Infect Dis Poverty 3:31
13. Alhassan A, Li Z, Poole CB et al (2015) Expanding the MDx toolbox for filarial diagnosis and surveillance. Trends Parasitol 31:391–400
14. Ibrahim F, Thio TH, Faisal T et al (2015) The application of biomedical engineering techniques to the diagnosis and management of tropical diseases: a review. Sensors (Basel) 15:6947–6995
15. Babayan SA, Allen JE, Taylor DW (2012) Future prospects and challenges of vaccines against filariasis. Parasite Immunol 34:243–253
16. Dakshinamoorthy G, von Gegerfelt A, Andersen H et al (2014) Evaluation of a multivalent vaccine against lymphatic filariasis in rhesus macaque model. PLoS One 9:e112982
17. Shenoy RK, Bockarie MJ (2011) Lymphatic filariasis in children: clinical features, infection burdens and future prospects for elimination. Parasitology 138:1559–1568
18. Otabil KB, Tenkorang SB (2015) Filarial hydrocele: a neglected condition of a neglected tropical disease. J Infect Dev Ctries 9:456–462
19. Streit T, Lafontant JG (2008) Eliminating lymphatic filariasis: a view from the field. Ann N Y Acad Sci 1136:53–63
20. Stanton MC, Smith EL, Martindale S et al (2015) Exploring hydrocoele surgery accessibility and impact in a lymphatic filariasis endemic area of southern Malawi. Trans R Soc Trop Med Hyg 109:252–261
21. Addiss DG, Brady MA (2007) Morbidity management in the Global Programme to Eliminate Lymphatic Filariasis: a review of the scientific literature. Filaria J 6:2
22. Vaqas B, Ryan TJ (2003) Lymphoedema: pathophysiology and management in resource-poor settings – relevance for lymphatic filariasis control programmes. Filaria J 2:4
23. Addiss DG (2013) Global elimination of lymphatic filariasis: a “mass uprising of compassion”. PLoS Negl Trop Dis 7:e2264
24. Babu S, Nutman TB (2012) Immunopathogenesis of lymphatic filarial disease. Semin Immunopathol 34:847–861
25. Vijayan VK (2007) Tropical pulmonary eosinophilia: pathogenesis, diagnosis and management. Curr Opin Pulm Med 13:428–433
26. Akuthota P, Weller PF (2012) Eosinophilic pneumonias. Clin Microbiol Rev 25:649–660
27. Ichimori K (2014) MDA-lymphatic filariasis. Trop Med Health 42:21–24
28. Hooper PJ, Chu BK, Mikhailov A et al (2014) Assessing progress in reducing the at-risk population after 13 years of the global programme to eliminate lymphatic filariasis. PLoS Negl Trop Dis 8:e3333
29. Cano J, Rebollo MP, Golding N et al (2014) The global distribution and transmission limits of lymphatic filariasis: past and present. Parasit Vectors 7:466
30. Raju K, Jambulingam P, Sabesan S et al (2010) Lymphatic filariasis in India: epidemiology and control measures. J Postgrad Med 56:232–238
31. Kimura E (2011) The Global Programme to Eliminate Lymphatic Filariasis: history and achievements with special reference to annual single-dose treatment with diethylcarbamazine in Samoa and Fiji. Trop Med Health 39:17–30
32. Anon (1996) Four TDR diseases can be “eliminated”. TDR News 1–2
33. Horton J, Witt C, Ottesen EA et al (2000) An analysis of the safety of the single dose, two drug regimens used in programmes to eliminate lymphatic filariasis. Parasitology 121(Suppl): S147–S160

34. Shenoy RK, Suma TK, Rajan K et al (1998) Prevention of acute adenolymphangitis in brugian filariasis: comparison of the efficacy of ivermectin and diethylcarbamazine, each combined with local treatment of the affected limb. Ann Trop Med Parasitol 92:587–594
35. WHO (2006) Informal consultation on preventing disability from lymphatic filariasis, WHO, Geneva. Wkly Epidemiol Rec 81:373–384
36. Das L, Subramanyam Reddy G et al (2003) Some observations on the effect of Daflon (micronized purified flavonoid fraction of *Rutaceae aurantiae*) in bancroftian filarial lymphoedema. Filaria J 2:5
37. Hewitt RI, Kushner S, White E et al (1947) Experimental chemotherapy of filariasis; effect of 1-diethyl-carbamyl-4-methylpiperazine hydrochloride against naturally acquired filarial infections in cotton rats and dogs. J Lab Clin Med 32:1314–1329
38. Dreyer G, Addiss D, Williamson J et al (2006) Efficacy of co-administered diethylcarbamazine and albendazole against adult *Wuchereria bancrofti*. Trans R Soc Trop Med Hyg 100:1118–1125
39. Noroes J, Dreyer G, Santos A et al (1997) Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti in vivo*. Trans R Soc Trop Med Hyg 91:78–81
40. Hawking F, Marques RJ (1967) Control of *Bancroftian* filariasis by cooking salt medicated with diethylcarbamazine. Bull World Health Organ 37:405–414
41. Ottesen EA (2006) Lymphatic filariasis: treatment, control and elimination. Adv Parasitol 61:395–441
42. Haarbrink M, Terhell AJ, Abadi GK et al (1999) Adverse reactions following diethylcarbamazine (DEC) intake in 'endemic normals', microfilaraemics and elephantiasis patients. Trans R Soc Trop Med Hyg 93:91–96
43. McLaughlin SI, Radday J, Michel MC et al (2003) Frequency, severity, and costs of adverse reactions following mass treatment for lymphatic filariasis using diethylcarbamazine and albendazole in Leogane, Haiti, 2000. Am J Trop Med Hyg 68:568–573
44. Lima AW, Medeiros Z, Santos ZC et al (2012) Adverse reactions following mass drug administration with diethylcarbamazine in lymphatic filariasis endemic areas in the Northeast of Brazil. Rev Soc Bras Med Trop 45:745–750
45. Richard-Lenoble D, Chandenier J, Gaxotte P (2003) Ivermectin and filariasis. Fundam Clin Pharmacol 17:199–203
46. Dreyer G, Addiss D, Noroes J et al (1996) Ultrasonographic assessment of the adulticidal efficacy of repeat high-dose ivermectin in bancroftian filariasis. Trop Med Int Health 1:427–432
47. Jayakody RL, De Silva CSS, Weerasinghe WMT (1993) Treatment of *bancroftian* filariasis with albendazole: evaluation of efficacy and adverse reaction. Trop Biomed 10:19–24
48. Ottesen EA, Ismail MM, Horton J (1999) The role of albendazole in programmes to eliminate lymphatic filariasis. Parasitol Today 15:382–386
49. Rao RU, Nagodavithana KC, Samarasekera SD et al (2014) A comprehensive assessment of lymphatic filariasis in Sri Lanka six years after cessation of mass drug administration. PLoS Negl Trop Dis 8:e3281
50. Critchley J, Addiss D, Gamble C et al (2005) Albendazole for lymphatic filariasis. Cochrane Database Syst Rev CD003753
51. Critchley J, Addiss D, Ejere H et al (2005) Albendazole for the control and elimination of lymphatic filariasis: systematic review. Trop Med Int Health 10:818–825
52. Ottesen EA (2002) Major progress toward eliminating lymphatic filariasis. N Engl J Med 347:1885–1886
53. Negesse Y, Lanoie LO, Neafie RC et al (1985) Loiasis: "Calabar" swellings and involvement of deep organs. Am J Trop Med Hyg 34:537–546
54. Boussinesq M (2006) Loiasis. Ann Trop Med Parasitol 100:715–731
55. Schwab AE, Churcher TS, Schwab AJ et al (2006) Population genetics of concurrent selection with albendazole and ivermectin or diethylcarbamazine on the possible spread of albendazole resistance in *Wuchereria bancrofti*. Parasitology 133:589–601

56. Yahathugoda TC, Weerasooriya MV, Sunahara T et al (2014) Rapid assessment procedures to detect hidden endemic foci in areas not subjected to mass drug administration in Sri Lanka. Parasitol Int 63:87–93
57. Beuria MK, Bal MS, Mandal NN et al (2002) Antigenemia at 10 years after diethylcarbamazine treatment of asymptomatic microfilaraemic individuals: marginal conversion to infection-free state. Parasite Immunol 24:109–111
58. Rebollo MP, Mohammed KA, Thomas B et al (2015) Cessation of mass drug administration for lymphatic filariasis in Zanzibar in 2006: was transmission interrupted? PLoS Negl Trop Dis 9:e0003669
59. Thomas G, Richards FO Jr, Eigege A et al (2009) A pilot program of mass surgery weeks for treatment of hydrocele due to lymphatic filariasis in central Nigeria. Am J Trop Med Hyg 80:447–451
60. Bockarie MJ, Pedersen EM, White GB et al (2009) Role of vector control in the global program to eliminate lymphatic filariasis. Annu Rev Entomol 54:469–487
61. Paily KP, Hoti SL, Das PK (2009) A review of the complexity of biology of lymphatic filarial parasites. J Parasit Dis 33:3–12
62. de Souza DK, Koudou B, Kelly-Hope LA et al (2012) Diversity and transmission competence in lymphatic filariasis vectors in West Africa, and the implications for accelerated elimination of *Anopheles*-transmitted filariasis. Parasit Vectors 5:259
63. Kumari AK, Yuvaraj J, Das LK (2012) Issues in delivering morbidity management for lymphatic filariasis elimination: a study in Pondicherry, South India. Scientific World J 2012:372618
64. Supali T, Djuardi Y, Pfarr KM et al (2008) Doxycycline treatment of *Brugia malayi*-infected persons reduces microfilaremia and adverse reactions after diethylcarbamazine and albendazole treatment. Clin Infect Dis 46:1385–1393
65. Rao R, Well GJ (2002) In vitro effects of antibiotics on *Brugia malayi* worm survival and reproduction. J Parasitol 88:605–611
66. Townson S, Hutton D, Siemienska J et al (2000) Antibiotics and *Wolbachia* in filarial nematodes: antifilarial activity of rifampicin, oxytetracycline and chloramphenicol against *Onchocerca gutturosa*, *Onchocerca lienalis* and *Brugia pahangi*. Ann Trop Med Parasitol 94:801–816
67. Schaberle TF, Schiefer A, Schmitz A et al (2014) Corallopyronin A – a promising antibiotic for treatment of filariasis. Int J Med Microbiol 304:72–78
68. Debrah AY, Mand S, Marfo-Debrekyei Y et al (2007) Macrofilaricidal effect of 4 weeks of treatment with doxycycline on *Wuchereria bancrofti*. Trop Med Int Health 12:1433–1441
69. Dreyer G, Noroes J, Figueredo-Silva J et al (2000) Pathogenesis of lymphatic disease in *bancroftian* filariasis: a clinical perspective. Parasitol Today 16:544–548
70. Hoerauf A (2008) Filariasis: new drugs and new opportunities for lymphatic filariasis and onchocerciasis. Curr Opin Infect Dis 21:673–681
71. Bulman CA, Bidlow CM, Lustigman S et al (2015) Repurposing auranofin as a lead candidate for treatment of lymphatic filariasis and onchocerciasis. PLoS Negl Trop Dis 9:e0003534
72. Casley-Smith JR, Jamal S, Casley-Smith J (1993) Reduction of filaritic lymphoedema and elephantiasis by 5,6 benzo-alpha-pyrone (coumarin), and the effects of diethylcarbamazine (DEC). Ann Trop Med Parasitol 87:247–258
73. WHO (1996) Coumarin (Lodema). WHO Pharm News 10:2
74. Sarma RV, Vallishayee RS, Rao RS et al (1988) Use of mebendazole in combination with DEC in *bancroftian* filariasis. Indian J Med Res 87:579–583
75. Farelli JD, Galvin BD, Li Z et al (2014) Structure of the trehalose-6-phosphate phosphatase from *Brugia malayi* reveals key design principles for anthelmintic drugs. PLoS Pathog 10: e1004245
76. Sharma RD, Bag S, Tawari NR et al (2013) Exploration of 2, 4-diaminopyrimidine and 2, 4-diamino-s-triazine derivatives as potential antifilarial agents. Parasitology 140:959–965

77. Mishra V, Parveen N, Singhal KC et al (2005) Antifilarial activity of *Azadirachta indica* on cattle filarial parasite *Setaria cervi*. Fitoterapia 76:54–61
78. Khunkitti W, Fujimaki Y, Aoki Y (2000) In vitro antifilarial activity of extracts of the medicinal plant *Cardiospermum halicacabum* against *Brugia pahangi*. J Helminthol 74:241–246
79. Al-Abd NM, Nor ZM, Al-Adhroey AH et al (2013) Recent advances on the use of biochemical extracts as filaricidal agents. Evid Based Complement Alternat Med eCAM 2013:986573
80. Sharma RD, Veerpathran AR, Dakshinamoorthy G et al (2010) Possible implication of oxidative stress in anti-filarial effect of certain traditionally used medicinal plants *in vitro* against *Brugia malayi* microfilariae. Pharmacognosy Res 2:350–354
81. Lakshmi V, Joseph SK, Srivastava S et al (2010) Antifilarial activity *in vitro* and *in vivo* of some flavonoids tested against *Brugia malayi*. Acta Trop 116:127–133
82. Azeez S, Babu RO, Aykkal R et al (2012) Virtual screening and *in vitro* assay of potential drug like inhibitors from spices against glutathione-S-transferase of filarial nematodes. J Mol Model 18:151–163
83. Ali M, Afzal M, Kaushik U et al (2014) Perceptive solutions to anti-filarial chemotherapy of lymphatic filariasis from the plethora of nanomedical sciences. J Drug Target 22:1–13
84. Rebollo MP, Bockarie MJ (2013) Toward the elimination of lymphatic filariasis by 2020: treatment update and impact assessment for the endgame. Expert Rev Anti Infect Ther 11:723–731
85. Johnston KL, Ford L, Taylor MJ (2014) Overcoming the challenges of drug discovery for neglected tropical diseases: the A WOL experience. J Biomol Screen 19:335–343
86. Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol 6:741–751
87. Foster J, Ganatra M, Kamal I et al (2005) The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. PLoS Biol 3:e121
88. Zimmer C (2001) *Wolbachia*. A tale of sex and survival. Science 292:1093–1095
89. Zeldenryk L, Gray M, Gordon S et al (2014) The use of focus groups to develop a culturally relevant quality of life tool for lymphatic filariasis in Bangladesh. Qual Life Res 23:299–309
90. Day KP (1991) The endemic normal in lymphatic filariasis: a static concept. Parasitol Today 7:341–343
91. Joseph SK, Ramaswamy K (2013) Single multivalent vaccination boosted by trickle larval infection confers protection against experimental lymphatic filariasis. Vaccine 31:3320–3326
92. Bal M, Das MK (1999) Antibody response to a filarial antigen fraction in individuals exposed to *Wuchereria bancrofti* infection in India. Acta Trop 72:259–274
93. Hitch WL, Hightower AW, Eberhard ML et al (1991) Analysis of isotype-specific antifilarial antibody levels in a Haitian pediatric population. Am J Trop Med Hyg 44:161–167
94. Dakshinamoorthy G, Samykutty AK, Munirathinam G et al (2013) Multivalent fusion protein vaccine for lymphatic filariasis. Vaccine 31:1616–1622
95. Arumugam S, Wei J, Ward D et al (2014) Vaccination with a genetically modified *Brugia malayi* cysteine protease inhibitor-2 reduces adult parasite numbers and affects the fertility of female worms following a subcutaneous challenge of Mongolian gerbils (*Meriones unguiculatus*) with *B. malayi* infective larvae. Int J Parasitol 44:675–679
96. Chandrashekar R, Rao UR, Subrahmanyam D (1985) Serum dependent cell-mediated immune reactions to *Brugia pahangi* infective larvae. Parasite Immunol 7:633–641
97. Samykutty A, Dakshinamoorthy G, Kalyanasundaram R (2010) Multivalent vaccine for lymphatic filariasis. Procedia Vaccinol 3:12–18
98. Sim BK, Kwa BH, Mak JW (1982) Immune responses in human *Brugia malayi* infections: serum dependent cell-mediated destruction of infective larvae *in vitro*. Trans R Soc Trop Med Hyg 76:362–370
99. Mackenzie CD, Oxenham SL, Liron DA et al (1985) The induction of functional mononuclear and multinuclear macrophages in murine *brugian* filariasis: morphological and immunological properties. Trop Med Parasitol 36:163–170

100. Kurniawan A, Yazdanbakhsh M, van Ree R et al (1993) Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. J Immunol 150:3941–3950
101. Malhotra I, Ouma JH, Wamachi A et al (2003) Influence of maternal filariasis on childhood infection and immunity to *Wuchereria bancrofti* in Kenya. Infect Immun 71:5231–5237, 111
102. Arasu P, Nutman TB, Steel C et al (1989) Human T-cell stimulation, molecular characterization and in situ mRNA localization of a *Brugia malayi* recombinant antigen. Mol Biochem Parasitol 36:223–231
103. Steel C, Guinea A, Ottesen EA (1996) Evidence for protective immunity to *bancroftian* filariasis in the Cook Islands. J Infect Dis 174:598–605
104. Sunish IP, Rajendran R, Mani TR et al (2007) Vector control complements mass drug administration against *bancroftian* filariasis in Tirukoilur, India. Bull World Health Organ 85:138–145
105. Morris CP, Evans H, Larsen SE et al (2013) A comprehensive, model-based review of vaccine and repeat infection trials for filariasis. Clin Microbiol Rev 26:381–421
106. Grieve RB, Wisnewski N, Frank GR et al (1995) Vaccine research and development for the prevention of filarial nematode infections. Pharm Biotechnol 6:737–768
107. Wong MM, Fredericks HJ, Ramachandran CP (1969) Studies on immunization against *Brugia malayi* infection in the rhesus monkey. Bull World Health Organ 40:493–501
108. Li BW, Wang Z, Rush AC et al (2012) Transcription profiling reveals stage- and function-dependent expression patterns in the filarial nematode *Brugia malayi*. BMC Genomics 13:184
109. Armstrong SD, Babayan SA, Lhermitte-Vallarino N et al (2014) Comparative analysis of the secretome from a model filarial nematode (*Litomosoides sigmodontis*) reveals maximal diversity in gravid female parasites. Mol Cell Proteomics 13:2527–2544
110. Sahoo MK, Sisodia BS, Dixit S et al (2009) Immunization with inflammatory proteome of *Brugia malayi* adult worm induces a Th1/Th2-immune response and confers protection against the filarial infection. Vaccine 27:4263–4271
111. Wongkamchai S, Chiangjong W, Sinchaikul S et al (2011) Identification of *Brugia malayi* immunogens by an immunoproteomics approach. J Proteomics 74:1607–1613
112. Gnanasekar M, Rao KV, He YX et al (2004) Novel phage display-based subtractive screening to identify vaccine candidates of *Brugia malayi*. Infect Immun 72:4707–4715
113. Dakshinamoorthy G, Kalyanasundaram R (2013) Evaluating the efficacy of rBmHATalphac as a multivalent vaccine against lymphatic filariasis in experimental animals and optimizing the adjuvant formulation. Vaccine 32:19–25
114. Dakshinamoorthy G, Munirathinam G, Stoicescu K et al (2013) Large extracellular loop of tetraspanin as a potential vaccine candidate for filariasis. PLoS One 8:e77394
115. Kalyanasundaram R, Balumuri P (2011) Multivalent vaccine formulation with BmVAL-1 and BmALT-2 confer significant protection against challenge infections with *Brugia malayi* in mice and jirds. Res Rep Trop Med 2011:45–56
116. Dakshinamoorthy G, Samykutty AK, Munirathinam G et al (2012) Biochemical characterization and evaluation of a *Brugia malayi* small heat shock protein as a vaccine against lymphatic filariasis. PLoS One 7:e34077
117. Gnanasekar M, Anand SB, Ramaswamy K (2008) Identification and cloning of a novel tetraspanin (TSP) homologue from *Brugia malayi*. DNA Seq 19:151–156
118. Joseph SK, Sambanthamoorthy S, Dakshinamoorthy G et al (2012) Protective immune responses to biolistic DNA vaccination of *Brugia malayi* abundant larval transcript-2. Vaccine 30:6477–6482
119. Gregory WF, Atmadja AK, Allen JE et al (2000) The abundant larval transcript-1 and -2 genes of *Brugia malayi* encode stage-specific candidate vaccine antigens for filariasis. Infect Immun 68:4174–4179

120. Anugraha G, Jeyaprita PJ, Madhumathi J et al (2013) Immune responses of *B. malayi* thioredoxin (TRX) and venom allergen homologue (VAH) chimeric multiple antigen for lymphatic filariasis. Acta Parasitol 58:468–477
121. Veerapathran A, Dakshinamoorthy G, Gnanasekar M et al (2009) Evaluation of *Wuchereria bancrofti* GST as a vaccine candidate for lymphatic filariasis. PLoS Negl Trop Dis 3:e457
122. Anand SB, Kodumudi KN, Reddy MV et al (2011) A combination of two *Brugia malayi* filarial vaccine candidate antigens (BmALT-2 and BmVAH) enhances immune responses and protection in jirds. J Helminthol 85:442–452
123. Vedi S, Dangi A, Hajela K et al (2008) Vaccination with 73kDa recombinant heavy chain myosin generates high level of protection against *Brugia malayi* challenge in jird and mastomys models. Vaccine 26:5997–6005
124. Shakya S, Singh PK, Kushwaha S et al (2009) Adult *Brugia malayi* approximately 34 kDa (BMT-5) antigen offers Th1 mediated significant protection against infective larval challenge in *Mastomys coucha*. Parasitol Int 58:346–353
125. Vanam U, Pandey V, Prabhu PR et al (2009) Evaluation of immunoprophylactic efficacy of *Brugia malayi* transglutaminase (BmTGA) in single and multiple antigen vaccination with BmALT-2 and BmTPX for human lymphatic filariasis. Am J Trop Med Hyg 80:319–324, 166
126. Li BW, Chandrashekar R, Weil GJ (1993) Vaccination with recombinant filarial paramyosin induces partial immunity to *Brugia malayi* infection in jirds. J Immunol 150:1881–1885
127. Zang X, Atmadja AK, Gray P et al (2000) The serpin secreted by *Brugia malayi* microfilariae, Bm-SPN-2, elicits strong, but short-lived, immune responses in mice and humans. J Immunol 165:5161–5169
128. Dixit S, Gaur RL, Sahoo MK et al (2006) Protection against L3 induced *Brugia malayi* infection in *Mastomys coucha* pre-immunized with BmAFII fraction of the filarial adult worm. Vaccine 24:5824–5831
129. Kushwaha S, Singh PK, Rana AK et al (2013) Immunization of *Mastomys coucha* with *Brugia malayi* recombinant trehalose-6-phosphate phosphatase results in significant protection against homologous challenge infection. PLoS One 8:e72585
130. Nag JK, Shrivastava N, Gupta J et al (2013) Recombinant translation initiation factor-1 of *Wolbachia* is an immunogenic excretory secretory protein that elicits Th2 mediated immune protection against *Brugia malayi*. Comp Immunol Microbiol Infect Dis 36:25–38
131. Singh PK, Kushwaha S, Rana AK et al (2014) Cofactor independent phosphoglycerate mutase of *Brugia malayi* induces a mixed Th1/Th2 type immune response and inhibits larval development in the host. Biomed Res Int 2014:590281
132. Anand SB, Murugan V, Prabhu PR et al (2008) Comparison of immunogenicity, protective efficacy of single and cocktail DNA vaccine of *Brugia malayi* abundant larval transcript (ALT-2) and thioredoxin peroxidase (TPX) in mice. Acta Trop 107:106–112
133. Dash Y, Ramesh M, Kalyanasundaram R et al (2011) Granuloma formation around filarial larvae triggered by host responses to an excretory/secretory antigen. Infect Immun 79:838–845
134. Shrivastava N, Singh PK, Nag JK et al (2013) Immunization with a multisubunit vaccine considerably reduces establishment of infective larvae in a rodent model of *Brugia malayi*. Comp Immunol Microbiol Infect Dis 36:507–519
135. Prince PR, Madhumathi J, Anugraha G et al (2014) Tandem antioxidant enzymes confer synergistic protective responses in experimental filariasis. J Helminthol 88:402–410, 178
136. Sunish IP, Kalimuthu M, Rajendran R et al (2015) Decline in lymphatic filariasis transmission with annual mass drug administration using DEC with and without albendazole over a 10 year period in India. Parasitol Int 64:1–4
137. Stolk WA, Stone C, de Vlas SJ (2015) Modelling lymphatic filariasis transmission and control: modelling frameworks, lessons learned and future directions. Adv Parasitol 87:249–291

138. Kisoka WJ, Tersbol BP, Meyrowitsch DW et al (2015) Community members' perceptions of mass drug administration for control of lymphatic filariasis in rural and urban Tanzania. J Biosoc Sci 19:1–19
139. Adhikari RK, Sherchand JB, Mishra SR et al (2014) Factors determining non-compliance to mass drug administration for lymphatic filariasis elimination in endemic districts of Nepal. J Nepal Health Res Counc 12:124–129
140. Rebollo MP, Sambou SM, Thomas B et al (2015) Elimination of lymphatic filariasis in the Gambia. PLoS Negl Trop Dis 9:e0003642

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Past, Present, and Future of Antifungal Drug Development

P.K. Shukla, Pratiksha Singh, Ravindra Kumar Yadav, Smriti Pandey, and Shome S. Bhunia

Abstract Fungi are eukaryotic, single cell or multicellular organisms which cause a wide range of human diseases ranging from superficial skin to invasive life-threatening infections. Over the last couple of decades the incidence of life-threatening fungal infections has increased seriously as the patients of AIDS, cancer, organ transplant and immune-compromised population have increased. Though a significant progress has been made in the discovery of antifungal agents in the form of polyenes, azoles and allylamines yet the antifungal therapy poses severe challenge because of the side effects, narrow spectrum of activity and recently development resistance among patients against the present antifungals. This chapter deals with the current antifungal agents, their spectrum of activity, mode of action, limitations, current challenges in antifungal therapy, and new avenues for future developments.

Keywords Allylamines, Antifungal therapy, Azole, Cell membrane, Ergosterol, Immunocompromise, Monoclonal antibodies, Pathogenic fungi, Polyenes

Contents

P.K. Shukla (✉), P. Singh, R.K. Yadav, and S. Pandey
Division of Microbiology, CSIR-Central Drug Research Institute, Lucknow 226031, India
e-mail: pk_shukla@cdr.res.in

S.S. Bhunia
Division of Medicinal & Process Chemistry, CSIR-Central Drug Research Institute, Lucknow 226031, India

1 Introduction

Fungi are one of the extensively spread organisms on earth and have great environmental and medical importance. The kingdom fungi contains about 1.5 million [1] different species which are either unicellular or multicellular eukaryotic, heterotrophic organisms that can be divided into biotrophs: which obtain their nutrients from a living host (plant or animal), saprotrophs: which obtained their nutrients from animals or dead plants, and necrotrophs: which infect a living host and kill host cells to obtain their nutrients [2].

Besides being beneficial organisms for humans in bio-production of alcohol and bakery, fungal species like *Aspergillus* sp., *Penicillium* sp., and *Acremonium* sp. are associated with the production of enzymes and antibiotics. Along with the above positive

impacts certain species adversely affect the crops and humans by producing diseases. A number of fungi have been reported as causal agents of human and animal infections and the first published record of infection in human is a case of oral manifestation of *Candida albicans* infection that was recorded in 1665 as a fatal disease [3].

In the atmosphere fungi are present from temperate to subtropical and tropical areas, and these organisms are mostly non-pathogenic and can cause infection under certain compromised conditions like immune suppression which may be due to various factors [4]. The fungi can cause infection of any part of the body starting from the hair of the scalp to nails of the toe web. However these infections are opportunistic in nature and the fungi causing these infections are categorized as opportunistic pathogens. The true pathogenic fungi are only four in number and these are *Coccidioides*, *Paracoccidiodes*, *Blastomyces*, and *Histoplasma* [5]. Fortunately the geographic distribution of these fungi is known to restricted area [6]. In case of superficial fungal infections the value is more of cosmetic in nature and man hour loss in terms and public nuisance. However the systemic infections pose a serious challenge in the form of early and accurate diagnosis as well as treatment [7].

A number of antifungal agents as described in this chapter are available in the market. Barring amphotericin B almost all the known antifungal agents are fungistatic in nature. Amphotericin B considered to be the gold standard of the antifungal agents is fungicidal; however its use is very much limited due to its side effects, particularly nephrotoxicity [5]. The fungal infections have emerged into prominence after the onset of AIDS and HIV infections where these infections may prove to be fatal to the host [6]. The number of antifungal agents is limited as compared to antibacterial drugs because of the fact that the fungus is an eukaryotic organism that parasitizes an eukaryotic host where the narrow range of physiologic difference between them cause difficulties in developing safe and broad spectrum antifungal agents. There are limited number of classes of antifungal agents to combat fungal infections with limitations of toxicity and development of drug resistance [8, 9].

2 Challenges in Antifungal Therapy

The major challenge in the treatment of mycoses is the timely and correct diagnosis of the disease. This is the first very important step which is mainly dependent on the clinical symptoms, which are very peculiar in case of superficial infections like raised erythematous margins of the lesions with prominent scaling and many times present with itching. However, in case of systemic infections the symptoms are very often common to those caused by other bacterial infections particularly in case of the infections of the lung. Then comes the step of obtaining the sample from the site of infection which may be achieved through scraping from the active sites of the infection in case of the involvement of the skin (margins), hair, nail, and sputum in case of lung infection, blood in case of systemic infection, etc. The samples thus collected are subjected to direct microscopic examination using wet mount, KOH preparations or fungal specific stains such as lactophenol cotton blue. In case of

deep seated infections biopsy is often required for establishing the correct diagnosis. From the obtained clinical specimen cultures are made generally in Sabouraud's dextrose agar at 30–53°C. Very often the fungi take longer periods to grow and thus result in the delay in diagnosis of mucoses.

Advances in biological techniques particularly the molecular one have opened avenues for diagnostic methods that are not dependent on culture of the organisms. Specific metabolites and molecular probes are often used for the detection and identification of fungal infections [10–13]. PCR (polymerase chain reaction) has exhibited its utility in the diagnosis of microbial infections inclusive of mycoses [14–18]. In the genome the most conserved region is the ribosomal DNA having capability of phylogenetic divergence [19]. The rRNA gene has a large subunit (LSU) 28S rRNA and small subunit (SSU) 18S rRNA and 5.8S rRNA. The internal transcribed spacer (ITS) region I (ITSI) and ITSII are found between SSU rRNA and 5.8S rRNA and between 5.8S rRNA and LSU rRNA respectively and are more variable than the rest of the ribosomal gene subunits. In addition the intergenicspacer (IGS) region I (IGSI) and IGSII occur between the LSU and SSU sequence [20]. Further the single-stranded conformation polymorphism (SSCP) technique to identify sequence variations in a single strand of DNA due to its adoption to a unique conformation under non-denaturing conditions [18] has been used by various researchers [21–24]. Such molecular approaches have the advantage of detecting fungi directly in the clinical specimen and provide much faster and more sensitive fungal detection than the conventional culture-based methods.

The next important step in the direction of therapy is in vitro sensitivity tests for the isolated fungal strain against the available antifungal agents. This is achieved by exposing the test fungus against the known concentrations of various antifungal agents and determining the minimal inhibitory concentration values. This may be achieved by either disc diffusion method or more precisely by the twofold serial dilution method as per guidelines of the CLSI (Earlier NCCLS). There are a number of antifungal agents available for the treatment of mycoses. However their usefulness has been limited either by their selective activity or more recently this situation is further complicated because of the development of resistance in the fungal pathogens against the existing antifungal agents.

3 Available Antifungal Drugs, Spectrum of Activity, and Development of Resistance

The availability of antifungal agents is limited for therapy and the use of these drugs is further restricted by the issue of safety, resistance, and their efficacy profiles. Understanding the mode of action of different antifungal agents is an important prerequisite to explore drug resistance mechanisms. The emergence of resistance against drugs is an evolutionary process based on natural selection of organisms

that enhances their ability to survive and multiply in presence of drug. Investment of a considerable amount of energy is required by competitive microbial communities for the production and elaboration of antimicrobial agents [25]. The evolution of resistance against antimicrobial agent is ubiquitous in nature and microbes evolve various strategies to combat the action of drugs. The development of new antibiotics is outpaced by the evolution of drug resistance due to which progressing our knowledge towards understanding evolutionary mechanisms gains utmost importance. The present antifungal arsenal has been discussed below.

4 Polyenes

The Polyene antibiotics discovered in late 1950s have a broad spectrum fungicidal activity and were isolated from different species of *Streptomyces* which are soil born [26] (Fig. 1). Chemically the polyenes are the molecules that contain polyhydroxylic lactone ring of 20–40 carbon atoms with 4–7 conjugated double bonds, that's why they are hydrophobic in nature. These are known to bind to the main component of fungal cell membrane, the ergosterol and result in the formation of transmembrane channels that allow the leakage of cell contents along with K+ and Na+ ions leading to the damage and death of the fungal cells [27]. The affinity of polyenes for ergosterol in fungal cell wall is higher than the affinity for cholesterol in mammalian cell; therefore they are less toxic to the latter. Yet this non-negligible toxicity cannot be ignored and explains the high toxicity associated with several side effects. Of the several polyene antibiotics only three, nystatin, natamycin, and amphotericin B, are in clinical use despite their side effects.

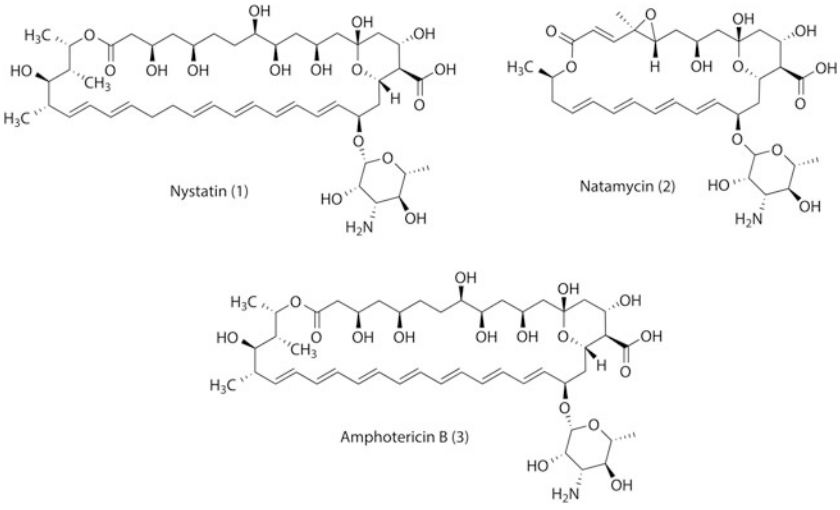

Fig. 1 Structure of polyene antibiotics

4.1 Nystatin

Nystatin (**1**) is the first antifungal agent introduced for the clinical use which was discovered by E.L. Hazen and R.F. Brown in 1944 while doing their research in the division of Laboratories and Research, New York State Department of Health which was published in 1950 [28, 29]. It was isolated from an actinomycete *Streptomyces noursei* which is commercially described as mycostatin and is active against many moulds and yeast infections [29, 30]. Nystatin is insoluble in water and sparingly soluble in organic solvents. It is unstable under moist conditions, heat, and light sensitive and therefore stored in cold and dark places [31]. Nystatin structure has been resolved by chemical degradation and X-ray crystallography [32]. It consists of a 38-membered macrolide lactone ring containing single tetraene and diene moieties separated by two methylene groups [33].

This drug is not absorbed through oral route but is effective topically for oropharyngealcandidosis. Nystatin was licensed for use in 1951 and due to its greater potential activity that caused toxicity in the system its use has been restricted to topical administration for superficial (mucosal) *Candida* infections of the oropharynx, esophagus, and intestinal tract.

Later on a liposomal preparation of nystatin was prepared that enhanced survival and reduced the tissue burden of *Aspergillus* in experimental neutropenic rabbits with invasive pulmonary aspergillosis and mice with disseminated aspergillosis [34, 35].

4.2 Natamycin

Natamycin (**2**) also known as pimaricin has been reported to be produced during fermentation process by a soil inhabiting microorganism *Streptomyces natalensis* [36]. It is sparingly soluble in water and has been found to exhibit antifungal activity at low concentrations. Natamycin is being used in the treatment of mycotic keratitis an infection of the cornea especially the cases caused by the species of *Aspergillus* and *Fusarium* [37]. It is normally used as topical antifungal agent in the form of cream or drops where it exhibits absorption in very low quantities in the body. This antibiotic is very little absorbed from the GI tract and therefore not recommended for use against systemic fungal infections [38].

4.3 Amphotericin B

Amphotericin B (**3**) is a polyene antifungal agent which is produced by *Streptomycin nodosus* [39]. According to the modern pharmacological standards, it is notified that amphotericin B, an antifungal agent, is a very old drug and since long times it was

the only therapeutic option for the treatment of invasive mycoses. This compound is amphoteric in nature with a primary amino group attached to the mycosamine ring and a carboxyl group on the macrocycle [40]. Amphotericin B forms deep yellow crystals that are sparingly soluble in organic solvents but insoluble in water [41].

Though it is not well absorbed after oral administration, it exhibits a wide spectrum of activity that is fungicidal in nature [42]. This drug can be used as an oral/topical formulation for the treatment of mucosal candidosis and intravenous amphotericin B for invasive fungal infections as a successful therapy [43]. It is proposed by most clinical medical mycologists as the drug of choice for all forms of invasive aspergillosis and cutaneous mucormycosis, blastomycosis, paracoccidioidomycosis, histoplasmosis, fusariosis, severe and moderate cryptococcal meningitis, coccidioidomycosis, candidosis, and *Candida* infections of the central nervous system [9]. The side effect of amphotericin B therapy causes serious nephrotoxicity where almost each patient contracts some defect in renal function [44].

The amphotericin B molecule is largely lipophilic and forms pore in the fungal membranes but does not cause pore formation in the mammalian cell membrane because its partition coefficient is lower for cholesterol which form the main constituent of mammalian cell membrane instead of ergosterol, which is found in fungal membrane. The drug gets saturated in fungal cell and leads to its lysis due to its higher partition coefficient for ergosterol. The fungicidal activity of amphotericin B is mediated by its binding with ergosterol that is supplemented by the secondary mechanism of membrane permeabilization through channel formation. In a recent study the cytocidal activity of amphotericin B has been attributed due to its ability to extract ergosterol from lipid bilayers by forming large, extramembranous aggregates [45–49]. Use of amphotericin B has certain limitations as its intravenous administration is associated with side effects such as fever, chills, headache, nausea, vomiting and nephrotoxicity. To overcome this problem different commercial lipid-based formulations of amphotericin B are available that cause less toxicity.

The clinically useful and established novel formulations are lipid combinations with amphotericin B, encapsulated in liposomes or in ribbon-like and disc-like lipid complexes while the others studied are amphotericin B–cochleate preparation and an arabinogalactan complex. To overcome the nephrotoxiciy of standard amphotericin B lipid formulation of amphotericin B can be used. The lipid formulation is very expensive as compared to the native formulation [25, 50]. Occurrence of resistance to polyenes in *C. albicans* is a rare event but recently increasing cases of resistance have been reported [51]. Filamentous fungi exhibit greater resistance to polyenes than yeasts. *Aspergillus terreus* is generally amphotericin B resistant whereas *A. fumigates* and *A. flavus* are becoming gradually more resistant [52]. Polyene resistance could be developed by reducing the substrate to which it binds, i.e., ergosterol content in plasma membrane. Mutation in *ERG3* gene lowers the ergosterol content of plasma membrane leading to accumulation of alternative sterols, causing amphotericin B resistance. The polyene resistance is also associated with increased catalase activity, which increases its oxidative tolerance [53].

A biochemical hypothesis for amphotericin B resistance has been given by Hamilton Miller that the altered sterol content of the resistant cells should bind to smaller amounts of polyene than do susceptible cells, hence may become resistant.

5 Azoles

Azoles were first introduced in 1960s as derivatives of N-substituted imidazole such as econazole, ketoconazole, miconazole, and clotrimazole, and is the most widely used class of antifungal agents [54] (Fig. 2). The azoles form a group of fungistatic agents with broad spectrum activity and are classified into two groups: the imidazoles and the triazoles. These antifungals inhibit the cytochrome P450 dependent enzyme lanosterol 14-alpha-demethylase that converts the lanosterol (the main sterol found in fungal cell wall) to ergosterol and thus results in the depleted ergosterol in the cell membrane causing cell death [55]. Azole antifungals are widely used in the treatment of systemic and topical (athletes foot, ringworm etc.) fungal infections. However azoles being fungistatic have a disadvantage due to recurrence of fungal infections.

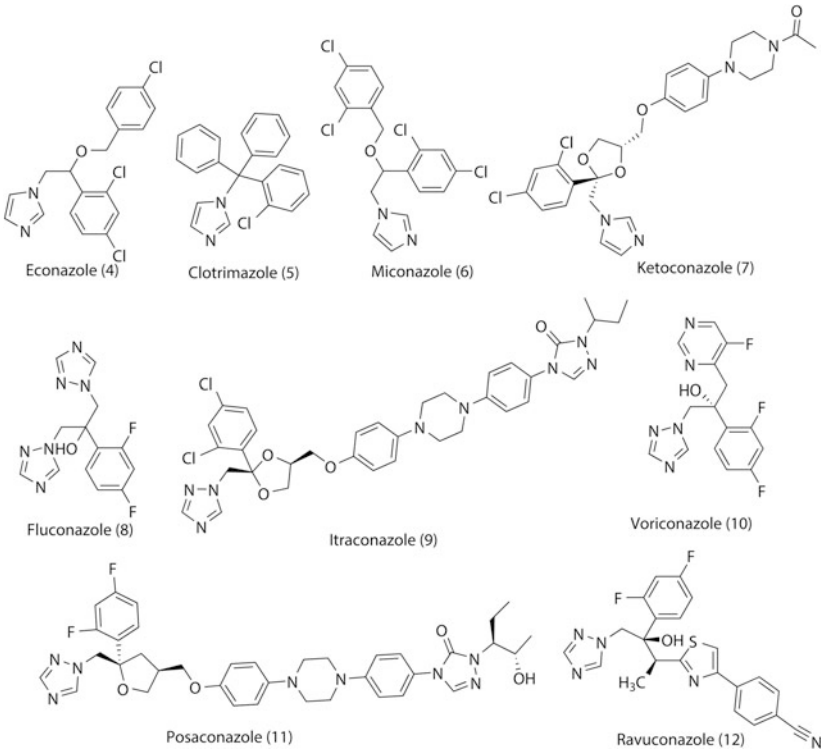

Fig. 2 Structure of azole class of antifungals

5.1 Econazole

Econazole nitrate chemically 1-[2-[(4-Chlorophenyl) methoxy]-2-(2,4-dichlorophenyl)-ethyl]-1*H*-imidazole (**4**) is a white crystalline nitric acid salt of econazole. It is slightly soluble in water, ether, and alcohol, sparingly soluble in chloroform, and soluble in methanol [56]. This antifungal is commonly used as the nitrate salt for antifungal therapy [57] mainly in the form of a cream to treat tinea corporis, tinea pedis, athlete's foot, tinea cruris, tinea versicolor and cutaneous candidiasis. However about 3% of treated patients have been reported to exhibit side effects like burning, itching, erthema, and pruritic rash [58].

5.2 Clotrimazole

Clotrimazole (**5**) was first described in 1969 from At Bayer Research Laboratories. 1-(*o*-Chloro-alpha,alpha-diphenyl benzyl)imidazole (clotrimazole) is a white crystalline solid that is sparingly soluble in water but soluble in alcohol and most organic solvents [59]. This antifungal is also known as Canesten or Lotrimin and is the first imidazole derivative which was developed for the treatment of human mycoses. It played an important role in the treatment of fungal infections such as vaginal yeast infections, oral thrush, ringworm, athlete foot, and jock itch. It is a vital medicine in the list of WHO [60].

Clotrimazole kills fungal cell by altering the permeability of fungal cell wall and binds to phospholipids in the cell membrane that inhibit the biosynthesis of ergosterol and sterols for the cell membrane production which results in loss of intracellular elements and cellular death [61]. Clotrimazole is a broad spectrum antifungal agent used in the treatment of infections caused by dermatophytes, yeasts, and *Malassezia furfur*.

5.3 Miconazole

Miconazole (**6**) is a synthetic imidazole antifungal agent that is poorly soluble in water and most of the organic solvents [62]. It also has some antibacterial action and antiparasitic properties. The mode of action of miconazole is inhibiting the synthesis of ergosterol [63]. It is used for the treatment of topical fungal infections including vaginal candidiasis [64], onychomycosis [65], tineapedis [66], and pityriasis versicolor [67]. It has also been moderately successful in the treatment of systemic mycoses [68].

5.4 *Ketoconazole*

Ketoconazole (**7**), discovered in 1976, is a member of imidazole synthetic compounds series which has a broad spectrum antifungal profile. 1-Acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2(1*H*-imidazole-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy] phenyl]piperazine is a weak basic compound that occurs as a white crystalline solid [69]. Ketoconazole is a racemic compound, consisting of the *cis*-2*S*,4*R* and *cis*-2*R*,4*S* isomers and it has seen that the 2*S*,4*R* isomer was more active than its 2*R*,4*S* enantiomer [70].

Ketoconazole was the first available compound for the oral treatment of systemic fungal infections in the early 1980s [71]. Ketoconazole is less soluble in water and administered orally [72] and in a range of formulations for topical administration such as creams (in treatment of cutaneous candidasis, pityriasis versicolor, candidal paronychia) and shampoos [73]. It shows toxic effects against yeast and interferes with other membrane lipids or enzymes. Ketoconazole inhibits the enzyme cytochrome P450 14-alpha-demethylase (P45014DM) which plays an important role in sterol biosynthesis pathway that leads from lanosterol to ergosterol [74].

High oral dose of ketoconazole may cause hepatotoxicity. Higher therapeutic doses may also produce endocrine abnormalities by reduction in circulating testosterone levels and blocks both testicular and adrenal androgen biosynthesis [75]. Ketoconazole is highly protein bound, has poor CNS penetration, and is not suitable for treating CNS infections. There is no intravenous preparation [76]. Oral ketoconazole is effective in patients with candidosis, coccidioidomycosis, blastomycosis, histoplasmosis, paracoccidioidomycosis, and cutaneous dermatophyte infections [77].

Later on the first generation triazoles such as fluconazole and itraconazole were introduced which are the imidazoles having five membrane ring atoms with one, two, and three nitrogen molecules. Fluconazole and itraconazole exhibited a broader antifungal activity spectrum as compared to the imidazoles and had a significant improved safety profile in comparison of amphotericin B and ketoconazole. Despite their prevalent use they face certain clinical limitations such as suboptimal level of activity spectrum, development of resistance and toxicity. In order to rectify these problems, several analogues have been derived. The second generation triazoles such as voriconazole, ravuconazole, and posaconazole possess higher potency and have increased efficacy against the emerging pathogens. Azoles perform their activity on cell membrane by inhibiting the ergosterol biosynthesis [78]. The major targets of most azoles are gene *ERG11* encoded cytochrome P450 lanosterol 14α-demethylase, it leads to generation of faulty intermediate namely 14-methylergosta-8, 24(28)-dien-3,6-diol, which is toxic and is responsible for inhibition of fungus [79]. Increase in azole resistance is mainly due to its fungistatic nature instead of fungicidal. Resistance against fluconazole among HIV patients with OPC is the direct consequence of excess use of itraconazole and fluconazole [80]. About one-third population of patients with AIDS has azole resistant

C. albicans in their oral tract [81]. *Candida* species employs various mechanisms to develop resistance against azoles as follows:

Over expression of efflux pumps: *C. albicans* overexpresses the efflux pumps in response to drug which results in efflux of drugs from cells thus reducing the drug concentration at action site. Two gene families namely *MDR* (Multi-Drug Resistance) genes of the major facilitator class and *CDR* genes belonging to the ATP-binding super cassette family. Up-regulation of *CDR* genes is responsible for resistance against most azoles while *MDR* encoded pumps exhibit narrow fluconazole specific spectrum [82].

Modification of target: Mutations in the *ERG11* gene, which encodes lanosterol 14α-demethylase, decrease azole affinity to the target site. Fluconazole has been used against a variety of mycotic infections and resistance to this antifungal has been documented. The two yeasts *Candida glabrata* and *C. krusei* with inherent low susceptibilities to fluconazole have been reported at a greater frequency from patients [83].

Up-regulation of target enzyme: *Candida* isolates overexpress the *ERG11* gene which results in reduced azole susceptibility [84]. The overexpression of gene results in accumulation of target molecules.

Development of alternative pathways: Organisms express alternative genes in order to bypass the pathway. Azole exposure results in ergosterol depletion from the membrane and leads to accumulation of toxic metabolite namely 14α-methyl 3, 6-diol. Additive mutation in *ERG3* gene prevents the formation of this toxic product from 14α-methyl fecosterol and leads to accumulation of nontoxic sterols [85].

5.5 *Fluconazole*

Fluconazole (**8**) was formulated in 1981 and marketed in 1990. It is a novel bi-striazole which is metabolically stable, water soluble, low lipophilicity, and plasma protein binding antifungal agent. Fluconazole acts by inhibiting ergosterol enzyme biosynthesis in fungal cells through inhibition of a cytochrome P450 enzyme dependent 14 alpha-sterol demethylase [86]. This leads to the accumulation of methylated sterols which break fungal membrane structure resulting in growth arrest. Fluconazole antifungal is administered orally, intravenously, or both and is used to treat broad spectrum of fungal infections and has a very low incidence of side effects. It is used to treat *Candida* infections of the vagina ("yeast infections"), mouth, throat, and bloodstream [87]. It is also used to prevent infections in people with weak immune systems, including those due to cancer chemotherapy, bone marrow transplantation patients, premature babies, and oropharyngeal candidosis, neutropenia, sporotrichosis infections [87, 88].

5.6 Itraconazole

Itraconazole (**9**) discovered in 1984 is another triazole antifungal agent with broad spectrum antifungal activity [89]. It contains a weakly basic 1,2,4-triazole and a non-basic 1,2,4-triazol-3-one moieties in its structure and requires an acidic environment for optimum solubilization and oral absorption [90].

It is insoluble in water and available in oral form. It is active against *Aspergillus*, *Candida*, and *Cryptococcus* species [91]. Itraconazole has been useful in the treatment of chronic cavitary pulmonary disease, extrapulmonary blastomycosis, disseminated non-meningeal histoplasmosis, osseous/articular and lymphocutaneoussporotrichosis in non-immunosuppressed patients [92]. Itraconazole has recently been repositioned as anticancer agent [93]. Traconazole is the only inhibitor in this class that has been exposed to reduce both hedgehog signaling pathway and angiogenesis. These different actions are unrelated to inhibition of the cytochrome P450 lenosterol 14 alpha demethylase. The anti-angiogenic action of itraconazole is associated with inhibition of glycosylation VEGFR2, phosphorylation, trafficking, and cholesterol biosynthesis pathways.

5.7 Voriconazole

Voriconazole (**10**) is a low molecular weight, water soluble broad spectrum triazole effective against the treatment of invasive aspergillosis and esophageal candidiasis [94, 95]. It shows activity against *Aspergillus* spp., *Fusarium* spp., *Candida* spp., *Cryptococcus neoformans*, Fusarium, and Scedosporium infections including the fluconazole resistant or less susceptible spp. of *C. glabrata* and *C. krusei* [96, 97]. It showed serious drug–drug interactions and side effects like skin rash and transaminase elevation and hallucinations [98–102].

5.8 Posaconazole

Posaconazole (**11**), a triazole antifungal drug, was approved by the US FDA in September 2006 for the prophylaxis of invasive *Aspergillus* and *Candida* infections in severely immune-compromised patients [103]. It shows in vitro activity against *Aspergillus*, *Candida* spp., *Cryptococcus* spp., and *Histoplasma* spp. and also effective against infections caused by the zygomycetes than voriconazole [8, 104]. The most common side effects of posaconazole are gastrointestinal complaints, nausea, vomiting, abdominal pain, headache, elevation of liver enzymes, and skin rash [105–107].

5.9 Ravuconazole

Ravuconazole (**12**), a triazole, is a broad spectrum antifungal agent. It shows activity against *Candida* spp. even isolates that are resistant to fluconazole, *Aspergillus*, *Cryptococcus*, and many dermatophytic fungi [107–109]. Ravuconazole shows long elimination half-life and high protein binding [110, 111].

5.10 Other Azoles

5.10.1 Imidazoles

Azoles being popular as antifungal agents have been considered for various modifications (Fig. 3). Among the imidazoles, a series of *N*-[(1,1′-biphenyl)-4-ylmethyl]-1*H*-imidazol-1-amine derivatives (**13**) reported by Setzu et al. [112] showed better antifungal activity with substitutions at 2-position (R_1) of the phenyl ring compared to substitution at the 4- position (R_2) when tested in *Candida neoformans*. However the most potent compound in the series with chloro substitutions at both 2 and 4 positions (R_1 and R_2) of the phenyl ring had a MIC value of 0.8 μg/mL against *Trichophyton rubrum* compared to miconazole (0.4 μg/mL). Imidazole modifications were also made by introducing nitro group at 5-position resulting in potent antifungal compounds. The analogs of **14** having R_1 substituted by morpholineor piperidine, R_2 and X substituted by H showed good activity against *Sclerophoma pityophila* [113]. Effective antifungal activity was also observed in another series of 5-nitro imidazoles having phenyl piperidine

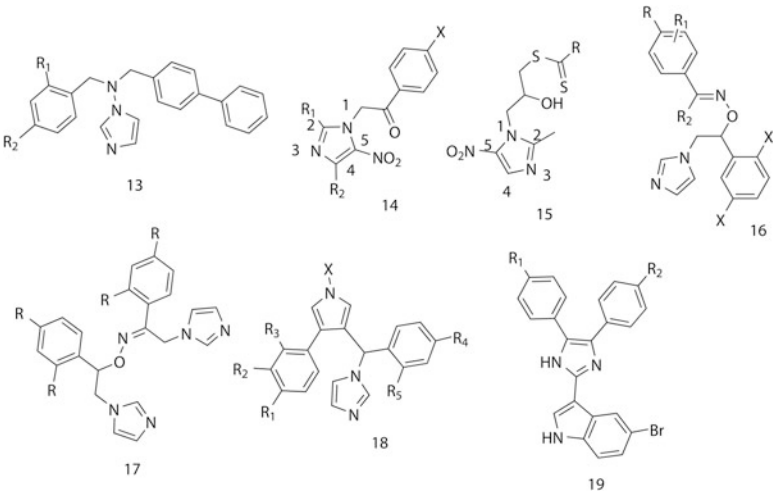

Fig. 3 Structure of imidazole containing molecules as antifungal agents

substitution at R separated by 2-hydroxypropyl methanedithioatespacer (**15**) [114]. The compound had MIC = 3 μg/mL against *Trichophyton tonsurans*. However the compound was less effective than miconazole (MIC = 0.2 μg/mL) or ornidazole (MIC = 0.8 μg/mL). In another imidazole containing series 2-(1*H*-imidazol-1-yl)-1-phenylethanone-*O*-2-(1*H*-imidazol-1-yl)-1-phenyl-ethyl oxime derivatives (**16**) were synthesized by inverting the oxime group present in oxiconazole [115]. The most active compound in the series having substitutions at R=Ethoxy morpholine, R_1=H, R_2=Me, and X=Cl is an effective antifungal compound against *C. glabrata* (MIC = 0.06 μg/mL), *C. parapsilosis* (MIC = 0.004 μg/mL), and *C. albicans* (MIC = 1 μg/mL). Another compound in the series where X=F also showed good antifungal activity in the above fungal species [*C. glabrata* (MIC = 0.25 μg/mL), *C. parapsilosis* (MIC = 0.03 μg/mL), and *C. albicans* (MIC = 8 μg/mL)]. In the same series modification of the R_2 to imidazole and substitution at R=F resulted in less active compound (**17**) with MIC values of 4, 8, 2 μg/mL in *C. glabrata*, *C. parapsilosis*, and *C. albicans*, respectively. In another series of imidazole derivatives having a pyrrole ring (**18**) it was observed that compounds having R_1=Cl, R_2=R_3=R_4=R_5=H, and R_3=CH_3 (MIC = 0.062 μg/mL), C_3H_7 (MIC = 0.016 μg/mL), CH_2–c–C_3H_5 (MIC = 0.016 μg/mL), CH_2=CH_2 (MIC = 0.032 μg/mL), CH_2–CH=CH_2 (MIC = 0.016 μg/mL), CH_2–CH=$C(CH_3)_2$ (MIC = 0.065 μg/mL) had comparable activity with miconazole (MIC = 0.062 μg/mL) and itraconazole (MIC = 0.062 μg/mL) and better than fluconazole (MIC = 0.25 μg/mL) in *C. albicans* [116]. In a series of 2,4,5- trisubstituted imidazoles (**19**), the best compounds had an indole moiety at the 2-position of the imidazole ring while the 4 and 5 positions were having substituted phenyl moiety . Three compounds [1: (R_1=R_2=F), 2: (R_1=Cl, R_2=H), 3:(R_1=Br, R_2=H)] in the series showed MIC = 8 μg/mL in *C. albicans* [117, 118].

5.10.2 Triazoles

Bile conjugates of fluconazole (**20**) (Fig. 4) have shown good antifungal activity when the R position of the steroid moiety is substituted by H or OH, the activity was in between 2.18 and 25 μg/mL when evaluated in different fungal species (*S. schenckii*, *C. albicans*, *C. parapsilosis*) [119]. The triazole derivatives of 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-cyclopropyl-*N*-substituted-amino)-2-propanol (**21**) were effective antifungal agents, most of them had broad antifungal activity with MIC_{80} less than 0.125 μg/mL [120]. The compounds having R=CH_3, CH_2CH_3, CH_2CHCH_2, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $(CH_2)_6CH_3$, $(CH_2)_7CH_3$ were the most potent ones having MIC_{80} in the range of 0.125–8 μg/mL against *C. albicans*, *C. parapsilosis*, *C. neoformans*, *C. tropicalis*, *T. rubrum*, *A. fumigatus*, *M. canis*, and *F. compacta*. Fluconazole at similar bioassay condition showed MIC_{80} range of 0.5–32 μg/mL in the fungal species mentioned above. In this series retention of antifungal activity was observed when the R group is substituted by benzyl group (**22**) having different substituents at the phenyl ring [X=H, 3F, 3Cl,

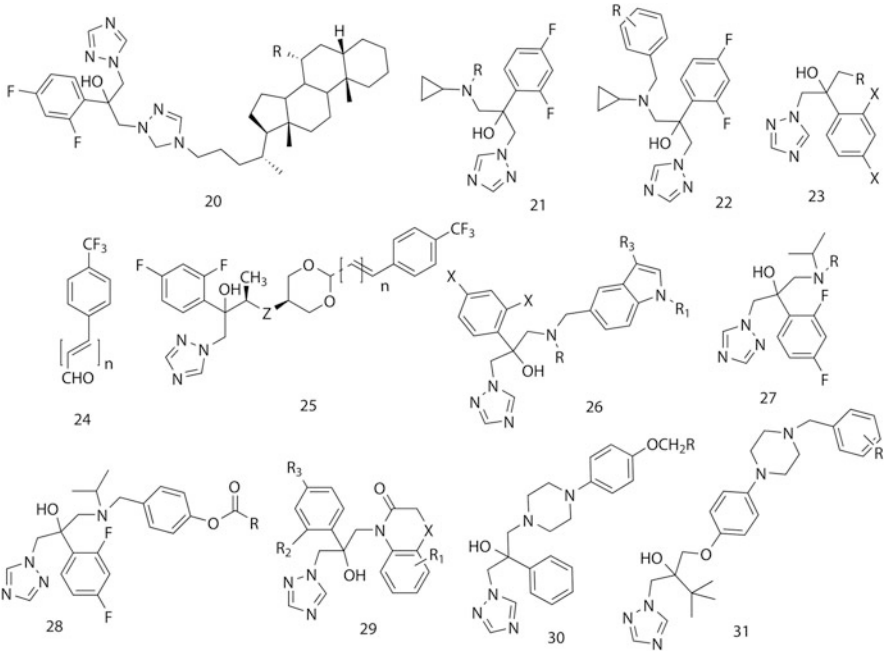

Fig. 4 Structure of molecules having triazole structure and fluconazole modifications

$3CH_3$, 4-NO_2, $2NO_2$, 2CN, 4CN, (2,4-Cl), $2CH_3$, $4CH_3$, 4F]. All these compounds had MIC_{80} value less than 0.125 μg/mL in *C. albicans*. Heterocyclic derivatives of fluconazole having N1-Indazole, indole, indoline, benzimidazole, azaindole, and benztriazole (**23**) were also synthesized where the R=N1-indazole and X=Cl, Cl substitution was the most potent candidate ($MIC_{80} = 0.0007$ μg/mL) than fluconazole ($MIC_{80} = 0.020$ μg/mL) against *C. albicans* [121]. In this series (**23**) better antifungal activity was observed by the replacement of N1-indazole by azaindole moiety having X=Cl, Cl ($MIC_{80} = 0.0031$ μg/mL) and X=F, F ($MIC_{80} = 0.007$ μg/mL). Another compound where R=3-ethoxycarbonylmethyl-1*H*-indole and X=Cl, Cl also showed good antifungal activity ($MIC_{80} = 0.006$ μg/mL). The syntheses of triazole derivatives with varying olefinic chain length for two series have been reported where in first case the optimum chain length of $n = 2$ having the structure **24** and varying olefinic chain length ($n = 0$–3) in structure **25** has shown excellent in vitro activity against *Candida*, *Cryptococcus*, and *Aspergillus* spp. with antifungal activity MIC ranging 0.016–0.125 μg/mL [122]. This is better than fluconazole that is having the MIC range 0.5–4 μg/mL in the above-mentioned species. A series of 1-[((hetero)aryl- or piperidinylmethyl) amino]-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols evaluated against *C. albicans* and *A. fumigatus* showed compound **26** having X=F, F, R=R_3=H, and R=N-Boc to be the most potent one ($MIC_{80} = 3$ ng/mL) and better than fluconazole ($MIC_{80} = 190$ ng/mL). In this series methyl substitution of the nitrogen atom in the linker reduces the activity 20 times

($MIC_{80} = 60$ ng/mL) when compared to **26** [123]. A series of triazole derivatives targeting lanosterol 14α-demethylase (CYP51) with a general structure 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-isoproyl-*N*-substituted-amino)-2-propanol depicted good antifungal activity when R=4-$H_3COC_6H_4$, 4-$H_5C_2OC_6H_4$ with MIC_{80} ranging 0.0156–1 μg/mL in **27**. In the same series different esters at 4-position of the phenyl ring having R=CH_3, CH_2CH_3, $CH_2CH_2CH_3$ in **28** had MIC_{80} in the range of 0.0156–64 μg/mL [124]. A series of fluconazole derivatives (**29**) with benzothiazinone substituent depicted slightly better antifungal activity. The compound **29** (X=S, R_1=H, R_2=R_3=F) (0.25 μg/mL) showed improved activity than the benzoxazinone replacement [X=O, R_1=H, R_2=R_3=F (0.5 μg/mL)] but both were found to be better than fluconazole (1 μg/mL) [125]. Another synthesized triazole containing compound (**30**) based on QSAR study with R=4-FC_6H_4, 4-$CONH_2C_6H_4$, 4-C_5H_4N was having comparable activity (0.0625–0.5 μg/mL) with itraconazole (1–2 μg/mL) when tested in *A. fumigatus*, *C. parapsilosis*, *C. tropicalis*, *C. neoformans*, *M. lauosum*, and *T. rubrum* with best activity in *M. lauosum* [126]. A series of triazole compounds having hydrophobic substitution or CN group with the general structure **31** (R=3,4- $(CH_3)_2$, 4-tBu, CN) was having comparable potency (0.125–64 μg/mL) with fluconazole (1–64 μg/mL) and itraconazole (0.125–1 μg/mL) [127]. A series of carboxylic acid esters of fluconazole showed higher activity than fluconazole against *C. albicans* (ATCC 14053) in SDB medium. The carboxylic acid esters of fluconazole having R=*O*-2-bromooctanoyl and *O*-11-bromoundecanoyl (**32**) (Fig. 5) have MIC values of 111 μg/mL and 198 μg/mL as compared to fluconazole that is having an MIC

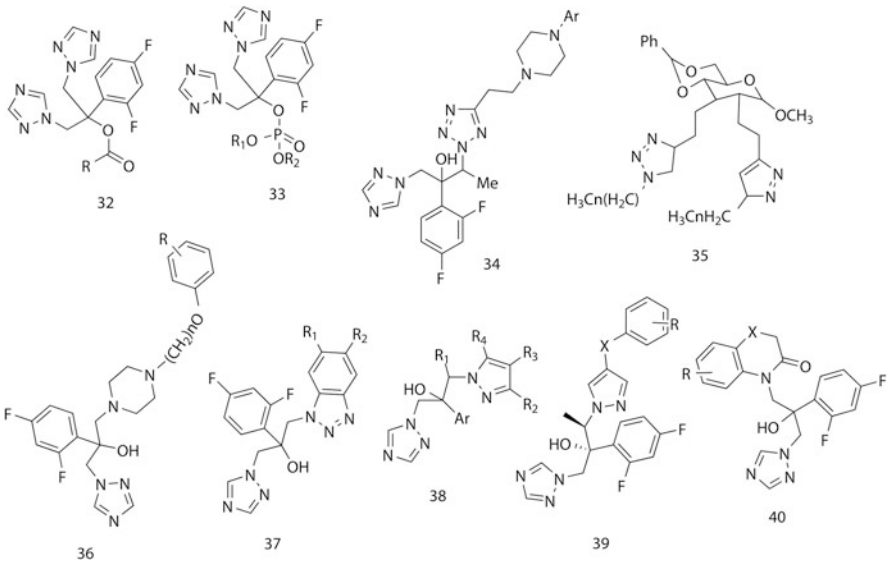

Fig. 5 Molecular structures having triazole moiety and fluconazole modifications

value greater than 4,444 μg/mL under similar bioassay conditions. Another series of fatty alcohol phosphate triester derivatives **33** has also been synthesized where compounds having R_1=$CNCH_2CH_2$: R_2=*n*-$C_{11}H_{23}$, R_1=$CNCH_2CH_2$: R_2=*n*-CH_2=CH–C_9H_{18}, R1=CH_3: R_2=*n*-$C_{11}H_{23}$, R_1=CH_3: R_2=*n*-CH_2=CH–C_9H_{18}, R_1=CH_3: R_2=*n*-C_8H_{17} have MIC values ranging from 12 to 1,658 μg/mL [128]. A series of triazole derivatives having 5-substituted tetrazole ring and having Ar =2-*n*BuOC_6H_6 attached to piperazine (**34**) is the most active with MIC values of 1.0–8.0 μg/mL, against *Candida* sp. [129]. In another series involving D-glucose derivatives of 1,2,3-Triazoles (**35**), chain length is important for antifungal activity with $n = 8$ having 14 times better activity than fluconazole with no activity when the chain length was increased to $n = 12$ [130]. Substituted 1,2,4-triazole and benzotriazole derivatives having phenoxypropyl piperazine side chains showed the linker length of three carbon atoms ($n = 3$) between piperazine and the phenyl ring to be crucial for antifungal activity (**36**). Compounds with R=H was having an MIC of 0.0156 μg/mL; however substitution at R by CH_3 (2,3,4 positions), 4-C$(CH_3)_3$, 4-Cl, 3-NO_2, 4-Br has good antifungal activity against *C. albicans* with MIC values ranging from 0.0156 to 0.25 μg/mL [131]. Benztriazole having no substitution (R_1=R_2=H) at 5, 6 positions was found to have an MIC value of 0.8 μg/mL while substitution at R_1=R_2=CH_3 and R_1=R_2=NO_2 was found to have same MIC value of 1.6 μg/mL in *C. albicans* (**37**) [129]. In the triazole series following the structural requirements in fluconazole a halogenated phenyl ring and tertiary alcoholic oxygen is preserved (**38**). In this series compounds having a phenyl ring with one halogen or trifluoro substituent were found to be active in *Candida* spp., *Aspergillus* spp., and *C. neoformans* with MIC ranging from 0.015 to 8 μg/mL. The most active compound in the series had an MIC $\leq$ 0.015 μg/mL in *C. parapsilopsis* while having good activity for *C. krusei* (MIC = 0.25 μg/mL) and *C. glabrata* (MIC = 1 μg/mL). A series of triazole molecules were synthesized where imidazole ring (A) was connected with variable spacer (X) to a substituted phenyl ring (**39**). The active compounds in the series were found to have X=C–C, C=C, C≡C, imidazolidine-2-one, 1*H*-imidazol-2(3*H*)-one, and R=4-Cl, 4-F with MIC_{80} ranging from 0.015 to 4 μg/mL in the *Candida* sp. (*C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. neoformans*). All the compounds have better activity in *C. albicans* with an MIC_{80} of $\leq$0.015 μg/mL as compared to fluconazole (MIC_{80} = 4 μg/mL) [132, 133]. A series of triazoles were synthesized where one triazole ring of fluconazole was modified into benzoxazinone (X=O, $n = 1$), benzothiazinone (X=S, $n = 1$), and benzoxazolinone (X=O; $n = 0$) moiety, with the most active compounds **40** having R=H, Cl and MIC = 0.06 μg/mL in *C. glabrata* [134].

6 Pyrimidine Analogue

5-Fluorocytosine or flucytosine (5-FC) (**41**) (Fig. 6), an antimetabolite, was first synthesized in 1957 and its antifungal property discovered in 1964 [135]. It is used for the treatment of invasive mycoses where it is effective against yeasts [136]. 5-FC is a fluorine analogue which inhibits nucleotide biosynthesis as it enters inside the fungal cells via cytosine permease and get deaminated to 5-fluorouracil (5-FU) by cytosine deaminase. 5-FU is a specific inhibitor of an enzyme essential for DNA synthesis namely thymidylate synthetase. This antifungal is selectively toxic to fungi as there is little or no cytosinedeaminase in mammalian cells [137]. The drug application is limited by the high prevalence of resistance in fungal species. Surveys conducted by Defever et al. and Stiller et al. [85, 137] on *C. albicans* estimated that 50–60% of the *Candida* isolates were susceptible, 30–40% were partially resistant along with 4–6% were highly resistant. 5-FC is administered in combination with other drugs such as fluconazole and amphotericin Bat present and rarely used as a sole agent. Resistance against 5-FC is developed due to mutational loss of permease activity. The resistance caused due to decreased uptake of 5-FC is prevalent in *C. glabrata* and *S. cerevisiae*, but this phenomenon is of least importance in case of *C. albicans* or *C. neoformans*. The mutational loss of the pyrimidine salvage enzymes forms the basis of resistance in laboratory or clinical strains of *C. neoformans* and *C. albicans* [138–140].

7 Allylamines

Allylamines form the newly developed class of ergosterol synthesis inhibitors. They are functionally and chemically very distinct from other classes of ergosterol binding antifungal agents [141] (Fig. 6). Allylamines inhibits the early steps of ergosterol biosynthesis leading to accumulation of squalene and absence of other

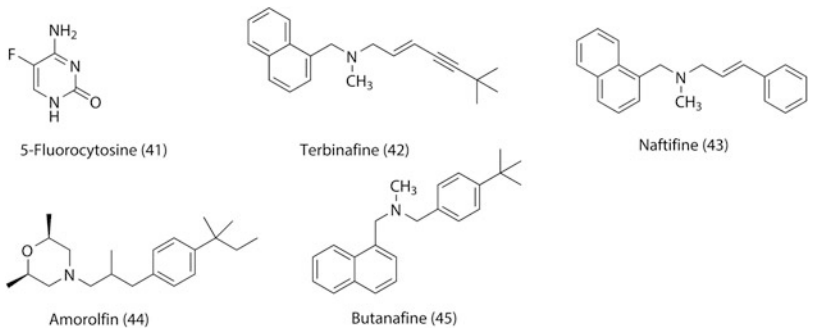

Fig. 6 Structure of pyrimidine and allylamine antifungal agents

sterol derivatives [142, 143]. Although clinical failures have been reported in treatment cases of allylamines yet human pathogenic fungi do not exhibit any associated resistance. Its resistance mechanism is poorly understood and further researches are required in this area. Important members of this group include naftifine and terbinafine.

7.1 Terbinafine

In Europe terbinafine (**42**) became available in 1991 whereas it got approval in the USA in 1996 [144]. Its hydrochloride salt is crystalline hydrophobic in nature but soluble in methanol, dichloromethane, and ethanol. This antifungal is mainly effective for dermatophytic fungi and used for superficial infections [145]. Terbinafineis recognized as inhibitor of fungal ergosterol biosynthesis by inhibiting squaleneepoxidase, an essential component of fungal cell. Fungal cell death is due to accumulation of squalene, which may increase permeability leading to disruption of cellular organization. Terbinafine hydrochloride may induce sub-acute cutaneous erythematous and people with this have been advised to know the possible risks with their physicians before the start of therapy [146].

A number of adverse drug reactions and side effects have been reported with oral terbinafine hydrochloride which may possibly due to longer duration of treatment and due to its extensive distribution in the body [144, 147].

7.2 Naftifine

Naftifine (**43**) is a synthetic, broad spectrum, allylamine antifungal agent which is used as a topical medication for the treatment of fungal infections. Naftifine hydrochloride is a white crystalline powder that is soluble in polar solvents such as ethanol and methylene chloride [148]. Naftifine hydrochloride, with potent in vitro antifungal activity against dermatophytes, was found to be effective against *tinea cruris*, *tinea corporis*, and *tinea pedis* as a topical agent [149]. It has shown very good activity against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Epidermophyton floccosum*, and *Microsporum canis*, *Microsporum audouini*, and *Microsporum gypseum*; and fungistatic activity against *Candida* species including *Candida albicans* [150, 151]. The mode of action of naftifine is not so clear but it seems to block the sterol biosynthesis via inhibition of the squalene 2,3-epoxidase enzyme [152]. This inhibition results in the accumulation of squalene, which is known to be toxic to fungi.

7.3 *Amorolfin*

Amorolfine (**44**) is a new topical water soluble antifungal drug of the morpholine derivatives. It inhibits D14 reductase and D 7-D8 isomerase which reduce ergosterol and accumulates in the fungal cytoplasmic membrane. This antifungal is used for the treatment of infections caused by dermatophyitic fungi and has been very effective in the treatment of onychomycosis [153, 154].

7.4 *Butenafine*

Butenafine (**45**) is a new synthetic benzylamine which has a broad spectrum of antifungal activity and used for the topical treatment of dermatophytoses caused fungi such as *Trichophyton mentagrophytes*, *Microsporum canis*, and *Trichophyton rubrum*. Its structure and mode of action are similar to allylamines as it inhibits sterol synthesis by blocking squalene epoxidation resulting in depletion of egosterol which is an essential lipid component of fungal cell membrane [147, 155]. The dermatophytes isolated from *Tinea cruris* have been found to be susceptible to both terbinafine and butenafine. The butenafine 1% cream has been found to exhibit supremacy over 1% terbinafine cream with statistically significant difference [156].

8 Indoles

Several compounds incorporating the indole moiety have also been reported as antifungal agents (Fig. 7). A series of 1*H*-Indole-4,7-diones derivatives have been synthesized by masking the indole nitrogen atom with CH_3 or with substituted phenyl groups (**46**, **47**). The compounds having substituted phenyl ring were active for *C. krusei*, *C. neoformans*, and *A. niger* with the most active compound having R_2=Cl (MIC = 0.8 μg/mL; *Candida krusei*) and a methyl ester attached to 3-position of the indole ring in **47**. A series of 5,6-bis(arylthio)-1*H*-indole-4,7-diones (**48**) showed moderate activity with an MIC range of 1.6–100 μg/mL with the most active compound (MIC = 1.6 μg/mL) having R_1=Cl, R_2=H for *Candida tropicalis*. The other substitutions such as R_1=CH_3, H and R_2=H, Cl, Br, I, OCH_3, CH_3; R_1=H, CH_3, F, Cl and R_2=H, Cl, Br, F, OH in all the 1*H*-Indole-4,7-dione series had potent antifungal activity with MICs ranging from 0.8 to 100 μg/mL [157]. The aminoguanidine derivatives of *N*-arylsulfonyl-3-acylindoles indicated that incorporation of electron donating groups at R_1 and R_2 improve antifungal activity. Variations were also made regarding the length of alkyl chain at R_3 (methyl, ethyl, propyl) (**49**). The compounds with R_1=4-Me, R_2=H, R_3=Me (*P. oryzae* = 79.64%, *A. alternata* = 79.15%, *B. sorokinianum* = 82.28%) and

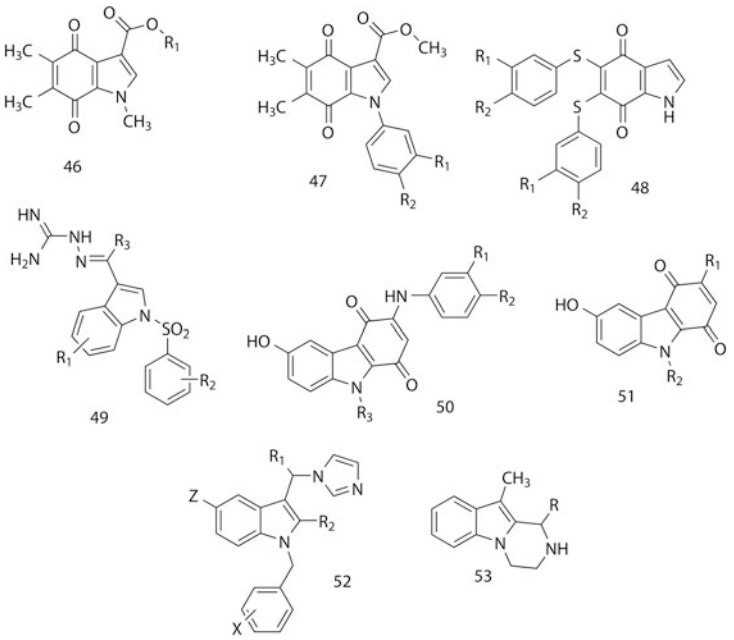

Fig. 7 Structure of indole antifungals

R_1=R_2=4-Me, R_3=Me (*P. oryzae* = 84.84%, *A. alternata* = 82.98%, *B. sorokinianum* = 80.58%) had good antifungal activity [158].

A series of compounds having indole fused with benzoquinone moiety having substitutions R_1=H, OH, F; R_2=CH_3O, H, CH_3, Br, Cl, I, F, OH, R_3=C_2H_5, CH_3, n-Pr (**50**) had potent antifungal activity with MIC 6.3–100 μg/mL in the *Candida* and *Aspergillus* sp. [159]. In another series of indole (**51**) substitution at R_1=CH_3CH_2S, H; R_2=C_2H_5, CH_3, *n*-Pr resulted in compounds with MIC of 1.6–100 μg/mL [159]. A series of 1-benzyl-3-(imidazol-1-ylmethyl)indole derivatives (**52**) showed that compound having Z=H, R_1=H, R_2=CH, and X=4-Cl to be the most potent in the series with an MIC of 1 μg/mL against *C. albicans* (CA980001). Compounds having Z=H/H/H/H/H (substitution for five compounds C1/C2/C3/C4/C5 at position Z), R_1=CH_3/H/i-propyl/H/*n*-butyl, R_2=H/H/H/H/H and X=4-Cl/4-F/4-Cl/2,4-diCl/4-Cl have MIC values of 3, 4,5,5,3.5 μg/mL respectively for the *C. albicans*. However none of these compounds are better than fluconazole (MIC = 0.02 μg/mL). Most of these compounds were less potent for *A. fumigatus* (AF980003) with the best compound (MIC = 8 μg/mL) having Z=Br, R_1=H, R_2=H, and X=2-Cl and 16 times less active than itraconazole [160]. Compounds having substituted-10-methyl-1,2,3,4-tetrahydro-pyrazino[1,2-a]indoles structure (**53**) with R=4-ClC_6H_4 was the most potent in the series having MIC values of 31.25, 15.62, and 31.25 μg/mL against *A. niger*, *A. fumigatus*, and *A. flavus*, respectively [161].

9 Quinolines

In a quinoline series (Fig. 8) compounds **54** having nitro substitutions at 5 and 7 positions of the quinoline ring and hydroxyl group at the 8 position had less antifungal activity ($MIC_{80} = 1.95$ μmol/L) compared to fluconazole ($MIC_{80} = 0.06$ μmol/L) against *C. albicans*. Two other compounds **55** and **56** had similar activity ($MIC_{80} = 1.95$ μmol/L) in *C. albicans*, the former had the quinoline ring substituted at position 8 by OH group and at position 2 by *N*-phenylethanimine moiety having 4′-OH substituent at the phenyl ring and the latter had same substitution at the quinoline ring (8-OH group) but a saturated linker with a methoxy group attached to the carbon next to the amine group with a phenyl ring having 2,5 diCl and 4-NO_2 substitution [162]. In another series of quinoline derivatives compounds having substitution at the 2-position by γ-pyridyl ring and at the C4 (R_1) and/or C8 (R_2) by methyl or isopropyl groups were found to be active. Substitutions at the same position by α-Furyl or α-thienyl group yielded inactive compounds (**57**). However some compounds having the γ-pyridyl ring were devoid of antifungal activity that indicated the importance of substituents at the C4/C8 position to be important for antifungal activity. The most active compounds in the series had C4=methyl and C8=methyl, isopropyl with an MIC value of 12.5 μg/mL [163]. The derivatives of norfloxacin (1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7(1-piperazinyl) quinoline-3-carboxylic acid) having R=3-(2,4-dichlorophenyl)propyl-2-en-1-one and 2-(2-methoxyphenoxy)ethyl-1-one (**58**) were found to inhibit the growth of *R. solani* by 83% and 94% at a concentration of 200 mg/L that is comparable to carbendazim (**59**) (100% inhibition under similar bioassay conditions) [164]. A series of 5-methyl benzothieno[3,2-b] quinolinium compounds were synthesized where two compounds having R=3-

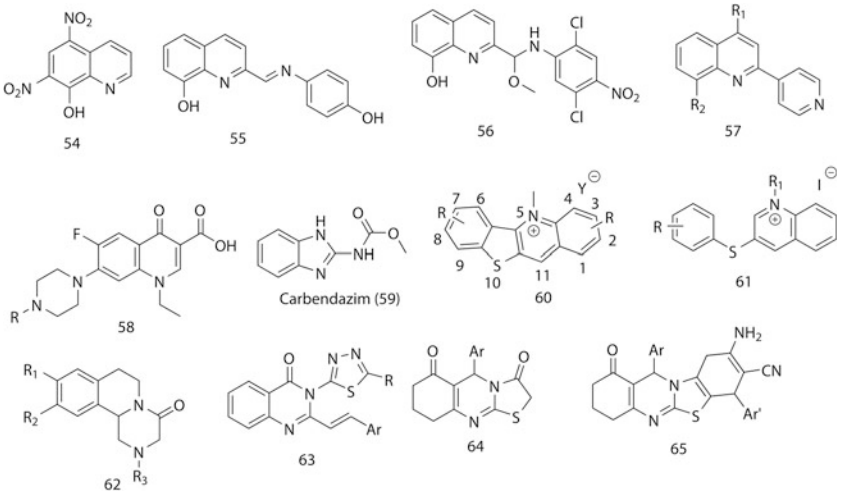

Fig. 8 Structure of quinoline, quinazoline, quinazolinone, and isoquinoline antifungals

OMe, 4-Cl and for both Y=OTf (**60**) [*C. neoformans* (IC_{50} = 6 μg/mL), *C. albicans* (IC_{50} = 1.5 μg/mL), *A. fumigatus* (IC_{50} = 0.4 μg/mL)] and R=4-Cl, Y=OTf [*C. neoformans* (IC_{50} = 4 μg/mL), *C. albicans* (not determined), *A. fumigatus* (IC_{50} = 6 μg/mL)] were observed to be active [165]. The seco analog (**61**) of the benzothienoquinoline (**60**) resulted in *N*-methyl-3-phenylthio-quinolinium salt. In this series the most active compound having R=H and R_1=5-cyclohexylpentyl group was found to be active in *C. neoformans* (IC_{50} = 0.5 μg/mL), *C. albicans* (IC_{50} = 2.7 μg/mL), *A. fumigatus* (IC_{50} = 8.6 μg/mL), *C. krusei* (IC_{50} = 0.7 μg/mL) [165]. In the isoquinoline analog hexahydro-4*H*-pyrazino[2,1-a]isoquinolin-4-one series the most potent compound had better activity than fluconazole (2–64 μg/mL) with R_1=H, R_2=F, R_3=$(CH_2)_8CH_3$ in **62** and an MIC range of 4–16 μg/mL against different fungal species such as *T. rubrum*, *C. neoformans*, *M. gypseum*, and *A. fumigatus* [166].

10 Quinazolines

In the quinazoline class (Fig. 8) the most potent compound (**63**) had R=*m*-ClC_6H_4 and Ar=*p*-$CH_3C_6H_4$ group having MIC values of 13.70, 17.07, 16.62 μg/mL against *A. nigers*, *C. albicans*, and *F. oxysporum*, respectively. At the same bioassay condition clotrimazole had slightly better activity (*A. nigers* = 12.98 μg/mL, *C. albicans* = 6.21 μg/mL, and *F. oxysporum* = 10.78 μg/mL) than the most potent compound in the series [167]. In the quinazoline class of compounds, the compound **64** (Ar=*p*-FC_6H_4) and **65** (Ar=*p*-FC_6H_4, Ar′=*p*-ClC_6H_4) showed less antifungal activity than Ticonazole (trosyd) [168].

11 Napthalenes

In a series of naphthalene derivatives (Fig. 9) compounds having R=7 or 8-NO_2 group at the naphthalene ring of **66** with X=S, Se showed better or comparable activity (MIC = 0.53–25 μg/mL) than fluconazole (MIC = 25 μg/mL) on *S. cerevisiae*. Better antifungal activity was also observed in *S. cerevisiae* (MIC = 3.12 μg/mL) when the NO_2 group was replaced by R=7-SO_2NH_2 and X=S, Se. One of the analogs of **66** having X=S and 7-SO_2NH_2 substitution was also active (MIC = 0.53 μg/mL) towards *C. neoformans* like fluconazole (MIC = 0.53 μg/mL) [169]. The butenafine derivative (**67**) with R=CH_3 (MIC = 0.125 μg/mL) had comparable activity to butenafine (MIC = 0.125 μg/mL) in *C. neoformans*. The terbinafine derivative (**68**) with R=CH_3 retained antifungal activity (MIC = 0.5 μg/mL) towards *C. neoformans* comparable to Butenafine (MIC = 0.25 μg/mL); however R=CH_2F, CHF_2, CF_3, and CN resulted in less active compounds [170].

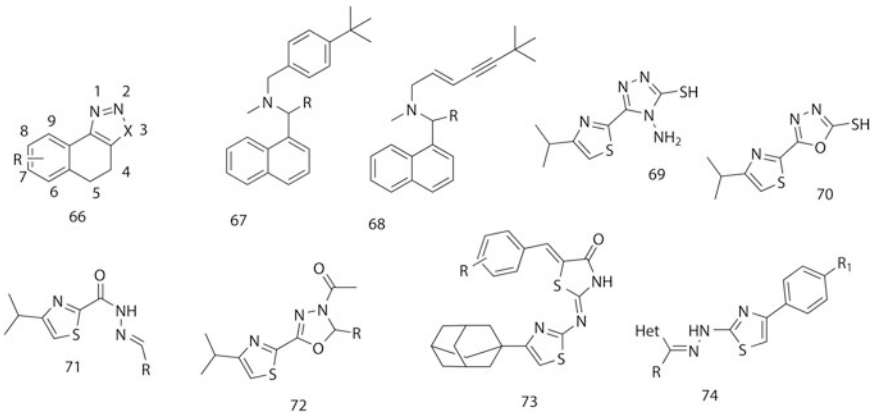

Fig. 9 Structure of naphthalene and thiazole antifungals

12 Thiazoles

In a series of thiazole derivatives (Fig. 9) compound having the structure **69** had an MIC of 8 μg/mL in *C. tropicalis* and in *A. niger*. The compound was also active in *S. cerevisiae* with MIC of 16 μg/mL. Another compound with structure (**70**) was also active in *C. tropicalis*. Compounds having the general structure of **71** with R=4-OH–C_6H_4 was active (MIC = 16 μg/mL) in *C. tropicalis* while R=2,3-di-ClC_6H_5 was active in *S. cerevisiae* with MIC of 16 μg/mL. Compounds with general structure **72** having R=C_6H_5, 3,4,5 -$(OCH_3)_3$–C_6H_2, 4-OH–C_6H_4, 2,3-diCl–C_6H_3 showed good activity with MIC ranging from 16 to 31.25 μg/mL in *S. cerevisiae*, *C. tropicalis*, and *A. niger* [171]. A series incorporating thiazole, thiazolidinone, and adamantine structures were synthesized where all the compounds were more potent than ketoconazole and bifonazole (**73**) under same biological assay condition. The various substituents at R=2-Cl, 3-Cl, 4-Cl, 2-NO_2, 3-NO_2, 4-NO_2, 4-OH, (4-OH and 3-OCH_3), (4-OH and 3,5-OCH_3) and 4-OCH_3 of **73** were having MIC in the range of 0.52–2.38 μg/mL in different fungal species (*P. funiculosum*, *P. ochrochloron*, *T. viride*, *A. fumigatus*, *A. niger*, *A. flavus*, *A. versicolor*, *F. fulvum*) [172, 173]. A series of [4-(4′-substituted-phenyl)thiazol-2-yl]hydrazine derivatives (**74**) showed better activity in *C. glabrata* and *C. albicans* with MIC values within 0.125–16 μg/mL. Under same assay conditions clotrimazole was found to have MIC values in the range of 2–8 μg/mL in both *C. glabrata* and *C. albicans* while fluconazole antifungal activity (MIC) varied from 4 to 16 μg/mL in *C. glabrata* and 4–64 μg/mL in *C. albicans*. The most active compounds for *C. albicans* (MIC = 0.125 μg/mL) had Het=Thiophen-2-yl, Pyridin-3-yl, Pyridin-4-yl, Benzodioxol-5-yl, Indol-3-yl, Coumarin-3-yl, R=H, CH_3 and R1=CH_3, OCH_3 [174].

13 Echinocandins

Echinocandins (**75**) (Fig. 10) are the most recent antifungals available for use. Echinocandins are water soluble, large hetrodimeric amphipathic polypeptides. This antifungal drug inhibit 1,3- β-D-glucansynthetase, resulting in damage of the cell wall of fungi, cell lysis, and cell death and are also called as "penicillin of antifungals" [175, 176]. Echinocandins are poorly absorbed through oral route; therefore they are administered intravenously to cure the localized and systemic fungal infections. It has a broad range of activity against all *Candida* species, also used in empirically in febrile neutropenia and stem cell transplant. At present medically used echinocandins like caspofungin, micafungin, and anidulafungin are semisynthetic derivatives with clinical use due to their solubility, antifungal spectrum, and pharmacokinetic properties [177].

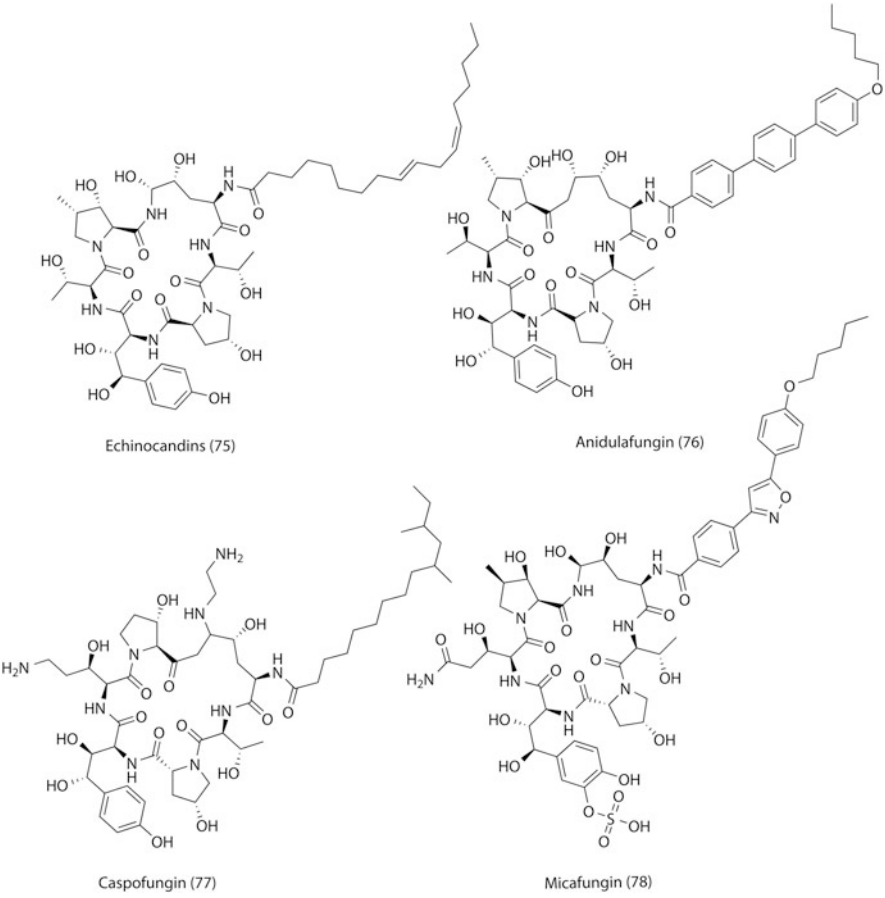

Fig. 10 Structure of echinocandin antifungals

13.1 Anidulafungin

Anidulafungin (**76**) is a semisynthetic lipopeptide antifungal approved by Food and Drug Administration. It was buildup by Eli Lily under clinical development at Vicuron Pharmaceuticals. It is the fermented product of the mold *Aspergillus nidulans*. Anidulafungin is used for the treatment of the persons who have high risk for serious fungal infections include patients with organ transplantation or hematopoietic stem cell transplantation, HIV infection/AIDS, malignancies, high-dose steroid therapy, and invasive A*spergillus* infections [178]. It inhibits β-1,3-D-glucan synthase as glucan is a major structural component of the cell wall of pathogenic fungi, resulting in cell death.

13.2 Caspofungin

Caspofungin (**77**) is a semi-synthetic water soluble lipopeptide antifungal drug which belongs to member of echinocandins. Caspofunginis is a fermented product of the fungus *Glareal-ozoyensis*. Caspofungin is administered intravenously and it inhibits the synthesis of component beta-(1,3)-D-glucan of fungal cell wall [179]. It is used for the treatment of fungal infections such as *Candida* infection (intra-abdominal abscesses, pleural cavity, perotonotis infections and esophagitis) and invasive aspergillosis [180].

13.3 Micafungin

Micafungin (**78**) is an echinocandin antifungal agent which was approved by FDA in March 2008. Micafungin is administered through intravenous route. Beta-(1,3)-D-glucanan is an essential component of fungal cell wall and the production of which is inhibited by micafungin. This drug is used in the treatment of infections caused by *Candida* sp. [181].

14 Miscellaneous

Diverse structural classes of compounds have been evaluated for antifungal activity. A series of benzoxazole derivatives (**79**) (Fig. 11) with fluorine substitution at different position of the phenyl ring were synthesized. All these compounds were synthesized as isosteric analogues of benzoheterocyclic-*N*-myristoyltransferase inhibitors. The most potent compound against *C. tropicalis* ($MIC_{80} = 0.0625$ μg/mL) had R=2-F substitution on the phenyl ring with better antifungal activity than

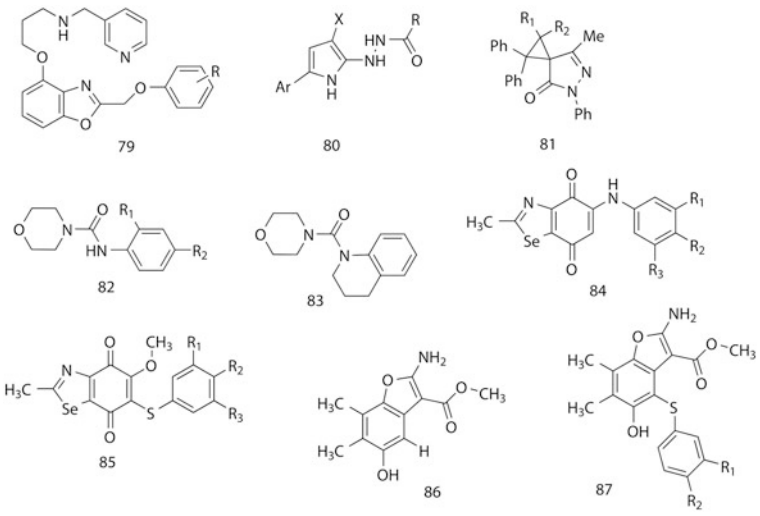

Fig. 11 Structure of different miscellaneous antifungals

fluconazole (*C. tropicalis*: $MIC_{80} = 4$ μg/mL) while another compound having R=2,3,4-trifluoro substitution in the phenyl ring had equipotent activity ($MIC_{80} = 0.25$ μg/mL) in *C. albicans*, *C. parasilosis*, and *C. tropicalis*. The compound (R=2,3,4-trifluoro substituted phenyl ring) had equivalent activity like fluconazole ($MIC_{80} = 0.25$ μg/mL) against *C. albicans* and better activity than fluconazole in *C. parasilosis* ($MIC_{80} = 4$ μg/mL) and *C. tropicalis* ($MIC_{80} = 4$ μg/mL) [182]. In a series of 2-Acylhydrazino-5-arylpyrroles (**80**) the most active compound with X=CN, Ar=4-OMePh and R=Et as substituent had an MIC of 0.39 μg/mL in *C. albicans* that is equipotent to amphotericin B (MIC = 0.39 μg/mL) and better than fluconazole (MIC = 0.78 μg/mL) under similar bioassay condition. The compound also showed good activity in other fungal species [*C. glabrata* (MIC = 0.78 μg/mL), *C. parapsilosis* (MIC = 0.78 μg/mL), *C. krusei* (MIC = 0.78 μg/mL)]. Substitution with R=iPr, 4-OMeBz when X is $-COOC_2H_5$ decreases activity drastically (MIC >100 μg/mL); however with X=CN fungal activity for R=iPr improved to great extent (MIC = 3.12 μg/mL) as observed against *C. albicans*. Hence the CN group is vital for antifungal activity [183]. A series of antifungal compounds having spiro[cyclopropane-1,4′-pyrazol-3-one] as the basic structural moiety (**81**) with R_1=H, CH_3 and R_2=CO_2Me, CO_2Et, CO_2iPr, CO_2tBt, CN, $CONEt_2$ had weak antifungal activity (MIC = 25 μg/mL) in *C. albicans* as compared to miconazole and itraconazole (MIC = 2 μg/mL) [184]. In a series of *N*-alkyl substituted urea derivatives two compounds having R1=F, R_2=H and R_1=H, R_2=F had MIC values of 3.1 and 3.5 μg/mL against *T. rubrum*as compared to ketoconazole (MIC = 3.9 μg/mL) on the same species (**82**). None of the compounds in the series are better than ketoconazole for *A. niger* (MIC = 7.8 μg/mL) except an analogue having the structure **83** had an MIC = 12.5 μg/mL [185]. A series of 5-Arylamino- and 6-arylthio-4,7-

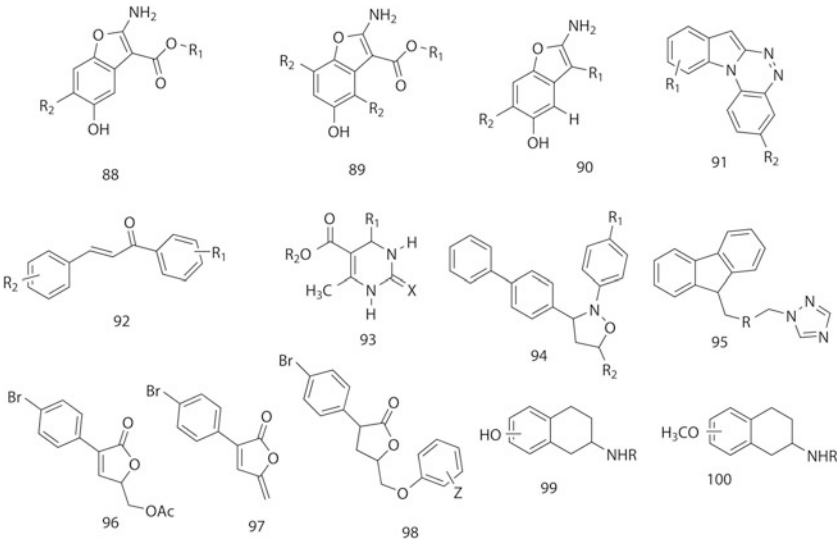

Fig. 12 Structure of different chemical class of antifungals

dioxobenzoselenazoles were synthesized where the best compound with R_1=R_3=Cl, R_2=H (**84**) had an MIC = 1.6 μg/mL better than 5-Fluorocytosine (MIC = 12.5 μg/mL) in *C. albicans*. The compound had MIC value of 3.2 μg/mL in *C. tropicalis* (5-Fluorocytosine : MIC = 12.5 μg/mL). The other active compounds in the series (**85**) had MIC values of 3.2 μg/mL (R_1=R_3=H, R_2=NO_2), 6.3 μg/mL (R_1=R_2=R_3=H), 6.3 μg/mL (R_1=R_3=H, R_2=F), 6.3 μg/mL (R_1=R_3=H, R_2=CH_3), 6.3 μg/mL (R_1=R_2=R_3=H) against *C. albicans*. The activity of other compounds in the series varied from 6.3 to 50 μg/mL in *C. tropicalis*, *C. krusei*, *A. niger*, and *A. flavus* [186]. A series of benzofuran compounds (**86–90**) (Figs. 11 and 12) with different substitutions R_1=H, CH_3, C_2H_5, CN and R_2=H, CH_3, Cl on the phenyl ring have good antifungal activity in *C. albicans*, *C. tropicalis*, *C. Krusei*, *A. niger*, *A. flavus*, and *C. neoformans* (MIC = 1.6–50-μg/mL). The two best compounds against *C. albicans* with R_1=CH_3, R_2=H (**89**) (Fig. 12) and R_1=C_2H_5, R_2=H (**89**) were equipotent (MIC = 1.6 μg/mL) and was better than 5-Fluorocytosine (MIC = 6.3 μg/mL) and fluconazole (MIC = 50 μg/mL). These two compounds were also active in *C. tropicalis* and *A. niger* with both having MIC of 3.2 μg/mL in the two fungal species [187]. In the benzotriazine series the most active compound having R_1=H, R_2=H (**91**) was more potent than hymexazol [188]. In the chalcones (**92**) compounds with R_1=H, 4-Br and R_2=H, 4-NO_2, 2-NO_2 had good antifungal activity with the potent compounds having electron withdrawing substituents at the para position of the phenyl ring [189].

In the pyrimidinone series three compounds having substitutions as R_1=C_6H_5, 4-$Me_2NC_6H_5$, 4-$Me_2NC_6H_5$ and R_2=C_2H_5 and X=S, S, O (**93**) had MIC = 0.35 μg/mL against *A. niger*. Another compound having R_1=2-HOC_6H_4, R_2=C_2H_5, X=O prevents the radical growth of *T. koningii* after 24 and 48 h completely (100%)

[190]. The isoxazolidine derivatives (**94**) having R_1=OCH_3, F and R_2=C_6H_5, $COOC_2H_5$, CH_2OH had MIC values ranging from 2.5 to 3 mM in *A. flavus* that is comparable to nystatin (3 mM) [191]. In the carbazole series introduction of azole (imidazole or 1,2,4-triazole) ring increased activity with better antifungal activity (2–4 μg/mL) for R=C_4H_8, C_2H_4 in (**95**) [192]. In the 3,5-disubstituted furanones series compounds with structure (**96**) and (**97**) had equal MIC values of 0.49 μmol/L in *C. albicans*. Other compounds in the series having Z=4-OCH_3, 4-I, 3-Br and 4-$COOCH_3$ had MIC values of 0.97, 0.48, 0.97, and 0.48 μmol/L in *C. albicans*. Modification in the phenyl ring with Z=3-COOH, 4-COOH, 4-OH (**98**) also resulted in active compounds against *C. albicans* with MIC values of 0.48, 0.97, and 0.97 μmol/L, respectively. Amphotericin B and fluconazole had MIC values of 0.03 and 1 μmol/L in the same assay system for *C. albicans* [193]. In the 2-amino tetraline series compounds with R=$(CH_2)_9CH_3$ had better antifungal activity with the two potent compounds having 5-OH and 5-OCH_3 substitutions in the phenyl ring having equal MIC values of 0.3125 μmol/L against *C. albicans* (**99**). Another compound having R=$(CH_2)_8CH_3$ and 6-OCH_3 was active (MIC = 0.0625 μmol/L) in *C. albicans* strain resistant to fluconazole (MIC >64 μmol/L) (**100**) [194].

15 Cationic Peptides

The cationic peptides are small cationic and amphipathic molecules isolated from plants, mammals, and microorganisms with antifungal activity with great potential for development as new therapeutic agents [195]. Cecropins isolated from the hemolymph of the giant silk moth (*Hyalophora cecropia*) is constituted by 35–37 residues with a strongly basic N-terminal linked to a neutral C-terminal by a flexible glycine–proline link. Both *Hyalophora* and *Drosophila* Cecropin (Cecropin A and B) inhibited growth of *S. cerevisiae*, *D. uninucleata*, *G. candidum*, and *M. anisopliae* in MICs ranging from 0.4 to 4 mM [196]. The LD_{50} value of Cecropin was also evaluated on germinating and non-germinating *A. flavus*, *A. fumigatus*, *A. niger*, *F. moniliforme*, and *F. oxysporum*. Cecropin B had LD_{50} values of 3.0, 0.5, 2, 0.2, and 1 μM in *A. flavus*, *A. fumigatus*, *A. niger*, *F. moniliforme*, and *F. oxysporum* respectively while for non-germinating *F. Moniliforme* and *F. oxysporum* the LD_{50} value was 0.2 μM for both species. Dermaseptin peptides found in skin secretions of Phyllomedusinae frogs reported in the same study had LD_{50} values of 4, 0.05, 2, 0.3, and 0.8 μM in *A. flavus*, *A. fumigatus*, *A. niger*, *F. moniliforme*, and *F. oxysporum* [197]. Indolicin, the shortest linearly occurring peptide consisting of 39% tryptophan and 23% proline (ILPWKWPWWPWRR), is found in the cytoplasmic granules of bovine neutrophil. Indolicin disrupt the structure of cell membranes as examined on interaction with *T. beigelii* [198]. Histatins are histidine rich peptides isolated from human saliva and had strong antifungal activity in different *Candida* spp. (C. *albicans*, *C. glabrata*, *C. guillermondii*, *C. krusei*, *C. lambica*, *C. parapsilosis*, *C. pseudo -tropicalis*, *C. stellatoidea*, and *C. tropicalis*) with histatin 5 showing the strongest

fungicidal activity against *C. albicans* (MIC = 100 μM) [199]. Magainins from *Xenopuslaevis* (the African frog) had antifungal activity against *Candida* spp., *C. neoformans*, and *Saccharomyces cerevisiae*. Magainin 2 acts as an antifungal against *C. neoformans* (MIC = 6.25 μg/mL), *C. glabrata* (MIC = 25.0 μg/ml), *C. tropicalis* (MIC, 12.5 μg/mL), and *C. krusei* (MIC = 12.5–25.0 μg/mL) with relatively low activity against *C. albicans* (MIC > 80 μg/mL) [200, 201]. Bombinin-H isolated from skin of *Bombina* genus are glycine rich peptides active against fungi, especially bombinin-like peptides-1 in *C. albicans* (MIC = 3–0.4-μM). Bombinins H2 and H4 also have antifungal activity against *C. albicans*, *C. guillermondii*, and *C. tropicalis*. Bombinin H2 had MIC values of 3.1, 1.3, 1.1 μM in C. *albicans*, *C. guillermondii*, and *C. tropicalis* respectively while Bombinin H4 had MIC values of 1.6, 0.7, and 0.6 μM for the above species [202, 203]. The antifungal activities of the amphibian cationic peptides have been reported elsewhere [204]. The cationic peptides bind to cholesterol and ergosterolin fungal cell membranes leading to fungal lysis [205]. Dolastatin 10, a synthetic cationic peptide, targeted at intracellular tubulin and inhibits microtubule assembly and tubulin-dependent GTP binding and have effective fungicidal activity against *C. neoformans* [206].

16 Monoclonal Antibodies

Since the fungi are eukaryotic organisms, a character shared with the host, it is difficult to develop a safe drug like antibacterials which are directed against prokaryotic organisms. In view of this an approach directed towards monoclonal antibodies against at least most common fungal pathogens like *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* is desirable. Identification and characterization of the proteins that are immunologically dominant and exhibit strong immune responses during mycoses could have vital repercussions for evolving new diagnostic, prophylactic, and therapeutic techniques for mycoses. Therefore efforts focused on the discovery of useful inhibitors of fungal specific, chitin, cell wall glucan and mannoprotein biosynthesis may play a very important role. In the absence of a safe and wide spectrum antimycotic agent, efforts may be directed for the development of monoclonal antibodies (MAbs). The MAbs have improved the specificity of immune procedures and have served as useful research methods and tools such as isolation, purification, and characterization of microbial antigens and development of assays methods for antibody and antigen detection [207–209]. In market there are many monoclonal antibodies available against a number of challenging diseases like cancer and many more diseases (Table 1). The antibodies are either developed in mouse which may be humanized, chimeric or in humans. The MAbs often exhibit adverse reactions like HAMA which is common for MAbs developed in mouse.

To overcome these types of side effects, an approach leading to the identification of active peptide sequences from the hypervariable regions of the hybridoma clone

Table 1 List of monoclonal antibodies approved for therapy

Antibody	Brand name	Approval date	Type	Indication
MuromonabCD3 [210]	Orthoclone OKT3	1986	Murine	Transplant rejection
Abciximab [211]	Reopro	1994	Chimeric	Cardiovascular disease
Daclizumab [212]	Zenapax	1997	Humanized	Transplant rejection
Rituximab [213]	Rituxan, Mabthera	1997	Chimeric	Non-Hodgkin lymphoma
Trastuzumab [214]	Herceptin	1998	Humanized	Brest cancer
Palivizumab [215]	Synagis	1998	Humanized	Respiratory syncytical virus
Infliximab [216]	Remicade	1998	Chimeric	Several autoimmune disorders
Basiliximab [217]	Simulect	1998	Chimeric	Transplant rejection
Gemtuzumab [218]	Mylotarg	2000	Humanized	Acute myelogenous leukemia
Alemtuzumab [219]	Campath	2001	Humanized	Chronic lymphocytic leukemia
Efalizumab [220]	Raptiva	2002	Humanized	Psoriasis
Adalimumab [221]	Humira	2002	Human	Several autoimmune disorders
Ibritumomab tiuxetan [222]	Zevalin	2002	Murine	Non-Hodgkin lymphoma (with yttrium-90 or indium-111)
Bevacizumab [223]	Avastin	2004	Humanized	Colorectal cancer, age-related macular degeneration
Cetuximab [224]	Erbitux	2004	Chimeric	Colorectal cancer, head and neck cancer
Omalizumab [225]	Xolair	2004	Humanized	Mainly allergy-related asthma
Natalizumab [226]	Tysabri	2006	Humanized	Multiple sclerosis and Crohn's disease
Panitumumab [227]	Vectibex	2006	Human	Colorectal cancer
Ranibizumab [228]	Lucentis	2006	Humanized	Macular degeneration
Eculizumab [229]	Soliris	2007	Humanized	Paroxysmal nocturnal hemoglobinuria
Certolizumab [230]	Cimzia	2008	Humanized	Crohn's disease
Ustekinumab [231]	Stelara	2009	Human	Psoriasis
Golimumab [232]	Simponi	2009	Human	Rheumatoid and psoriatic arthritis, ankylosing spondylitis
Canakinumab [233]	Ilaris	2009	Human	Muckle–Wells syndrome

(continued)

Table 1 (continued)

Antibody	Brand name	Approval date	Type	Indication
Ofatumumab [234]	Arzerra	2009	Human	Chronic lymphocytic leukemia
Tocilizumab [235]	RoActemra, Actemra	2010	Humanized	Rheumatoid arthritis
Denosumab [236]	Prolia	2010	Human	Bone loss
Ipilimumab [237]	Yervoy	2011	Human	Metastatic melanoma
Belimumab [238]	Benlysta	2011	Human	Systemic lupus erythematosus
Brentuximab				
Vedotin [239]	Adcetris	2011	Chimeric	Hodgkin lymphoma, systemic anaplastic large cell lymphoma
Pertuzumab [240]	Perjeta	2012	Humanized	Breast cancer
Adotrastuzumab Emtansine [241]	Kadcyla	2013	Humanized	Breast cancer
Obinutuzumab [242]	Gazyva	2013	Humanized	Chronic lymphocytic leukemia
Siltuximab [243]	Sylvant	2014	Chimeric	Castleman disease
Vedolizumab [244]	Entyvio	2014	Humanized	Ulcerative colitis, Crohn's disease
Ramucirumab [245]	Cyramza	2014	Human	Gastric cancer
Secukinumab [246]	Cosentyx	2015	Human	Psoriasis

may be helpful. This way a library of peptide sequences may be synthesized and evaluated for antifungal activity which may have specific activity against fungi. The peptide sequences thus generated may not only have specific antifungal activity but may also result in specific diagnostic tolls.

17 Conclusions

Fungal diseases are global health problem with rising prevalence of infections in immunocompromised hosts related to cases of cancer, AIDS, diabetes, cystic fibrosis and in invasive surgical procedures. The three major fungal diseases in immunocompromised subjects are candidosis, aspergillosis, and cryptococcosis. Azoles, the most common clinically antifungals among the other candidates (polyenes, pyrimidines, allylamines, and echinocandins), suffer from developing

resistance with drug–drug interactions and drug toxicity. This chapter presented the most common antifungals used for human health and also a brief update about the latest developments in antifungal agents.

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References

1. Hawksworth DL (2004) Fungal diversity and its implications for genetic resource collections. Stud Mycol 50:9–18
2. Carris LM, Little CR, Stiles CM (2012) Introduction to fungi. Plant Health Instructor. doi:10.1094/PHI-I-2012-0426-01
3. Martin DS, Jones CP (1940) Further studies on the practical classification of the Monilias. J Bacteriol 39(5):609–630
4. Sobel JD, Vazquez J (1990) Candidemia and systemic candidiasis. Semin Respir Infect 5: 123–137
5. Rippon JW (1982) Medical mycology: the pathogenic fungi and the pathogenic actinomycetes. Saunders, Philadelphia
6. Stein DK, Sugar AM (1989) Fungal infections in the immunocompromised host. Diagn Microbiol Infect Dis 12:221S–228S
7. Larriba G, Rubio Coque JJ, Ciudad A, Andaluz E (2000) Candida albicans molecular biology reaches its maturity. Int Microbiol 3:247–252
8. Carrillo-Munoz AJ, Giusiano G, Ezkurra PA, Quindos G (2006) Antifungal agents: mode of action in yeast cells. Rev Esp Quimioter 19:130–139
9. Andriole VT (1999) Current and future antifungal therapy: new targets for antifungal agents. J Antimicrob Chemother 44:151–162
10. Ahmad S, Khan Z, Mustafa AS, Khan ZU (2002) Seminested PCR for diagnosis of candidemia: comparison with culture, antigen detection, and biochemical methods for species identification. J Clin Microbiol 40:2483–2489
11. Fujita S, Lasker BA, Lott TJ, Reiss E, Morrison CJ (1995) Microtitration plate enzyme immunoassay to detect PCR amplified DNA from *Candida* species in blood. J Clin Microbiol 33:962–967
12. Iwastu TM, Miyaji M, Taguchi H, Okamoto S (1982) Evaluation of skin test for chromoblastomycosis using antigen prepared from cultural filtrates of *Fonsecaea pedrosoi*, *Phlalophora verrucosa*, *Wangiella dermatitidis* and *Exophiala jeanselmei*. Mycopathologia 77:59–64
13. Wu Z, Tsumura Y, Blomquist G, Wang X (2003) 18S rRNA gene variation among common airborne fungi, and development of specific oligonucleotide probes for the detection of fungal isolate. Appl Environ Microbiol 69:5389–5397
14. Ferrer C, Colom F, Frases S, Mulet E, Abad JL, Alio JL (2001) Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8S ribosomal DNA typing in ocular infections. J Clin Microbiol 39:2873–2879
15. Ferrer C, Munoz G, Alio JL, Colom F (2002) Polymerase chain reaction diagnosis in fungal keratitis caused by *Alternaria alternata*. Am J Ophthalmol 133:398–399
16. Holmberd K, Feroze F (1996) Evaluation of an optimized system for random amplified polymorphic DNA (RAPD)-analysis for genotypic mapping of *Candida albicans* strains. J Clin Lab Anal 10:59–69
17. Hui M, Ip M, Chan PK, Chin ML, Cheng AF (2000) Rapid identification of medically important *Candida* to species level by polymerase chain reaction and single-strand conformational polymorphism. Diagn Microbiol Infect Dis 38:95–99

18. Humphreis SE, Gudnason V, Whittall R, Day INM (1997) Single stranded conformation polymorphism analysis with high throughput modifications and its use in mutation detection in familial hypercholesterolemia. Clin Chem 43:427–435
19. Iwen PC, Hinrichs SH, Rupp ME (2002) Utilization of the internal transcribed spacer region as molecular targets to detect and identify human fungal pathogens. Med Mycol 40:87–109
20. White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322
21. Gillman LM, Gunton J, Turenne CY, Wolfe J, Kabani AM (2001) Identification of *Mycobacterium* species by multiple-fluorescence PCR-single-strand conformation polymorphism analysis of the 16S rRNA gene. J Clin Microbiol 39:3085–3091
22. Kumeda Y, Asao T (1996) Single-strand conformation polymorphism analysis of PCR-amplified ribosomal DNA internal transcribed spacers to differentiate species of *Aspergillus* section Flavi. Appl Environ Microbiol 62:2947–2952
23. Mora D, Ricci G, Gugliemetti S, Daffonchio D, Fortina MG (2003) 16S-23S rRNA intergenic spacer region sequence variation in *Streptococcus thermophilus* and related dairy streptococci and development of a multiplex ITS-SSSP analysis for their identification. Microbiology 149:807–813
24. Rath PM, Ansorg R (2000) Identification of medically important *Aspergillus* species by single stranded conformational polymorphism (SSCP) of the PCR-amplified intergenic spacer region. Mycoses 43:381–386
25. Pfaller MA, Messer SA, Boyken L, Tendolkar S, Hollis RJ, Diekema DJ (2004) Geographic variation in the susceptibilities of invasive isolates of *Candida glabrata* to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002. J Clin Microbiol 42:3142–3146
26. Hazen EL (1960) Nystatin. Ann N Y Acad Sci 89:258–266
27. Mayers DL (2009) Antimicrobial drug resistance: mechanism of drug resistance vol. 1. Humana Press/Springer, Totowa/New York, p 299
28. Hazen EL, Brown R (1950) Two antifungal agents produced by a soil actinomycete. Science 112:423
29. Hazen EL, Brown R (1951) Fungicidin, an antibiotic produced by a soil actinomycete. Proc Soc Exp Biol Med 76:93
30. Harris EJ, Pritzker HG, Laski B, Eisen A, Steiner JW, Shack L (1958) The effect of nystatin (mycostatin) on neonatal candidiasis (thrush)- a method of eradicating thrush from hospital nurseries. Can Med Assoc J 79(11):891–896
31. Sklenář Z, Ščigel V, Horáčkova K, Slanař O (2013) Compounded preparations with nystatin for oral and oromucosal administration. Acta Pol Pharm Drug Res 70:759–762
32. Lencelin JM et al (1988) Tetrahedron Lett 29:2827
33. Pandey RC, Rinehart KL (1976) J Antibiot 29:1035
34. Groll AH, Gonzalez CE, Giri N et al (1999) Liposomal nystatin against experimental pulmonary aspergillosis in persistently neutropenic rabbits: efficacy, safety and non-compartmental pharmacokinetics. J Antimicrob Chemother 44(3):397–401
35. Wallace TL, Paetznick V, Cossum PA, Lopez-Berestein G, Rex JH, Anaissie E (1997) Activity of liposomal nystatin against disseminated *Aspergillus fumigatus* infection in neutropenic mice. Antimicrob Agents Chemother 41(10):2238–2243
36. Farid MA, El-Enshasy HA, El-Diwany AI, El-Sayed ESA (2000) Optimization of the cultivation medium for natamycin production by *Streptomyces natalensis*. J Basic Microbiol 40(3):157–166
37. Lalitha P, Kumar VR, Prajna NV, Fothergill AW (2008) *In vitro* natamycin susceptibility of ocular isolates of *Fusarium* and *Aspergillus* species: comparison of commercially formulated natamycin eye drops to pharmaceutical-grade powder. J Clin Microbiol 46(10):3477–3478
38. Vandeputte P, Ferrari S, Coste AT (2012) Antifungal resistance and new strategies to control fungal infections. Int J Microbiol 2012:1–27. doi:10.1155/2012/713687

39. Caffrey P, Lynch S, Flood E, Finnan S, Oliynyk M (2001) Amphotericin biosynthesis in *Streptomyces nodosus*: deductions from analysis of polyketide synthase and late genes. Chem Biol 8(7):713–723
40. Matsumori N, Sawada Y, Murata M (2005) Mycosamine orientation of amphotericin B controlling interaction with ergosterol: sterol-dependent activity of conformation-restricted derivatives with an amino-carbonyl bridge. J Am Chem Soc 127:10667–10675
41. Barratt G, Bretagne S (2007) Optimizing efficacy of amphotericin B through nano-modification. Int J Nanomedicine 2:301–313
42. Ogita A, Fujita KI, Tanaka T (2012) Enhancing effects on vacuole-targeting fungicidal activity of amphotericin B. Front Microbiol 3:100
43. Gallis H, Drew RH, Pickard WW (1990) Amphotericin B: 30 years of clinical experience. Rev Infect Dis 12(2):308–329
44. Laniado-Laborín R, Cabrales-Vargas MN (2009) Amphotericin B: side effects and toxicity. Rev Iberoam Micol 26(4):223–227
45. Czub J, Baginski M (2006) Modulation of amphotericin B membrane interaction by cholesterol and ergosterol--a molecular dynamics study. J Phys Chem B 110(33):16743–16753
46. Palacios DS, Dailey I, Siebert DM, Wilcock BC, Burke MD (2011) Synthesis-enabled functional group deletions reveal key underpinnings of amphotericin B ion channel and antifungal activities. Proc Natl Acad Sci U S A 108(17):6733–6738
47. Gray KC, Palacios DS, Dailey I et al (2012) Amphotericin primarily kills yeast by simply binding ergosterol. Proc Natl Acad Sci U S A 109(7):2234–2239
48. Wilcock BC, Endo MM, Uno BE, Burke MD (2013) C2-OH of amphotericin B plays an important role in binding the primary sterol of human cells but not yeast cells. J Am Chem Soc 135(23):8488–8491
49. Anderson TM, Clay MC, Cioffi AG et al (2014) Amphotericin forms an extramembranous and fungicidal sterol sponge. Nat Chem Biol 10(5):400–406
50. Messer SA, Jones RN, Fritsche TR (2006) International surveillance of *Candida* spp. and *Aspergillus* spp.: report from the SENTRY Antimicrobial Surveillance Program (2003). J Clin Microbiol 44:1782–1787
51. Sokol-Anderson ML, Brajtburg J, Medoff G (1986) Amphotericin B-induced oxidative damage and killing of *Candida albicans*. J Infect Dis 154:76–83
52. Maertens JA (2004) History of the development of azole derivatives. Clin Microbiol Infect 10(Suppl 1):1–10
53. Odds FC, Brown AJ, Gow NA (2003) Antifungal agents: mechanisms of action. Trends Microbiol 11:272–279
54. Fromtling RA (1988) Overview of medically important antifungal azole derivatives. Clin Microbiol Rev 1:187–217
55. Sheehan DJ, Hitchcock CA, Sibley CM (1999) Current and emerging azole antifungal agents. Clin Microbiol Rev 12:40–79
56. Elkasabgy NA (2014) Ocular supersaturated self-nanoemulsifying drug delivery systems (S-SNEDDS) to enhance econazole nitrate bioavailability. Int J Pharm 460:33–44
57. Thienpont D, Van Cutsem J, Van Nueten JM, Niemegeers CJ, Marsboom R (1975) Bilogical and toxicological properties of econazole, a broad-spectrum antimycotic. Arzneimittelforschung 25:224–230
58. Heel RC, Brogden RN, Speight TM, Avery GS (1978) Econazole: a review of its antifungal activity and therapeutic efficacy. Drugs 16(3):177–201
59. Waitz JA, Moss EL, Weinstein MJ (1971) Chemotherapeutic evaluation of clotrimazole (Bay b 5097, 1 (o-chloro- - -diphenylbenzyl) imidazole). Appl Microbiol 22:891–898
60. World Health Organization (2013) WHO model list of essential medicines. World Health Organization. October 2013. Edition 18. http://www.who.int/medicines/publications/essentialmedicines/en/index.html. Retrieved 22 Apr 2014
61. Haller I (1985) Mode of action of clotrimazole: implications for therapy. Am J Obstet Gynecol 152(7 Pt 2):939–944

62. Rai VK, Dwivedi H, Yadav NP, Chanotiya CS, Saraf SA (2014) Solubility enhancement of miconazole nitrate: binary and ternary mixture approach. Drug Dev Ind Pharm 40:363–9045
63. Morita T, Nozawa Y (1985) Effects of antifungal agents on ergosterol biosynthesis in *Candida albicans* and *Trichophyton mentagrophytes*: differential inhibitory sites of naphthiomate and miconazole. J Invest Dermatol 85:434–437
64. Puolakka J, Tuimala R (1983) Comparison between oral ketoconazole and topical miconazole in the treatment of vaginal candidiasis. Acta Obstet Gynecol Scand 62:575–577
65. Rollman O (1982) Treatment of onychomycosis by partial nail avulsion and topical miconazole. Dermatologica 165:54–61
66. Brugmans JB, Van Cutsem JM, Thienpont DC (1970) Treatment of long-term tinea pedis with miconazole. Arch Dermatol 102:428–432
67. Van Cutsem J, Reyntjens A (1978) Miconazole treatment of pityriasis versicolor a review. Mykosen 21(3):87–91
68. Sung JP, Grendahl JG, Levine HB (1977) Intravenous and intrathecal miconazole therapy for systemic mycoses. West J Med 126:5–13
69. Balata G, Mahdi M, Bakera RA (2010) Improvement of solubility and dissolution properties of ketoconazole by solid dispersions and inclusion complexes. Asian J Pharm Sci 5:1–12
70. Rotstein DM, Kertesz DJ, Walker KAM et al (1992) J Med Chem 35:2818
71. Hume AL, Kerkering TM (1983) Ketoconazole. Drug Intell Clin Pharm 17:169–174
72. Terrell CL (1999) Antifungal agents. Part II. The azoles. Mayo Clin Proc 74:78–100
73. Gary G (2013) Optimizing treatment approaches in seborrheic dermatitis. J Clin Aesthet Dermatol 6:44–49
74. Venkateswarlu K, Kelly SL (1996) Biochemical characterisation of ketoconazole inhibitory action on *Aspergillus fumigatus*. FEMS Immunol Med Microbiol 16:11–20
75. Wood A (1994) Oral azole drugs as systemic antifungal therapy. N Engl J Med 330:263–272
76. Perfect JR, Durack DT (1985) Penetration of imidazoles and triazoles into cerebrospinal fluid of rabbits. J Antimicrob Chemother 16:81–86
77. Van Tyle JH (1984) Ketoconazole. Mechanism of action, spectrum of activity, pharmacokinetics, drug interactions, adverse reactions and therapeutic use. Pharmacotherapy 4:343–373
78. Akins RA (2005) An update on antifungal targets and mechanisms of resistance in *Candida albicans*. Med Mycol 43:285–318
79. Sanglard D, Odds FC (2002) Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect Dis 2:73–85
80. Albertson GD, Niimi M, Cannon RD, Jenkinson HF (1996) Multiple efflux mechanisms are involved in *Candida albicans* fluconazole resistance. Antimicrob Agents Chemother 40: 2835–2841
81. Orozco AS, Higginbotham LM, Hitchcock CA, Parkinson T, Falconer D, Ibrahim AS, Ghannoum MA, Filler SG (1998) Mechanism of fluconazole resistance in *Candida krusei*. Antimicrob Agents Chemother 42:2645–2649
82. Kelly SL, Lamb DC, Kelly DE, Manning NJ, Loeffler J, Hebart H, Schumacher U, Einsele H (1997) Resistance to fluconazole and cross-resistance to amphotericin B in *Candida albicans* from AIDS patients caused by defective sterol delta 5,6-desaturation. FEBS Lett 400:80–82
83. Bossche HV, Marichal P, Odds FC (1994) Molecular mechanisms of drug resistance in fungi. Trends Microbiol 2:393–400
84. Romani L (2004) Immunity to fungal infections. Nat Rev Immunol 4:1–23
85. Stiller RL, Bennett JE, Scholer HJ, Wall M, Polak A, Stevens DA (1982) Susceptibility to 5-fluorocytosine and prevalence of serotype in 402 *Candida albicans* isolates from the United States. Antimicrob Agents Chemother 22:482–487
86. Zervos M, Meunier F (1993) Fluconazole (diflucan): a review. Int J Antimicrob Agents 3: 147–170
87. Philpott-Howard JN, Wade JJ, Mufti GJ, Brammer KW, Ehninger G (1993) Randomized comparison of oral fluconazole versus oral polyenes for the prevention of fungal infection in

patients at risk of neutropenia. Multicentre Study Group. J Antimicrob Chemother 31:973–984
88. Martin MV (1999) The use of fluconazole and itraconazole in the treatment of *Candida albicans* infections: a review. J Antimicrob Chemother 44:429–437
89. Willems L, Geest VD, De Beule K (2001) Itraconazole oral solution and intravenous formulations: a review of pharmacokinetics and pharmacodynamics. J Clin Pharm Ther 26: 159–169
90. Jaruratanasirikul S, Kleepkaew A (1997) Influence of an acidic beverage (Coca-Cola) on the absorption of itraconazole. Eur J Clin Pharmacol 66:235–237
91. Odds FC, Oris M, Dorsselaer PV, Gerven FV (2000) Activities of an intravenous formulation of itraconazole in experimental disseminated *Aspergillus, Candida*, and *Cryptococcus* infections. Antimicrob Agents Chemother 44:3180–3183
92. Kauffman CA (1996) Role of azoles in antifungal therapy. Clin Infect Dis 22(2):S148–S153
93. Aftab BT, Dobromilskaya I, Liu JO, Rudin CM (2011) Itraconazole inhibits angiogenesis and tumor growth in non-small cell lung cancer. Cancer Res 71:6764–6772
94. Saravolatz LD, Johnson LB, Kauffman CA (2003) Voriconazole: a new triazole antifungal agent. Clin Infect Dis 36:630–637
95. Van Duin D, Cleare W, Zaragoza O, Nosanchuk JD, Casadevall A (2014) Effects of voriconazole on *Cryptococcus neoformans*. Antimicrob Agents Chemother 48:2014–2020
96. Rafael Z, Javier P (2008) Adv Sepsis 6:90
97. Ghannoum MA, Kuhn DM (2002) Eur J Med Res 7:242
98. Denning DW, Ribaud P, Milpied H, Raoul N, Eckhard T, Andrea H (2002) Clin Infect Dis 34:563
99. Pascual A, Calandra T, Bolay S et al (2008) Clin Infect Dis 46:201
100. Lewis RE (2008) Clin Infect Dis 46:212
101. Zonios DL, Gea-Banacloche J, Childs R (2008) Clin Infect Dis 47:e7–e10
102. Pasqualotto AC, Xavier MO, Andreolla HF, Linden R (2010) Voriconazole therapeutic drug monitoring: focus on safety. Expert Opin Drug Saf 9:125–137
103. Kauffman CA, Malani AN, Easley C, Kirkpatrick P (2007) Posaconazole. Nat Rev Drug Discov 6(3):183–184
104. Ullmann AJ, Lipton JH, Vesole DH (2007) N Engl J Med 356:335
105. Keating GM (2005) Drugs 65:1553
106. Torres HA, Hachem RY, Chemaly RF, Kantoyiannis DP, Raad I (2005) Lancet Infect Dis 5:775
107. Yamazumi T, Pfaller MA, Messer SA (2000) Antimicrob Agents Chemother 44:6
108. Mikamo H, Yin XH, Hayasaki Y et al (2002) Penetration of ravuconazole, a new triazole antifungal, into rat tissues. Chemotherapy 48:7–9
109. Pfaller MA, Messer SA, Hollis RJ (2002) Antimicrob Agents Chemother 46:1723
110. Pasqualotto AC, Denning DW (2008) New and emerging treatments for fungal infections. J Antimicrob Chemother 61(Suppl 1):19–30. doi:10.1093/jac/dkm428
111. Marino MR, Mummanei V, Norton J, et al (2001) Ravuconazole exposure-response relationship in HIV-patients with oropharyngeal candidiasis. In: Abstracts of the forty-first interscience conference on Antimicrobial Agents and Chemotherapy, Chicago. American Society for Microbiology, Washington, DC. Abstract J-1622
112. Giovanna Setzu M, Stefancich G, La Colla P, Castellano S (2002) Synthesis and antifungal properties of N-[(1,1′-biphenyl)-4-ylmethyl]-1H-imidazol-1-amine derivatives. Farmaco 57: 1015–1018
113. Günay NS, Çapan G, Ulusoy N, Ergenç N, Ötük G, Kaya D (1999) 5-Nitroimidazole derivatives as possible antibacterial and antifungal agents. Farmaco 54:826–831
114. Olender D, Żwawiak J, Lukianchuk V, Lesyk R, Kropacz A, Fojutowski A, Zaprutko L (2009) Synthesis of some N-substituted nitroimidazole derivatives as potential antioxidant and antifungal agents. Eur J Med Chem 44:645–652

115. Rossello A, Bertini S, Lapucci A, Macchia M, Martinelli A, Rapposelli S, Herreros E, Macchia B (2002) Synthesis, antifungal activity, and molecular modeling studies of new inverted oxime ethers of oxiconazole. J Med Chem 45:4903–4912
116. Di Santo R, Tafi A, Costi R, Botta M, Artico M, Corelli F, Forte M, Caporuscio F, Angiolella L, Palamara AT (2005) Antifungal agents. 11. N-substituted derivatives of 1-[(aryl)(4-aryl-1H-pyrrol-3-yl)methyl]-1H-imidazole: synthesis, anti-Candida activity, and QSAR studies. J Med Chem 48:5140–5153
117. Lorus Therapeutic, Inc. (2011) 2,4,5-trisubstituted imidazoles and their use as anti-microbial agents. US7884120
118. Lorus Therapeutic, Inc. (2013) 2,4,5-trisubstituted imidazoles and their use as anti-microbial agents. US8394815
119. Pore VS, Aher NG, Kumar M, Shukla PK (2006) Design and synthesis of fluconazole/bile acid conjugate using click reaction. Tetrahedron 62:11178–11186
120. Zhao QJ, Song Y, Hu HG, Yu SC, Wu QY (2007) Design, synthesis and antifungal activity of novel triazole derivatives. Chin Chem Lett 18:670–672
121. Lebouvier N, Pagniez F, Duflos M, Le Pape P, Na YM, Le Baut G, Le Borgne M (2007) Synthesis and antifungal activities of new fluconazole analogues with azaheterocycle moiety. Bioorg Med Chem Lett 17:3686–3689
122. Uchida T, Somada A, Kagoshima Y, Konosu T, Oida S (2008) Carbon analogs of antifungal dioxane-triazole derivatives: synthesis and in vitro activities. Bioorg Med Chem Lett 18: 6538–6541
123. Guillon R, Giraud F, Logé C, Le Borgne M, Picot C, Pagniez F, Le Pape P (2009) Design of new antifungal agents: synthesis and evaluation of 1-[(1H-indol-5-ylmethyl)amino]-2-phenyl-3-(1H-1,2,4-triazol-1-yl)propan-2-ols. Bioorg Med Chem Lett 19:5833–5836
124. Dan ZG, Zhang J, Yu SC, Hu HG, Chai XY, Sun QY, Wu QY (2009) Design and synthesis of novel triazole antifungal derivatives based on the active site of fungal lanosterol 14a-demethylase (CYP51). Chin Chem Lett 20:935–938
125. Borate HB, Maujan SR, Sawargave SP, Chandavarkar MA, Vaiude SR, Joshi VA, Wakharkar RD, Iyer R, Kelkar RG, Chavan SP, Kunte SS (2010) Fluconazole analogues containing 2H-1,4-benzothiazin-3(4H)-one or 2H-1,4-benzoxazin-3(4H)-one moieties, a novel class of anti-Candida agents. Bioorg Med Chem Lett 20:722–725
126. He QQ, Liu CM, Li K, Cao YB (2007) Design, synthesis of novel antifungal triazole derivatives with high activities against *Aspergillus fumigatus*. Chin Chem Lett 18:421–423
127. He QQ, Li K, Cao YB, Dong HW, Zhao LH, Liu CM, Sheng CQ (2007) Design, synthesis and molecular docking studies of novel triazole antifungal compounds. Chin Chem Lett 18: 663–666
128. Nam N-H, Sardari S, Selecky M, Parang K (2004) Carboxylic acid and phosphate ester derivatives of fluconazole: synthesis and antifungal activities. Bioorg Med Chem 12: 6255–6269
129. Upadhayaya RS, Jain S, Sinha N, Kishore N, Chandra R, Arora SK (2004) Synthesis of novel substituted tetrazoles having antifungal activity. Eur J Med Chem 39:579–592
130. Wei JJ, Jin L, Wan K, Zhou CH (2011) Synthesis of novel D-glucose-derived benzyl and alkyl 1,2,3-triazoles as potential antifungal and antibacterial agents. Bull Korean Chem Soc 32:229–238
131. Che X, Sheng C, Wang W, Cao Y, Xu Y, Ji H, Dong G, Miao Z, Yao J, Zhang W (2009) New azoles with potent antifungal activity: design, synthesis and molecular docking. Eur J Med Chem 44:4218–4226
132. Daewoong Pharmaceutical Co. (2011) Antifungal triazole derivatives. US7968579
133. Daewoong Pharmaceutical Co. (2011) Antifungal triazole derivatives, method for the preparation thereof and pharmaceutical composition containing same. US8063229
134. Council of Scientific & Industrial Research and FDC Ltd. (2012) Antifungal compounds containing benzothiazinone, benzoxazinone, or benzoxazolinone and process thereof. US8129369

135. Loyse A, Dromer F, Day J, Lortholary O, Harrison TS (2013) Flucytosine and cryptococcosis: time to urgently address the world wide accessibility of a 50-year-old antifungal. J Antimicrob Chemother 68:2435–2444
136. Perumalla S, Pedireddi V, Sun C (2013) Design, synthesis, and characterization of new 5-flucytosine salts. Mol Pharm 10:2462–2466
137. Defever KS, Whelan WL, Rogers AL, Beneke ES, Veselenak JM, Soll DR (1982) *Candida albicans* resistance to 5-fluorocytosine: frequency of partially resistant strains among clinical isolates. Antimicrob Agents Chemother 22:810–815
138. Hector RF, Domer JE, Carrow EW (1982) Immune responses to *Candida albicans* in genetically distinct mice. Infect Immun 38:1020–1028
139. Polak A, Scholer HJ (1975) Mode of action of 5-fluorocytosine and mechanisms of resistance. Chemotherapy 21:113–130
140. Whelan WL, Kerridge D (1984) Decreased activity of UMP pyrophosphorylase associated with resistance to 5-fluorocytosine in *Candida albicans*. Antimicrob Agents Chemother 26: 570–574
141. Hector RF (1993) Compounds active against cell walls of medically important fungi. Clin Microbiol Rev 6:1–21
142. Cassone A, Bernardis FD, Torososantucci A (2005) An outline of the role of anti-Candida antibodies within the context of passive immunization and protection from candidiasis. Curr Mol Med 5:377–382
143. Cassone A, Mason RE, Kerridge D (1981) Lysis of growing yeast-form cells of *Candida albicans* by echinocandin: a cytological study. Sabouraudia 19:97–110
144. Gupta AK, Shear NH (1997) Terbinafine: an update. J Am Acad Dermatol 37:979–988
145. Darkes MJM, Scott LJ, Goa KL (2003) Terbinafine: a review of its use in onychomycosis in adults. Am J Clin Dermatol 4:39–65
146. Callen JP, Hughes P, Kulp-Shorten C (2001) Subacute cutaneous lupus erythematosus induced or exacerbated by terbinafine: a report of 5 cases. Arch Dermatol 137L:1196–1198
147. Ryder NS (1992) Terbinafine: mode of action and properties of the squalene epoxidase inhibition. Br J Dermatol 126(Suppl 39):2–7
148. Georgopoulos A, Petranyi G, Mieth H, Drews J (1981) In vitro activity of naftifine, a new antifungal agent. Antimicrob Agents Chemother 19:386–389
149. Venugopal PV, Venugopal TV (1994) Antidermatophytic activity of allylamine derivatives. Indian J Pathol Microbiol 37:381–388
150. Gupta AK, Ryder JE, Cooper EA (2008) Naftifine: a review. J Cutan Med Surg 12:51–58
151. Ghannoum M et al (2013) In vitro antifungal activity of naftifine hydrochloride against dermatophytes. Antimicrob Agents Chemother 57:4369–4372
152. Ryder NS, Dupont MC (1985) Inhibition of squalene epoxidase by allylamine antimycotic compounds. A comparative study of the fungal and mammalian enzymes. Biochem J 230: 765–770
153. Regli P, Ferrari H (1989) In vitro action spectrum of a new antifungal agent derived from morpholine: amorolfin. Pathol Biol 37:617–620
154. Hänel H, Smith-Kurtz E, Pastowsky S (1991) Therapy of seborrheic eczema with an antifungal agent with an antiphlogistic effect. Mycoses 34(Suppl 1):91–93
155. Singal A (2008) Butenafine and superficial mycoses: current status. Expert Opin Drug Metab Toxicol 4:999–1005
156. Das S, Barbhuniya JN, Biswas I, Bhattacharya S, Kundu PK (2010) Studies on comparison of the efficacy of terbinafine 1% cream and butenafine 1% cream for the treatment of Tinea cruris. Indian Dermatol Online J 1:8–9
157. Ryu C-K, Lee JY, Park R-E, Ma M-Y, Nho J-H (2007) Synthesis and antifungal activity of 1H-indole-4,7-diones. Bioorg Med Chem Lett 17:127–131
158. Xu H, Wang Y-Y (2010) Antifungal agents. Part 5: synthesis and antifungal activities of aminoguanidine derivatives of N-arylsulfonyl-3-acylindoles. Bioorg Med Chem Lett 20: 7274–7277

159. Ryu C-K, Lee S-Y, Kim NY, Hong JA, Yoon JH, Kim A (2011) Synthesis and antifungal evaluation of 6-hydroxy-1H-carbazole-1,4(9H)-diones. Bioorg Med Chem Lett 21:427–430
160. Na Y-M, Borgne ML, Pagniez F, Baut GL, Pape PL (2003) Synthesis and antifungal activity of new 1-halogenobenzyl-3-imidazolylmethylindole derivatives. Eur J Med Chem 38:75–87
161. Tiwari RK, Verma AK, Chhillar AK, Singh D, Singh J, Kasi Sankar V et al (2006) Synthesis and antifungal activity of substituted-10-methyl-1,2,3,4-tetrahydropyrazino[1,2-a]indoles. Bioorg Med Chem 14:2747–2752
162. Musiol R, Jampilek J, Buchta V, Silva L, Niedbala H, Podeszwa B et al (2006) Antifungal properties of new series of quinoline derivatives. Bioorg Med Chem 14:3592–3598
163. Meléndez Gómez CM, Kouznetsov VV, Sortino MA, Álvarez SL, Zacchino SA (2008) In vitro antifungal activity of polyfunctionalized 2-(hetero)arylquinolines prepared through imino Diels–Alder reactions. Bioorg Med Chem 16:7908–7920
164. Yu Z, Shi G, Sun Q, Jin H, Teng Y, Tao K et al (2009) Design, synthesis and in vitro antibacterial/antifungal evaluation of novel 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7 (1-piperazinyl)quinoline-3-carboxylic acid derivatives. Eur J Med Chem 44:4726–4733
165. Boateng CA, Eyunni SVK, Zhu XY, Etukala JR, Bricker BA, Ashfaq MK et al (2011) Benzothieno[3,2-b]quinolinium and 3-(phenylthio)quinolinium compounds: synthesis and evaluation against opportunistic fungal pathogens. Bioorg Med Chem 19:458–470
166. Tang H, Zheng C, Lv J, Wu J, Li Y, Yang H et al (2010) Synthesis and antifungal activities in vitro of novel pyrazino [2,1-a] isoquinolin derivatives. Bioorg Med Chem Lett 20:979–982
167. Jatav V, Kashaw S, Mishra P (2008) Synthesis, antibacterial and antifungal activity of some novel 3-[5-(4-substituted phenyl) 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones. Med Chem Res 17:169–181
168. Abdel-Gawad SM, El-Gaby MSA, Ghorab MM (2000) Synthesis and antifungal activity of novel pyrano[2′,3′:4,5]thiazolo[2,3-b]quinazolines, pyrido[2′,3′:4,5]thiazolo[2,3-b] quinazolines and pyrazolo[2′,3′:4,5]thiazolo[2,3-b]quinazolines. Farmaco 55:287–292
169. Jalilian AR, Sattari S, Bineshmarvasti M, Daneshtalab M, Shafiee A (2003) Synthesis and in vitro antifungal and cytotoxicity evaluation of substituted 4,5-dihydronaphtho[1,2-d] [1,2,3]thia(or selena)diazoles. Farmaco 58:63–68
170. Fuglseth E, Otterholt E, Høgmoen H, Sundby E, Charnock C, Hoff BH (2009) Chiral derivatives of Butenafine and Terbinafine: synthesis and antifungal activity. Tetrahedron 65:9807–9813
171. Mallikarjuna BP, Sastry BS, Suresh Kumar GV, Rajendraprasad Y, Chandrashekar SM, Sathisha K (2009) Synthesis of new 4-isopropylthiazole hydrazide analogs and some derived clubbed triazole, oxadiazole ring systems – a novel class of potential antibacterial, antifungal and antitubercular agents. Eur J Med Chem 44:4739–4746
172. Omar K, Geronikaki A, Zoumpoulakis P, Camoutsis C, Soković M, Ćirić A et al (2010) Novel 4-thiazolidinone derivatives as potential antifungal and antibacterial drugs. Bioorg Med Chem 18:426–432
173. Pitta E, Tsolaki E, Geronikaki A, Petrovic J, Glamoclija J, Sokovic M et al (2015) 4-Thiazolidinone derivatives as potent antimicrobial agents: microwave-assisted synthesis, biological evaluation and docking studies. MedChemComm 6:319–326
174. Chimenti F, Bizzarri B, Bolasco A, Secci D, Chimenti P, Granese A et al (2011) Synthesis and biological evaluation of novel 2,4-disubstituted-1,3-thiazoles as anti-Candida spp. agents. Eur J Med Chem 46:378–382
175. Stan CD, Tuchiluş C, Stan CI (2002) Echinocandins--new antifungal agents. Rev Med Chir Soc Med Nat Iasi 118:528–536
176. Sucher AJ, Chahine EB, Balcer HE (2009) Echinocandins: the newest class of antifungals. Ann Pharmacother 43:1647–1657
177. Spampinato C, Leonardi D (2013) Candida infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. Biomed Res Int 2013:1–13
178. Vazquez J, Sobel JD (2006) Anidulafungin: a novel echinocandin. Clin Infect Dis 43: 215–222

179. Denning DW (2003) New drug classes echinocandin antifungal drugs. Lancet 362: 1142–1151
180. Letscher-Bru V, Herbrecht R (2003) Caspofungin: the first representative of a new antifungal class. J Antimicrob Chemother 51:513–521
181. Chandrasekar PH, Sobel JD (2006) Micafungin: a new echinocandin. Clin Infect Dis 42: 1171–1178
182. Sheng C, Xu H, Wang W, Cao Y, Dong G, Wang S et al (2010) Design, synthesis and antifungal activity of isosteric analogues of benzoheterocyclic N-myristoyltransferase inhibitors. Eur J Med Chem 45:3531–3540
183. Onnis V, De Logu A, Cocco MT, Fadda R, Meleddu R, Congiu C (2009) 2-Acylhydrazino-5-arylpyrrole derivatives: synthesis and antifungal activity evaluation. Eur J Med Chem 44: 1288–1295
184. Maruoka H, Kashige N, Eishima T, Okabe F, Fujioka T, Miake F et al (2008) Synthesis and antifungal activity of spiro[cyclopropane-1,4′-pyrazol-3-one] derivatives. J Heterocycl Chem 45:1883–1887
185. Zheng Q-Z, Cheng K, Zhang X-M, Liu K, Jiao Q-C, Zhu H-L (2010) Synthesis of some N-alkyl substituted urea derivatives as antibacterial and antifungal agents. Eur J Med Chem 45:3207–3212
186. Ryu C-K, Han J-Y, Jung O-J, Lee S-K, Lee JY, Jeong SH (2005) Synthesis and antifungal activity of noble 5-arylamino- and 6-arylthio-4,7-dioxobenzoselenazoles. Bioorg Med Chem Lett 15:679–682
187. Ryu C-K, Song AL, Lee JY, Hong JA, Yoon JH, Kim A (2010) Synthesis and antifungal activity of benzofuran-5-ols. Bioorg Med Chem Lett 20:6777–6780
188. Xu H, Fan L-L (2011) Antifungal agents. Part 4: synthesis and antifungal activities of novel indole[1,2-c]-1,2,4-benzotriazine derivatives against phytopathogenic fungi in vitro. Eur J Med Chem 46:364–369
189. López SN, Castelli MV, Zacchino SA, Domínguez JN, Lobo G, Charris-Charris J et al (2001) In vitro antifungal evaluation and structure–activity relationships of a new series of chalcone derivatives and synthetic analogues, with inhibitory properties against polymers of the fungal cell wall. Bioorg Med Chem 9:1999–2013
190. Singh OM, Singh SJ, Devi MB, Devi LN, Singh NI, Lee S-G (2008) Synthesis and in vitro evaluation of the antifungal activities of dihydropyrimidinones. Bioorg Med Chem Lett 18: 6462–6467
191. Ravi Kumar KR, Mallesha H, Basappa, Rangappa KS (2003) Synthesis of novel isoxazolidine derivatives and studies for their antifungal properties. Eur J Med Chem 38: 613–619
192. Zhang F-F, Gan L-L, Zhou C-H (2010) Synthesis, antibacterial and antifungal activities of some carbazole derivatives. Bioorg Med Chem Lett 20:1881–1884
193. Šenel P, Tichotová L, Votruba I, Buchta V, Špulák M, Kuneš J et al (2010) Antifungal 3,5-disubstituted furanones: from 5-acyloxymethyl to 5-alkylidene derivatives. Bioorg Med Chem 18:1988–2000
194. Yao B, Ji H, Cao Y, Zhou Y, Zhu J, Lü J et al (2007) Synthesis and antifungal activities of novel 2-aminotetralin derivatives. J Med Chem 50:5293–5300
195. Hilchie AL, Wuerth K, Hancock REW (2013) Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. Nat Chem Biol 9:761–768
196. Rodrigues EG, Dobroff AS, Taborda CP, Travassos LR (2009) Antifungal and antitumor models of bioactive protective peptides. An Acad Bras Cienc 81:503–520
197. Ekengren S, Hultmark D (1999) Drosophila cecropin as an antifungal agent. Insect Biochem Mol Biol 29:965–972
198. De Lucca AJ, Bland JM, Jacks TJ, Grimm C, Walsh TJ (1998) Fungicidal and binding properties of the natural peptides cecropin B and dermaseptin. Med Mycol 36:291–298

199. Lee DG, Kim HK, Kim SA, Park Y, Park SC, Jang SH et al (2003) Fungicidal effect of indolicidin and its interaction with phospholipid membranes. Biochem Biophys Res Commun 305:305–310
200. Raj PA, Edgerton M, Levine MJ (1990) Salivary histatin 5: dependence of sequence, chain length, and helical conformation for candidacidal activity. J Biol Chem 265:3898–3905
201. Zasloff M (1987) Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci U S A A84:5449–5453
202. Giacometti A, Cirioni O, Barchiesi F, Del Prete MS, Scalise G (1999) Antimicrobial activity of polycationic peptides. Peptides 20:1265–1273
203. Mangoni ML, Grovale N, Giorgi A, Mignogna G, Simmaco M, Barra D (2000) Structure-function relationships in bombinins H, antimicrobial peptides from Bombina skin secretions. Peptides 21:1673–1679
204. Simmaco M et al (2003) Defense peptides in the amphibian immune system. In: Ascenzi P, Polticelli F, Visca P (eds) Bacterial, plant, and animal toxins. Research Signpost, Kerala
205. Hancock REW, Rozek A (2002) Role of membranes in the activities of antimicrobial cationic peptides. FEMS Microbiol Lett 206:143–149
206. Pettit RK, Pettit GR, Hazen KC (1998) Specific activities of dolastatin 10 and peptide derivatives against *Cryptococcus neoformans*. Antimicrob Agents Chemother 42:2961–2965
207. Cassone A, Torosantucci A, Boccanera M, Pellengrini G, Palma C, Malavasi G (1988) Production and characterization of a monoclonal antibody to a cell surface, gluco-mannoprotein constituent of *Candida albicans* and other pathogenic *Candida* species. J Med Microbiol 27:233–238
208. De Wit MYL, Klaster PR (1988) Purification and characterization of a 36kDa antigen of *Mycobacterium leprae*. J Gen Microbiol 134:1541–1548
209. Chaturvedi AK, Kavishwar A, Shiva Keshava GB, Shukla PK (2005) Monoclonal immunoglobulin G1 directed against *Aspergillus fumigatus* cell wall glycoprotein protects against experimental murine aspergillosis. Clin Diagn Lab Immunol 12:1063–1068
210. Sgro C (1995) Side-effects of a monoclonal antibody, muromonab CD3/orthoclone OKT3: bibliographic review. Toxicology 105:23–29
211. Kettner SC et al (1999) Use of abciximab-modified thrombelastography in patients undergoing cardiac surgery. Anesth Analg 89:580–584
212. Zhang Y et al (2014) Daclizumab reduces CD25 levels on T cells through monocyte-mediated trogocytosis. Mult Scler 20:156–164
213. Borker A, Choudhary N (2011) Rituximab. Indian Pediatr 48:627–632
214. Boekhout AH, Beijnen JH, Schellens JHM (2011) Trastuzumab. Oncologist 16:800–810
215. Scott LJ, Lamb HM (1999) Palivizumab. Drugs 58:303–305
216. Valle E, Gross M, Bickston SJ (2001) Infliximab. Expert Opin Pharmacother 2:1015–1025
217. Onrust SV, Wiseman LR (1999) Basiliximab. Drugs 57:207–213, discussion 214
218. McGavin JK, Spencer CM (2001) Gemtuzumab ozogamicin. Drugs 61:1317–1324
219. Frampton JE, Wagstaff AJ (2003) Alemtuzumab. Drugs 63:1229–1243, discussion 1245–6
220. Savk E (2007) Efalizumab. Anti-inflamm Anti-Allergy Agents Med Chem 6:205–210
221. Mease PJ (2007) Adalimumab in the treatment of arthritis. Ther Clin Risk Manag 3:133–148
222. Witzig TE et al (2002) Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol 20:2453–2463
223. Mukherji SK (2010) Bevacizumab (Avastin). AJNR Am J Neuroradiol 31:235–236
224. Graham J, Muhsin M, Kirkpatrick P (2004) Cetuximab. Nat Rev Drug Discov 3:549–550
225. Corren J et al (2009) Safety and tolerability of omalizumab. Clin Exp Allergy 39:788–797
226. Selewski DT, Shah GV, Segal BM, Rajdev PA, Mukherji SK (2010) Natalizumab (Tysabri). Am J Neuroradiol 31:1588–1590
227. Saltz L, Easley C, Kirkpatrick P (2006) Panitumumab. Nat Rev Drug Discov 5:987–988

228. Blick SK, Keating GM, Wagstaff AJ (2007) Ranibizumab. Drugs 67:1199–1206, discussion 1207–9
229. Davis J (2008) Eculizumab. Am J Health Syst Pharm 65:1609–1615
230. Goel N, Stephens S (2010) Certolizumab pegol. MAbs 2:137–147
231. Cingoz O (2009) Ustekinumab. MAbs 1:216–221
232. Mazumdar S, Greenwald D (2009) Golimumab. MAbs 1:422–431
233. Dhimolea E (2010) Canakinumab. MAbs 2:3–13
234. Keating MJ, Dritselis A, Yasothan U, Kirkpatrick P (2010) Ofatumumab. Nat Rev Drug Discov 9:101–102
235. Venkiteshwaran A (2009) Tocilizumab. MAbs 1:430–435
236. Cummings SR et al (2009) Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med 361:756–765
237. Sondak VK, Smalley KSM, Kudchadkar R, Grippon S, Kirkpatrick P (2011) Ipilimumab. Nat Rev Drug Discov 10:411–412
238. Sanz I, Yasothan U, Kirkpatrick P (2011) Belimumab. Nat Rev Drug Discov 10:335–336
239. Ansell SM (2014) Brentuximab vedotin. Blood 124:3197–3200
240. Zagouri F et al (2013) Pertuzumab in breast cancer: a systematic review. Clin Breast Cancer 13:315–324
241. Diéras V, Bachelot T (2014) The success story of trastuzumab emtansine, a targeted therapy in HER2-positive breast cancer. Target Oncol 9:111–122
242. Shah A (2014) Obinutuzumab: a novel anti-CD20 monoclonal antibody for previously untreated chronic lymphocytic leukemia. Ann Pharmacother 48:1356–1361
243. Rhee VF et al (2010) Siltuximab, a novel anti-interleukin-6 monoclonal antibody, for Castleman's disease. J Clin Oncol 28:3701–3708
244. Mosli MH, Feagan BG (2013) Vedolizumab for Crohn's disease. Expert Opin Biol Ther 13: 455–463
245. Javle M, Smyth EC, Chau I (2014) Ramucirumab: successfully targeting angiogenesis in gastric cancer. Clin Cancer Res 20:5875–5881
246. Sanford M, McKeage K (2015) Secukinumab: first global approval. Drugs 75:329–338

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Neglected Tropical Bacterial Diseases

Anil Kumar Saxena and Chandra Sourabh Azad

Abstract Neglected tropical diseases (NTDs) belong to a diverse group of communicable diseases caused by pathogens including helminthes, protozoa, bacteria, and viruses. The NTDs prevail in tropical and subtropical conditions in 149 countries and affect billions of people, resulting in an economic burden of billions of dollars every year. The major neglected tropical bacterial diseases (NTBDs) are Leprosy, Buruli ulcer, and Trachoma. Leprosy and Buruli ulcer are caused by members of the *Mycobacterium* genus viz *M. leprae* and *M. ulcerans* and are responsible for the most severe medical impact in the tropics. Trachoma is the result of infection of the eye with *Chlamydia trachomatis* and it is responsible for the visual impairment of about 1.8 million people, of whom 0.5 million are irreversibly blind. In this chapter the above major NTBDs are discussed in terms of their epidemiology, pathogenic vector, metabolism, genomic and immunological analysis, classification, treatment, resistance, and vaccine development.

Keywords Buruli ulcer, *Chlamydia trachomatis*, Leprosy, *Mycobacterium leprae*, *Mycobacterium ulcerans*, Neglected tropical bacterial diseases, Trachoma, Treatment, Vaccine

A.K. Saxena (✉)
Division of Medicinal and Process Chemistry, Central Drug Research Institute, Lucknow 226031, India
e-mail: anilsak@gmail.com

C.S. Azad
"Hygeia" Centre of Excellence in Pharmaceutical Sciences (CEPS), GGS Indraprastha University, Sec. 16-C, Dwarka, New Delhi 110078, India
e-mail: csazad9@gmail.com

Contents

Abbreviations

BB	Borderline borderline
BL	Borderline lepromatous
BT	Borderline tuberculoid
BU	Buruli ulcer
CMI	Cell-mediated immunity
DC	Dendritic cell
DC-SIGN	DC specific intercellular adhesion molecule-grabbing nonintegrin
DDS	Dapsone
DF5HT	Deoxyfructo-5-hydroxytryptamine
DHPS	Dihydropteroate synthase
EB	Elementary body
ENL	Erythema nodosum leprosum
Hsp	Heat shock protein
IL-12	Ligand-associated interleukin 12
LL	Lepromatous leprosy
MB	Multibacillary leprosy

MDT	Multidrug therapy
MHC	Major histocompatibility complex
MOMP	Major outer membrane protein
NK cells	Natural killer cells
NTBD	Neglected tropical bacterial disease
NTD	Neglected tropical disease
ORF	Open reading frame
PB	Paucibacillary leprosy
PBMC	Peripheral blood mononuclear cell
PGL	Phenolic glycolipid
PmpD	Polymorphic membrane protein D
PSSHE	Persistent serpentine supravenous hyperpigmented eruption
RB	Reticulate body
TCA	Tricarboxylic acid
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TSC	Thiosemicarbazone
TT	Tuberculoid leprosy
WHO	World Health Organization

1 Introduction

The neglected tropical diseases (NTDs) are mostly chronic parasitic infections affecting human health in the developing countries. The parasites responsible for such diseases are viruses, bacteria, protozoa, and helminthes. Recently, Fenwick [1] reported 12 "core" NTDs: Human African trypanosomiasis, Buruli ulcer, leprosy, Chagas' disease, trachoma, dracunculiasis, ascariasis, trichuriasis, leishmaniasis, schistosomiasis, onchocerciasis, and lymphatic filariasis. Bacterial infections are particularly prevalent in the tropical regions. According to the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), the four major bacterial infections are Buruli ulcer, leprosy, trachoma, and yaws. Widespread occurrence of these diseases is also observed in the temperate regions as a result of migration of human carriers from tropical zones. In temperate regions, inappropriate diagnosis caused by misinterpretation of signs and symptoms coupled with improper therapy and preventive measures results in further progression of these diseases. In this context, tuberculosis and leprosy are the major diseases, affecting millions worldwide.

Mycobacterium sp. is responsible for more human infections than other bacteria, with mycobacteriosis having severe impact in tropical countries. Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is the most well-known mycobacterium infection in humans. When it comes to social stigma, the mycobacterium infection known as leprosy can be considered the leader of all bacterial infections. Furthermore, atypical mycobacteria are responsible for Buruli ulcer, a commonly occurring skin disease. Trachoma caused by *Chlamydia trachomatis* is responsible for visual

impairment in 1.8 million people worldwide, including 0.5 million with irreversible blindness. Furthermore, the high death rates associated with anthrax and yaws have led to these diseases being deliberately used in bioterrorism. The other significant bacterial infections of tropical regions are pyomyositis, bartonellosis, and the sexually transmitted diseases lymphogranuloma venereum and granuloma inguinale [2]

2 Leprosy

2.1 Introduction

Leprosy was first reported in India (600 BC), where the disease is known as kustha (meaning "eating away" in Sanskrit). It was described as being dissimilar to vitiligo [3, 4]. The prehistoric medical texts of *Sushruta*, *Vagbhata*, and *Charaka* report that Indian physicians saw leprosy as a disease that could be alleviated and cured. The *Sushruta Samhita* recommended treatment of leprosy with oil from the chaulmoogra tree; this was the backbone of treatment until the introduction of sulfones [5, 6]. Leprosy is a chronic infection by *Mycobacterium leprae* [7, 8] and is also known as Hansen's disease, named after Gerhard Amauer Hansen, who discovered *M. leprae* in 1873 [9]. *M. leprae* multiplies slowly, with an incubation period of 2.9–5.3 years. The symptoms may take as long as 20 years to appear. Leprosy is characterized by infection of the skin, upper respiratory passage and nerves, and associated immunological damage. The nerve damage is responsible for repeated ulceration and paralysis affecting hands, feet, and eyes. If detected early it can be reversed with steroid treatment; otherwise, permanent nerve damage causes significant morbidity [7]. To improve the social stigma associated with the disease, in 1948 the International Leprosy Association abandoned the word "leper" for describing leprosy patients. Now, the term Hansen's disease is more usually used for leprosy [10]. The disability and related social stigma associated with leprosy has resulted in a significant barrier to full participation of individual leprosy patients in society, resulting in socioeconomic burden for those associated and for society.

2.2 Transmission and Epidemiology

Leprosy transmission does not require a tropical environment. Historically, the disease has affected people in nearly every corner of the world, including countries traversed by the Arctic Circle. At present, its higher prevalence in the tropical region is best attributed to socioeconomic factors [11]. Infected humans are the most common source of leprosy, although infected nonhumans are also known. Humans with untreated leprosy may have billions of organisms per gram of tissue, which are shed from nasal mucosa. Healthy bearers of *M. leprae* in the nasal mucosa may also be a factor in the transmission of leprosy. Rates of population nasal carriage of

M. leprae DNA, based on polymerase chain reaction (PCR) studies, are much higher than the registered prevalence rates of leprosy [12]. The mode of entry for *M. leprae* into a host is ambiguous, but the only seriously considered sites are the upper respiratory tract and skin. Rees and McDougall reported the infection of immunodeficient mice through aerosols containing *M. leprae*, and the nasal route was considered to be the most important [13]. It is assumed that *M. leprae* settles in the lymph node and from there roams to the skin and nerves. Experimental models and clinical examples of transmission through the skin have also been reported [14]. Naturally acquired leprosy was firstly perceived in armadillos in 1974 and infected up to half of the armadillos in some regions [15]. Similarly, native leprosy has been detected in monkeys and chimpanzees in West Africa and elsewhere [16–18]. Humans can acquire leprosy from wild infected armadillos and possibly from other zoonotic sources in other geographical areas [19, 20]. There is no evidence of leprosy transmission by insects or any other vectors.

The global registered prevalence of leprosy has decreased markedly since 1985, when around 5.4 million cases were registered for treatment. A total of 213,899 new cases of leprosy were reported to WHO at the end of the first quarter of 2015, from 121 countries, the vast majority of cases occurring in the eastern Mediterranean, Africa, South-East Asia, the Americas, and the western Pacific. The countries that reported the most new cases were India, Brazil, Indonesia, Nigeria, Democratic Republic of Congo, and Ethiopia. The prevalence rate of 0.31 per 10,000 population in the first quarter of 2015 differs marginally from the data of 2014 (0.32 per 10,000 population) (Fig. 1) [21]. The approval and widespread use of multidrug therapy

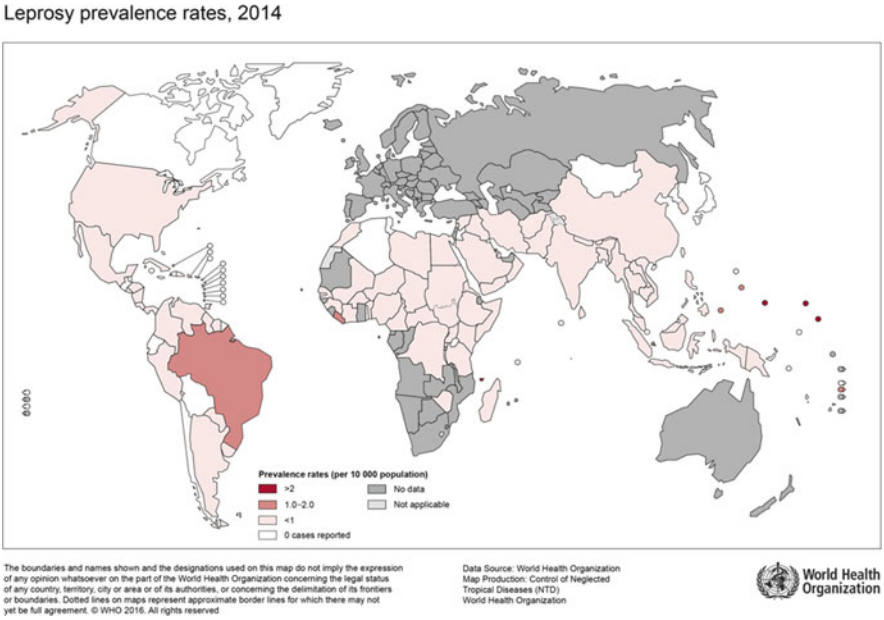

Fig. 1 Registered cases of leprosy per 10,000 population (prevalence rate) in 2014

and large-scale public health campaigns from 1982 onwards has contributed to the drastic decrease in the number of leprosy patients. Over 14 million people have been diagnosed with leprosy since 1985. A significant proportion of these have ongoing disability as a result of leprosy-related nerve damage. A probable 3 million people are living with leprosy-caused physical impairments and disabilities and the related social stigma.

2.3 Bacteriology

2.3.1 Cellular Morphology

M. leprae is a strong acid-fast rod-shaped organism with rounded ends and parallel sides. Its length varies from 1 to 8 μm and the diameter is 0.3 μm (Fig. 2) [22]. *M. leprae* looks very similar to *Mycobacterium tuberculosis* in size and shape, and is an obligatory intracellular parasite that cannot be cultivated in vitro, although it can be grown in the nine-banded armadillo (*Dasypus novemcinctus*) and in the footpad of mice (Shepard's hind footpad inoculation method). *M. leprae* can be recognized in stained biopsies, smears of nasal secretions, and in slit-skin smears. *M. leprae* has a tropism for infection of skin keratinocytes and histocytes, with the purpose of entering the Schwann cells of the peripheral nerves. This leads to axonal atrophy and dysfunction, with segmental demyelination via laminin-binding protein [23–25]. The main targets of the bacteria are macrophages, where it forms large clumps (globi). The bacteria prevent lysosome and phagosome fusion to avoid degradation [26]. *M. leprae* has a slow doubling time of 14 days and it remains viable for up to 2 months. The slow replication time is a result of the restricted intake of nutrients through its waxy walls. The cell walls of *M. leprae* are

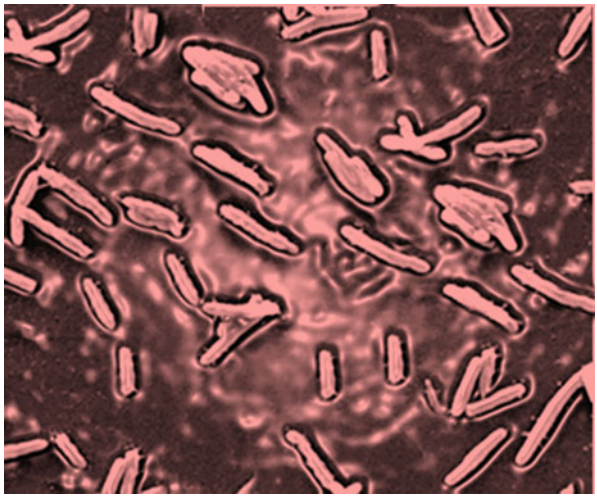

Fig. 2 Suspension of nude-mouse footpad-derived *M. leprae* under the scanning electron microscope

made up of a covalently linked peptidoglycan–arabinogalactan–mycolic acid complex, similar to other mycobacterial cell walls. The immunological specificity of *M. leprae* is a result of the presence of phenolic glycolipid-1 (PGL-1) a dominant lipid in the cell wall. The interaction of *M. leprae* with the laminin of Schwann cells requires the involvement of PGL-1, as suggested by Vincent and coworkers [27].

2.3.2 Metabolism

Knowledge of metabolic pathways gives new insights for the development of new antileprotic drugs. Genomic studies have revealed the involvement of the Embden–Meyerhof–Parnas (EMP) pathway for the oxidation of glucose to pyruvate for generation of energy in *M. leprae*. Energy is produced in the form of ATP, which is produced by acetyl-CoA via glycolysis through the Krebs cycle. Lipid degradation and glyoxylate shunt are the major pathways for energy production in *M. leprae* and *M. tuberculosis* according to biochemical studies and genome analysis [28, 29]. Five different membrane proteins of *M. leprae* import lipids into the cell; these lipids are the major source of carbon. *M. leprae* is not able to generate ATP from the oxidation of NADH because of the absence of anaerobic and microaerophilic electron transfer systems and a severely truncated aerobic respiratory chain [30]. However, the anabolic capabilities of *M. leprae* are less affected [31].

2.3.3 Genomics and Proteomics

Cole and coworkers sequenced the genome of *M. leprae* and published the results in *Nature* [30]. An extreme case of reductive evolution has been observed in the genome of *M. leprae* in comparison with *M. tuberculosis* [32, 33]. This is revealed by a major reduction in G+C content (66% for *M. tuberculosis* versus 58% for *M. leprae*) and smaller genome size (4.4 Mb for *M. tuberculosis* versus 3.3 Mb for *M. leprae*). The most salient feature of the *M. leprae* genome is the presence of 1,133 pseudogenes (mutational gene loss), compared with only six for *M. tuberculosis* [34]. This suggests that genes nonessential for survival of *M. leprae* are eliminated during the process of evolution. This reduction in the genome has led to elimination of several metabolic pathways, resulting in *M. leprae* with specific growth requirements.

M. leprae cannot acquire iron from the extracellular environment as a result of deletion of the entire *mbt* operon and, thus, cannot use either the membrane-associated or excreted form of mycobactin T. Furthermore, *M. leprae* cannot utilize iron because of the absence of genes responsible for iron acquisition in its genome. Hence, *M. leprae* is dependent only on the intracellular iron, which is regulated through genes encoding iron-containing proteins or complexes such as ferredoxin (*fdxCD*), cytochrome *c* (*ccsAB*), a hemoglobin-like oxygen carrier (*glbO*), the heme group (*hem* genes), and the iron storage protein bacterioferritin (*bfrA* and

ideR) [35]. The genomic analysis thus explains the obligatory intracellular nature of *M. leprae* and provides better understanding of its pathogenicity, which may be useful in drug discovery and development programs.

2.4 Pathogenesis and Immune Response

The host immunological response to *M. leprae* is crucial in determining either clinico-pathological manifestation or immunity and the type of disease. A protective immune response in leprosy depends on cell-mediated immunity (CMI) for killing *M. leprae*. Antibodies are produced to *M. leprae* antigen, and are abundant in lepromatous leprosy, but do not appear to have any useful role in the elimination of these organisms.

2.4.1 Innate Immunity

An effective innate immune response and the low virulence of the leprosy bacillus retard the development of leprosy. Dendritic cells (DCs) may be the first cells to encounter the bacilli at the site of *M. leprae* invasion of the host (e.g., nasal mucosa or skin abrasion). DCs uptake *M. leprae* and the consequential local production of cytokines and chemokines regulates inflammation and is recognized to affect the course of adaptive CMI into a type 1 T helper (Th1) or Th2 cell response [36]. Even though DCs are identified as operational presenters of *M. leprae* antigens, expression of major histocompatibility complex (MHC) classes I and II is downregulated in monocyte-derived DCs infected with *M. leprae* bacilli [37]. In contrast, DCs stimulated with *M. leprae* membrane antigens upregulate production of both MHC class II and CD40 ligand-associated interleukin-12 (IL-12), suggesting that entire live bacilli may suppress the interaction of DCs and T cells [38]. *M. leprae*-infected DCs express PGL-1 on the surface. PGL-1 exhibits immunosuppressive properties and masks the DC-expressed PGL-1 with specific antibody. It also upregulates both gamma interferon (IFN-γ) production and the proliferative response by T cells [39]. Macrophage-derived DCs are more effective antigen-presenting cells; additionally, they are highly susceptible to killing by *M. leprae* membrane-specific $CD8^+$ cytotoxic T cells [40]. Higher levels of $CD1^+$ DCs are found in tuberculoid leprosy (TT) lesions than in lepromatous leprosy (LL) lesions [41]. Langerhans cells are a subset of DCs and are initiators of the immune response in the skin. LL patients have considerably fewer Langerhans cells, in both the lesion and healthy skin, than uninfected controls or TT patients [42]. On the other hand, patients with TT have an amplified number of Langerhans cells in lesions, suggesting the active infiltration of these cells to these sites. The analysis of leprosy biopsy samples has shown that monocytes and DCs in TT lesions express many more Toll-like receptors (TLR1 and TLR2) than those in LL lesions: Th1-type cytokines were generally associated with TLR1 and TLR2 activation, and Th2-type cytokines were involved in

inhibition of activation. In vitro studies indicated that *M. leprae* 19-kDa and 33-kDa lipoproteins could activate monocytes and monocyte-derived DCs through TLR2 [43]. Massone et al. performed a retrospective immunohistochemical study and showed that plasmacytoid DCs are not involved in the immune response against *M. leprae*, whereas FoxP3-positive cells (markers of regulatory T cells) were present in 95% of the cases, with an average density of 2.9% of the infiltrate [44].

Receptors of Innate Immunity

The pathogen-associated molecular pattern exhibited by microorganisms is detected by the pattern recognition receptors expressed on the immune cells at the site of exposure. The calcium-dependent or C-type lectins, such as DC-specific intercellular adhesion molecule-grabbing nonintegrin (DC-SIGN, also known as CD209), mannose receptor CD206, and langerin (CD207) form a group of pattern recognition receptors. The binding of CD206 to specific carbohydrate groups on pathogens assists internalization, resulting in antigen processing and presentation. CD206 is primarily expressed on cells of the myeloid lineage, especially mature macrophages, which facilitate the uptake of virulent mycobacteria [45]. DC209 expressed on DCs via binding with mannose-containing structures recognizes the pathogens [46]. In a similar manner, CD207 expressed by Langerhans cells has a single calcium-dependent carbohydrate recognition domain and identifies only *N*-acetylglucosamine, mannose, and fucose [47]. The TLRs with extracellular leucine-rich motifs comprise a second category of pattern recognition receptors. They are phylogenetically conserved transmembrane proteins with ten subtypes, among which TLR2 homodimers, TLR2–TLR1 heterodimers, and TLR4 are crucial for mycobacteria recognition. The TLRs play a crucial role in the production of cytokines IL-12 [48] (induction of Th1-type immunity) and tumor necrosis factor alpha (TNF-α) [49]. TNF-α is also responsible for tissue destruction in leprosy. Another group of receptors responsible for uptake of mycobacteria are the C′ receptors. Phagocytosis of *M. leprae* is mediated by complement receptor 1 (CR1) and CR3 present on the monocyte surface and by CR1, CR3, and CR4 on macrophages [50].

2.4.2 Adaptive Immunity

T cell lineage cells play a crucial role in resistance to *M. leprae*. LL patients are not prone to cancer or the adaptable infections that affect persons with immunodeficiency diseases. Resistance to leprosy has been estimated to be present in 95% of the population. Protection may occur early, with no obvious signs of disease. The strong immune response may control the development of leprosy but simultaneously damage the tissues (i.e., CMI-associated granulomatous inflammation leading to peripheral nerve injury).

T-Lymphocyte Subsets

1. *$CD4^+$ and $CD8^+$ cells*: TT lesions exhibit mostly $CD4^+$ helper cells with a $CD4^+$ to $CD8^+$ ratio of 1.9:1, which is 2:1 in normal peripheral blood [51]. Cytotoxic T cells play a crucial role in the localization, activation, and maturation of macrophages, leading to restriction or elimination of the pathogen. $CD8^+$ cells are harbored at the periphery of the TT lesion, whereas $CD4^+$ cells remains distributed throughout the lesion [52]. However, $CD8^+$ T cells are dispersed throughout LL lesions rather than at the periphery, with a $CD4^+$ to $CD8^+$ ratio of 0.6:1. The $CD8^+$ suppressor cells may downregulate the activation of macrophages and suppression of CMI.
2. *T regulatory cells*: These cells constitute 5–10% of all CD4 T cells in peripheral blood and typically express CD25 and the fork-head family transcription factor P3 (FoxP3). FoxP3-positive cells are present in 95% of leprosy cases, with average density of 2.9% of the infiltrate [44].
3. *CD1-restricted T cells*: Human CD1 molecules bind ligands via hydrophobic interaction in an antigen-binding pocket designed to accommodate the chains of lipids and glycolipids. In vitro and in vivo studies confirmed that CD1 molecules are crucial for mycobacterial lipid presentation in the immune response to *M. leprae*. The mycobacterium-reactive double-negative T cell lines derived from the skin lesion of a leprosy patient responded to mycobacterial subcellular fractions in the presence of CD1-expressing antigen-presenting cells. In contrast, lipoarabinomannan-depleted soluble cell wall fraction was not capable of inducing detectable T cell proliferation. Lipoarabinomannan also induces these T cells to secrete large amounts of IFN-γ. The $CD1^+$ cells are commonly positive for CD83, a marker for mature DCs, and have a strong connection between CMI in leprosy and CD1 expression [53].
4. *Cytotoxic cells*: Cytotoxic cells can be classified into T cells and natural killer (NK) cells:

   (a) Both $CD8^+$ and $CD4^+$ T cells can lyse *M. leprae*-infected macrophages and function as class I- and class II-restricted cytotoxic T cells, respectively [54–56]. The target cells are lysed by cytotoxic T lymphocytes, which is mediated by perforin and cytotoxic granules (granzyme B), a serine protease found in NK cells and cytotoxic T cells [57]. The perforin is released by T cytotoxic cells whenever they come into contact with the target cell, leading to pore formation in the target cell membrane. This allows granzyme B entry to the cell, followed by activation of caspases, and resulting death of target cells. The occurrence of granulysin is more common in TT skin lesions than in LL lesions. *M. leprae*-infected macrophages target cells destroyed by cytotoxic T cells may contribute to defense from leprosy as an adjunct to ongoing attempts at intracellular killing or growth inhibition mediated by IFN-γ-activated macrophages. It has been demonstrated that the presence of viable *M. leprae* inside the cell for a long time can cause impairment of

several important functions of infected macrophages, especially the ability to be activated by stimulation with IFN-γ [58, 59].

(b) NK cells in a variety of neoplastic and pathogen-infected target cells are responsible for non-MHC-restricted cytotoxicity. Although antigen specificity is absent in the cytotoxicity of NK cells and the more active IL-2-stimulated lymphokine-activated killer cells, they are directed against macrophages and Schwann cells infected by *M. leprae*. Administration of IL-2 into LL lesions seems to recruit NK cells by promoting migration to lesion foci, with subsequent local clearance of the bacilli [60, 61].

Macrophages

Macrophages are the primary host cells for *M. leprae* and have major functions in antigen processing and presentation, secretion of cytokine/chemokines, and microbicidal activity. *M. leprae* can survive in normal mouse macrophages but can be killed or inhibited by IFN-γ-activated macrophages in vitro [58, 62]. In normal macrophages, only live *M. leprae* blocks phagosome–lysosome fusion. Phagosomes in activated macrophages having *M. leprae* are able to fuse with secondary lysosomes. Through generation of reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates (RNIs), macrophages can inhibit or kill the invading pathogens.

Cytokines

The CMI response is crucial for host resistance to infection by mycobacteria and is thought to be regulated by a balance between the type 1 cytokines (IL-2, IL-12, TNF-α, IFN-γ) and the type 2 cytokines (IL-4, IL-6, and IL-10). The production of IL-10 during bacterial infection has been shown to suppress production of inflammatory mediators and aid the development of Th2 immunity. The Th1/Th2 paradigm suggests that Th1 and Th2 cells are responsible for the cellular and humoral immune response, depending on the functional discrimination of T-helper cells based on the pattern of cytokine production [63]. It has been observed that a Th2 cytokine pattern is produced by peripheral blood mononuclear cells (PBMCs) and T cell lines in lepromatous patients afflicted by *M. leprae* in vitro, whereas a Th1 cytokine pattern by PBMCs and T cell lines is observed in tuberculoid patients. $CD4^+$ clones isolated from TT lesions secrete mainly IFN-γ, whereas $CD4^+$ clones from LL lesions secrete IL-4. Large amounts of IL-4 are produced from $CD8^+$ clones from LL patients. Resistance to *M. leprae* is developed in tuberculoid lesions, as a result of the high expression of IL-12 and IL-18 [64].

2.5 Classification of Leprosy

Leprosy can be classified as borderline tuberculoid (BT), tuberculoid (TT), borderline lepromatous (BL), borderline borderline (BB), and lepromatous leprosy (LL) types.

2.5.1 Initial Changes

The initial indication of skin infection with *M. leprae* is the multiplication of bacilli within the fixed cells of the skin. Initially, there is focal damage at blood vessels near the site of *M. leprae* entry. *M. leprae* spreads centripetally along the fibers of cutaneous nerves and proliferates within Schwann cells, which then burst and release bacteria into endoneural spaces, where they are engulfed by histiocytes.

2.5.2 Indeterminate Leprosy

The earliest lesions are usually indeterminate leprosy and present as inadequately defined macules, mildly hypopigmented in dark skin and slightly erythematous in lighter skin. Indeterminate leprosy can last for months, resolve, or progress to TT, borderline leprosy, or LL, depending on the host immune response to the infection. Histological examination shows foci of inflammatory cellular exudates, mainly in the region of the finest nerve fibers of the dermal plexuses (perineural paravascular inflammation), which predominantly contain lymphocytes and histiocytes, with or without scarce acid-fast bacilli. These changes are nonspecific unless acid-fast bacilli are found in nerves, arrectores pilorum muscles, or the subepidermal area [65].

2.5.3 Tuberculoid Leprosy

A proper CMI response produced by initial interaction between bacteria and dermal histiocytes results in migration from indeterminate leprosy towards TT, which is defined by skin lesions and nerve damage. Histologically, TT is characterized by the transformation of histiocytes into groups of epithelioid cells, which may combine to form giant cells. Well-constrained foci of these cells are frequently surrounded by a zone of lymphocytes and are known as epithelioid granulomata. Efficient mycobacterial phagocytosis and antigen presentation results in an effective Th1 immune response of cell-mediated type; therefore few, if any, recognizable acid-fast bacilli are seen histologically. Nerve bundles become swollen by proliferation of Schwann cells, which develop into epithelioid cells.

2.5.4 Lepromatous Leprosy

The initial skin lesions are small hypochromic macules with indistinct edges. If left untreated, they form copper colored papules or nodules known as leproma. Macrophages proliferate in LL lesions and can become foamy because of a sufficient quantity of scantily processed mycobacterial lipid material; however, failure of phospholipase activity results in inadequate antigen processing. Lymphocytes are very few. In mature lepromatous disease, chronic inflammatory tissue with bacteria-filled cells dominates the dermis, whereas the subepidermal zone of the dermis is clear of infiltrate. Histoid leprosy is an unusual nodular form of LL. The histopathological characteristics are well-formed lesions; polygonal, spindle-shaped, and foamy histiocytes; and a large number of solid staining acid-fast bacilli.

2.5.5 Borderline Leprosy

Borderline leprosy has three major subgroups: borderline tuberculoid (BT), midborderline (BB), and borderline lepromatous (BL). Borderline leprosy corresponds to a transition state between lepromatous and tuberculoid leprosy. BT is defined by the occurrence of several large asymmetrical and hypoesthetic lesions with peripheral macules or infiltration of the skin. Borderline–borderline (BB) leprosy is defined by the existence of several non-anesthetic annular lesions with indistinct edges. BL is defined by the presence of more than ten bilateral and non-anesthetic lepromas and annular lesions. An inflammatory reaction is observed in the superficial layers of the dermis, consisting of small round cells, histiocytes, and clumps of epithelioid cells but without giant cells.

2.5.6 Classification by WHO

According to WHO, patients can be divided into three groups: (1) paucibacillary leprosy (PB), (2) paucibacillary single-lesion leprosy (PBSLL), and (3) multibacillary leprosy (MB). PB and MB are initially identified through skin smears. Appropriate patient classification and dose regimen in most leprosy programs depend on the number of nerve and skin lesions: PBSLL, one skin lesion; PB, two to five skin lesions; and MB, more than five skin lesions. A patient with a positive skin smear must be considered as having MB, for which the maximum-tolerated dose (MTD) regimen for MB is followed [66].

2.6 Nerve Damage Mechanism

The ability of *M. leprae* to cause nerve damage is based on its unique propensity to invade Schwann cells. The specificity of *M. leprae* for Schwann cells is co-related

to the tissue-specific expression of laminin-2 on Schwann cells. The PHL-1 of *M. leprae* has been shown to bind to the G-domain of the α2 chain of laminin-2 on the membrane of Schwann cells [27]. Laminin-binding protein 21 (LBP 21, 21-kDa histone-like protein) also facilitates the intracellular entry of *M. leprae* into the Schwann cell by binding [67, 68]. The receptors expressed on monocytes and macrophages might also mediate intracellular uptake by *M. leprae*. On monocytes, expression of PGL-1 leads to *M. leprae* phagocytosis via serum complement 3 and complement receptor CR3 [50]. On macrophages, CR1 and CR4 assist phagocytosis of *M. leprae* [69]. Another phagocytic receptor expressed on macrophages is the mannose receptor, which binds mannose and other carbohydrates [70, 71]. Nerve injury in leprosy involves both myelinated and unmyelinated nerves [72, 73]. Biopsy specimens taken from the affected nerves of leprosy patients has revealed perineural and intraneural inflammation and, in myelinated fibers, eventual demyelination. Axonal degeneration and regeneration can be observed with fibrosis, which leads to an empty matrix. Nerves occasionally undergo caseation and become difficult to recognize [74]. T cells activated by Schwann cell processing possess direct cytotoxicity. Presentation of *M. leprae* proteins and peptides to MHC class II-restricted $CD4^+$ Th1 cells, in turn, leads to inflammatory damage to the nerve [75]. TLRs are involved in immunological communications between the host and *M. leprae*. Schwann cell killing through the activation of TLR2 by *M. leprae* has been examined in vitro [76]. In tuberculoid disease, granulomatous inflammation develops at sites of infection, including the endoneurium, and may destroy infected nerve fibers. On the other hand, in lepromatous disease, prolific bacterial growth occurs inside Schwann cells and endoneural macrophages [77]. Persisting mycobacterial antigens (both protein and glycolipid) have been identified in nerves and these contribute to chronic ongoing neural inflammation [78]. Schwann cells exposed in vitro to necrotic neurons produce pro-inflammatory cytokines such as TNF-α and nitric oxide, which are potent inflammatory mediators [79]. TNF-α has been detected in nerve lesions and also promotes nerve damage [80]. Transforming growth factor beta (TGF-β), a downregulatory cytokine, has also been detected in the biopsies of infected hosts [81]. *M. leprae* can also deregulate mitogen-activated protein kinases (MAPKs) and monofilament proteins, which could lead to structural nerve damage [78]. These processes produce Wallerian and axonal damage in TT [82]. Patients with LL can show a very high number of bacteria but comparatively little inflammation. Acute and chronic neuritis may occur as a result of induction of an immunologic response to dead *M. leprae* in the nerve cells [23].

2.7 Treatment of Leprosy

M. leprae is naturally resistant to most of the usually prescribed antibiotics because of the high number of lipids in its cell wall, which prevent penetration by antibiotics, especially hydrophilic compounds (β-lactams, glycopeptides, fusidic acid, and chloramphenicol) [83]. Chaulmoogra oil, extracted from the seeds of the

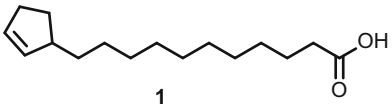

Fig. 3 Structure of hydnocarpic acid

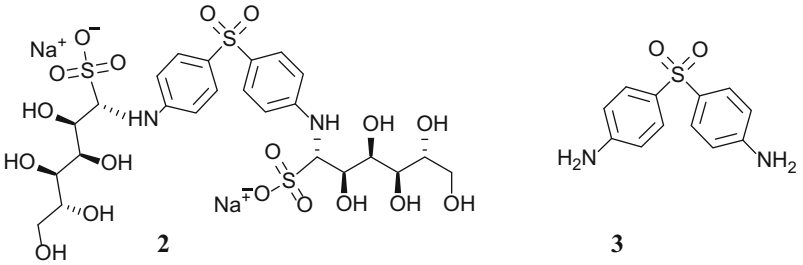

Fig. 4 Structures of sulfones used in the treatment of leprosy

Hydanocarpus wightiana tree, was the first leprosy treatment [84]. One of its compounds, hydnocarpic acid ($C_{16}H_{28}O_2$) (**1**; see Fig. 3), exhibits in vitro activity against some mycobacteria species, but is inactive against *M. leprae* [85].

2.7.1 Chemotherapy of Leprosy

The drugs presently used to treat leprosy can be divided into two main groups, first-line and second-line. First-line drugs are dapsone, rifampicin, and clofazimine. Second-line drugs are the fluoroquinolones, minocycline, and clarithromycin.

Dapsone

The modern era of leprosy chemotherapy began in 1940, when Faget showed the advantage of promin (sodium glucosulfone, **2**; see Fig. 4) in the treatment of leprosy [86].

Patients were intravenously administrated 5 mL of 30% promin solution, which causes severe pain to the patient. Promin and other sulfones cannot be used as substitutes for dapsone when intolerance develops, because this is a general reaction to sulfones, and is not specific to dapsone [87]. In 1950, a more effective sulfone, dapsone (4, 4′-diaminodiphenyl sulfone, **3**; see Fig. 4) became the standard chemotherapy drug for leprosy and was used across the globe for treating both MB and PB forms of the disease [88]. Its mode of action has been clearly established [89]. Dapsone competes with *para*-aminobenzoate (PABA) to bind with dihydropteroate synthase (DHPS) and inhibit the bacterial synthesis of dihydrofolic acid. DHPS is encoded by *folP1* and is involved in folic acid synthesis

[90, 91]. Dapsone is also known as a myeloperoxidase inhibitor. Myeloperoxidase is the enzyme that converts hydrogen peroxide into hypochlorous acid, which is the most potent oxidant generated by neutrophils during respiratory burst and causes significant tissue damage during inflammation, [92]. Therefore, dapsone also modulates the immune response by acting as a scavenger of active oxygen species. This mechanism of action is well correlated with its use in the treatment of dermatitis herpetiformis rheumatoid arthritis [93]. Dapsone may also have potential use in the treatment of Alzheimer's disease and stroke, because its myeloperoxidase inhibition mechanism has been suggested as a neuron-sparing mechanism for reducing inflammation in neurodegenerative diseases [94]. Chlorproguanil-dapsone is more potent than pyrimethamine-sulfadoxine and could be introduced in the near future to delay the onset of antifolate resistance and as "salvage therapy" for pyrimethamine-sulfadoxine failure in the treatment of malaria [95]. Long-term dapsone monotherapy led to treatment failure and the emergence of dapsone-resistant strains of *M. leprae* in the 1970s [96, 97]. Major adverse effects are uncommon in dapsone chemotherapy. Dapsone hypersensitivity is characterized by fever, hepatitis, and exfoliative dermatitis, which may be life threatening. Dapsone hypersensitivity generally develops after 4–6 weeks of treatment and, if symptoms develop, treatment must be discontinued and urgent medical attention sought. Mild hemolytic anemia is common in dapsone treatment, but severe hemolysis occurs in patients with glucose-6-phosphate dehydrogenase deficiency so dapsone should be avoided for these patients.

Rifampicin

Rifampicin (**4**; see Fig. 5), also known as rifampin, is an antibiotic used to treat a number of bacterial infections, mainly tuberculosis and leprosy. It is a semisynthetic compound from the naturally occurring rifamycins produced by *Nocardia mediterranea* [98, 99]. Although rifamycin (**5**) is poorly active, it is spontaneously oxidized and hydrolyzed to yield the active rifamycin S. Upon reduction, rifamycin S yields the hydroquinone form called rifamycin SV (**6**), which was the first member of this class to be used as an antibiotic. It was first introduced for the treatment of leprosy in 1970 and is currently the key bactericidal component of all recommended MDT regimens [100]. A single dose of rifampicin can reduce the number of viable bacilli to undetectable levels within a few days, with killing rates measured in excess of 99.9% after 1 month [101]. Rifampicin acts by specifically inhibiting bacterial DNA-dependent RNA synthesis by bacterial DNA-dependent RNA polymerase, leading to a massive failure in protein synthesis [102]. Rifampicin targets the β-subunit of RNA polymerase, which is encoded by *rpoB* [103]. The rifampicin binds in a pocket of the RNA polymerase β-subunit deep within the DNA/RNA channel, but more than 12 Å away from the active site (Fig. 6). Rifampicin acts by directly blocking the path of the elongating RNA by formation of a phosphodiester bond in the RNA backbone [104, 105]. Various other derivatives of rifampicin have been synthesized to achieve more potent molecules such as

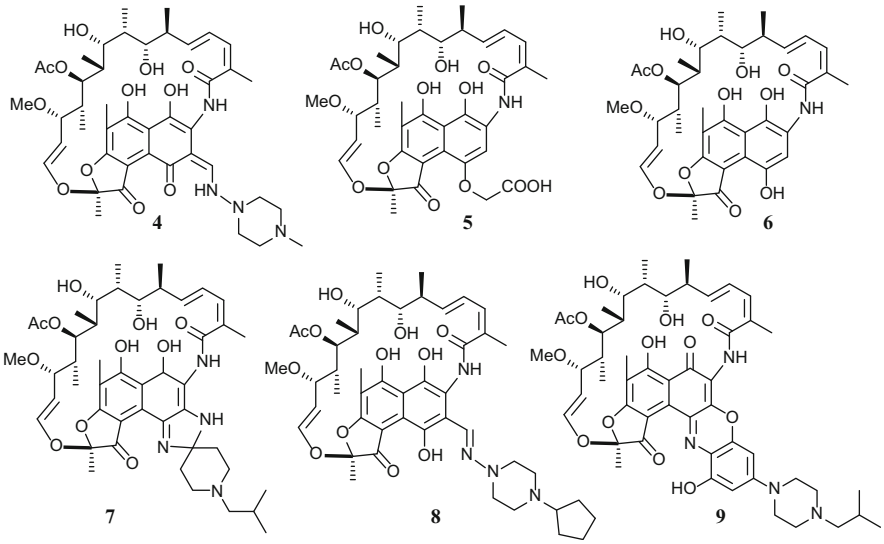

Fig. 5 Structures of rifampicin and its derivatives found active against *M. leprae*

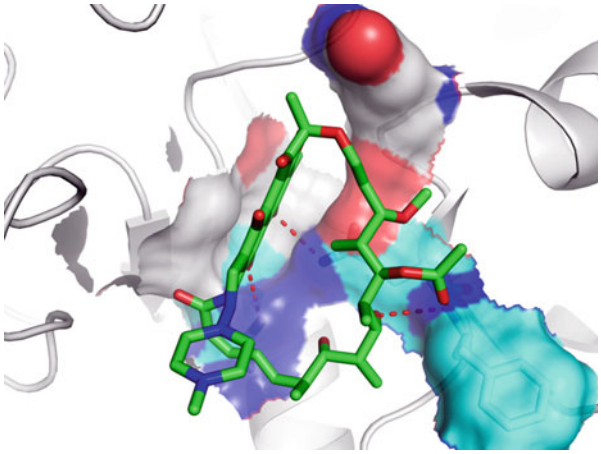

Fig. 6 Binding of rifampicin in the active site of RNA polymerase

rifabutin [LM 427 (**7**)], rifapentine [DL 473 (**8**)], and benzoxazinorifamycin [Rifalazil, KRM 1648 (**9**)] (Fig. 5). These molecules display stronger bactericidal activities against *M. leprae* than rifampicin [106–111]. However, there is no difference in antileprotic activity between patients treated with rifampicin 600 mg daily and those treated with rifamycin derivatives. These compounds can be used in chemotherapy only if they are active against rifampicin-resistant strains of *M. leprae*. Unfortunately, the claim that rifabutin is active against rifampicin-resistant *M. leprae* [106] has not been confirmed in supplementary experiments.

The major side effects associated with rifampicin are renal failure, thrombocytopenia, influenza-like syndrome, and hepatitis. Rifampicin resistance in leprosy is very rare. Rifampicin resistance in *M. leprae* also correlates with missense mutations within the *rpoB* gene (S425L mutation) [112]. Substitutions within codon Ser456 have been shown to be the most frequent mutations associated with the development of the rifampicin-resistant phenotype in *M. leprae* [113]. Docking studies have been very useful in the determination of ligand binding site [114–118]. A recent docking study demonstrated stable binding of rifampicin through two hydrogen bonds with the His420 residue of native *rpoB* than with mutant *rpoB*, where one hydrogen bond was found with Ser406. The difference in binding energies signifies that rifampicin is less effective in the treatment of patients with the S425L variant [119]

Clofazimine

Clofazimine (lamprene) [(3-*p*-chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino) phenazine] (**10**, Fig. 7) is a fat-soluble iminophenazine drug that has been used in multidrug therapy (MDT) for leprosy treatment since 1962. Clofazimine stabilizes the lysosomal membrane, which results in the inhibition of macrophages. Clofazimine also inhibits the motility of neutrophils [120], transformation of lymphocytes [121], proliferation of PBMCs induced by mitogen [122], and complement-mediated solubilization of preformed immune complexes in vitro [123]. The Kv1.3 (KCNA3) channel blocker activity of clofazimine in human T cells has opened a new direction for the treatment of rheumatoid arthritis, multiple sclerosis, and type 1 diabetes [124]. The immunosuppressive activity of clofazimine has been reported to be effective in treating autoimmune diseases such as psoriasis [125], Miescher's granulomatous cheilitis [126], Crohn's disease, and ulcerative colitis [127]. In a recent application of computer-aided drug design for the integration and optimization of drug discovery and preclinical development, clofazimine has been repositioned as an anti-trypanocidal agent [128]. Clofazimine has also been shown to inhibit the growth of triple-negative breast cancer cells via inhibition of canonical Wnt signaling [129]. The mechanism of action of clofazimine has not yet been fully elucidated and may be multifactorial. However, it has been suggested to work through its preferential binding with mycobacterial DNA, which appears to occur mainly at the base sequences containing guanine.

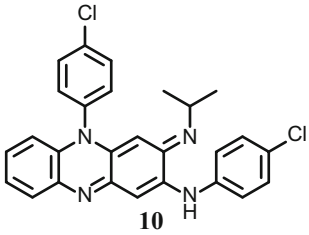

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Fig. 7 Structure of clofazimine

This may explain clofazimine's preference for the G+C-rich genomes of mycobacteria over human DNA [130]. Clofazimine-sensitive cellular mechanisms also include mitochondrial depolarization [131], upregulation of cellular phospholipase A_2 (which is toxic and inhibits bacterial proliferation), caspase activation [132], and induction of apoptosis [133].

Clofazimine is also a functional inhibitor of acid sphingomyelinase [134]. Clofazimine accumulates extremely in macrophages, forming insoluble, intracellular crystal-like drug inclusions during long-term oral dosing. These inclusions can modulate innate immune signaling by inhibiting TNF-α and boosting IL-1RA secretion [131]. To develop more potent phenazine derivatives, several derivatives have been synthesized, and some of them are significantly more active in vitro against *M. leprae* than clofazimine [135, 136], nevertheless none of them has shown activity vivo [137]. The lack of positive in vivo results could be a result of the pharmacokinetic properties, in particular the low lipophilicity of the derivatives, which is also responsible for skin coloration. It would be challenging to develop a nonpigmenting phenazine with higher in vivo activity against *M. leprae*, which could be used as a substitute for clofazimine in the treatment of leprosy.

Clofazimine is relatively nontoxic drug, but it produces skin pigmentation (pink to brownish) in leprosy patients and discoloration of most bodily fluids and secretions. These changes clear within 6–12 months after stopping treatment. Other associated skin-related side effects are icthyosis and pruritis. The anti-inflammatory activity of clofazimine is of great importance in leprosy because it suppresses erythema nodosum leprosum (ENL), an extensive inflammatory reaction occurring during treatment. In higher doses, clofazimine produces more skin pigmentation. Occasionally, clofazimine causes crystal deposition in the intestinal tract, which leads to severe gastrointestinal side effects that may mimic an acute abdomen [138]. Clofazimine-resistant leprosy has been reported a few times over the years. No molecular method is available for detection of clofazimine resistance [139, 140]. In a recent study conducted in *M. tuberculosis*, mutations in the *rv0678* gene, which encodes a transcription repressor of efflux pump MmpS5–MmpL5, causes overexpression of the efflux pump, which results in development of cross-resistance to both clofazimine and bedaquiline (TMC207) [141]. Shuo Zhang et al. characterized clofazimine mutants isolated in vitro from *M. tuberculosis* H37Rv and found two new genes (*rv1979c* and *rv2535c*) that are associated with clofazimine resistance in *M. tuberculosis* [142].

Fluoroquinolone Derivatives

Fluoroquinolones (Fig. 8), a new class of compounds with limited toxicity and characterized by broad spectrum antimicrobial activity, including against mycobacteria, have been introduced for the chemotherapy of leprosy. Ciprofloxacin (**11**) is more active than other commercially available fluoroquinolone derivatives, but is virtually inactive against *M. leprae* in mice, even at a dose of 150 mg/kg daily, most likely because of its lower in vitro activity against *M. leprae* [143] and

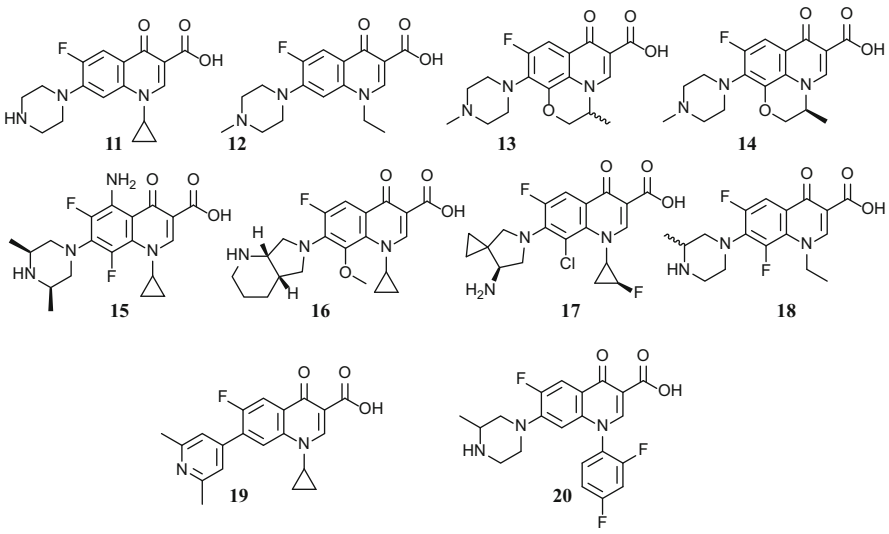

Fig. 8 Structures of antileprotic fluoroquinolones

unfavorable pharmacokinetic properties [144]. Pefloxacin (**12**) was the first fluoroquinolone to show promising activity against *M. leprae*. Pefloxacin was developed in 1979 and was approved in France in 1985 (http://www.bailii.org/ew/cases/EWHC/Patents/2008/2413.html). Ofloxacin (**13**) has the strongest in vitro and in vivo activities against *M. leprae*. Ofloxacin is a synthetic antibiotic of the second-generation fluoroquinolones. Ofloxacin was first patented in 1982 and approved by the FDA in December 1990. Ofloxacin is a racemic mixture of 50% levofloxacin (**14**) (the biologically active component) and 50% dextrofloxacin. Franzblau et al. reported that sparfloxacin is more active against *M. leprae* than ofloxacin [145]. Clinical trials of sparfloxacin (**15**) showed that the effect of 200 mg daily was similar to that of ofloxacin 400 mg daily, although the treatments have not yet been compared in the same trial [146]. Moxifloxacin (**16**) is a fourth-generation synthetic fluoroquinolone antibacterial agent that proved highly effective in all trial patients. A single 400-mg dose of moxifloxacin resulted in significant ($P \leq 0.006$) killing, of 82–99% of *M. leprae* bacilli, with a mean of 91% [147]. The in vitro antibacterial activity of sitafloxacin (**17**) (DU-6859a) against *M. leprae* was evaluated and found to be potent, with a minimum inhibitory concentration of 0.1875 μg/mL [148, 149]. The fluoroquinolones are rapidly developing, with many new compounds appearing that might prove to be more active against *M. leprae*.

Levofloxacin (**14**) possesses twofold greater antileprotic activity than ofloxacin and exhibits synergistic activity with rifabutin and other rifamycin analogs against *M. leprae* [150]. Other quinolones such as lomefloxacin (**18**), WIN 57273 (**19**), and temafloxacin (**20**) (Fig. 8) show good bactericidal activity in mice, but their effect on leprosy patients has yet to be studied [151]. The antileprotic effects of these

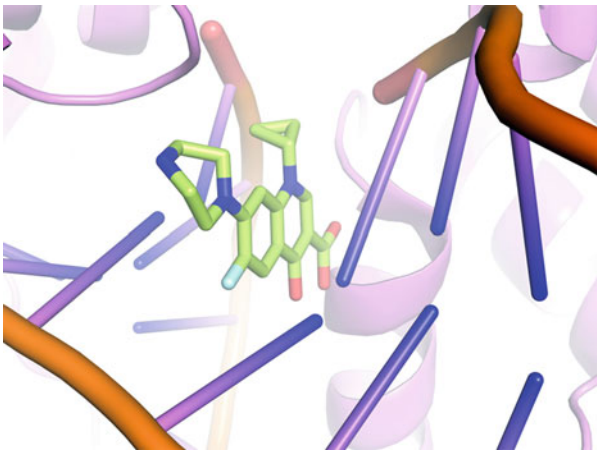

Fig. 9 Structure of bacterial DNA gyrase complexed with DNA and ciprofloxacin molecule (*green*); Protein Data Bank ID: 2XCT

fluoroquinolones appear to be a result of interactions with both DNA gyrase and topoisomerase IV, a related type II topoisomerase (Fig. 9). The DNA gyrase is more sensitive in Gram-negative bacteria, and topoisomerase IV more sensitive in Gram-positive bacteria. First and second generation fluoroquinolones selectively inhibit the topoisomerase II ligase domain, leaving the two nuclease domains intact; this results in DNA fragmentation via the nuclease activity of the intact enzyme domains (Fig. 9) [152]. Third and fourth generation fluoroquinolones have greater selectivity for the topoisomerase IV ligase domain, resulting in enhanced Gram-positive coverage. Some fluoroquinolone compounds have been shown to inhibit the synthesis of mitochondrial DNA [153].

In general, fluoroquinolones are tolerated, with side effects ranging from mild to moderate [154]. The major side effects associated with fluoroquinolone therapy are nausea, diarrhea, and other gastrointestinal complaints; skin rash may also occur. A variety of undesired CNS-related effects have been reported, including insomnia, headaches, dizziness, nervousness, and hallucinations. Quinolones are associated with an increased risk of tendinitis and tendon rupture in all age groups [155]. The FDA held a joint meeting on 5 November 2015, and asked the committee to review side effects in fluoroquinolone therapy (e.g., tendonitis and tendon rupture, peripheral neuropathy, and cardiac arrhythmia) to determine whether the use of these drugs justifies the associated risk [156]. The first ofloxacin-resistant *M. leprae* was described in 1994 and other cases have been found subsequently [157]. In most resistant strains of *M. tuberculosis*, *gyrA* mutations at highly conserved regions are responsible for the development of ofloxacin resistance. The *gyrA* gene is highly similar to that of *M. tuberculosis*, and missense mutations within codon Ala91 of this region have been found in the majority of ofloxacin-resistant strains of *M. leprae* [158].

Minocycline

Minocycline (7-dimethylamino-6-demethyl-6-deoxy-tetracycline) (**21**; Fig. 10) is prepared semisynthetically from natural tetracycline antibiotics and is the only tetracycline active against *M. leprae*. Although minocycline was synthesized by Lederle Laboratories in 1966, its activity against *M. leprae* was only confirmed in 1987 [159, 160]. Minocycline antileprotic activity may be a result of its lipophilic properties, allowing it to penetrate the cell wall more effectively than other tetracyclines [161]. In clinical trials, minomycin at 100 mg daily dose level displayed powerful bactericidal activity in previously untreated LL patients. After 28 and 56 days treatment, more than 99.9% of the *M. leprae* had been killed [162]. Minocyclin activity is additive when combined with other antileprosy drugs such as dapsone and rifampin. Minocycline is used for single lesion paucibacillary leprosy with rifampicin and ofloxacin in MDT.

The mechanism of action of minocycline against *M. leprae* is not clear but is considered to be similar to that of all tetracyclines, which are well-known protein synthesis inhibiters. The tetracyclines bind reversibly to the 30S ribosomal subunit, blocking the binding of aminoacyl-tRNA to the mRNA ribosome complex [163]. A recent study by Regen et al. showed that minocyclin could be involve in the inhibition of local cytochrome P450 (CYP450)-mediated degradation [164]. Minocycline is also known for its indirect inhibition of inducible nitric oxide synthase (NOS), which is a known retrograde neurotransmitter [165]. Minocycline has been confirmed to have neurorestorative as well as neuroprotective properties. Minocycline is proven to be beneficial in neurodegenerative diseases such as Huntington's disease, Parkinson's disease, and schizophrenia [166]. Minocyclin also has an anti-inflammatory effect, which is a result of its inhibition of apoptosis via attenuation of TNF-α, downregulating pro-inflammatory cytokine output [167]. Minocyclin is associated with side effects such as gastrointestinal upset, photosensitivity, and dizziness and is not recommended for children and pregnant women. Persistent serpentine supravenous hyperpigmented eruption (PSSHE) appears in LL after minocycline treatment [168]. There is no evidence of minocyclin resistance when used alone. The molecular mechanism of resistance to minocycline in *M. leprae* is still unknown, and the resistant strains have not been identified. Three different mechanisms have been suggested for tetracycline resistance: (1) energy-driven integral membrane protein-dependent efflux of tetracycline, (2) enzymatic inactivation of tetracycline, or (3) ribosomal protection by a soluble protein [169].

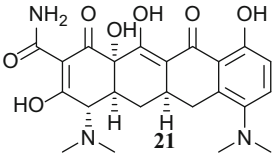

Fig. 10 Structure of minocycline

Clarithromycin

Clarithromycin or 6-*O* methylerythromycin A (**22**) is a semisynthetic macrolide antibiotic derived from erythromycin A (**23**) (Fig. 11) and displays significant activity against *M. leprae* in humans [162, 170]. In this category, erythromycin was the first drug to be tried for the treatment of leprosy, but was found to be ineffective in mice as a result of poor gastrointestinal absorption. In LL patients, daily administration of 500 mg of clarithromycin kills 99% of viable *M. leprae* within 28 days, and 99.9% by 56 days. Its mechanism of action is thought to be similar to that of erythromycin. Clarithromycin is first metabolized to 14-OH clarithromycin, which is active and works synergistically with its parent compound.

Clarithromycin initiates action by penetrating the bacteria cell wall and reversibly binding to domain V of the 23S rRNA of the 50S subunit of the ribosome, blocking translocation of aminoacyl tRNA and polypeptide synthesis. Clarithromycin also inhibits hepatic microsomal CYP3A4 isoenzyme and P-glycoprotein, an energy-dependent drug efflux pump [171, 172]. Clarithromycin can be taken orally because of its stability in the gastric acid medium. Clarithromycin has good absorption and diffusion through most tissues and phagocytes and is transported actively to the infection site. A missense mutation in the gene encoding 23S rRNA of bacteria and mycobacteria causes decreased binding of clarithromycin to ribosomes, with development of resistance [173, 174]. In the case of *M. leprae*, this resistance mechanism has not yet been confirmed as You et al. reported that no mutation of the 23S rRNA gene was observed in a clarithromycin-resistant strain of *M. leprae* [175].

Other Antileprotic Agents

There are a few novel therapies being investigated using antileprotic drugs, and preclinical studies are in progress for several new drugs (e.g., dialkyldithiocarbamates, bipyridyl analogs, diaryl-quinolines, and ansamycins) that have already been tested for other mycobacteria. The macrolide derivatives

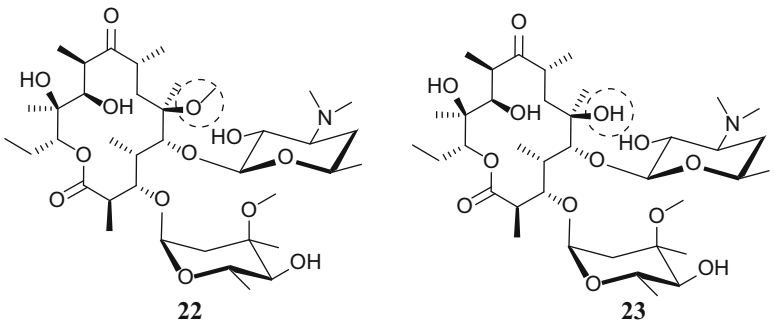

Fig. 11 Structures of clarithromycin and erythromycin

roxithromycin and fosfomycin have anti-inflammatory and immunomodulatory activities in addition to their anti-*M. leprae* activity [176], which may be of advantage in antileprosy treatment [177].

There are several reports of failures and ongoing research in antileprotic drug discovery. Some of these are related to fusidic acid, dihydrofolate reductase inhibitors (brodimoprim, K-130), cephalosporins (β-lactams), mycobacterial ribonucleotide reductase (MRR) inhibitors, deoxyfructo-5-hydroxytryptamine, and aminoglycosides.

Fusidic acid (**24**; Fig. 12) is active against *M. leprae* both in axenic medium and in macrophage culture, as determined in the BACTEC 460 system [178]. In a clinical trial, doses of 500 mg daily for 12 weeks/750 mg daily for 4 weeks followed by 500 mg daily for 8 weeks were given to lepromatous patients. After 8–12 weeks of treatment, all patients showed various degrees of clinical improvement and significant decline in the number of *M. leprae* in skin smears; however, the results of footpad inoculations showed that fusidic acid only displays weak bactericidal activity against *M. leprae* [179]. Therefore, the future of fusidic acid as an antileprosy drug remains uncertain.

The most important dihydrofolate reductase (DHFR) inhibitors are K-130 (**25**), brodimoprim (**29**), and epiroprim (**28**) (see Fig. 12). The X-ray structure of *E. coli* DHFR shows a positively charged arginine moiety in the cavity of the active center [180]. Trimethoprim (**27**) analogs bearing more negative substituents lead to enhanced activity against the isolated enzyme. However, such compounds exhibit permeability problems in intact bacteria. Synthesis of such as K-130, which is a hybrid of trimethoprim and dapsone through a linker is more lipophilic with negatively polarized but unionized groups binds with DHFR and additional binding sites than trimethoprim through the its dapsone part. The hybrid demonstrated higher activity than either trimethoprim or dapsone against *M. lufu*. After 4 months

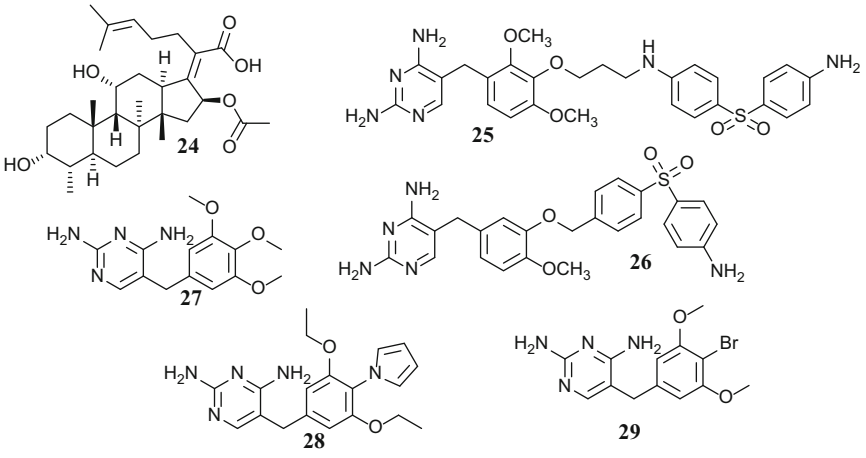

Fig. 12 Structures of fusidic acid and antileprotic DHFR inhibitors

of treatment with 0.03% K-130 alone, no viable *M. leprae* were detected. Brodimoprim stopped multiplication of *M. leprae* at a diet concentration of 0.1%, and at 0.05% in combination with dapsone (0.001%). Other experiments on *M. leprae* suspensions, measuring the inhibition of tritiated thymidine uptake and ATP levels, showed similar inhibition by both K-130 (**25**) and K-128 (**26**) [181, 182]. In vivo experiments revealed that combination of 0.01 mg/L of dapsone and 1 mg/L of brodimoprim resulted in 100% inhibition of the metabolic activity of *M. leprae*, and the effect was synergistic [183]. The first clinical trials in Paraguay and Ethiopia showed that combinations of brodimoprim and dapsone and of brodimoprim/dapsone plus rifampicin were highly effective for the treatment of leprosy. The tolerance of the regimens used was generally good [184]. An in vivo study of epiroprim, either singly or in combination with dapsone, against *M. leprae* using a mouse footpad model showed that a concentration of 0.05% in the diet completely inhibited the growth of both dapsone-sensitive and dapsone-resistant strains of *M. leprae* [185].

Cephalosporins, like penicillins, are β-lactam antibiotics that work by inhibiting the bacterial transpeptidase enzyme responsible for cell wall synthesis. Cephaloridine (**30**) was found to be effective against *M. leprae* in mice at doses of 300 mg/kg daily [186]. It was observed that the order of growth inhibition was cefoxitin (**31**) ≅ cephaloridine (**30**) >7-aminocephalosporanic acid (**32**), cephaloglycin (**33**), and cefuroxime (**34**) (see Fig. 13 for structures) [187]. Unfortunately, some of the cephalosporins are exceptionally expensive and further scientific effort may lead to cheaper and orally more effective β-lactam antibiotics.

Thiacetazone (**35**; Fig. 14) is known for its activity against *M. leprae*. It has been found that related thiosemicarbazones (TSC) are metal ion chelaters and act as inhibitors of the iron-containing bacterial enzyme MRR [188]. The toxicity of acylpyridine-TSCs was significantly reduced by replacing the thioamide group by different N-heterocycles. This led to the development of a new compound, PH22 (**36**), with increased antibacterial activity and chelating properties. The mode of action of PH22 derivatives is thought to be the result of inhibition of DNA synthesis

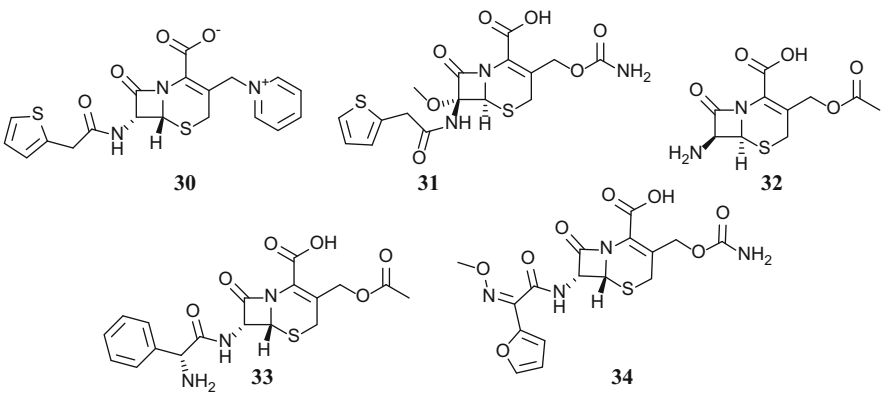

Fig. 13 Structures of antileprotic β-lactam antibiotics

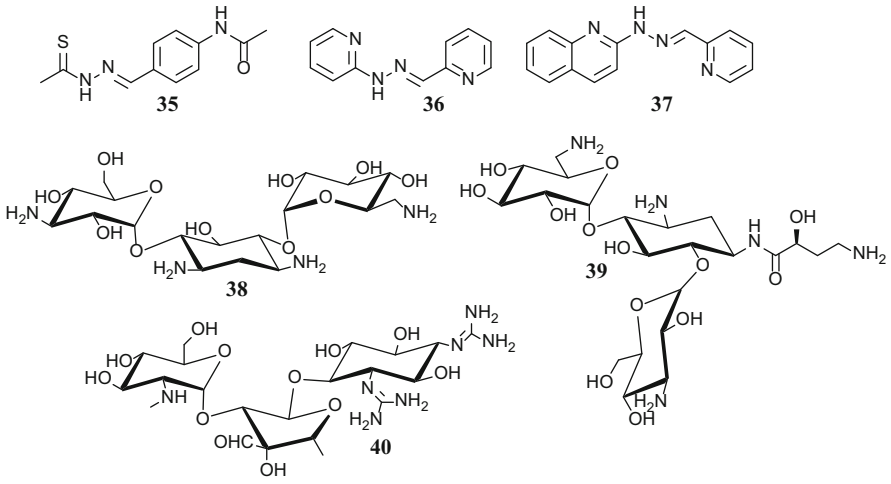

Fig. 14 Structures of antileprotic hydrazones and aminoglycosides

by inhibition of MRR. Another important molecule active against *M. leprae* is PQ22 (**37**).

Deoxyfructo-5-hydroxytryptamine (DF5HT) is a human metabolite, first detected in 1981 [189]. This metabolite shows antileprotic activity in vitro and in the mouse footpad system and was found to be bacteriostatic [190]. DF5HT is also active against bacilli in Schwann cells of a nerve culture, and in macrophages from multibacillary patients. Experimental results demonstrated enhanced lymphocyte–macrophage interaction promoted by the drug, which could be the result of stimulation of the CMI. In a clinical trial conducted in India, a dose of 10 mg/kg was given to six patients with MB for 6 months. Improvement occurred in five out of six patients, both in clinical and histological terms. In some cases, DF5HT showed the ability to clear bacilli faster than dapsone. This could be the result of an immunostimulating effect [191]. Scientists are also looking at other human metabolites, such as deoxyfructo-5hydroxytryptophan, deoxyfructo-serotonine, and some lipid-soluble derivatives of DF5HT [192]. The time taken for resorption and penetration into *M. leprae*-infected tissue is very dissimilar for these metabolites, and their application could be advantageous in the treatment of MB.

Aminoglycosides exert an antileprotic effect by binding the 30S ribosomal subunit; some also bind to the 50S subunit and result in the inhibition of protein synthesis and damage to the RNA translation function. Aminoglycosides have shown good activity in an animal model and in clinical trials conducted in Malaysia [193–195]. The two important compounds of this class, kanamycin (**38**) and amikacin (**39**) (Fig. 14), have shown potential activity against *M. leprae* [196]. Unfortunately, aminoglycosides are not absorbed if administered orally; intraperitoneal injections have been used in animal studies but are not very practical for larger scale administration. Furthermore, the high doses needed may cause renal and acoustovestibular damage in chronic therapy. At a dose level of 100 mg/kg,

both agents (kanamycin and amikacin) are bactericidal. At lower doses, kanamycin loses its activity but streptomycin is still effective [197]. Streptomycin (**40**) was found to combine synergistically with rifampin, even if administered only once a month. It is anticipated that monthly streptomycin might present an alternative to clofazimine in MDT, and could be used for MB patients once a month with rifampin [187].

2.7.2 Therapeutic Strategies and Drug Resistance

In order to improve treatment efficacy and drug resistance in *M. leprae*, WHO recommended MDT for leprosy in 1981. Initially, patients with MB were treated daily with dapsone and clofazimine along with a monthly dose of rifampin for 2 years or until the skin smear was negative. Modifications in the recommendations and diagnostic criteria have been made several times since 1981. Since 1998, WHO has recommended 1 year of treatment for MB and 6 months of treatment for PB. Additionally, WHO recommends that patients with a single lesion be treated with a single combination dose of rifampin (600 mg), ofloxacin (400 mg), and minocycline (100 mg) [66], Children with a single lesion should take half the adult dose of the three medications. However, it has been suggested that because of lack of long-term follow-up, this recommendation has to be considered experimental. Other drugs (i.e., rifabutin, ofloxacin, sparfloxacin, levofloxacin, minocycline, and clarithromycin) with antimycobacterial properties can also be used if needed as substitutes in the current therapeutic programs.

Treatment regimes as recommended by WHO		
6 month regimen for paucibacillary leprosy		
	Dapsone (daily in mg)	Rifampicin (monthly in mg)
Adult 50–70 kg	100	600[a] (once)
Child 10–14 years[b]	50	450[a] (once)

[a]Under supervision
[b]Adjust dose appropriately for a child of less than 10 years, for example, dapsone 25 mg daily and rifampicin 300 mg given once a month under supervision

12 month regimen for multibacillary leprosy			
	Dapsone (daily dose in mg)	Rifampicin (monthly dose in mg)[a]	Clofazimine
Adult 50–70 kg	100	600 (once)	50 mg (daily) and 300 mg[a] (once in a month)
Child 10–14 years[b]	50	450 mg (alternate days)	50 mg (daily) and 150 mg[a] (once in a month)

[a]Under medical supervision
[b]Adjust dose appropriately for a child of less than 10 years, for example, dapsone 25 mg daily, rifampicin 300 mg given once a month under supervision, clofazimine 50 mg given twice a week, and clofazimine 100 mg given once a month under supervision

Single lesion paucibacillary leprosy (one-time dose of three medications taken together)			
	Rifampicin (mg)	Ofloxacin (mg)	Minocycline (mg)
Adult 50–70 kg	600	400	100
Child 5–14 years[a]	300	200	50

[a]Not recommended for pregnant women or children of less than 5 years

MDT has been very practical and successful in the treatment of both MB and PB. There has been a considerable decrease in the overall number of registered cases globally; however, MDT has not been able to reduce the number of newly registered cases and the problem of drug resistance still persists. Some 19% of *M. leprae* isolates from biopsied samples were resistant to dapsone, rifampin, or clofazimine at various concentrations. It was also observed that 6.23% of the isolates were resistant to more than one drug in the mouse footpad susceptibility assay [198]. Several instances of *M. leprae* multidrug-resistant strains have been reported [113], for which ofloxacin and minocycline have been recommended. Although there is a lack of knowledge about the mechanism of resistance of *M. leprae* to antileprosy drugs, current understanding of drug-resistant cases reported for *M. tuberculosis* and other bacteria indicates that resistance in *M. leprae* could be a result of chromosomal mutation. This type of mutation is generally found in patients that had undergone inappropriate or inadequate drug therapy. It has been estimated that a mutation frequency of 10^{-6} and 10^{-7} to 10^{-8} in a population may be possible for dapsone and rifampin or ofloxacin, respectively [199, 200]. Clofazimine resistance rates in *M. leprae* are relatively low. Because undiagnosed MB patients can have large bacterial loads ($>10^{11}$ *M. leprae*), it is possible that a patient could contain up to thousands of rifampin- or ofloxacin-resistant organisms and 10^5 dapsone-resistant organisms. Inappropriate therapy for these patients could result in the spread of one or more resistant phenotypes.

2.7.3 Vaccination

In addition to WHO-recommended MDT, there are many indications that further efforts are required to prevent the re-emergence of leprosy and to eradicate it. These efforts include an effective vaccine with potential for both prophylactic and therapeutic use. An antileprosy vaccination can be immunotherapeutic or immunoprophylactic. The main objective in immunotherapy is to deactivate the mechanisms leading to immunopathology and to amplify intracellular mechanisms by which bacilli are killed. On the other hand, the immunoprophylactic approach is characterized by restoration of the host recognition of shared mycobacterial antigens to promote Th1 responses, to induce $CD8^+$ cytotoxic cells, and to downregulate the proportion of T cells producing Th2 interleukins. The vaccine being studied is *M. bovis* BCG, but its failure to protect certain populations clearly indicates that there is pressing need for an improved vaccine against leprosy. Other vaccines being used or explored are Mycobacterium w., Mycobacterium ICRC (*M. avium* intracellulare), and heat-killed *M. leprae*, *M. lufu*, and *M. habana*.

Researchers have identified the multiple antigens that are recognized by the T cells of leprosy patients, although it is currently unclear how or if their responses are affected during treatment [201, 202]. The BCG has been found to have good potential in highly endemic countries such as India and Brazil [203–205]. The comparative genomics of different mycobacterial species have been explored, resulting in the identification of new vaccine targets, which provides new hope in the direction of a new leprosy vaccine.

3 Buruli Ulcer

3.1 Introduction

Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, is the third most deadly mycobacterial infection, after tuberculosis and leprosy. BU is characterized by an indolent necrotizing infection of the skin, subcutaneous tissue, and bone. In the early stage of BU infection, a painless nodule is formed on the skin and the infection generally swells; later, the nodule later turns into an ulcer. In the later stage of infection, the ulcer becomes larger inside than at the surface of the skin, which becomes swollen as a result of the infection [206]. BU is more common in humid rural tropical areas and predominantly affects children aged between 5 and 15 years. Sir Albert Cook is supposed to have been the first to identified skin ulcers with BU in 1897, in Uganda. Later, in 1948, the disease was found in Bairnsdale, Australia, and the etiological agent was identified [207, 208]. The disease is sometimes called Bairnsdale or Searles' ulcer in Australia, and Kumusi ulcer in Papua New Guinea. The name Buruli ulcer was first used by Clancey and coworkers after correlating the geographic location of their observations in Buruli County, Uganda (now called Nakasongola District) [209]. In distinction to tuberculosis and leprosy, BU is related to environmental factors and is thus considered noncommunicable [210]. In July 1998, WHO organized the first international conference on Buruli ulcer control and research, and recognized BU as a re-emerging infectious disease in West and Central Africa with an important public health impact [211]. In the endemic region, BU causes public health and psychosocial problems to a very high extent because of potential disabling sequelae, estimated to occur in at least 60% of patients [212]. The number of patients with BU now exceeds those with leprosy or tuberculosis in some West African countries, which indicates its catastrophic effects [213]. Despite a re-emergence of BU over the past 10–20 years, the disease has for decades remained largely ignored in terms of funding and research by many national and public health programs [214].

3.2 Transmission and Epidemiology

There are two main theories related to the mode of transmission of *M. ulcerans*: (1) that infection is developed via direct inoculation of the skin from contaminated soil, vegetation, or water and (2) that infection is developed indirectly from the bites of insects that have become contaminated from the environment. The main risk factors associated with BU are proximity to a water source, poor wound care, and having uncovered skin. In the African endemic region, aquatic insects are thought to be potential vectors for transmission of the disease to humans [215, 216]. Marsollier and coworkers showed that *M. ulcerans* was transmitted to mice via the bites of aquatic insects having *M. ulcerans* in the salivary glands [217]. Portaels et al. isolated *M. ulcerans* in pure culture from an environmental specimen for the first time [218]. Even though this theory is widely accepted, there is little evidence to support the role of this type of aquatic insect as a major vector. The incubation period of Buruli ulcer was previously estimated to be between 4 and 13 weeks [219]. Recently, the mean incubation period was calculated at 4.5 months (18 weeks) [220]. The existence of colonies of native possums (small tree dwelling marsupials) with high concentrations of *M. ulcerans* in their gastrointestinal tracts and feces suggests that Buruli ulcer in this region may be a zoonosis and that humans are a spillover host [221–223]. Because there is no published evidence for direct person-to-person transmission of *M. ulcerans*, it is necessary to analyze whether contamination of an environmental reservoir with *M. ulcerans* from chronic ulcers plays a role in local transmission [224].

BU has been reported in more than 33 countries, mainly the rural wetlands of tropical and subtropical countries, especially in terrain that has seasonal flooding. A few reports have also come from tropical latitudes in southern Australia, China, and Japan. Incidence rates are currently highest in West and Central Africa (Benin, Cameroon, and Côte d'Ivoire). Other endemic countries are the Democratic Republic of the Congo and Ghana. In 2014, there were 2,251 new cases of Buruli ulcer globally and 2,151 of them were from the African region (Fig. 15). Since 2009, when 5,000 cases were reported, there has been a considerable reduction of more than 50% in the number of new cases [225]. The re-emergence of BU could be a result of environmental changes such as deforestation, topographic human-made alterations (dams, irrigation systems), and increasing numbers of people carrying out manual basic agriculture in wetlands [226, 227]. In Africa, about 48% of those infected are children under 15 years, whereas this number is 10% in Australia and 19% in and Japan. The genders are affected equally and no racial susceptibility is known. Most of the lesions are formed on the limbs, with highest frequencies on the lower extremities. Lesions in humans also arise at the sites of antecedent trauma, which is possibly the most common means by which *M. ulcerans* is introduced into the skin or subcutaneous tissue from surface contamination [228]. Reported trauma can be as severe as gunshot or landmine wounds or as slight as a hypodermic injection [229]. There are multiple reports of patients with Buruli ulcer in nonendemic countries (e.g., France, Germany, Canada, and the USA).

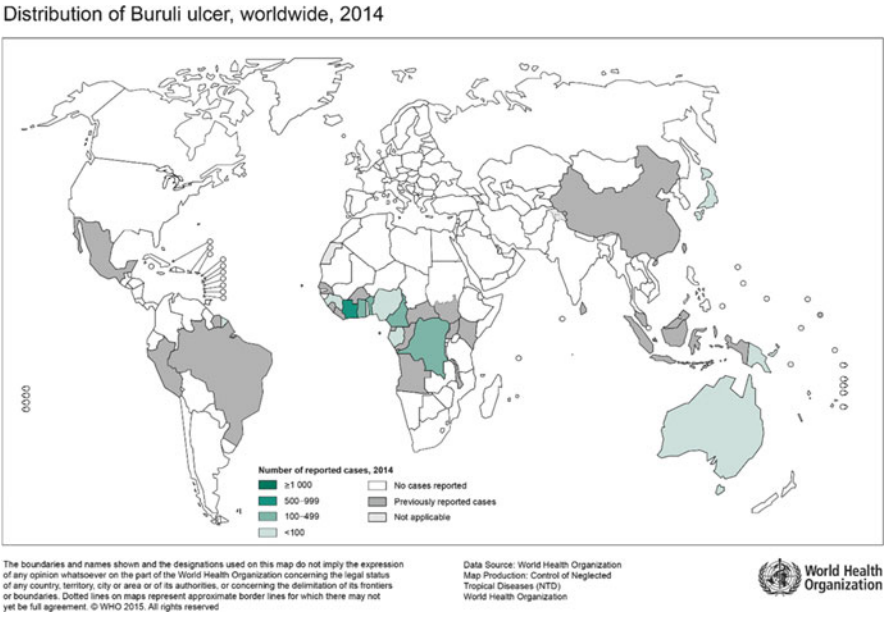

Fig. 15 Geographical distribution of Buruli ulcer worldwide, 2014

3.3 Bacteriology

M. ulcerans is an acid-fast (HCl), alcohol-fast, rod-shaped bacillus that is characterized by its slow-growing nature, optimally on routine mycobacteriologic media such as Löwenstein–Jensen medium or Brown and Buckle media at 30–32°C. The bacteria was first isolated and characterized in 1948 in Australia by MacCallum and associates [208]. *M. ulcerans* is microaerophilic (low, 2.5%, oxygen concentration required for growth), exquisitely sensitive to UV radiation, and sensitive to temperatures of 37°C or higher. These properties are in good agreement with the concept that in nature, especially tropical environments, the organism proliferates in stagnant water as a saprophyte, symbiont commensal, or parasite [230]. *M. ulcerans* is an environmental mycobacterium closely related to *M. marinum*, with >97% genetic homology and identical mycosides [231, 232]. *M. marinum* infections take place worldwide and are described by granulomatous inflammation and intracellular infection [233]. There are two main genomic differences between *M. ulcerans* and *M. marinum*. The first difference is the unique virulence plasmid pMUM, which encodes the enzymes that synthesize a novel type 1 polyketide called mycolactone [234]. Mycolactone is a necrotizing immunosuppressive soluble polyketide cytotoxin that exhibits the unique clinical and histopathological features of Buruli ulcer. Mycolactone inhibits the local immune response by diffusing into the surrounding tissues and inducing tissue necrosis [235, 236]. The second genetic difference is the presence of high copy-

number insertion sequences (ISs), IS2404 and IS2606. These ISs are the target of a *M. ulcerans*-specific PCR, which is now the gold standard for diagnosis of Buruli ulcer across the globe. The IS2404 PCR is extremely sensitive and specific, because of the high number of *M. ulcerans* cells present in most lesions and the high copy number of the PCR target inside each cell [237].

3.3.1 Metabolism

Studies of the metabolic pathways of *M. ulcerans* support the prominence of lipid degradation for central carbon metabolism, and have uncovered intact glycolysis and pentose phosphate pathways in lieu of the Entner–Doudoroff pathway [231]. *M. ulcerans* has a divided tricarboxylic acid (TCA) cycle; it converts α-ketoglutarate to succinate via α-ketoglutarate decarboxylase, which is evidenced by the absence of α-ketoglutarate dehydrogenase [238]. The TCA cycle incorporates the glyoxylate shunt, and thus *M. ulcerans* metabolism can lead to two-carbon degradation products (acetyl-CoA) and propionyl-CoA from β-oxidation of host fatty acids. The methyl citrate pathway metabolism of propionyl-CoA can occur in several ways. *M. ulcerans* lacks methylisocitrate lyase, which causes a reduction in the metabolism of propionic acid with a detrimental effect on growth [239]. *M. ulcerans* also lacks the nitrate and fumarate reductase systems, resulting in its aerobic rather than anaerobic nature. *M. ulcerans* has maintained >400 putative oxidoreductases, dehydrogenases, and mono- and dioxygenases, signifying that a robust and complex respiratory potential remains in aerobic conditions [231, 240].

The secondary metabolites and cell envelope of *M. ulcerans* have been more extensively studied than those of other mycobacteria. It has been found by genomics that, as a result of depletion of genes encoding polyketide synthase (PKS), *M. ulcerans* lacks the *pks2* locus required for the production of sulfolipids [241]. *M. ulcerans* liberates the energy (NADP) and substrates (malonyl-CoA and methylmalonyl- CoA) for the production of mycolactone. *M. ulcerans* has retained a significant anabolic lipid, which is essential for preservation of the mycobacterial cell wall. *M. ulcerans* has been shown to produce diunsaturated, methoxy, and ketomycolic acids and other lipids synthesized by fatty acid synthases I and II [242]. *M. ulcerans* also produces a highly abundant and apolar cell wall, whose lipids include diesters of phthiodiolone [243, 244]. In *M. ulcerans*, the β-diol backbone of phthiodiolones is produced from C_{16}–C_{18} fatty acids by five type I PKSs encoded by the genes *ppsA* to *ppsE*. These PKSs process *p*-hydroxyphenylalkanoate by producing phenolphthiodiolones, which undergo further modification by glycosylation to generate immune modulators, virulence factors, and phenolic glycolipids (PGLs) [232, 245]. *M. ulcerans* contains both mevalonate and nonmevalonate pathways for polyprenoid synthesis [246]. The polyprenyl phosphate forms lipid-linked sugar intermediates formed by sequential condensation of isopentenyl diphosphate and dimethylallyl diphosphate, which are required by bacteria during cell wall biosynthesis.

3.3.2 Genomics and Proteomics

M. ulcerans and *M. marinum* have more or less identical genomes, but are phenotypically different. *M. ulcerans* produces no photochromogenic pigments and replicates slowly, whereas *M. marinum* replicates twofold every 6–11 h with no mycolactone production. Stinear et al. reported the complete genome sequences from a strain of clinical *M. marinum* (strain M) and an African epidemic strain of *M. ulcerans* (strain Agy99, isolated from Ghana in 1999). This genomic analysis established the reductive evolution of *M. ulcerans* from *M. marinum* [231, 233, 247]. Computer-based genomic comparison of *M. marinum* and *M. ulcerans* established similar genetic compositions for these bacteria, with more than 4,000 orthologous and syntenic protein-coding DNA sequences (CDSs) with average 98.3% sequence identity. This study also confirmed a loss of 1.1 Mb of DNA in *M. ulcerans* as a result of deletions. Many chromosome rearrangements had occurred and insertion sequence elements were identified, especially IS2606 (91 copies) and IS2404 (213 copies), which disrupt more than 110 genes [231]. *M. ulcerans* strains have 11 chromosomal CDSs that seem to be bacterium specific and might, in combination with mycolactone, resulting in the pathogenesis associated with BU. In conclusion, it is better to say that *M. ulcerans* has evolved by lateral gene transfer and reductive evolution, procurement of the virulence plasmid pMUM001, huge expansion of IS2606 and IS2404 content, extensive formation of pseudogenes, rearrangements of the genome, and deletion of genes.

PE and PPE are protein families particularly found in the cell envelope of mycobacterium, but their function has not been elucidated. The PE and PPE proteins are predominantly rich in glycine and alanine, have characteristic N-terminal domains, and are influenced by amino acid content. The reductive genome of *M. ulcerans* leads to a decrease in PE and PPE proteins from 281 in *M. marinum* to 115 in *M. ulcerans*, supplemented by reduction in the related ESX secretion systems and their effector proteins [248]. The members of the ESAT-6 (6 kDa early secretory T cell antigenic target) protein family and specific effectors such as EspA (ESX-1 secretion-associated protein A) are exported by the ESX loci, which encodes type VII secretion systems [249, 250]. The genes encoding LipY (an immunodominant PPE protein with triacylglycerol hydrolase activity) have been deleted in *M. ulcerans*. Furthermore, the number of ESX secretomes is depleted from five systems to three [231, 251]. As a result of this, there is a loss of expression of ESAT-6 (considered important in granuloma formation and pathogenesis) and the EspA 1068 effector proteins, which are reduced to 2 paralogs in *M. ulcerans* from 18 in *M. marinum* [250, 252]. Huber and coworkers revealed that some strains of *M. ulcerans* display a similar pattern to that of *M. ulcerans* Agy99, whereas others have acquired loss-of-function mutations or independent deletions in these regions, suggesting that loss of the ESX loci is an advantage for mycolactone producers [253]. *M. ulcerans* also synthesizes phenolphthiodiolone (highly apolar and abundant polyketide-derived methyl-branched lipid intermediate, responsible for PGL synthesis) but cannot make PGL, because the gene for the

glycosyl transferase that adds the rhamnosyl moiety (locus tag mUL_1998) is inactive [232].

3.4 Pathogenesis and Immune Response

3.4.1 Mycolactone and Pathogenesis

Mycolactones are polyketide-derived macrolides with poor immunogenic potential [254]. Mycolactone A/B is the most active and prevalent, and is representative of *M. ulcerans* strains from Africa [255]. If mycolactone A/B (**41**¸ Fig. 16) is added externally, there is intense cytotoxic activity in vitro, affecting macrophages, monocytes, lymphocytes, neutrophils, fibroblasts, adipose cells, epithelial cells, and DCs [236, 254, 256, 257]. Mve-Obiang showed that 0·01 ng/mL of mycolactone A/B is enough to induce cell death in L929 cells [255]. The apoptogenic activity of mycolactone is linked to cytotoxicity, because Buruli ulcer lesions are reported to show massive apoptosis [258]. In 1999, mycolactone was isolated and shown to cause cell-cycle arrest in cultured L929 murine fibroblasts [235]. Mycolactone is composed of a 12-membered ring (macrolide) to which two polyketide-derived side chains are attached. Mycolactone is structurally similar to immunosuppressants such as rapamycin, FK506, and cyclosporin A. The characteristic mixture of mycolactone congeners differs in clinical isolates from different geographical areas [255]. Mycolactone A/B has the highest activity in mammalian cell lines. It exists as a 3:2 equilibrium mixture of Z-$\Delta^{4',5'}$ and E-$\Delta^{4',5'}$ isomers, as shown in Fig. 16 [259].

Stinear et al. reported the identification of a giant plasmid (pMUM001) bearing mycolactone-producing enzymes. The plasmid pMUM001 is composed of 174,155 base pairs (bp), with 62.8% G+C content. Its 81 protein-coding DNA sequences bear a cluster of genes for complete mycolactone synthesis [234, 260]. A type II thioesterase and a FabH-like ketosynthase may play important roles in the chain termination and transfer of the mycolactone acyl side chain to the

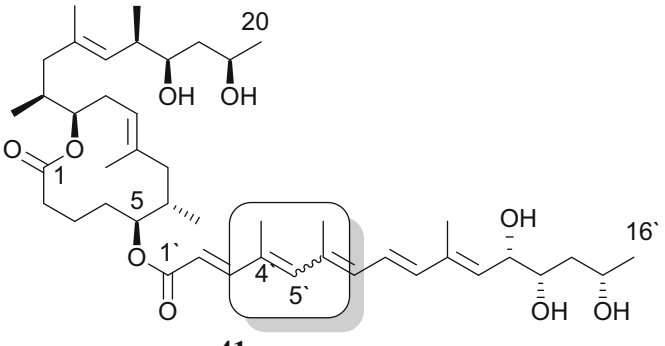

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Fig. 16 Mycolactone A/B as Z-$\Delta^{4',5'}$/E-$\Delta^{4',5'}$ = 3:2 dynamic equilibrium mixture

core [254]. The discovery of pMUM001 had a great impact on mycobacterial research because mycobacterial plasmids had never been directly linked to virulence. The role of mycolactone in *M. ulcerans* survival in nature has not yet been clarified. A painless lesion and poor acute inflammatory cellular infiltration are characteristics of BU infection. The mechanism of loss of pain sensation (hypoesthesia) is related to nerve degeneration, which occurs through invasion of bacilli or mycolactone at the perineural and endoneurial level [261, 262]. Recently, it has been shown that mycolactone activates type 2 angiotensin II receptors, which results in hypoesthesia through potassium-dependent hyperpolarization of neurons [263]. Mycolactone-exposed cells undergo cytoskeletal rearrangement in vitro as a result of a modification in actin dynamics and binding of mycolactone to Wiskott–Aldrich syndrome protein (WASP) and neural WASP [264]. Mycolactone has different effects on DCs and T cells, signifying that it might bind to a different receptor and interfere with distinct signaling pathways. Mycolactone also inhibits the cytokine production and function of the Sec61 translocon (responsible for translocation of proteins into the endoplasmic reticulum) and affects the prepro-α factor, IL-6, and β lactamase (conventional secreted proteins; two nonmammalian model proteins and a cytokine used routinely in translocation assays); TNF and thrombomodulin (both type I membrane proteins); and Cox-2 (an enzyme resident in the endoplasmic reticulum) [265, 266].

Scherr and coworkers studied both naturally and non-naturally occurring variants and established the structure–activity relationship of synthetic mycolactones. They showed the importance of the lower C5-O-linked polyunsaturated acyl side chain and the C-linked upper side chain. Greater variation in cytotoxic activity against mammalian cells was observed as a result of changes in the lower side chain compared with changes in the upper side chain. Mycolactone A/B had no antimicrobial activity against Gram-negative and Gram-positive bacteria and was not active against Dictyostelium and Saccharomyces [267]. Hence, generation of additional mycolactone variants is required to obtain a better understanding of the pathogenic effect. A nontoxic variant that competes with mycolactone for receptor binding could eventually lead to the development of novel anti-BU drugs.

3.4.2 *M. ulcerans*-Associated Immune Response

Mycolactone is involved in immune suppression by affecting both the innate and adaptive immune responses. Infection is associated with granulomas, initiated by infected macrophages in mycolactone-negative strains of *M. ulcerans* [268]. According to Schutte et al., antibiotic treatment for 8 weeks leads to an active inflammatory process in skin compartments as a result of reversal of local immunosuppression [269]. Mycolactone has been shown to interfere with phagocytosis by J774 macrophages [268]. An intramacrophage growth phase is also observed, which is in accordance with the development of cell-mediated and delayed-type hypersensitivity responses in BU patients [270]. Toraddo et al. showed that mycolactone modulates macrophage microbicidal activity as a result of dose-dependent inhibition of the IFN-γ-induced protective mechanisms,

including phagosome maturation/acidification and increased NO production, leading to increased bacterial load [271]. Mycolactone also inhibits production of chemokines, cytokines, and other secreted immune modulators and intracellular effector molecules such as Cox-2 and IL-1β [257, 272]. Mycolactone had no effect on the TLR-dependent activation of the MAPK and nuclear factor (NF)-κB signaling pathways.

At noncytotoxic concentrations (<50 ng/mL), mycolactone inhibits the phenotypic and functional maturation of DCs and, in response to TLR ligands, exerts a selective effect on the secretion of inducible chemokines and cytokines by DCs [273]. This suppressive effect inhibits the capacity of DCs to prime cellular immune responses, resulting in the initiation of adaptive immune responses.

Because mycolactone inhibits production of IL-2 by activated T cells, its immunosuppressive properties extend to lymphocytes [274]. The production of PMA/ionophore-induced chemokines and cytokines by primary human $CD4^+$ T lymphocytes is strongly inhibited by mycolactone. Mycolactone also strongly inhibits the production of many Th1, Th2, and Th17 cytokines by T cells [275]. The large dose (50 or 100 μg) used for subcutaneous administration of mycolactone causes T cell homing in vivo, resulting in massive depletion of T cells in the peripheral lymph nodes, which is associated with defective expression of L-selectin [276]. Moreover, mycolactone reduces the levels of L-selectin, because it also reduces the expression of microRNA *let-7b*. Recently, caspase-3 was described as a target of *let-7b* in mesenchymal stem cells, signifying that reduced *let-7b* contributes to apoptosis in T cells [277].

The main characteristic of Buruli ulcer is that lesions are painless, despite extensive tissue necrosis. En et al. showed that the nerves in the perineurium are invaded by *M. ulcerans* and that invasion can extend further to the endoneurium, with degeneration of vacuoles in Schwann cells involved in the formation of myelin. Mycolactone at a dose level of 100 μg induced nerve damage, hemorrhage in neurons, neutrophilic infiltration with loss of nuclei in Schwann cells, and vacuolar changes in myelin [262, 278]. These studies suggest a direct role of mycolactone in the destruction of nerves; however, no evidence of neural degeneration was observed in mycolactone-induced analgesia [263].

3.5 Buruli Ulcer Manifestation and Classification

Buruli ulcer at the skin level manifests as papules, nodules, plaques, edema, and ulcers, defined as follows:

A *papule* is usually a painless, non-tender, sometimes itchy, raised intradermal skin lesion of <1 cm in diameter. The surrounding skin may be reddened. Papules are commonly observed in Australia and can be confused with an insect bite (Fig. 17a).

A *nodule* is a lesion 1–2 cm in diameter that extends from the skin into the subcutaneous tissue. It is typically firm and painless, but may be itchy. The

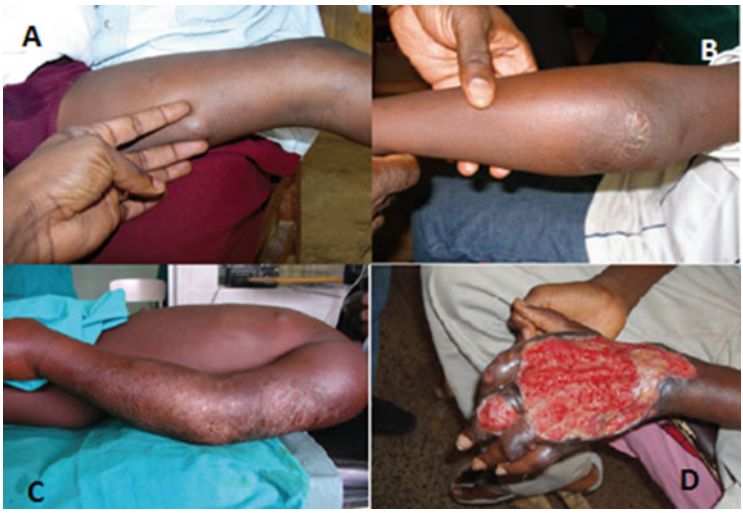

Fig. 17 Skin level manifestations of Buruli ulcer: (**a**) papule, (**b**) nodule, (**c**) plaque, and (**d**) ulcer [279]

surrounding skin may be discolored, often hypopigmented, in comparison with adjacent areas. Nodules are commonly seen in Africa (Fig. 17b).

A *plaque* is a firm, non-tender, painless, elevated, well-demarcated, indurated lesion >3 cm in diameter with irregular edges. The skin around the lesion is often also discolored; in dark-skinned people it is generally hypopigmented (Fig. 17c).

Edema is diffuse, often extensive, firm, usually non-pitting swelling with ill-defined margins, which involves part or all of a limb or other part of the body. There may be changes in color over the affected area of skin. It may be painful.

An *ulcer* is a painless, deep ulcer extending into the subcutaneous fatty tissue. It has undermined edges and variable induration extending from the margin of the ulcer into the surrounding healthy skin, usually with good differentiation on palpation. The surrounding skin may also be edematous. The floor of the ulcer may have a white, cotton wool-like appearance as a result of necrotic slough. Untreated ulcers are painless, unless there is secondary bacterial infection. When there is more than one ulcer and the ulcers are close together, they often communicate beneath normal-looking skin and could spread over a sizeable distance (Fig. 17d).

Osteitis/osteomyelitis is a complication of severe cases, usually resulting from contiguous spread of infection from covering non-ulcerative or ulcerative disease, especially on the forearm or lower leg. Some cases could be the consequence of hematogenous spread of *M. ulcerans*. Joints and small bones are often involved.

WHO has introduced a classification system based on lesion size and treatment recommendation. The two reasons behind this classification are (1) small lesions

are more likely to heal with only antibiotic treatment and (2) small lesions reflect the health-promoting impact of early diagnosis and can therefore be used to monitor progress [279]. Classification is based on three categories:

- *Category I*: a single lesion <5 cm in diameter including the indurated areas defined by palpation. Most category I lesions heal completely with antibiotic treatment.
- *Category II*: a single lesion measuring 5–15 cm in diameter including the indurated areas defined by palpation. Some category II lesions heal completely with antibiotic treatment.
- *Category III*: a single lesion >15 cm in diameter, multiple lesions, lesion(s) at a critical site (eye, breast, genitalia), and osteomyelitis. Category III ulcers are typically managed, in addition to antibiotics, by surgery (debridement and skin grafting) to achieve an acceptable rate of healing. Multiple small lesions and lesions at critical sites may heal with antibiotics alone. Treatment indications may differ according to the subcategory:
  (a) A single lesion >15 cm in diameter and osteomyelitis (complete antibiotics before surgery)
  (b) Lesions at critical sites (complete antibiotics and carefully avoid surgery if possible)
  (c) Small multiple lesions (complete antibiotics, if possible, before considering surgery)

3.6 Treatment

The traditional approach for treatment of BU has been surgery to remove necrotic tissue and subsequent skin grafting to repair the defect [280, 281]. This approach is therapeutic for many small lesions, but relapse rates in some centers can be as high as 18–47% [282]. In Australia, a relapse rate of up to 32% was reported for those treated only with surgery [283]. In 2012, WHO published "*Treatment of Mycobacterium ulcerans disease (Buruli ulcer): Guidance for health workers*" [279]. WHO currently recommends 8 weeks of multidrug therapy with a combination of rifampicin, streptomycin, clarithromycin, and moxifloxacin for the treatment of BU.

3.6.1 Chemotherapy

Rifampicin: As described in the section "Rifampicin," rifampicin is a semisynthetic derivative of rifamycin. It has bactericidal action against tubercle bacilli in cellular locations, as already described. The recommended dose of rifampicin is 10 mg/kg body weight (8–12 mg/kg) daily or three times weekly; maximum daily dose is 600 mg. Vitamin K should be co-administered with rifampicin during pregnancy to reduce the risk of postnatal hemorrhage.

Streptomycin: As described in the section "Other Antileprotic Agents," streptomycin is an aminoglycoside bactericidal antibiotic (antimycobacterial) drug. The recommended dose of streptomycin in adults is 15 mg/kg body weight (12–18 mg/kg) daily or two or three times weekly; maximum daily dose is 1,000 mg. Streptomycin causes auditory nerve impairment and nephrotoxicity in the fetus and should not be used in pregnancy.

Clarithromycin: As described in the section "Clarithromycin," clarithromycin is a semisynthetic macrolide antibiotic derived from erythromycin A. It displays significant activity against *M. ulcerans* in humans. Clarithromycin is converted to 14-OH clarithromycin by rapid first-pass hepatic metabolism that is less active against *M. ulcerans*. The conversion of clarithromycin to 14-OH clarithromycin increases for co-administration with rifampicin, whereas the blood concentration of rifampicin increases slightly. Of all the macrolide drugs, clarithromycin and its metabolites have the best oral bioavailability (50%) and can thus be administered orally. The recommended dose of clarithromycin for adults is 7.5 mg/kg twice daily using immediate-release tablets and 15 mg/kg once daily for extended-release capsules. For children, the recommended dose is $<$ 20 kg body weight using immediate-release tablets or 7.5 mg/kg once or twice daily for pediatric suspension. Clarithromycin should not be used during pregnancy except under clinical circumstances in which no alternate therapy is available.

Moxifloxacin: As described in the section "Fluoroquinolones Derivatives," moxifloxacin is a synthetic fourth-generation fluoroquinolone antibiotic. The presence of a methoxy group at the C8 position of the fluoroquinoline ring makes it less likely that Gram-positive bacteria will become resistant. Multiple mutations in bacteria are necessary for the development of moxifloxacin resistance; hence, resistance to moxifloxacin develops slowly. The recommended dose is 400 mg once daily for all indications. No dosage adjustments are essential for gender, aged populations, low body weight adults, mild or moderate hepatic insufficiency, renal impairment, or patients on chronic dialysis.

3.6.2 Combination Therapy

According to WHO guidelines and published results [279], clarithromycin is the preferred oral companion drug to rifampicin. The combination use of ciprofloxacin instead of moxifloxacin is based on published in vitro results of its activity against *M. ulcerans* [284, 285], and on clinical experience with its use in combination with rifampicin [286–288]. However, use of ciprofloxacin alone has not been studied in clinical trials and it is not currently recommended by WHO. In a mouse model, clarithromycin combined with a fluoroquinolone antibiotic showed effectiveness, and can be used if rifampicin is contraindicated or not tolerated [289]. The effectiveness of rifampicin and amikacin and their combination was estimated in the experimental treatment of mice. After 7 weeks of treatment with this combination, no viable bacilli

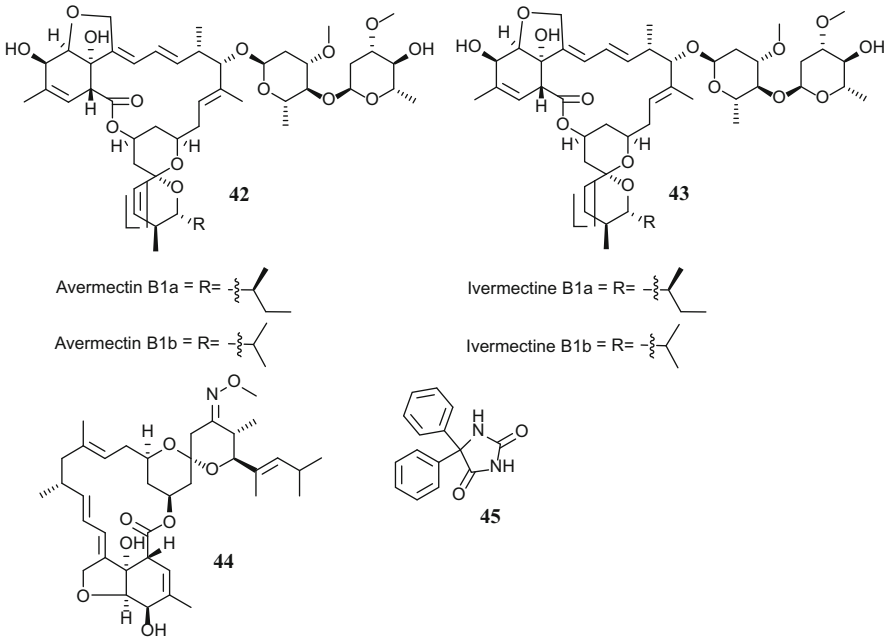

Fig. 18 Structures of drugs found to be active against *M. ulcerans*

were found in the infected tissues and these remained uninfected during the following 6 months with no sign of relapse [290]. The healing of BU lesions is slow and can continue for up to 12 months after completion of the recommended 8-week antibiotic regimen if skin defects are large, particularly if the diagnosis of BU was delayed [291]. A recent study related to prolonged streptomycin administration revealed significant persistent hearing loss; nephrotoxicity was also present in both adults and children but appeared to be transient [292]. Omansen et al. showed that the avermectins (**42**), ivermectin (**43**), and moxidectin (**44**) (see Fig. 18). inhibited growth of *M. ulcerans* at 4–8 μg/mL and showed dose-dependent killing in culture-based and bioluminescence assays. The avermectins are low-priced and already in use for treatment of river blindness. Thus, there may be a chance to repurpose a well-tolerated drug for the treatment of BU [293].

3.6.3 Thermotherapy and Other Methods

M. ulcerans grows best at 30–33°C and not above 37°C and this property has been explored for therapeutic purposes. A temperature of approximately 40°C maintained in the ulcerated area for 68 days managed to cure all treated patients. There was no evidence of local recurrence during follow-up periods of up to

22 months [294]. In 2007, a cheap phase-change material device, widely used in commercial pocket heat pads, was explored as a prospective treatment for ulcerative lesions. In patients with small ulcers, complete wound healing occurred without further intervention, whereas patients with larger defects underwent skin grafting after successful heat treatment. All patients were relapse-free 1.5 years after treatment [295].

Ointments generating topical nitrogen oxides promoted healing in one small controlled trial and could be a treatment of choice in resource-poor regions [296]. Nitric oxide has also been shown to kill *M ulcerans* in vitro [297]. Phenytoin (**45**) powder appears to promote healing, possibly as a result of accelerated fibrogenesis [298]. The effectiveness of hyperbaric oxygen (2·5 kPa of partial pressure) in humans need to be confirmed through controlled trials, although its effectiveness has been proven in mice infected with *M. ulcerans* [299, 300].

3.7 Vaccine

There is no specific vaccine against BU. BCG vaccination may provide some protection against *M. ulcerans* infections for 6–12 months and appears to prevent BU-related osteomyelitis in adults and children [301]. BCG vaccines are used to boost the immune response by the use of recombinant BCG overexpressing protective antigens [302, 303]. Sometimes, boosting through adjuvanted subunit protein vaccines [304–306] or viral vectors expressing *M. tuberculosis* antigens is also used [307]. The limited protection of BCG vaccination is a result of antigenic differences between BCG and mycobacterial strains of *M. ulcerans*. The use of a live, attenuated mycolactone-deficient *M. ulcerans* strain could be another alternative. The advantage of this approach is the expression of all antigens produced by virulent *M. ulcerans* bacteria, except the mycolactone toxin. As a result, a broad spectrum of humoral and cellular *M. ulcerans*-specific immune responses are induced [308]. In *M. ulcerans*, many proteins are highly homologous with other mycobacterial proteins; for example, 84% amino acid sequence identity was found between antigen 85A (Ag85A) of *M. ulcerans* and *M. tuberculosis* [309]. Delay in BU progression in mice was achieved by the use of a DNA vaccine coding for Ag85A from either *M. tuberculosis* or *M. ulcerans*, with better protection in the case of *M. ulcerans* [310]. Protection against *M. ulcerans* in mice was observed by the use of DNA vaccination with *M. ulcerans* heat shock protein (Hsp)65 [311]. However, this Hsp is immunogenic and homologous with human Hsp60, which is a serious drawback for vaccine development [312]. To develop a vaccine, there is a need for identification of specific *M. ulcerans* antigens. Some protein-coding sequences of plasmid pMUM001 may encode surface-exposed antigens and, if these surface-exposed antigens turn out to be more than hypothetical, they could have implications for serodiagnosis as well as for vaccine development [210].

4 Trachoma

4.1 Introduction

Trachoma, also known as granular conjunctivitis, Egyptian ophthalmia [313], and blinding trachoma, is an infectious disease caused by the obligate intracellular bacterium *Chlamydia trachomatis*. The trachoma, described in the Egyptian Ebers Papyrus in 1500 BC, is one of the ancient diseases of humankind [314, 315]. Repeated infection over many years leads to chronic sequelae, with pain, discomfort, and permanent damage to the cornea of the eye, leading to irreversible visual impairment or blindness. In fact, trachoma is the one of the most common cause of blindness worldwide after cataracts, age-related macular degeneration, corneal opacity, diabetic retinopathy, and vitamin A deficiency (in children) [316]. The infection is transmitted through eye contact and the nasal discharge of infected people, predominantly young children. It is also transmitted by flies having contact with infected people. In trachoma, a severely scarred eyelid (trachomatous conjunctival scarring) turns inwards, causing the eyelashes to rub against the eyeball (trachomatous trichiasis) resulting in constant pain and light intolerance. Women are two to three times more prone to blindness than men. Although trachoma is more related to the blindness caused by *C. trachomatis*, it is important to consider its role in sexually transmitted diseases. Chlamydial urogenital infections can cause cervicitis, urethritis, pelvic inflammatory disease, and infertility. Trachoma mostly occurs in Africa and Asia, but is also found in South America and Australia. Nowadays, due to improvements in living standards, trachoma has completely disappeared from the developed world. However, it still has catastrophic effects on people in less developed countries. The main causes for the spread of trachoma in these areas are crowded living conditions, poor sanitation, and scarcity of clean water and toilets [313, 317].

4.2 Transmission and Epidemiology

The symptoms of trachoma in aged peoples are a result of their exposure to trachoma when young. This is relevant to the fact that, after disappearance of trachoma, late sequelae (including trichiasis) can still occur for decades [318, 319]. Cross-sectional and longitudinal studies have shown that a lower risk of trachoma is associated with a clean face [320]. Facial cleanliness also decreases the severity of trachoma, probably by reducing the probability of transmission [321]. Because water is necessary for face washing, its source is closely related to the prevalence of active trachoma. Crowded conditions and close contact enable exchange of infected secretions, especially among children. Eye-seeking flies have been supposed to be physical vectors for *C. trachomatis* and it has also been identified in trapped flies; however, transmission through flies is not certain [322, 323]. Whether the presence of a latrine and its distance from the house affect the chances of trachoma is not clear, although the absence or distance of latrines might reduce breeding sites for the eye-seeking fly *Musca sorbens* [324].

Trachoma is endemic in many of the remote and poorest areas of Asia, Africa, the Middle East, and Australia (Fig. 19) [325]. An intensive global trachoma mapping effort is ongoing at present and should be completed soon [326]. WHO classes 51 countries as endemic for trachoma and estimates that 232 million people live in these endemic areas, with most blinding trachoma in Africa. Active trachoma affects an estimated 21 million people, with visual impairment of about 1.8 million people, of whom 0.5 million are irreversibly blind. In 2013, in the 29 endemic countries of the Africa region, 204,000 cases of trichiasis were operated upon and 55 million people were treated with antibiotics for trachoma [317, 327]. The estimated number of people affected by trachoma has fallen from 360 million people in 1985 to approximately 80 million people today (Table 1).

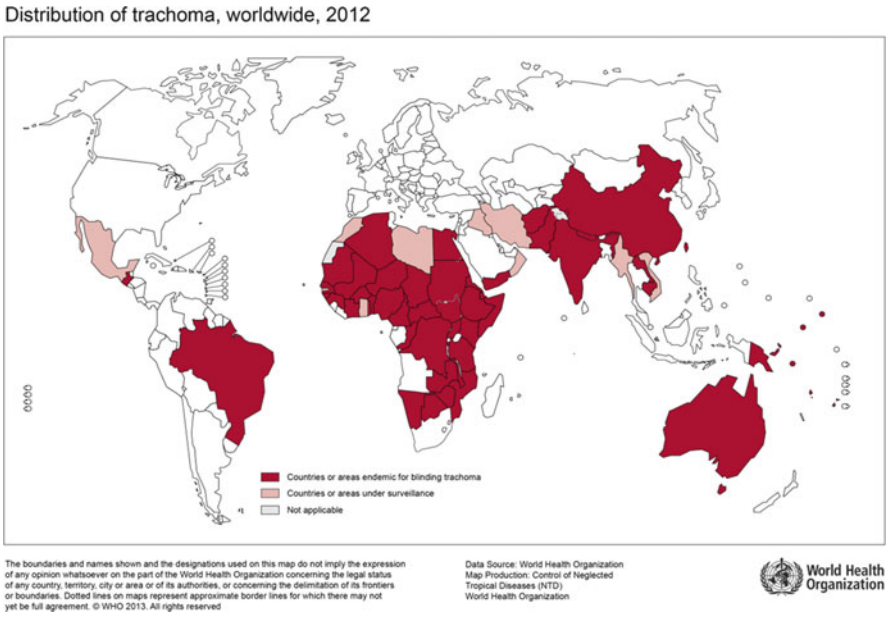

Fig. 19 Global distribution of trachoma, 2012

Table 1 WHO estimates of the global burden of trachoma, by year

Year	Active trachoma	Blindness	Trichiasis
1956	400	–	–
1971	400–500	1–2	–
1981	500	6–7	–
1985	360	6–9	–
1994	146	5.9	–
1996	–	–	10.6
2003	84.0	1.6	7.6
2007	40.6	–	8.2
2011	21.4	2.2	7.3

Data are millions of individuals

Some reports suggest the low rate of prevalence in China and India, in spite of their large populations. Follicular or severe inflammatory trachoma is mostly prevalent in children aged less than 5 years, and the prevalence can reach 60% or more [328–330]. Active inflammation symptoms increase with age, meaning that up to 90% of infected people over 25 years old could have scarring [331].

4.3 Bacteriology

In 1907, the presence of *Chlamydia* was defined for the first time in conjunctival epithelial cells from patients with trachoma, but *C. trachomatis* was not isolated in pure culture until 1957 [332, 333]. Collier and coworkers described experimental inoculation of a human volunteer with a virus isolated from a trachomatous patient [334]. *C. trachomatis* is nonmotile and ovoid in shape. The bacteria are not spore forming, but the elementary bodies turn into spores when released into the host [335]. It has a cytoplasmic membrane and outer membrane analogous to those of Gram-negative bacteria; nevertheless, it lacks a peptidoglycan cell wall. *C. trachomatis* requires a eukaryotic host cell in order to remain viable because it cannot synthesize its own ATP [336]. There are two *C. trachomatis* biovars (variant prokaryotic strains that differs physiologically and/or biochemically from other strains in a particular species), namely the trachoma and the lymphogranuloma venereum biovars [337]. Both biovars lead to the 19 different serotypes or serovars. The serovars A–K are assigned to the trachoma biovar, and serovars L1, L2, L2a, and L3 to the lymphogranuloma venereum biovar. The serovars A, B, Ba, and C cause trachoma by infecting the epithelial cells of the eye. Serovars D–K are responsible for sexually transmitted genital-tract infections [338]. The serovar specificity depend on epitope variance of the major outer membrane protein (MOMP), which constitutes 60% of the surface proteins [339]. MOMP characterization is important for differentiation between the *C. trachomatis* serovars. The *C. trachomatis* strains are recognized by monoclonal antibodies (mAbs) to epitopes in the VS4 region of MOMP [340], although these mAbs may similarly crossreact with two other *Chlamydia* species, *C. suis* and *C. muridarum*. *C. trachomatis* has a single chromosome of about 1 Mbp and a multicopy plasmid that functions as a virulence factor [341].

4.3.1 Chlamydial Life Cycle

A significant factor in the chronic nature of these infections is related to the unique developmental life cycle of chlamydial bacteria. *C. trachomatis* can adopt two distinct forms in its life cycle: the small, metabolically inactive, extracellular elementary body (EB) and the larger, metabolically active, intracellular reticulate body (RB). In *C. trachomatis*, EBs are usually smaller than the replicative stage RBs (0.3 μm for EBs compared with 1.0 μm for RBs) [342–344]. EBs are the

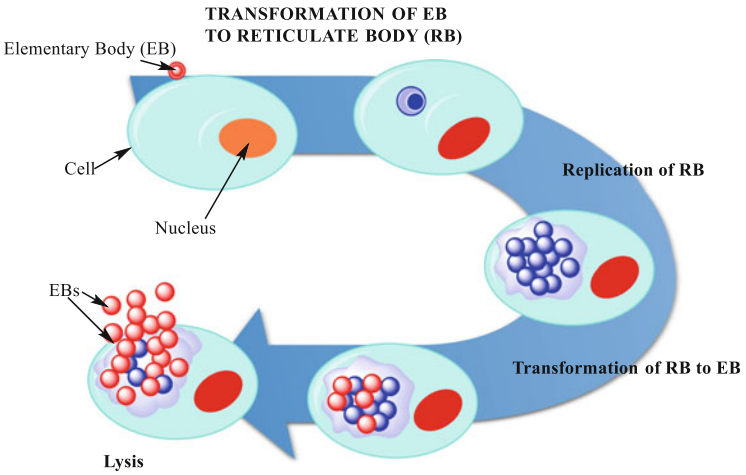

Fig. 20 Schematic representation of chlamydial life cycle

infectious form of *C. trachomatis*, whereas RBs are the replicating form. The developmental life cycle alternates over a 48–72 h period between EB and RB [345]. The life cycle of *C. trachomatis* begins with attachment and entry of infectious EBs into host epithelial cells. Heparan sulfate proteoglycans are suggested to be involved in this process [346]. In the host cell after endocytosis, EBs persist within a membrane-bound compartment named an inclusion, where they start to differentiate into RBs (Fig. 20). The stability of the EB cell wall is a result of outer membrane proteins that are highly crosslinked with disulfide bonds, including MOMP and two cysteine-rich proteins OmcA and OmcB [347]. The breaking of disulfide bonds takes place during EB to RB transformation, which leads to a more fluid and permeable membrane that allows transport of nutrients and ATP from the host, which is necessary for the transformation steps [348]. In the host cells, inclusions utilize most of the cytoplasm and become several thousand times larger through binary fission of the contained RBs. This is followed by the fusion of inclusions and differentiation of RBs back into EBs. Simultaneously, DNA condensation takes place, leading to an overall reduction in DNA size. The newly formed EBs are eventually released by cell lysis and/or extrusion to attack uninfected cells. It is difficult for the immune system to eliminate the pathogen, because *C. trachomatis* exists most of the time in intracellular inclusions that are not exposed to lysosomal enzymes and even inhibits the fusion of phagosomes with lysosomes [349].

4.3.2 Metabolism

Genome sequencing revealed that *Chlamydia* is not an "energy parasite" as had previously been believed, because they possess the necessary pathways for ATP

generation in addition to ATP/translocases for acquiring ATP from the host [343, 350]. *C. trachomatis* generates ATP via substrate-level phosphorylation by the enzymes phosphoglycerate kinase and pyruvate kinase, resulting from metabolism of glucose-6-phosphate to pyruvate via glycolysis [351, 352]. *C. trachomatis* has a glycolytic pathway and a linked TCA cycle. The TCA cycle is incomplete in *C. trachomatis* because of the lack of three enzymes: aconitase, citrate synthase, and isocitrate dehydrogenase [352–356]. Genomics revealed that acetyl-CoA resulting from pyruvate in glycolysis and acetyl-CoA derived from fatty acid degradation cannot enter the TCA cycle [357, 358]. As a result of this, constant metabolite exchange with the host is mandatory for TCA cycle operation [353]. The *C. trachomatis* succinate dehydrogenase subunit C is not functional because it lacks the functionally residues required for the binding of hemes and for interaction with vitamin K_2 [359, 360]. The succinate dehydrogenase or complex II of the respiratory chain acts as a regulator of the TCA cycle and of oxidative phosphorylation. *C. trachomatis* has the capacity to produce ATP by oxidative phosphorylation using the respiratory chain, which consists of a Na^+-translocating NADH dehydrogenase, a V-type ATPase, succinate dehydrogenase, and cytochrome bd oxidase. *C. trachomatis* is auxotrophic for most amino acids, cofactors, and purine and pyrimidine nucleotides and therefore depends on the import of host-derived compounds [352–356].

Jiangwei Yao et al. showed that fatty acid synthase II and FabI are essential for chlamydial replication, in contradiction to the results of Saka et al., who suggested that the fatty acids and phospholipids of chlamydial membranes are obtained from the host [361, 362]. There is growing evidence for the occurrence of some degree of metabolic activity in the EB stage [363, 364]. The concept of chlamydial EBs as metabolically inert or spore-like may therefore be in need of modification.

4.3.3 Genomics and Proteomics

C. trachomatis has a genome that consists of 1,042,519 nucleotide base pairs and approximately 894 likely protein-coding sequences; the average size of the genes is 1,050 bp [354]. *C. trachomatis* has few repetitive elements and pseudogenes, reflecting its long-term co-adaptation to life within eukaryotic hosts [365]. The *C. trachomatis* strains have an extrachromosomal plasmid, which was shown by sequencing to be a 7,493-bp plasmid with a copy number approximately fourfold greater than that of the chromosome [366, 367]. The chlamydial plasmids are nonconjugative and nonintegrative. They do not encode antibiotic resistance genes and do not display signs of genetic flexibility [368]. The plasmids from different *Chlamydia* species show nucleotide sequence identity of 69–99%, with very high identity within *C. trachomatis* [366, 369]. The *C. trachomatis* plasmid brings a set of five open reading frames (ORFs), which share identity with episomal maintenance genes common to other plasmids, and a set of three genes encoding proteins of unknown function, each of which is *Chlamydia* specific. Some *C. trachomatis* strains are deficient in these plasmids, and the consequences help

in detection of the *C. trachomatis* strain. *C. trachomatis* strains lacking plasmids have an unusual inclusion morphology, no glycogen, and show no alteration in antibiotic sensitivity. The plasmid of *C. trachomatis* is a favored target for DNA-based diagnosis. The chlamydial plasmid is a common target for nucleic acid amplification test (NAAT)-based diagnosis of human infections [370]. The glycogen biosynthesis gene *glgA* is downregulated in plasmid-negative strains [368].

4.4 Immune Response in Trachoma

In trachoma, cytokines and chemokines are produced after infection (released by infected epithelial cells) and are responsible for both adaptive and innate immune responses against *C. trachomatis* [371]. Several pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, and IFN-γ, were found to be involved [372]. The recruitment of phagocyting neutrophils and macrophages is induced by IL-1 and TNF-α [373]. *Chlamydia* infections are recognized by host pattern recognition receptors (PRRs) that recognize chlamydial lipopolysaccharide (LPS) via TLR4 [374–377] and heat shock protein Hsp60 through TLR2 and TLR4 [378–383]. In addition to TLRs, cytosolic PRRs recognize chlamydial pathogen-associated molecular patterns (PAMPS). *Chlamydia* infection also leads to production of reactive oxygen species (ROS), and K^+ efflux activates ROS production [384–386]. During the initiation of *Chlamydia* infection, there is an acute localized inflammatory response, mainly mediated by polymorphonuclear leukocytes (PMNs) and mononuclear leukocytes, recruited by cytokines and chemokines [387, 388]. *Chlamydia*-infected cells secrete the pro-inflammatory cytokines, chemokines, and interferons such as IL-1α, IL-1β, GM-CSF, IL-8, IFN-α, and IFN-γ. The adaptive immune response at the site of infection is due to immature DC infiltration [389]. The expression of chemokines that are chemotactic for natural killer (NK) cells is significantly induced at the site of infection [389, 390]. The intracellular localization of *C. trachomatis* in inclusions restricts its exposure to antibodies and replication, resulting in prolonged infection [391]. The inhibition of apoptosis of infected host cells and the induction of apoptosis in T cells by secretion of TNF-α both lead to the avoidance of immune responses [392, 393]. *C. trachomatis* releases cytoplasmic proteases that downregulate the expression of MHC I and II, thus inhibiting the activation of T cell responses [394].

4.5 Classification and Grading of Trachoma

After an incubation period of about 5–10 days, the infected phase of trachoma begins with conjunctivitis characterized by the formation of lymphoid follicles, irritation, and a watery discharge. The trachoma conjunctivitis may extent to the

upper margin of the cornea, where vascularization occurs during healing, forming shallow grooves called Herbert's pits [337]. Repeated conjunctivitis can lead to fibrosis, which causes permanent scarring of the upper tarsal conjunctiva, the contraction of which leads to trichiasis (rubbing of eyelashes against the cornea) and entropion (inward folding of the eyelids).

Trachoma can be assessed by the WHO grading system, which categorizes trachoma into five stages [395, 396]. The eyelids and cornea are diagnosed first for corneal opacity or turned eyelashes. The normal conjunctiva is thin, smooth, pink, and transparent with large deep-lying blood vessels running vertically. Examination of the conjunctiva over the stiffer part of the upper lid (tarsal conjunctiva) is carried out by turning over (everted) the upper eyelid (Fig. 21a). The five stages are as follows:

- *TF – Trachomatous inflammation, follicular*: The presence of five or more follicles in the upper tarsal conjunctiva. Follicles are round swellings that are paler than the surrounding conjunctiva, appearing white, grey, or yellow. Follicles must be at least 0.5 mm in diameter (Fig. 21b).
- *TI – Trachomatous inflammation, intense*: Pronounced inflammatory thickening of the tarsal conjunctiva that obscures more than half of the normal deep tarsal vessels. The tarsal conjunctiva appears red, rough, and thickened. There are usually numerous follicles, which may be partially or totally covered by the thickened conjunctiva (Fig. 21c).
- *TS – Trachomatous scarring*: The presence of scarring in the tarsal conjunctiva. Scars are easily visible as white lines, bands, or sheets in the tarsal conjunctiva. They are glistening and fibrous in appearance. Scarring, especially diffuse fibrosis, may obscure the tarsal blood vessels (Fig. 21d).

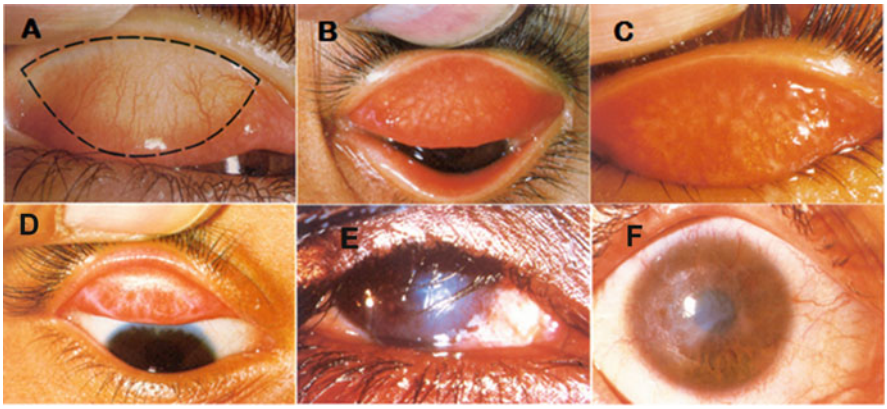

Fig. 21 Different grades of trachoma according to WHO guidelines: (**a**) area of upper eyelid examined, (**b**) trachomatous inflammation – follicular, (**c**) trachomatous inflammation – intense, (**d**) trachomatous scarring, (**e**) trachomatous trichiasis, and (**f**) corneal opacity

- *TT – Trachomatous trichiasis*: At least one eyelash rubs on the eyeball. Evidence of recent removal of inturned eyelashes should also be graded as trichiasis (Fig. 21e).
- *CO – Corneal opacity*: Easily visible corneal opacity over the pupil. The pupil margin is blurred viewed through the opacity. Such corneal opacities cause significant visual impairment (less than 6/18 or 0.3 vision), and therefore visual acuity should be measured if possible (Fig. 21f).

4.6 Treatment

Treatment of trachoma includes surgery and the usage of antibiotics. To prevent corneal opacification, the abrasive action of lashes on the cornea must be stopped by surgical correction of the eyelid margin, with epilation as an acceptable short-term option. The two procedures recommended by WHO are bilamellar tarsal rotation and posterior lamellar tarsal rotation (or Trabut) [397]. Antibiotics are used to target the source of the trachoma (i.e., *C. trachomitis*) and to eliminate bacteria inside the host. Because both of the above methods require an adequate amount of money, which is not available in the endemic countries, it is important to follow the rule "prevention is better than the cure" in affected communities.

4.6.1 WHO SAFE Program

The blindness caused by trachoma can be prevented by precautionary actions and early treatment, before the incidence of scarring. In 1997, WHO established the Alliance for Global Elimination of Trachoma by the year 2020 (GET 2020), a partnership with the aim of eliminating blindness caused by trachoma [398]. The GET 2020 alliance recommends interventions for prevention and management of trachoma, known as the SAFE program. SAFE is an acronym for surgery for trichiasis, antibiotics for active disease, facial cleanliness to reduce transmission, and environmental improvements [337]. Surgery includes epilation for infections with few inturned eyelashes, and bilamellar tarsal rotation and posterior lamellar tarsal rotation (or Trabut) for more severe trichiasis [397, 399]. However, high relapse rates decrease the success of surgery [400]. Treatment with antibiotics is desirable to prevent relapse, reduce the severity of disease, and decrease transmission rates. WHO recommends the application of tetracycline ointment twice daily for 6 weeks, as well as a single oral dose of azithromycin (20 mg/kg) [401]. Face washing removes infectious ocular secretions and also reduces the risk of being targeted by eye-seeking flies. Several studies have shown that proper sanitation and control of flies by insecticides are important factors in the control of trachoma [322, 402, 403].

4.6.2 Antibiotic Treatment

The spread of trachoma was controlled by the use of sulfonamides in North America in 1930–1940; nevertheless, their use has been stopped as a result of severe side effects such as the Stevens–Johnson syndrome [404, 405]. Afterwards, oral treatment with tetracycline (**46**), doxycycline (**47**), and minocycline (Fig. 22) were recommended for periods of up to 1 year, but adverse effects were observed in children and fetuses [406–408]. Doxycycline treatment was discontinued because it leads to permanent discoloration of teeth during childhood development [409]. However, oral erythromycin has been used for the management of trachoma, but it was also found to be associated with gastrointestinal side effects [410]. Today, topical tetracycline eye ointment is the most commonly used antibiotic in the treatment of trachoma and is a part of the SAFE protocol.

Tetracycline

The recommended treatment for active trachoma is 1% topical tetracycline (achromycin) ointment, twice daily for 6 weeks [411]. This treatment has proven successful, but is difficult because the ointment is applied onto the inner surface of the lower eyelids. Furthermore, the use of ointments momentarily leads to blurred vision and stinging eyes [412]. Consequently, compliance is poor and trained staff are usually needed for effective application. Tetracyclines are broad-spectrum antibiotics that exhibit activity against a wide range of Gram-positive and Gram-negative bacteria. Tetracycline is a natural product produced by the strains *Streptomyces aureofaciens* and *Streptomyces rimosus* [413]. Tetracyclines are polyketide antibiotics with a linear tetracyclic core (rings A to D) of complex stereochemistry having several adjacent chiral centers. The core scaffold of the tetracyclines is produced by the programmed synthesis determined by type II polyketide synthases, and tailoring reactions introduce functional groups with specific configurations [414]

Tetracycline is a bacteriostatic compound associated with the reversible inhibition of protein synthesis by binding with the bacterial ribosome and stopping the association of aminoacyl-tRNA with the ribosomal acceptor site. In *C. trachomatis*, tetracycline is known to cross the outer membrane in the form of positive ion

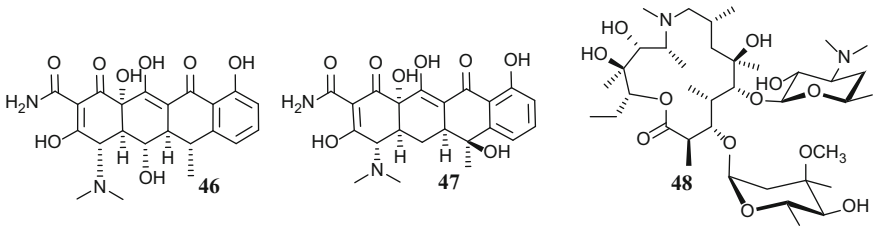

Fig. 22 Structures of antibiotics used in the management of trachoma

coordinated complexes, through the OmpF and OmpC porin channels. Subsequently, the complexes aggregate in the periplasm and released a lipophilic molecule that diffuses through the cytoplasmic membrane via an energy-dependent mechanism. Once in the cytoplasm, they encounter higher internal pH and divalent metal concentrations, become chelated to Mg^{2+}, and bind the ribosome in a chelated complex form [415–417]. The ribosomal protein S7 is part of the high-affinity binding site; the proteins S3, S8, S14, and S19 are also considered to be important in the binding [418]. The hydrophilic side of the molecule may be responsible for the chemical interactions with 16S rRNA, leaving space on the hydrophobic side susceptible to chemical substitutions, which is well-tolerated by tetracycline [413, 419]. Side effects from tetracyclines are not common, but of particular note is phototoxicity. Tetracyclines are teratogens and can cause teeth discoloration in the fetus. Therefore, tetracycline is not recommended for oral use in children under 8 years of age.

Azithromycin

The WHO recommends a single oral dose of azithromycin (**48**) of 20 mg/kg up to 1 g and this is now the standard medication for mass treatment in trachoma-endemic regions [420]. Although azithromycin is an expensive drug, mass distribution is possible because of Pfizer's continuing large-scale donation program [421]. The short duration of treatment, safety, and better compliance has made azithromycin the first-line medication in treating active trachoma [420]. Furthermore, with oral azithromycin, systemic treatment is necessary, which has proven to be more effective in preventing relapse of trachoma [412].

The azithromycin synthesized in 1980 (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin) is an azalide, a subclass of macrolides [422, 423]. In addition to its activity against Gram-negative pathogens (e.g., *C. trachomatis*), it has immunomodulatory properties and, for this reason, is used to treat diseases distinct from infections. Azithromycin is a semisynthetic analog of erythromycin in which an extra nitrogen atom has been inserted into the macrocyclic lactone (azalide), resulting in a higher degree of structural stability, excellent tissue penetration, low toxicity, and a long half-life of approximately 68 h [424]. Azithromycin reversibly inhibits bacterial protein synthesis by targeting 23S rRNA of the 50S ribosomal subunit of bacterial ribosomes close to the peptidyl-transferase center , where it prevents both the formation of peptide bonds and the translocation of peptidyl tRNA, leading to the inhibition of translation [425, 426]. The binding site involves hydrogen bond interactions between C5 mono- or disaccharide side chains of azithromycin and the nitrogen bases of nucleotide residues in domain V of 23S rRNA [426]. The hydrophobic interaction of the lactone ring with the ribosome may account for more than 25% of the free binding energy of the drug, which indicates that the lactone ring interacts hydrophobically with a crevice formed by rRNA bases 2,057–2,059 [427].

Azithromycin is stable in gastric acidic medium. It is readily absorbed, passively through organic ion channels in the small intestinal lumen and/or actively through ATP-dependent phosphoglycoprotein transport channels [428, 429]. The absorption of azithromycin takes place in the blood circulation; azithromycin interacts weakly with the phase I protein cytochrome P450-3A (CYP3A), but without significant alteration [430]. Azithromycin is actively transported to the site of infection, due to its high concentration in phagocytes. The concentration of azithromycin in the tissues may be much higher than in plasma as a result of ion trapping and its high hydrophobicity. The most common side effects are nausea, abdominal ache, diarrhea, and vomiting. Azithromycin treatment may lead to pseudomembranous colitis. Rarely, patients develop cholestatic hepatitis or delirium. The use of some macrolides leads to prolonged cardiac repolarization and QT interval with risk of cardiac arrhythmia and torsades de pointes. Such effects of azithromycin cannot be ignored in patients at increased risk for prolong QT interval [431]. However, chlamydial infection relapse rates are high when only individual cases are treated, probably due to re-infection by untreated family members. Thus, community-inclusive management of antibiotics is needed [432].

4.7 Bacterial Resistances

Antibiotic resistance is an ongoing global medical emergency and we are in great need of new antibacterial agents to combat promptly emerging resistant pathogens. The use of antibiotics at a large scale raises the concern of the emergence of resistant *C. trachomatis* strains. Currently, there are no examples of stable tetracycline resistance in clinical strains of *C. trachomatis* at extraocular sites. However, the swine pathogen *Chlamydia suis* is commonly tetracycline resistant, both in America and Europe. In tested US strains, this resistance is mediated by a genomic island carrying a tet(C) allele [371, 433]. There is no report of macrolide resistances in *C. trachomatis*, which was recently confirmed in a study conducted by West et al. in Tanzania [434]. There may be some other factors of persistent infection that are in the need of evaluation. The development of resistance in other azithromycin-sensitive bacteria such as *Streptococcus pneumoniae* in the nasopharynx after mass drug treatment has been reported [435, 436]. Improvements in environmental and education factors are required to eradicate trachoma completely, in addition to the mass administration of antibiotics. Environmental risk factors include water supply, fecal and refuse disposal, animal pens within households, and fly density. Effective control of trachoma needs prolonged effort and is best integrated with local health systems and other development sectors. Antibiotic treatment strategies show limitations in controlling trachoma as a result of recurrence. The reasons could be the typical development cycle of *C. trachomatis*, the extended kinetics of disease pathogenesis, and limited proper diagnosis. Thus, an effective vaccination would help to resolve an infection quickly and provide immunity [437].

4.8 Vaccine

Development of a vaccine against *C. trachomatis* infections includes several ongoing efforts. In initial trials, killed whole *C. trachomatis* EBs, recombinant cLPS, and cHsp70 were used [438]. These methods brought partial, provisional protection and, in some cases, more severe disease-associated symptoms were observed probably as a result of adverse effects initiated by the immune system [339]. Extensive research was carried out to determine the potential of major outer membrane protein (MOMP) as a subunit vaccine candidate. MOMP consists of four variable domains, which comprise the serovar-specific epitopes, and five constant domains that are highly conserved between the different serovars and contain several conserved CD4 and CD8 T cell epitopes [439–441]. MOMP has been used in several vaccine studies, but efforts to induce protection using MOMP peptides, MOMP, or plasmids expressing MOMP have not been promising, although strong cell-mediated and humoral immune responses were observed after vaccination. An increased level of IgA and IgG and longer-lived CMI led to reduction, but not prevention, of infection in immunized primates [442–444]. Kari and coworkers showed that systemically administered native MOMP as a subunit vaccine produced significant reduction in ocular shedding in nonhuman primates [445].

The polymorphic membrane protein D (PmpD) is a major protective antigen on the surface of chlamydial EBs that could generate neutralizing antibodies and seems to display the potential to become an effective vaccine candidate [446]. The N-terminal (N-pmpD) translocates to the surface of bacteria, where it noncovalently binds the components of the outer membrane. Antibodies raised against N-pmpD blocked chlamydial infectivity in epithelial cells, indicating that N-pmpD works as an adhesin [447]. This shows that anti-PmpD antibodies prevent infection [447, 448]. PmpD may play a role in regulation of host gene expression, leading to apoptosis of uninfected epithelial cells and T cell suppression [446]. There is a need to investigate the effects responsible for the beneficial immune response by PmpD as it is a multifunctional protein with the potential to trigger different pathological pathways. DNA vaccines have been proposed as viable alternatives to conventional live or peptide vaccines [449]. The potential of DNA-encoded chlamydial antigens or epitopes to induce Th1-type immunity against *Chlamydia* in mice has been reported [450, 451]. The virus vectors, as well as DCs, have been examined and were reported to be promising for development of a vaccine [452, 453]. New ongoing advanced research, with developments in vaccine adjuvants and delivery mechanisms, promises that a *C. trachomatis* vaccine may soon be within the reach.

5 Conclusion

This chapter has discussed the relevant biology and approaches for the treatment of the three major NTBDs: leprosy, BU, and trachoma. There is a lack of research programs in the area of NTBDs and current progress in the clinical management of

NTDs is mainly the result of major efforts by WHO. The WHO Strategic Plan for Leprosy Elimination 2005 had the elimination of leprosy as its main goal, demarcated as a reduction in prevalence to less than one case per 10,000 population. The main principle of leprosy control is "morbidity control," that is, timely detection of new cases, their management with effective chemotherapy in the form of MDT, prevention of disability, and rehabilitation. The Innovative and Intensified Disease Management (IDM) program of WHO was founded to control NTDs (e.g., BU) by combining expertise in disease-specific areas with cross-cutting issues such as surveillance, capacity building, advocacy, and research with the aim of eliminating or controlling the disease. WHO also established the SAFE strategy and the association for the Global Elimination of Blinding Trachoma (GET 2020) and expect to eliminate trachoma by the year 2020. Although the continuous efforts of WHO in the management of these diseases have been commendable, there are still several barriers to cross before achieving the goals.

Clinical management of these three diseases mainly depends on the use of antibiotics. The major limitation of antibiotics is their use for the management of NTBDs with HIV infection. Furthermore, chemotherapy with antibiotics involves the risk of emerging resistance to antibiotics, which has been classified by WHO as one of the biggest threats to human health. Hence, there is a continuous need to search for new chemical entities to combat this growing problem of antibiotic-resistant infections. Drug development in the area of NTBDs is very limited because of the low profit to pharmaceutical industries. In addition to antibiotics, alternative inexpensive methods of treatment must be explored, such as heat therapy and hyperbaric oxygen, which have both shown promising results in the management of BU. Development of a vaccine will certainly help researchers and medical practitioners to eradicate these diseases. The statement "prevention is better than the cure" applies in the Third Word because of the high poverty rate, low hygiene, and poor environment. Furthermore, clinical management is mainly dependent on the work of WHO. These three illustrations of neglected bacterial diseases indicate that they need more attention and extensive research for their control and eradication.

CDRI communication no: 9210

References

1. Fenwick A (2012) The global burden of neglected tropical diseases. Public Health 126:233–236
2. Lupi O, Madkan V, Tyring SK (2006) Tropical dermatology: bacterial tropical diseases. J Am Acad Dermatol 54:559–578
3. Browne SG (1985) The history of leprosy. In: Leprosy. Longman Group, Edinburgh, pp 1–14
4. Monot M, Honoré N, Garnier T, Araoz R, Coppée J-Y, Lacroix C, Sow S, Spencer JS, Truman RW, Williams DL (2005) On the origin of leprosy. Science 308:1040–1042
5. Trautman JR (1984) A brief history of Hansen's disease. Bull N Y Acad Med 60:689

6. Frank G (1991) Leprosy, racism, and public health: social policy in chronic disease control. ZACHARY GUSSOW. Am Ethnol 18:383–384
7. Walker SL, Lockwood DNJ (2007) Leprosy. Clin Dermatol 25:165–172
8. Avula B, Khan SI, Tekwani BL, Dhammika Nanayakkara N, McChesney JD, Walker LA, Khan IA (2011) Analysis of primaquine and its metabolite carboxyprimaquine in biological samples: enantiomeric separation, method validation and quantification. Biomed Chromatogr 25:1010–1017
9. Hansen GA, Looft C (1895) Leprosy: in its clinical and pathological aspects. Am J Med Sci 110:586
10. Meyers W (1995) Mycobacterial infections of the skin. In: Tropical pathology. Springer, Berlin, pp 291–377
11. Guerrant RL, Walker DH, Weller PF (2011) Tropical infectious diseases: principles, pathogens and practice. Elsevier, Edinburgh
12. Hatta M, van Beers SM, Madjid B, Djumadi A, de Wit MY, Klatser PR (1995) Distribution and persistence of *Mycobacterium leprae* nasal carriage among a population in which leprosy is endemic in Indonesia. Trans R Soc Trop Med Hyg 89:381–385
13. Rees R, McDougall A (1977) Airborne infection with *Mycobacterium leprae* in mice. J Med Microbiol 10:63–68
14. Brandsma J, Yoder L, Macdonald M (2005) Leprosy acquired by inoculation from a knee injury. Lepr Rev 76:175–179
15. Walsh G, Storrs E, Meyers W, Binford C (1977) Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*): recent epizootiologic findings. J Reticuloendothel Soc 22:363–367
16. Donham KJ, Leininger JR (1977) Spontaneous leprosy-like disease in a chimpanzee. J Infect Dis 136:132–136
17. Meyers WM, Gormus BJ, Walsh GP, Baskin GB, Hubbard GB (1991) Naturally acquired and experimental leprosy in nonhuman primates. Am J Trop Med Hyg 44:24–27
18. Wolf RH, Gormus BJ, Martin LN, Baskin GB, Walsh GP, Meyers WM, Binford CH (1985) Experimental leprosy in three species of monkeys. Science 227:529–531
19. Lumpkin LR, Cox GF, Wolf JE (1983) Leprosy in five armadillo handlers. J Am Acad Dermatol 9:899–903
20. West BC, Todd JR, Lary CH, Blake LA, Fowler ME, King JW (1988) Leprosy in six isolated residents of northern Louisiana: time-clustered cases in an essentially nonendemic area. Arch Intern Med 148:1987–1992
21. World Health Organization (2015) Global leprosy update, 2014: need for early case detection. Wkly Epidemiol Rec 90(36): 461–476
22. Rastogi N, Legrand E, Sola C (2001) The mycobacteria: an introduction to nomenclature and pathogenesis. Rev Sci Tech 20:21–54
23. Scollard DM, Truman RW, Ebenezer GJ (2015) Mechanisms of nerve injury in leprosy. Clin Dermatol 33:46–54
24. White C, Franco-Paredes C (2015) Leprosy in the 21st century. Clin Microbiol Rev 28:80–94
25. Polycarpou A, Walker SL, Lockwood DN (2013) New findings in the pathogenesis of leprosy and implications for the management of leprosy. Curr Opin Infect Dis 26:413–419
26. Truman RW, Krahenbuhl JL (2001) Viable *M. leprae* as a research reagent. Int J Lepr Other Mycobact Dis 69:1–12
27. Ng V, Zanazzi G, Timpl R, Talts JF, Salzer JL, Brennan PJ, Rambukkana A (2000) Role of the cell wall phenolic glycolipid-1 in the peripheral nerve predilection of *Mycobacterium leprae*. Cell 103:511–524
28. Zu Bentrup KH, Miczak A, Swenson DL, Russell DG (1999) Characterization of activity and expression of isocitrate lyase in *Mycobacterium avium* and *Mycobacterium tuberculosis*. J Bacteriol 181:7161–7167
29. McKinney JD, Höner zu Bentrup K, Muñoz-Elías EJ, Miczak A, Chen B, Chan W-T, Swenson D, Sacchettini JC, Jacobs WR, Russell DG (2000) Persistence of *Mycobacterium*

tuberculosis in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature 406:735–738
30. Cole S, Eiglmeier K, Parkhill J, James K, Thomson N, Wheeler P, Honore N, Garnier T, Churcher C, Harris D (2001) Massive gene decay in the leprosy bacillus. Nature 409:1007–1011
31. Wheeler PR (2003) Leprosy–clues about the biochemistry of *Mycobacterium leprae* and its host-dependency from the genome. World J Microbiol Biotechnol 19:1–16
32. Eiglmeier K, Parkhill J, Honore N, Garnier T, Tekaia F, Telenti A, Klatser P, James KD, Thomson NR, Wheeler PR (2001) The decaying genome of *Mycobacterium leprae*. Lepr Rev 72:387–398
33. Monot M, Honoré N, Garnier T, Zidane N, Sherafi D, Paniz-Mondolfi A, Matsuoka M, Taylor GM, Donoghue HD, Bouwman A (2009) Comparative genomic and phylogeographic analysis of *Mycobacterium leprae*. Nat Genet 41:1282–1289
34. Cole ST (1998) Comparative mycobacterial genomics. Curr Opin Microbiol 1:567–571
35. Wheeler PR (2001) The microbial physiologist's guide to the leprosy genome. Lepr Rev 72:399–407
36. Ribeiro-Rodrigues R (2012) Host response to *M. leprae*. Springer, Mailand
37. Gulia A, Fried I, Massone C (2010) New insights in the pathogenesis and genetics of leprosy. F1000 Med Rep 2: 30. doi: 10.3410/M2-30
38. Maeda Y, Gidoh M, Ishii N, Mukai C, Makino M (2003) Assessment of cell mediated immunogenicity of *Mycobacterium leprae*-derived antigens. Cell Immunol 222:69–77
39. Hashimoto K, Maeda Y, Kimura H, Suzuki K, Masuda A, Matsuoka M, Makino M (2002) *Mycobacterium leprae* infection in monocyte-derived dendritic cells and its influence on antigen-presenting function. Infect Immun 70:5167–5176
40. Kimura H, Maeda Y, Takeshita F, Takaoka L, Matsuoka M, Makino M (2004) Upregulation of T-cell-stimulating activity of mycobacteria-infected macrophages. Scand J Immunol 60:278–286
41. Sieling PA, Jullien D, Dahlem M, Tedder TF, Rea TH, Modlin RL, Porcelli SA (1999) CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. J Immunol 162:1851–1858
42. Gimenez M, GIGLI I, Tausk F (1989) Differential expression of Langerhans cells in the epidermis of patients with leprosy. Br J Dermatol 121:19–26
43. Krutzik SR, Ochoa MT, Sieling PA, Uematsu S, Ng YW, Legaspi A, Liu PT, Cole ST, Godowski PJ, Maeda Y (2003) Activation and regulation of toll-like receptors 2 and 1 in human leprosy. Nat Med 9:525–532
44. Massone C, Nunzi E, Ribeiro-Rodrigues R, Talhari C, Talhari S, Schettini APM, Parente JNT, Brunasso AM, Puntoni M, Clapasson A (2010) T regulatory cells and plasmocytoid dendritic cells in Hansen disease: a new insight into pathogenesis? Am J Dermatopathol 32:251–256
45. Schlesinger L (1993) Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. J Immunol 150:2920–2930
46. van Kooyk Y, Geijtenbeek TB (2003) DC-SIGN: escape mechanism for pathogens. Nat Rev Immunol 3:697–709
47. Kissenpfennig A, Aït-Yahia S, Clair-Moninot V, Stössel H, Badell E, Bordat Y, Pooley JL, Lang T, Prina E, Coste I (2005) Disruption of the langerin/CD207 gene abolishes Birbeck granules without a marked loss of Langerhans cell function. Mol Cell Biol 25:88–99
48. Brightbill HD, Libraty DH, Krutzik SR, Yang R-B, Belisle JT, Bleharski JR, Maitland M, Norgard MV, Plevy SE, Smale ST (1999) Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. Science 285:732–736
49. Underhill DM, Ozinsky A, Smith KD, Aderem A (1999) Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc Natl Acad Sci 96:14459–14463

50. Schlesinger LS, Horwitz MA (1991) Phenolic glycolipid-1 of *Mycobacterium leprae* binds complement component C3 in serum and mediates phagocytosis by human monocytes. J Exp Med 174:1031–1038
51. Modlin RL, Melancon-Kaplan J, Young S, Pirmez C, Kino H, Convit J, Rea TH, Bloom BR (1988) Learning from lesions: patterns of tissue inflammation in leprosy. Proc Natl Acad Sci 85:1213–1217
52. Modlin RL, Hofman FM, Taylor CR, Rea TH (1983) T lymphocyte subsets in the skin lesions of patients with leprosy. J Am Acad Dermatol 8:182–189
53. Sieling P, Chatterjee D, Porcelli S, Prigozy T, Mazzaccaro R, Soriano T, Bloom B, Brenner M, Kronenberg M, Brennan P et al (1995) CD1-restricted T cell recognition of microbial lipoglycan antigens. Science 269:227–230
54. Hancock GE, Molloy A, Kale AB, Kiessling R, Becx-Bleumink M, Cohn ZA, Kaplan G (1991) *In vivo* administration of low-dose human interleukin-2 induces lymphokine-activated killer cells for enhanced cytolysis *in vitro*. Cell Immunol 132:277–284
55. Chiplunkar S, De Libero G, Kaufmann S (1986) *Mycobacterium leprae*-specific Lyt-2+ T lymphocytes with cytolytic activity. Infect Immun 54:793–797
56. Kaleab B, Ottenoff T, Converse P, Halapi E, Tadesse G, Rottenberg M, Kiessling R (1990) Mycobacterial-induced cytotoxic T cells as well as nonspecific killer cells derived from healthy individuals and leprosy patients. Eur J Immunol 20:2651–2659
57. Shi L, Kraut R, Aebersold R, Greenberg A (1992) A natural killer cell granule protein that induces DNA fragmentation and apoptosis. J Exp Med 175:553–566
58. Sibley LD, Franzblau SG, Krahenbuhl JL (1987) Intracellular fate of *Mycobacterium leprae* in normal and activated mouse macrophages. Infect Immun 55:680–685
59. Sibley LD, Krahenbuhl JL (1988) Induction of unresponsiveness to gamma interferon in macrophages infected with *Mycobacterium leprae*. Infect Immun 56:1912–1919
60. Chiplunkar S, Deshmukh M, Samson P, Butlin R, Bhatki W, Chulawalla R, Deo M, Gangal S (1990) Natural killer-cell-mediated and antibody-dependent cellular cytotoxicity in leprosy. Int J Lepr Other Mycobact Dis 58:334–341
61. Steinhoff U, Wand-Württenberger A, Bremerich A, Kaufmann S (1991) Mycobacterium leprae renders Schwann cells and mononuclear phagocytes susceptible or resistant to killer cells. Infect Immun 59:684–688
62. Ramasesh N, Adams L, Franzblau S, Krahenbuhl J (1991) Effects of activated macrophages on *Mycobacterium leprae*. Infect Immun 59:2864–2869
63. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348–2357
64. Salgame P, Abrams JS, Clayberger C, Goldstein H, Convit J, Modlin RL, Bloom BR (1991) Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. Science 254:279–282
65. Ridley D (1987) Skin biopsy in leprosy. Histological interpretation and clinical application, 2nd edn. Ciba, Basle, p 63
66. World Health Organization (1998) WHO model prescribing information: drugs used in leprosy. World Health Organization, Geneva
67. Shimoji Y, Ng V, Matsumura K, Fischetti VA, Rambukkana A (1999) A 21-kDa surface protein of *Mycobacterium leprae* binds peripheral nerve laminin-2 and mediates Schwann cell invasion. Proc Natl Acad Sci 96:9857–9862
68. Rambukkana A (2001) Molecular basis for the peripheral nerve predilection of *Mycobacterium leprae*. Curr Opin Microbiol 4:21–27
69. Schlesinger L, Horwitz M (1991) Phagocytosis of *Mycobacterium leprae* by human monocyte-derived macrophages is mediated by complement receptors CR1 (CD35), CR3 (CD11b/CD18), and CR4 (CD11c/CD18) and IFN-gamma activation inhibits complement receptor function and phagocytosis of this bacterium. J Immunol 147:1983–1994

70. Schlesinger LS, Kaufman TM, Iyer S, Hull SR, Marchiando LK (1996) Differences in mannose receptor-mediated uptake of lipoarabinomannan from virulent and attenuated strains of *Mycobacterium tuberculosis* by human macrophages. J Immunol 157:4568–4575
71. Knight JC, Keating BJ, Kwiatkowski DP (2004) Allele-specific repression of lymphotoxin-α by activated B cell factor-1. Nat Genet 36:394–399
72. Hagge DA, Robinson SO, Scollard D, McCormick G, Williams DL (2002) A new model for studying the effects of *Mycobacterium leprae* on Schwann cell and neuron interactions. J Infect Dis 186:1283–1296
73. Job CK (1989) Nerve damage in leprosy. Int J Lepr Other Mycobact Dis 57:532–539
74. Scollard DM (2008) The biology of nerve injury in leprosy. Lepr Rev 79:242–253
75. Spierings E, de Boer T, Wieles B, Adams LB, Marani E, Ottenhoff TH (2001) *Mycobacterium leprae*-specific, HLA class II-restricted killing of human Schwann cells by CD4+ Th1 cells: a novel immunopathogenic mechanism of nerve damage in leprosy. J Immunol 166:5883–5888
76. Oliveira RB, Ochoa MT, Sieling PA, Rea TH, Rambukkana A, Sarno EN, Modlin RL (2003) Expression of toll-like receptor 2 on human Schwann cells: a mechanism of nerve damage in leprosy. Infect Immun 71:1427–1433
77. Harboe M, Aseffa A, Leekassa R (2005) Challenges presented by nerve damage in leprosy. Lepr Rev 76:5–13
78. Save M, Shetty V, Shetty K, Antia N (2004) Alterations in neurofilament protein (s) in human leprous nerves: morphology, immunohistochemistry and Western immunoblot correlative study. Neuropathol Appl Neurobiol 30:635–650
79. Lee H, Jo E-K, Choi S-Y, Oh SB, Park K, Kim JS, Lee SJ (2006) Necrotic neuronal cells induce inflammatory Schwann cell activation via TLR2 and TLR3: implication in Wallerian degeneration. Biochem Biophys Res Commun 350:742–747
80. Khanolkar-Young S, Rayment N, Brickell P, Katz D, Vinayakumar S, Colston M, Lockwood D (1995) Tumour necrosis factor-alpha (TNF-α) synthesis is associated with the skin and peripheral nerve pathology of leprosy reversal reactions. Clin Exp Immunol 99:196–202
81. Lockwood D, Suneetha L, Sagili KD, Chaduvula MV, Mohammed I, van Brakel W, Smith W, Nicholls P, Suneetha S (2011) Cytokine and protein markers of leprosy reactions in skin and nerves: baseline results for the North Indian INFIR cohort. PLoS Negl Trop Dis 5: e1327
82. Shetty V, Antia N (1988) Nerve damage in leprosy. Int J Lepr Other Mycobact Dis 56:619
83. Reibel F, Cambau E, Aubry A (2015) Update on the epidemiology, diagnosis, and treatment of leprosy. Med Mal Infect 45:383–393
84. Skinsnes O (1972) Origin of chaulmoogra oil--another version. Int J Lepr Other Mycobact Dis 40:172
85. Jacobsen PL, Levy L (1973) Mechanism by which hydnocarpic acid inhibits mycobacterial multiplication. Antimicrob Agents Chemother 3:373–379
86. Bennett BH, Parker DL, Robson M (2008) Leprosy: steps along the journey of eradication. Public Health Rep 123:198
87. Singh R (2002) Synthetic drugs. Mittal, New Delhi
88. Lowe J (1950) Treatment of leprosy with diamino-diphenyl sulphone by mouth. Lancet 255:145–150
89. Wozel G, Barth J (1988) Current aspects of modes of action of dapsone. Int J Dermatol 27:547–552
90. Dallas WS, Gowen J, Ray PH, Cox M, Dev I (1992) Cloning, sequencing, and enhanced expression of the dihydropteroate synthase gene of *Escherichia coli* MC4100. J Bacteriol 174:5961–5970
91. Williams DL, Spring L, Harris E, Roche P, Gillis TP (2000) Dihydropteroate synthase of *Mycobacterium leprae* and dapsone resistance. Antimicrob Agents Chemother 44:1530–1537
92. Kettle AJ, Winterbourn CC (1991) Mechanism of inhibition of myeloperoxidase by anti-inflammatory drugs. Biochem Pharmacol 41:1485–1492

93. Uetrecht J (1994) Myeloperoxidase as a generator of drug free radicals. Biochem Soc Symp 61:163–170
94. Diaz-Ruiz A, Zavala C, Montes S, Ortiz-Plata A, Salgado-Ceballos H, Orozco-Suarez S, Nava-Ruiz C, Pérez-Neri I, Perez-Severiano F, Ríos C (2008) Antioxidant, antiinflammatory and antiapoptotic effects of dapsone in a model of brain ischemia/reperfusion in rats. J Neurosci Res 86:3410–3419
95. Bukirwa H, Garner P, Critchley J (2004) Chlorproguanil-dapsone for treating uncomplicated malaria. Cochrane Database Syst Rev CD004387 doi: 10.1002/14651858.CD004387.pub2
96. Pettit J, Rees R (1964) Sulphone resistance in leprosy: an experimental and clinical study. Lancet 284:673–674
97. Pearson T, Rees R, Waters M (1975) Sulphone resistance in leprosy: a review of one hundred proven clinical cases. Lancet 306:69–72
98. Perlman D (1977) Structure-activity relationships among the semisynthetic antibiotics. Academic, New York
99. Corcoran JW, Hahn FE (1975) Mechanism of action of antimicrobial and antitumor agents. Springer, New York
100. Rees R, Pearson J, Waters M (1970) Experimental and clinical studies on rifampicin in treatment of leprosy. Br Med J 1:89
101. Levy L, Shepard C, Fasal P (1975) The bactericidal effect of rifampicin on *M. leprae* in man: a) single doses of 600, 900 and 1200 mg; and b) daily doses of 300 mg. Int J Lepr Other Mycobact Dis 44:183–187
102. Calvori C, Frontali L, Leoni L, Tecce G (1965) Effect of rifamycin on protein synthesis. Nature 207:417–418
103. Jin DJ, Gross CA (1988) Mapping and sequencing of mutations in the *Escherichia coli* rpoB gene that lead to rifampicin resistance. J Mol Biol 202:45–58
104. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA (2001) Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 104:901–912
105. Feklistov A, Mekler V, Jiang Q, Westblade LF, Irschik H, Jansen R, Mustaev A, Darst SA, Ebright RH (2008) Rifamycins do not function by allosteric modulation of binding of Mg2+ to the RNA polymerase active center. Proc Natl Acad Sci 105:14820–14825
106. Hastings R, Richard V, Jacobson R (1984) Ansamycin activity against rifampicin-resistant *Mycobacterium leprae*. Lancet 323:1130
107. Pattyn S, Saerens E (1977) Activity of three new rifamycin derivates on the experimental infection by *Mycobacterium leprae*. Ann Soc Belg Med Trop 57:169–173
108. Pattyn S (1987) Rifabutin and rifapentine compared with rifampin against *Mycobacterium leprae* in mice. Antimicrob Agents Chemother 31:134
109. Ji B, Chen J, Lu X, Wang S, Ni G, Hou Y, Zhou D, Tang Q (1986) Antimycobacterial activities of two newer ansamycins, R-76-1 and DL 473. Int J Lepr Other Mycobact Dis 54:563–577
110. Tomioka H, Saito H, Hidaka T (1993) *In vivo* antileprosy activity of the newly synthesized benzoxazinorifamycin, KRM-1648. Int J Lepr Other Mycobact Dis 61:255–258
111. Saito H, Tomioka H, Sato K, Dekio S (1994) Therapeutic efficacy of benzoxazinorifamycin, KRM-1648, in combination with other antimicrobials against *Mycobacterium leprae* infection induced in nude mice. Int J Lepr Other Mycobact Dis 62:43
112. Hasanoor Reja A, Biswas N, Biswas S, Lavania M, Chaitanya VS, Banerjee S, Maha Patra P, Gupta UD, Patra PK, Sengupta U (2015) Report of rpoB mutation in clinically suspected cases of drug resistant leprosy: a study from Eastern India. Indian J Dermatol Venereol Leprol 81:155
113. Williams DL, Gillis TP (2012) Drug-resistant leprosy: monitoring and current status. Lepr Rev 83:269

114. Saxena M, Bhunia SS, Saxena AK (2012) Docking studies of novel pyrazinopyridoindoles class of antihistamines with the homology modelled H1-receptor. SAR QSAR Environ Res 23:311
115. Bhunia SS, Roy KK, Saxena AK (2011) Profiling the structural determinants for the selectivity of representative factor-Xa and thrombin inhibitors using combined ligand-based and structure-based approaches. J Chem Inf Model 51:1966
116. Bhunia SS, Singh S, Saxena S, Saxena AK (2015) Pharmacophore modeling, docking and molecular dynamics studies on caspase-3 activators binding at β-tubulin site. Curr Comput Aided Drug Des 11:72
117. Azad CS, Bhunia SS, Krishna A, Shukla PK, Saxena AK (2015) Novel glycoconjugate of 8-fluoro norfloxacin derivatives as gentamicin-resistant *Staphylococcus aureus* inhibitors: synthesis and molecular modelling studies. Chem Biol Drug Des 86:440
118. Saxena M, Bhunia SS, Saxena AK (2015) Molecular modelling studies on 2-substituted octahydropyrazinopyridoindoles for histamine H2 receptor antagonism. SAR QSAR Environ Res 26:739
119. Nisha J, Shanthi V (2015) Computational simulation techniques to understand rifampicin resistance mutation (S425L) of rpoB in *M. leprae*. J Cell Biochem 116:1278–1285
120. Van Rensburg C, Gatner E, Imkamp F, Anderson R (1982) Effects of clofazimine alone or combined with dapsone on neutrophil and lymphocyte functions in normal individuals and patients with lepromatous leprosy. Antimicrob Agents Chemother 21:693–697
121. Anderson R, Smit MJ (1993) Clofazimine and B669 inhibit the proliferative responses and Na+, K+-adenosine triphosphatase activity of human lymphocytes by a lysophospholipid-dependent mechanism. Biochem Pharmacol 46:2029–2038
122. Mohd A, Parwaz Khan AA, Bano S, Siddiqi K (2011) Interaction of clofazimine with divalent metal ions: a fluorescence quenching study. J Dispers Sci Technol 32:1465–1469
123. Kashyap A, Sehgal VN, Sahu A, Saha K (1992) Anti-leprosy drugs inhibit the complement-mediated solubilization of pre-formed immune complexes *in vitro*. Int J Immunopharmacol 14:269–273
124. Faouzi M, Starkus J, Penner R (2015) State-dependent blocking mechanism of Kv1. 3 channels by the antimycobacterial drug clofazimine. Br J Pharmacol 172:5161–5173
125. Chuaprapaisilp T, Piamphongsant T (1978) Treatment of pustular psoriasis with clofazimine. Br J Dermatol 99:303–305
126. Podmore P, Burrows D (1986) Clofazimine: an effective treatment for Melkersson-Rosenthal syndrome or Miescher's cheilitis. Clin Exp Dermatol 11:173–178
127. Kelleher D, O'Brien S, Weir D (1982) Preliminary trial of clofazimine in chronic inflammatory bowel-disease. Gut 23:A449–A450
128. Bellera CL, Balcazar DE, Vanrell MC, Casassa AF, Palestro PH, Gavernet L, Labriola CA, Gálvez J, Bruno-Blanch LE, Romano PS (2015) Computer-guided drug repurposing: identification of trypanocidal activity of clofazimine, benidipine and saquinavir. Eur J Med Chem 93:338–348
129. Koval A, Vlasov P, Shichkova P, Khunderyakova S, Markov Y, Panchenko J, Volodina A, Kondrashov F, Katanaev V (2014) Anti-leprosy drug clofazimine inhibits growth of triple-negative breast cancer cells via inhibition of canonical Wnt signaling. Biochem Pharmacol 87:571–578
130. Arbiser JL, Moschella SL (1995) Clofazimine: a review of its medical uses and mechanisms of action. J Am Acad Dermatol 32:241–247
131. Yoon GS, Sud S, Keswani RK, Baik J, Standiford TJ, Stringer KA, Rosania GR (2015) Phagocytosed clofazimine biocrystals can modulate innate immune signaling by inhibiting TNFα and boosting IL-1RA secretion. Mol Pharm 12:2517–2527
132. Fukutomi Y, Maeda Y, Makino M (2011) Apoptosis-inducing activity of clofazimine in macrophages. Antimicrob Agents Chemother 55:4000–4005

133. Pourgholami MH, Lu Y, Wang L, Stephens RW, Morris DL (2004) Regression of Novikoff rat hepatocellular carcinoma following locoregional administration of a novel formulation of clofazimine in lipiodol. Cancer Lett 207:37–47
134. Kornhuber J, Muehlbacher M, Trapp S, Pechmann S, Friedl A, Reichel M, Mühle C, Terfloth L, Groemer TW, Spitzer GM et al (2011) Identification of novel functional inhibitors of acid sphingomyelinase. PLoS One 6:e23852
135. Franzblau S, O'sullivan J (1988) Structure-activity relationships of selected phenazines against *Mycobacterium leprae in vitro*. Antimicrob Agents Chemother 32:1583–1585
136. Franzblau SG, White KE, O'Sullivan JF (1989) Structure-activity relationships of tetramethylpiperidine-substituted phenazines against *Mycobacterium leprae in vitro*. Antimicrob Agents Chemother 33:2004–2005
137. Van Landingham RM, Walker LL, O'Sullivan JF, Shinnick TM (1993) Activity of phenazine analogs against *Mycobacterium leprae* infections in mice. Int J Lepr Other Mycobact Dis 61:406
138. Walker SL, Withington SG, Lockwood DNJ (2014) Leprosy. In: Farrar J, Hotez PJ, Junghanss T, Kang G, Lalloo D, White N (eds) Manson's tropical diseases, 23rd edn. Elsevier Saunders, Philadelphia, pp 506–518
139. Saunderson PR (2015) Drug-resistant *M. leprae*. Clin Dermatol. doi:10.1016/j.clindermatol.2015.1010.1019
140. Andries K, Villellas C, Coeck N, Thys K, Gevers T, Vranckx L, Lounis N, de Jong BC, Koul A (2014) Acquired resistance of *Mycobacterium tuberculosis* to bedaquiline. PLoS One 9:e102135
141. Hartkoorn RC, Uplekar S, Cole ST (2014) Cross-resistance between clofazimine and bedaquiline through upregulation of MmpL5 in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 58:2979–2981
142. Zhang S, Chen J, Cui P, Shi W, Zhang W, Zhang Y (2015) Identification of novel mutations associated with clofazimine resistance in *Mycobacterium tuberculosis*. J Antimicrob Chemother 70:2507–2510
143. Franzblau SG, White KE (1990) Comparative *in vitro* activities of 20 fluoroquinolones against *Mycobacterium leprae*. Antimicrob Agents Chemother 34:229–231
144. Guelpa-Lauras C-C, Perani EG, Giroir A-M, Grosset JH (1987) Activities of pefloxacin and ciprofloxacin against *Mycobacterium leprae* in the mouse. Liver 1:17–28
145. Franzblau SG, Parrilla MLR, Chan GP (1993) Sparfloxacin is more bactericidal than ofloxacin against *Mycobacterium leprae* in mice. Int J Lepr Other Mycobact Dis 61:66
146. Chan GP, Garcia-Ignacio BY, Chavez VE, Livelo J, Jimenez C, Parrilla M, Franzblau S (1994) Clinical trial of sparfloxacin for lepromatous leprosy. Antimicrob Agents Chemother 38:61–65
147. Pardillo FEF, Burgos J, Fajardo TT, Cruz ED, Abalos RM, Paredes RMD, Andaya CES, Gelber RH (2008) Powerful bactericidal activity of moxifloxacin in human leprosy. Antimicrob Agents Chemother 52:3113–3117
148. Dhople AM, Namba K (2003) In-vitro activity of sitafloxacin (DU-6859a), either singly or in combination with rifampin analogs, against *Mycobacterium leprae*. J Infect Chemother 9:12–15
149. Keating GM (2011) Sitafloxacin. Drugs 71:731–744
150. Dhople AM, Ibanez MA (1995) The in-vitro activities of novel benzoxazinorifamycins against *Mycobacterium leprae*. J Antimicrob Chemother 35:463–471
151. Gelber R, Iranmanesh A, Murray L, Siu P, Tsang M (1992) Activities of various quinolone antibiotics against *Mycobacterium leprae* in infected mice. Antimicrob Agents Chemother 36:2544–2547
152. Aldred KJ, Kerns RJ, Osheroff N (2014) Mechanism of quinolone action and resistance. Biochemistry 53:1565–1574

153. Suto MJ, Domagala JM, Roland GE, Mailloux GB, Cohen MA (1992) Fluoroquinolones: relationships between structural variations, mammalian cell cytotoxicity and antimicrobial activity. J Med Chem 35:4745–4750
154. Owens RC, Ambrose PG (2005) Antimicrobial safety: focus on fluoroquinolones. Clin Infect Dis 41:S144–S157
155. Sarro A, Sarro G (2001) Adverse reactions to fluoroquinolones. An overview on mechanistic aspects. Curr Med Chem 8:371–384
156. FDA (2015) Joint Meeting of the Antimicrobial Drugs Advisory Committee (AMDAC) and the Drug Safety and Risk Management Advisory Committee (DSaRM), 5 November 2015. www.fda.gov. Retrieved Dec 2015
157. Cambau E, Sougakoff W, Jarlier V (1994) Amplification and nucleotide sequence of the quinolone resistance-determining region in the gyrA gene of mycobacteria. FEMS Microbiol Lett 116:49–54
158. Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, Cole ST, Jacobs WR, Telenti A (1994) Cloning and nucleotide sequence of *Mycobacterium tuberculosis* gyrA and gyrB genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother 38:773–780
159. Redin G (1966) Antibacterial activity in mice of minocycline, a new tetracycline. Antimicrob Agents Chemother 6:371
160. Gelber RH (1987) Activity of minocycline in *Mycobacterium leprae*-infected mice. J Infect Dis 156:236–239
161. Ji B, Sow S, Perani E, Lienhardt C, Diderot V, Grosset J (1998) Bactericidal activity of a single-dose combination of ofloxacin plus minocycline, with or without rifampin, against *Mycobacterium leprae* in mice and in lepromatous patients. Antimicrob Agents Chemother 42:1115–1120
162. Ji B, Jamet P, Perani EG, Bobin P, Grosset JH (1993) Powerful bactericidal activities of clarithromycin and minocycline against *Mycobacterium leprae* in lepromatous leprosy. J Infect Dis 168:188–190
163. Taylor DE, Chau A (1996) Tetracycline resistance mediated by ribosomal protection. Antimicrob Agents Chemother 40:1
164. Regen F, Hildebrand M, Le Bret N, Herzog I, Heuser I, Hellmann-Regen J (2015) Inhibition of retinoic acid catabolism by minocycline: evidence for a novel mode of action? Exp Dermatol 24:473–476
165. Sadowski T, Steinmeyer J (2001) Minocycline inhibits the production of inducible nitric oxide synthase in articular chondrocytes. J Rheumatol 28:336–340
166. Chaudhry IB, Hallak J, Husain N, Minhas F, Stirling J, Richardson P, Dursun S, Dunn G, Deakin B (2012) Minocycline benefits negative symptoms in early schizophrenia: a randomised double-blind placebo-controlled clinical trial in patients on standard treatment. J Psychopharmacol 26:1185–1193
167. Giuliani F, Hader W, Yong VW (2005) Minocycline attenuates T cell and microglia activity to impair cytokine production in T cell-microglia interaction. J Leukoc Biol 78:135–143
168. SAKIA UN (2015) Persistent serpentine supravenous hyperpigmented eruption in lepromatous leprosy after minocycline. Lepr Rev 86:191–194
169. Speer BS, Shoemaker NB, Salyers AA (1992) Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. Clin Microbiol Rev 5:387–399
170. Gelber RH (1995) Successful treatment of a lepromatous patient with clarithromycin. Int J Lepr Other Mycobact Dis 63:113
171. Sturgill MG, Rapp RP (1992) Clarithromycin: review of a new macrolide antibiotic with improved microbiologic spectrum and favorable pharmacokinetic and adverse effect profiles. Ann Pharmacother 26:1099–1108
172. Rapp RP, McCraney SA, Goodman NL, Shaddick DJ (1994) New macrolide antibiotics: usefulness in infections caused by mycobacteria other than *Mycobacterium tuberculosis*. Ann Pharmacother 28:1255–1263

173. Vester B, Douthwaite S (2001) Macrolide resistance conferred by base substitutions in 23S rRNA. Antimicrob Agents Chemother 45:1–12
174. Meier A, Heifets L, Wallace RJ, Zhang Y, Brown BA, Sander P, Bottger EC (1996) Molecular mechanisms of clarithromycin resistance in *Mycobacterium avium*: observation of multiple 238 rDNA mutations in a clonal population. J Infect Dis 174:354–360
175. You E-Y, Kang TJ, Kim S-K, Lee S-B, Chae G-T (2005) Mutations in genes related to drug resistance in *Mycobacterium leprae* isolates from leprosy patients in Korea. J Infect 50:6–11
176. Gidoh M (1999) [The control leprous peripheral neuropathy and chemotherapy]. Nihon Hansenbyo Gakkai Zasshi 68:83–86
177. Illarramendi X, de Oliveira MLWR, Sales AM, da Costa Nery JA, Sarno EN (2013) Considerations on clinical trials of leprosy treatment: need of novel drug combinations. Clin Invest 3:617–635
178. Franzblau SG, Biswas AN, Harris EB (1992) Fusidic acid is highly active against extracellular and intracellular *Mycobacterium leprae*. Antimicrob Agents Chemother 36:92–94
179. Franzblau SG, Chan GP, Garcia-Ignacio BG, Chavez VE, Livelo JB, Jimenez CL, Parrilla M, Calvo RF, Williams DL, Gillis TP (1994) Clinical trial of fusidic acid for lepromatous leprosy. Antimicrob Agents Chemother 38:1651–1654
180. Baker D, Beddell C, Champness J, Goodford P, Norrington F, Smith D, Stammers D (1981) The binding of trimethoprim to bacterial dihydrofolate reductase. FEBS Lett 126:49–52
181. Seydel J, Rosenfeld M, Sathish M, Wiese M, Schaper K-J, Hachtel G, Haller R, Kansy M, Dhople A (1986) Strategies in the development of new drugs and drug combinations against leprosy, demonstrated on the example of folate and gyrase inhibitors. Lepr Rev 57:235–253
182. Seydel JK, Wiese M, Walter R, Kansy M, Schaper KJ, Sethi N, Chandra S, Dhople AM, Saxena AK (1994) In: Sushil Kumar, Sen AK, Dutta GP, Sharma RN (eds) Tropical diseases molecular biology and control strategies. Publication & Information Directorate, New Delhi, p 214
183. Dhople AM (1999) *In vitro* activity of epiroprim, a dihydrofolate reductase inhibitor, singly and in combination with brodimoprim and dapsone, against *Mycobacterium leprae*. Int J Antimicrob Agents 12:319–323
184. Seydel J (1993) *In vitro* and *in vivo* results of brodimoprim and analogues alone and in combination against *E. coli* and mycobacteria. J Chemother 5:422–429
185. Dhople AM (2002) *In vivo* activity of epiroprim, a dihydrofolate reductase inhibitor, singly and in combination with dapsone, against *Mycobacterium leprae*. Int J Antimicrob Agents 19:71–74
186. Gaugas J (1967) Antimicrobial therapy of experimental human leprosy (*Myco. leprae*) infection in the mouse foot pad. Lepr Rev 38:225–230
187. Baker RJ (1990) The need for new drugs in the treatment and control of leprosy. Int J Lepr Other Mycobact Dis 58:78–97
188. Schaper K-J, Seydel J, Rosenfeld M, Kazda J (1986) Development of inhibitors of mycobacterial ribonucleotide reductase. Lepr Rev 57:254–264
189. Mester L, Szabados L, Mester M, Yadav N (1980) Maillard type carbonyl-amine reactions *in vivo* and their physiological effects. Prog Food Nutr Sci 5:295–314
190. Antia N, Upleker M, Ambrose E, Mahadevan P, Mester L (1988) Effect of deoxyfructoserotonin (DFS) on lepromatous leprosy. Lancet 331:619–622
191. Ambrose E, Antia N, Birdi T, Mahadevan P, Mester L, Mistry N, Mukherjee R, Shetty V (1985) The action of deoxyfructose serotonin on intracellular bacilli and on host response in leprosy. Lepr Rev 56:199–208
192. Mester de Parajd L, Balakrishnan S, Saint-Andre P, Mester de Parajd M (1981) Deoxyfructoserotonin: a new drug with anti-leprosy activity. Ann Microbiol 133:427–432
193. Faget G, Erickson P (1946) Use of streptomycin in the treatment of leprosy. Int J Lepr 15:146–153
194. DREISBACH J, Cochrane R (1958) A study of the effect of streptohydrazid on lepromatous leprosy over a period of about three years. Lepr Rev 29:136–142

195. Gelber R, Gibson J (1979) Killing potential of various aminoglycoside antibiotics for *Mycobacterium-leprae*. Int J Lepr Other Mycobact Dis 47:684–685
196. Gelber R, Henika P, Gibson J (1984) The bactericidal activity of various aminoglycoside antibiotics against *Mycobacterium leprae* in mice. Lepr Rev 55:341–347
197. Gelber RH (1987) Further studies of the killing of *M. leprae* by aminoglycosides: reduced dosage and frequency of administration. Int J Lepr Other Mycobact Dis 55:78–82
198. Ebenezer G, Norman G, Joseph G, Daniel S, Job C (2001) Drug resistant-*Mycobacterium leprae*--results of mouse footpad studies from a laboratory in South India. Indian J Lepr 74:301–312
199. Honoré N, Roche PW, Grosset JH, Cole ST (2001) A method for rapid detection of rifampicin-resistant isolates of *Mycobacterium leprae*. Lepr Rev 72:441–448
200. Musser JM (1995) Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin Microbiol Rev 8:496–514
201. Sampaio LH, Stefani MM, Oliveira RM, Sousa AL, Ireton GC, Reed SG, Duthie MS (2011) Immunologically reactive *M. leprae* antigens with relevance to diagnosis and vaccine development. BMC Infect Dis 11:26
202. Stefani MM, Guerra JG, Sousa AL, Costa MB, Oliveira ML, Martelli CT, Scollard DM (2009) Potential plasma markers of type 1 and type 2 leprosy reactions: a preliminary report. BMC Infect Dis 9:75
203. Zodpey S, Bansod B, Shrikhande S, Maldhure B, Kulkarni S (1999) Protective effect of Bacillus Calmette Guerin (BCG) against leprosy: a population-based case-control study in Nagpur, India. Lepr Rev 70:287–294
204. Zodpey S, Ambadekar N, Thakur A (2005) Effectiveness of Bacillus Calmette Guerin (BCG) vaccination in the prevention of leprosy: a population-based case-control study in Yavatmal District, India. Public Health 119:209–216
205. Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC, Hijjar MA, Cruz AA, Sant'Anna C, Bierrenbach AL (2005) Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet 366:1290–1295
206. Nakanaga K, Yotsu RR, Hoshino Y, Suzuki K, Makino M, Ishii N (2013) Buruli ulcer and mycolactone-producing mycobacteria. Jpn J Infect Dis 66:83–88
207. Cook AR (1897) Mengo hospital notes. Makerere Medical School Library, Kampala
208. MacCallum P, Tolhurst JC, Buckle G, Sissons H (1948) A new mycobacterial infection in man. J Pathol Bacteriol 60:93–122
209. Clancey J, Dodge O, Lunn H, Oduori M (1961) Mycobacterial skin ulcers in Uganda. Lancet 278:951–954
210. Wansbrough-Jones M, Phillips R (2006) Buruli ulcer: emerging from obscurity. Lancet 367:1849–1858
211. WHO (2008) Buruli ulcer: progress report: 2004–2008. Wkly Epidemiol Rec 83:144–154
212. Aujoulat I, Johnson C, Zinsou C, Guédénon A, Portaels F (2003) Psychosocial aspects of health seeking behaviours of patients with Buruli ulcer in Southern Benin. Trop Med Int Health 8:750–759
213. Debacker M, Aguiar J, Steunou C, Zinsou C, Meyers WM, Guédénon A, Scott JT, Dramaix M, Portaels F (2004) *Mycobacterium ulcerans* disease (Buruli ulcer) in rural hospital, Southern Benin, 1997-2001. Emerg Infect Dis 10:1391–1398
214. Moran M, Guzman J, Ropars A-L, McDonald A, Jameson N, Omune B, Ryan S, Wu L (2009) Neglected disease research and development: how much are we really spending. PLoS Med 6:e1000030
215. Johnson P, Azuolas J, Lavender CJ, Wishart E, Stinear TP, Hayman JA, Brown L, Jenkin GA, Fyfe J (2007) *Mycobacterium ulcerans* in mosquitoes captured during outbreak of Buruli ulcer, Southeastern Australia. Emerg Infect Dis 13:1653–1660
216. Portaels F, Elsen P, Guimaraes-Peres A, Fonteyne P-A, Meyers WM (1999) Insects in the transmission of *Mycobacterium ulcerans* infection. Lancet 353:986

217. Marsollier L, Robert R, Aubry J, Saint André J-P, Kouakou H, Legras P, Manceau A-L, Mahaza C, Carbonnelle B (2002) Aquatic insects as a vector for *Mycobacterium ulcerans*. Appl Environ Microbiol 68:4623–4628
218. Portaels F, Meyers WM, Ablordey A, Castro AG, Chemlal K, de Rijk P, Elsen P, Fissette K, Fraga AG, Lee R (2008) First cultivation and characterization of *Mycobacterium ulcerans* from the environment. PLoS Negl Trop Dis 2:e178
219. The Uganda Buruli Group (1971) Epidemiology of *Mycobacterium ulcerans* infection (Buruli ulcer) at Kinyara, Uganda. Trans R Soc Trop Med Hyg 65:763–775
220. Trubiano JA, Lavender CJ, Fyfe J, Bittmann S, Johnson P (2013) The incubation period of Buruli ulcer (*Mycobacterium ulcerans* infection). PLoS Negl Trop Dis 7:e2463
221. Carson C, Lavender CJ, Handasyde KA, O'Brien CR, Hewitt N, Johnson PD, Fyfe JA (2014) Potential wildlife sentinels for monitoring the endemic spread of human Buruli ulcer in South-East Australia. PLoS Negl Trop Dis 8:e2668
222. O'Brien CR, Handasyde KA, Hibble J, Lavender CJ, Legione AR, McCowan C, Globan M, Mitchell AT, McCracken HE, Johnson PD (2014) Clinical, microbiological and pathological findings of *Mycobacterium ulcerans* infection in three Australian Possum species. PLoS Negl Trop Dis 8:e2666
223. Huang GKL, Johnson PD (2014) Epidemiology and management of Buruli ulcer. Expert Rev Anti Infect Ther 12:855–865
224. Junghanss T, Johnson RC, Pluschke G (2014) *Mycobacterium ulcerans* disease. In: Farrar J, Hotez PJ, Junghanss T, Kang G, Lalloo D, White N (eds) Manson's tropical diseases, 23rd edn.Elsevier Saunders, Philadelphia, pp 519–531
225. WHO (2015) Buruli ulcer (*Mycobacterium ulcerans* infection) fact sheet N° 199, World Health Organization, Geneva. http://www.who.int/mediacentre/factsheets/fs199/en/
226. Portaels F, Johnson P, Meyers WM (2001) Buruli ulcer: diagnosis of *Mycobacterium ulcerans* disease. World Health Organization, Geneva. https://extranet.who.int/iris/restricted/handle/10665/67000. Accessed December 2015
227. Merritt RW, Walker ED, Small PL, Wallace JR, Johnson PD, Benbow ME, Boakye DA (2010) Ecology and transmission of Buruli ulcer disease: a systematic review. PLoS Negl Trop Dis 4:e911
228. Stienstra Y, Van Der Graaf W, Te Meerman G, The T, De Leij L, Van der Werf T (2001) Susceptibility to development of *Mycobacterium ulcerans* disease: review of possible risk factors. Trop Med Int Health 6:554–562
229. Meyers WM, Shelly WM, Connor DH, Meyers EK (1974) Human *Mycobacterium ulcerans* infections developing at sites of trauma to skin. Am J Trop Med Hyg 23:919–923
230. Portaels F, Chemlal K, Elsen P, Johnson P, Hayman J, Hibble J, Kirkwood R, Meyers W (2001) *Mycobacterium ulcerans* in wild animals. Rev Sci Tech 20:252–264
231. Stinear TP, Seemann T, Pidot S, Frigui W, Reysset G, Garnier T, Meurice G, Simon D, Bouchier C, Ma L (2007) Reductive evolution and niche adaptation inferred from the genome of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer. Genome Res 17:192–200
232. Daffé M, Varnerot A, Lévy-Frébault VV (1992) The phenolic mycoside of *Mycobacterium ulcerans*: structure and taxonomic implications. J Gen Microbiol 138:131–137
233. Stinear TP, Seemann T, Harrison PF, Jenkin GA, Davies JK, Johnson PD, Abdellah Z, Arrowsmith C, Chillingworth T, Churcher C (2008) Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*. Genome Res 18:729–741
234. Stinear TP, Mve-Obiang A, Small PL, Frigui W, Pryor MJ, Brosch R, Jenkin GA, Johnson PD, Davies JK, Lee RE (2004) Giant plasmid-encoded polyketide synthases produce the macrolide toxin of *Mycobacterium ulcerans*. Proc Natl Acad Sci USA 101:1345–1349
235. George KM, Chatterjee D, Gunawardana G, Welty D, Hayman J, Lee R, Small P (1999) Mycolactone: a polyketide toxin from *Mycobacterium ulcerans* required for virulence. Science 283:854–857

236. George KM, Pascopella L, Welty DM, Small P (2000) A *Mycobacterium ulcerans* toxin, mycolactone, causes apoptosis in guinea pig ulcers and tissue culture cells. Infect Immun 68:877–883
237. Eddyani M, Fraga AG, Schmitt F, Uwizeye C, Fissette K, Johnson C, Aguiar J, Sopoh G, Barogui Y, Meyers WM (2009) Fine-needle aspiration, an efficient sampling technique for bacteriological diagnosis of nonulcerative Buruli ulcer. J Clin Microbiol 47:1700–1704
238. Tian J, Bryk R, Itoh M, Suematsu M, Nathan C (2005) Variant tricarboxylic acid cycle in *Mycobacterium tuberculosis*: identification of α-ketoglutarate decarboxylase. Proc Natl Acad Sci USA 102:10670–10675
239. Muñoz-Elías EJ, McKinney JD (2005) *Mycobacterium tuberculosis* isocitrate lyases 1 and 2 are jointly required for *in vivo* growth and virulence. Nat Med 11:638–644
240. Brosch R, Pym AS, Gordon SV, Cole ST (2001) The evolution of mycobacterial pathogenicity: clues from comparative genomics. Trends Microbiol 9:452–458
241. Portevin D, de Sousa-D'Auria C, Houssin C, Grimaldi C, Chami M, Daffé M, Guilhot C (2004) A polyketide synthase catalyzes the last condensation step of mycolic acid biosynthesis in mycobacteria and related organisms. Proc Natl Acad Sci 101:314–319
242. Daffe M, Laneelle M, Lacave C (1991) Structure and stereochemistry of mycolic acids of *Mycobacterium marinum* and *Mycobacterium ulcerans*. Res Microbiol 142:397–403
243. Daffe M, Laneelle M, Roussel J, Asselineau C (1983) Specific lipids from *Mycobacterium ulcerans*. Ann Microbiol 135:191–201
244. Onwueme KC, Vos CJ, Zurita J, Soll CE, Quadri LE (2005) Identification of phthiodiolone ketoreductase, an enzyme required for production of mycobacterial diacyl phthiocerol virulence factors. J Bacteriol 187:4760–4766
245. Reed MB, Domenech P, Manca C, Su H, Barczak AK, Kreiswirth BN, Kaplan G, Barry CE (2004) A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature 431:84–87
246. Rohdich F, Bacher A, Eisenreich W (2004) Perspectives in anti-infective drug design. The late steps in the biosynthesis of the universal terpenoid precursors, isopentenyl diphosphate and dimethylallyl diphosphate. Bioorg Chem 32:292–308
247. Yip MJ, Porter JL, Fyfe JA, Lavender CJ, Portaels F, Rhodes M, Kator H, Colorni A, Jenkin GA, Stinear T (2007) Evolution of *Mycobacterium ulcerans* and other mycolactone-producing mycobacteria from a common *Mycobacterium marinum* progenitor. J Bacteriol 189:2021–2029
248. Demangel C, Stinear TP, Cole ST (2009) Buruli ulcer: reductive evolution enhances pathogenicity of *Mycobacterium ulcerans*. Nat Rev Microbiol 7:50–60
249. Abdallah AM, van Pittius NCG, Champion PAD, Cox J, Luirink J, Vandenbroucke-Grauls CM, Appelmelk BJ, Bitter W (2007) Type VII secretion—mycobacteria show the way. Nat Rev Microbiol 5:883–891
250. Brodin P, Rosenkrands I, Andersen P, Cole ST, Brosch R (2004) ESAT-6 proteins: protective antigens and virulence factors? Trends Microbiol 12:500–508
251. Mishra KC, De Chastellier C, Narayana Y, Bifani P, Brown AK, Besra GS, Katoch VM, Joshi B, Balaji KN, Kremer L (2008) Functional role of the PE domain and immunogenicity of the *Mycobacterium tuberculosis* triacylglycerol hydrolase LipY. Infect Immun 76:127–140
252. Fortune S, Jaeger A, Sarracino D, Chase M, Sassetti C, Sherman D, Bloom B, Rubin E (2005) Mutually dependent secretion of proteins required for mycobacterial virulence. Proc Natl Acad Sci USA 102:10676–10681
253. Huber CA, Ruf M-T, Pluschke G, Käser M (2008) Independent loss of immunogenic proteins in *Mycobacterium ulcerans* suggests immune evasion. Clin Vaccine Immunol 15:598–606
254. Hong H, Demangel C, Pidot SJ, Leadlay PF, Stinear T (2008) Mycolactones: immunosuppressive and cytotoxic polyketides produced by aquatic mycobacteria. Nat Prod Rep 25:447–454

255. Mve-Obiang A, Lee RE, Portaels F, Small P (2003) Heterogeneity of mycolactones produced by clinical isolates of *Mycobacterium ulcerans*: implications for virulence. Infect Immun 71:774–783
256. Oswald E, Nougayrède J-P, Taieb F, Sugai M (2005) Bacterial toxins that modulate host cell-cycle progression. Curr Opin Microbiol 8:83–91
257. Simmonds RE, Lali FV, Smallie T, Small PL, Foxwell BM (2009) Mycolactone inhibits monocyte cytokine production by a posttranscriptional mechanism. J Immunol 182:2194–2202
258. Walsh DS, Meyers WM, Portaels F, Lane JE, Mongkolsirichaikul D, Hussem K, Gosi P, Myint KSA (2005) High rates of apoptosis in human *Mycobacterium ulcerans* culture-positive Buruli ulcer skin lesions. Am J Trop Med Hyg 73:410–415
259. Kishi Y (2011) Chemistry of mycolactones, the causative toxins of Buruli ulcer. Proc Natl Acad Sci 108:6703–6708
260. Stinear TP, Pryor MJ, Porter JL, Cole ST (2005) Functional analysis and annotation of the virulence plasmid pMUM001 from *Mycobacterium ulcerans*. Microbiology 151:683–692
261. Goto M, Nakanaga K, Aung T, Hamada T, Yamada N, Nomoto M, Kitajima S, Ishii N, Yonezawa S, Saito H (2006) Nerve damage in *Mycobacterium ulcerans*-infected mice: probable cause of painlessness in Buruli ulcer. Am J Pathol 168:805–811
262. En J, Goto M, Nakanaga K, Higashi M, Ishii N, Saito H, Yonezawa S, Hamada H, Small PL (2008) Mycolactone is responsible for the painlessness of *Mycobacterium ulcerans* infection (Buruli ulcer) in a murine study. Infect Immun 76:2002–2007
263. Marion E, Song O-R, Christophe T, Babonneau J, Fenistein D, Eyer J, Letournel F, Henrion D, Clere N, Paille V (2014) Mycobacterial toxin induces analgesia in Buruli ulcer by targeting the angiotensin pathways. Cell 157:1565–1576
264. Guenin-Macé L, Veyron-Churlet R, Thoulouze M-I, Romet-Lemonne G, Hong H, Leadlay PF, Danckaert A, Ruf M-T, Mostowy S, Zurzolo C (2013) Mycolactone activation of Wiskott-Aldrich syndrome proteins underpins Buruli ulcer formation. J Clin Invest 123:1501–1502
265. Zimmermann R, Eyrisch S, Ahmad M, Helms V (2011) Protein translocation across the ER membrane. Biochim Biophys Acta Biomembr 1808:912–924
266. Sarfo FS, Phillips R, Wansbrough-Jones M, Simmonds RE (2015) Recent advances: role of mycolactone in the pathogenesis and monitoring of *Mycobacterium ulcerans* infection/Buruli ulcer disease. Cell Microbiol. doi:10.1111/cmi.12547
267. Scherr N, Gersbach P, Dangy J-P, Bomio C, Li J, Altmann K-H, Pluschke G (2013) Structure-activity relationship studies on the macrolide exotoxin mycolactone of *Mycobacterium ulcerans*. PLoS Negl Trop Dis 7:e2143
268. Adusumilli S, Mve-Obiang A, Sparer T, Meyers W, Hayman J, Small PLC (2005) *Mycobacterium ulcerans* toxic macrolide, mycolactone modulates the host immune response and cellular location of *M. ulcerans in vitro* and *in vivo*. Cell Microbiol 7:1295–1304
269. Schütte D, Um-Boock A, Mensah-Quainoo E, Itin P, Schmid P, Pluschke G (2007) Development of highly organized lymphoid structures in Buruli ulcer lesions after treatment with rifampicin and streptomycin. PLoS Negl Trop Dis 1:e2
270. Torrado E, Fraga AG, Castro AG, Stragier P, Meyers WM, Portaels F, Silva MT, Pedrosa J (2007) Evidence for an intramacrophage growth phase of *Mycobacterium ulcerans*. Infect Immun 75:977–987
271. Torrado E, Fraga AG, Logarinho E, Martins TG, Carmona JA, Gama JB, Carvalho MA, Proença F, Castro AG, Pedrosa J (2010) IFN-γ–dependent activation of macrophages during experimental infections by *Mycobacterium ulcerans* is impaired by the toxin mycolactone. J Immunol 184:947–955
272. Hall BS, Hill K, McKenna M, Ogbechi J, High S, Willis AE, Simmonds RE (2014) The pathogenic mechanism of the *Mycobacterium ulcerans* virulence factor, mycolactone, depends on blockade of protein translocation into the ER. PLoS Pathog 10:e1004061

273. Coutanceau E, Decalf J, Martino A, Babon A, Winter N, Cole ST, Albert ML, Demangel C (2007) Selective suppression of dendritic cell functions by *Mycobacterium ulcerans* toxin mycolactone. J Exp Med 204:1395–1403
274. Boulkroun S, Guenin-Macé L, Thoulouze M-I, Monot M, Merckx A, Langsley G, Bismuth G, Di Bartolo V, Demangel C (2010) Mycolactone suppresses T cell responsiveness by altering both early signaling and posttranslational events. J Immunol 184:1436–1444
275. Phillips R, Sarfo FS, Guenin-Macé L, Decalf J, Wansbrough-Jones M, Albert ML, Demangel C (2009) Immunosuppressive signature of cutaneous *Mycobacterium ulcerans* infection in the peripheral blood of patients with Buruli ulcer disease. J Infect Dis 200:1675–1684
276. Guenin-Macé L, Carrette F, Asperti-Boursin F, Le Bon A, Caleechurn L, Di Bartolo V, Fontanet A, Bismuth G, Demangel C (2011) Mycolactone impairs T cell homing by suppressing microRNA control of L-selectin expression. Proc Natl Acad Sci 108:12833–12838
277. Ham O, Lee S-Y, Lee CY, Park J-H, Lee J, Seo H-H, Cha M-J, Choi E, Kim S, Hwang K-C (2015) Let-7b suppresses apoptosis and autophagy of human mesenchymal stem cells transplanted into ischemia/reperfusion injured heart 7by targeting caspase-3. Stem Cell Res Ther 6:1–11
278. En J, Ishii N, Goto M (2011) Role of mycolactone in the nerve damage of Buruli ulcer (*Mycobacterium ulcerans* infection). Nihon Hansenbyo Gakkai Zasshi 80:5–10
279. World Health Organization (2012) Treatment of *Mycobacterium ulcerans* disease (Buruli ulcer): guidance for health workers. World Health Organization, Geneva. http://www.who.int/buruli/information/antibiotics/en
280. Debacker M, Aguiar J, Steunou C, Zinsou C, Meyers WM, Portaels F (2005) Buruli ulcer recurrence, Benin. Emerg Infect Dis 11:584–589
281. Van der Werf TS, Van der Graaf WT, Tappero JW, Asiedu K (1999) *Mycobacterium ulcerans* infection. Lancet 354:1013–1018
282. Teelken M, Stienstra Y, Ellen D, Quarshie E, Klutse E, van der Graaf W, van der Werf TS (2003) Buruli ulcer: differences in treatment outcome between two centres in Ghana. Acta Trop 88:51–56
283. O'Brien DP, Walton A, Hughes AJ, Friedman ND, McDonald A, Callan P, Rhadon R, Holten I, Athan E (2013) Risk factors for recurrent *Mycobacterium ulcerans* disease after exclusive surgical treatment in an Australian cohort. Med J Aust 198:436–439
284. Thangaraj H, Adjei O, Allen B, Portaels F, Evans M, Banerjee D, Wansbrough-Jones M (2000) *In vitro* activity of ciprofloxacin, sparfloxacin, ofloxacin, amikacin and rifampicin against Ghanaian isolates of *Mycobacterium ulcerans*. J Antimicrob Chemother 45:231–233
285. Saito H, Ishii N (2001) Antibacterial activities of new fluoroquinolones against *Mycobacterium ulcerans*. J Antimicrob Chemother 47:30
286. O'Brien DP, McDonald A, Callan P, Robson M, Friedman ND, Hughes A, Holten I, Walton A, Athan E (2012) Successful outcomes with oral fluoroquinolones combined with rifampicin in the treatment of *Mycobacterium ulcerans*: an observational cohort study. PLoS Negl Trop Dis 6:e1473
287. Friedman ND, Athan E, Hughes AJ, Khajehnoori M, McDonald A, Callan P, Rahdon R, O'Brien DP (2013) *Mycobacterium ulcerans* disease: experience with primary oral medical therapy in an Australian cohort. PLoS Negl Trop Dis 7:e2315
288. O'Brien DP, Athan E, Hughes A, Johnson PD (2008) Successful treatment of *Mycobacterium ulcerans* osteomyelitis with minor surgical debridement and prolonged rifampicin and ciprofloxacin therapy: a case report. J Med Case Rep 2:123
289. Ji B, Chauffour A, Robert J, Lefrançois S, Jarlier V (2007) Orally administered combined regimens for treatment of *Mycobacterium ulcerans* infection in mice. Antimicrob Agents Chemother 51:3737–3739
290. Marsollier L, Prévot G, Honoré N, Legras P, Manceau A-L, Payan C, Kouakou H, Carbonnelle B (2003) Susceptibility of *Mycobacterium ulcerans* to a combination of amikacin/rifampicin. Int J Antimicrob Agents 22:562–566

291. Nienhuis WA, Stienstra Y, Thompson WA, Awuah PC, Abass KM, Tuah W, Awua-Boateng NY, Ampadu EO, Siegmund V, Schouten JP (2010) Antimicrobial treatment for early, limited *Mycobacterium ulcerans* infection: a randomised controlled trial. Lancet 375:664–672
292. Klis S, Stienstra Y, Phillips RO, Abass KM, Tuah W, van der Werf TS (2014) Long term streptomycin toxicity in the treatment of Buruli ulcer: follow-up of participants in the BURULICO drug trial. PLoS Negl Trop Dis 8:e2739
293. Omansen TF, Porter JL, Johnson PD, van der Werf TS, Stienstra Y, Stinear TP (2015) In-vitro activity of avermectins against *Mycobacterium ulcerans*. PLoS Negl Trop Dis 9:e0003549
294. Meyers WM, Shelly WM, Connor DH (1974) Heat treatment of *Mycobacterium ulcerans* infections without surgical excision. Am J Trop Med Hyg 23:924–929
295. Junghanss T, Boock AU, Vogel M, Schuette D, Weinlaeder H, Pluschke G (2009) Phase change material for thermotherapy of Buruli ulcer: a prospective observational single centre proof-of-principle trial. PLoS Negl Trop Dis 3:e380
296. Phillips R, Adjei O, Lucas S, Benjamin N, Wansbrough-Jones M (2004) Pilot randomized double-blind trial of treatment of *Mycobacterium ulcerans* disease (Buruli ulcer) with topical nitrogen oxides. Antimicrob Agents Chemother 48:2866–2870
297. Phillips R, Kuijper S, Benjamin N, Wansbrough-Jones M, Wilks M, Kolk A (2004) *In vitro* killing of *Mycobacterium ulcerans* by acidified nitrite. Antimicrob Agents Chemother 48:3130–3132
298. Adjei O, Evans M, Asiedu A (1998) Phenytoin in the treatment of Buruli ulcer. Trans R Soc Trop Med Hyg 92:108–109
299. Krieg R, Wolcott J, Confer A (1975) Treatment of *Mycobacterium ulcerans* infection by hyperbaric oxygenation. Aviat Space Environ Med 46:1241–1245
300. Krieg R, Wolcott J, Meyers W (1979) *Mycobacterium ulcerans* infection: treatment with rifampin, hyperbaric oxygenation, and heat. Aviat Space Environ Med 50:888–892
301. Portaels F, Aguiar J, Debacker M, Guedenon A, Steunou C, Zinsou C, Meyers W (2004) *Mycobacterium bovis* BCG vaccination as prophylaxis against *Mycobacterium ulcerans* osteomyelitis in Buruli ulcer disease. Infect Immun 72:62–65
302. Horwitz MA, Harth G, Dillon BJ, Masleša-Galić S (2005) Enhancing the protective efficacy of *Mycobacterium bovis* BCG vaccination against tuberculosis by boosting with the *Mycobacterium tuberculosis* major secretory protein. Infect Immun 73:4676–4683
303. Magalhaes I, Sizemore DR, Ahmed RK, Mueller S, Wehlin L, Scanga C, Weichold F, Schirru G, Pau MG, Goudsmit J (2008) rBCG induces strong antigen-specific T cell responses in rhesus macaques in a prime-boost setting with an adenovirus 35 tuberculosis vaccine vector. PLoS One 3:e3790
304. Von Eschen K, Morrison R, Braun M, Ofori-Anyinam O, De Kock E, Pavithran P, Koutsoukos M, Moris P, Cain D, Dubois M-C (2009) The candidate tuberculosis vaccine Mtb72F/AS02A: tolerability and immunogenicity in humans. Hum Vaccin 5:475–482
305. Skeiky YA, Dietrich J, Lasco TM, Stagliano K, Dheenadhayalan V, Goetz MA, Cantarero L, Basaraba RJ, Bang P, Kromann I (2010) Non-clinical efficacy and safety of HyVac4: IC31 vaccine administered in a BCG prime–boost regimen. Vaccine 28:1084–1093
306. Van Dissel JT, Soonawala D, Joosten SA, Prins C, Arend SM, Bang P, Tingskov PN, Lingnau K, Nouta J, Hoff ST (2011) Ag85B–ESAT-6 adjuvanted with IC31® promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in volunteers with previous BCG vaccination or tuberculosis infection. Vaccine 29:2100–2109
307. McShane H, Pathan AA, Sander CR, Keating SM, Gilbert SC, Huygen K, Fletcher HA, Hill AV (2004) Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. Nat Med 10:1240–1244
308. Einarsdottir T, Huygen K (2011) Buruli ulcer. Hum Vaccin 7:1198–1203

309. Tanghe A, Van Vooren J-P, Portaels F, Huygen K (2001) Protective efficacy of a DNA vaccine encoding antigen 85A from *Mycobacterium bovis* BCG against Buruli ulcer. Infect Immun 69:5403–5411
310. Tanghe A, Dangy J-P, Pluschke G, Huygen K (2008) Improved protective efficacy of a species-specific DNA vaccine encoding mycolyl-transferase Ag85A from *Mycobacterium ulcerans* by homologous protein boosting. PLoS Negl Trop Dis 2:e199
311. Coutanceau E, Legras P, Marsollier L, Reysset G, Cole ST, Demangel C (2006) Immunogenicity of *Mycobacterium ulcerans* Hsp65 and protective efficacy of a *Mycobacterium leprae* Hsp65-based DNA vaccine against Buruli ulcer. Microbes Infect 8:2075–2081
312. Nguyen TTH, Bezouska K, Vavrincova P, Sedlacek P, Hromadnikova I (2008) Humoral response against *Mycobacterium bovis* Hsp65 derived fragments in children and young people with various disorders. J Immunoass Immunochem 29:281–298
313. Dawson CR, Schachter J (2013) Trachoma. Oxford University Press, Oxford
314. Bechtle M, Chen S, Efferth T (2010) Neglected diseases caused by bacterial infections. Curr Med Chem 17:42–60
315. Bryan CP (1930) The papyrus Ebers. Bles, London (translated from the German version)
316. WHO (2014) Visual impairment and blindness. Fact sheet N°282. World Health Organization, Geneva. http://www.who.int/mediacentre/factsheets/fs282/en/
317. WHO (2013) Blinding trachoma fact sheet N°382. World Health Organization, Geneva. http://www.who.int/mediacentre/factsheets/fs382/en/
318. Tabbara KF, Al-Omar OM (1997) Trachoma in Saudi Arabia. Ophthalmic Epidemiol 4:127–140
319. Khandekar R, Mohammed AJ (2007) The prevalence of trachomatous trichiasis in Oman (Oman eye study 2005). Ophthalmic Epidemiol 14:267–272
320. Taylor HR (2008) Trachoma: a blinding scourge from the Bronze Age to the twenty-first century. Centre for Eye Research Australia, East Melbourne
321. West S, Muñoz B, Lynch M, Kayongoya A, Chilangwa Z, Mmbaga B, Taylor HR (1995) Impact of face-washing on trachoma in Kongwa, Tanzania. Lancet 345:155–158
322. Emerson PM, Bailey RL, Mahdi OS, Walraven GE, Lindsay SW (2000) Transmission ecology of the fly Musca sorbens, a putative vector of trachoma. Trans R Soc Trop Med Hyg 94:28–32
323. Miller K, Pakpour N, Yi E, Melese M, Alemayehu W, Bird M, Schmidt G, Cevallos V, Olinger L, Chidambaram J (2004) Pesky trachoma suspect finally caught. Br J Ophthalmol 88:750–751
324. Emerson PM, Lindsay SW, Alexander N, Bah M, Dibba S-M, Faal HB, Lowe K, McAdam KP, Ratcliffe AA, Walraven GE (2004) Role of flies and provision of latrines in trachoma control: cluster-randomised controlled trial. Lancet 363:1093–1098
325. Smith JL, Flueckiger RM, Hooper PJ, Polack S, Cromwell EA, Palmer SL, Emerson PM, Mabey DC, Solomon AW, Haddad D (2013) The geographical distribution and burden of trachoma in Africa. PLoS Negl Trop Dis 7:e2359
326. Solomon AW, Pavluck AL, Courtright P, Aboe A, Adamu L, Alemayehu W, Alemu M, Alexander ND, Kello AB, Bero B (2015) The global trachoma mapping project: methodology of a 34-country population-based study. Ophthalmic Epidemiol 22:214–225
327. Burton MJ, Mabey D (2009) The global burden of trachoma: a review. PLoS Negl Trop Dis 3: e460
328. West SK, Munoz B, TURNER VM, Mmbaga B, TAYLOR HR (1991) The epidemiology of trachoma in central Tanzania. Int J Epidemiol 20:1088–1092
329. Ngondi J, Onsarigo A, Adamu L, Matende I, Baba S, Reacher M, Emerson P, Zingeser J (2005) The epidemiology of trachoma in Eastern Equatoria and Upper Nile States, Southern Sudan. Bull World Health Organ 83:904–912
330. Ngondi J, Gebre T, Shargie EB, Adamu L, Ejigsemahu Y, Teferi T, Zerihun M, Ayele B, Cevallos V, King J (2009) Evaluation of three years of the SAFE strategy (surgery,

antibiotics, facial cleanliness and environmental improvement) for trachoma control in five districts of Ethiopia hyperendemic for trachoma. Trans R Soc Trop Med Hyg 103:1001–1010
331. Courtright P, Sheppard J, Schachter J, Said M, Dawson C (1989) Trachoma and blindness in the Nile Delta: current patterns and projections for the future in the rural Egyptian population. Br J Ophthalmol 73:536–540
332. Halberstaedter L, von Prowazek S (1907) Ueber Zelleinschlüsse parasitärer Natur beim trachom. Arb K GesundhAmte 26:44–47
333. Tang F, Chang H, Huang Y, Wang K (1957) Studies on the etiology of trachoma with special reference to isolation of the virus in chick embryo. Chin Med J (Engl) 75:429–447
334. Collier L, Duke-Elder S, Jones BR (1958) Experimental trachoma produced by cultured virus. Br J Ophthalmol 42:705
335. Cho N-J, Potroz MG (2015) Natural products for the treatment of trachoma and *Chlamydia trachomatis*. Molecules 20:4180–4203
336. Byrne GI (2003) *Chlamydia* uncloaked. Proc Natl Acad Sci 100:8040–8042
337. Burton MJ (2007) Trachoma: an overview. Br Med Bull 84:99–116
338. Andreasen AA, Burton MJ, Holland MJ, Polley S, Faal N, Mabey DC, Bailey RL (2008) *Chlamydia trachomatis* ompA variants in trachoma: what do they tell us? PLoS Negl Trop Dis 2:e306
339. Grayston JT, Wang S-P, Yeh L-J, Kuo C-C (1985) Importance of reinfection in the pathogenesis of trachoma. Rev Infect Dis 7:717–725
340. Ortiz L, Angevine M, Kim S-K, Watkins D, DeMars R (2000) T-cell epitopes in variable segments of *Chlamydia trachomatis* major outer membrane protein Elicit Serovar-specific immune responses in infected humans. Infect Immun 68:1719–1723
341. Clarke IN (2011) Evolution of *Chlamydia trachomatis*. Ann N Y Acad Sci 1230:E11–E18
342. Matsumoto A (1988) Structural characteristics of chlamydial bodies. In: Barron AL (ed) Microbiology of *Chlamydia*. CRC, Boca Raton
343. Moulder JW (1991) Interaction of chlamydiae and host cells *in vitro*. Microbiol Rev 55:143
344. AbdelRahman YM, Belland RJ (2005) The chlamydial developmental cycle. FEMS Microbiol Rev 29:949–959
345. Brunham RC, Rey-Ladino J (2005) Immunology of *Chlamydia* infection: implications for a *Chlamydia trachomatis* vaccine. Nat Rev Immunol 5:149–161
346. Chen JC-R, Stephens RS (1997) *Chlamydia trachomatis* glycosaminoglycan-dependent and independent attachment to eukaryotic cells. Microb Pathog 22:23–30
347. Hackstadt T, Todd W, Caldwell H (1985) Disulfide-mediated interactions of the chlamydial major outer membrane protein: role in the differentiation of chlamydiae? J Bacteriol 161:25–31
348. Beatty WL, Morrison RP, Byrne GI (1994) Persistent chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis. Microbiol Rev 58:686–699
349. Kalayoglu MV (2002) Ocular chlamydial infections: pathogenesis and emerging treatment strategies. Curr Drug Targets Infect Disord 2:85–91
350. Hatch T, Al-Hossainy E, Silverman J (1982) Adenine nucleotide and lysine transport in *Chlamydia psittaci*. J Bacteriol 150:662–670
351. Iliffe-Lee ER, McClarty G (1999) Glucose metabolism in *Chlamydia trachomatis*: the 'energy parasite' hypothesis revisited. Mol Microbiol 33:177–187
352. McClarty G (1999) Chlamydial metabolism as inferred from the complete genome sequence. In: Chlamydia: intracellular biology, pathogenesis, and immunity. American Society for Microbiology, Washington, pp 69–100
353. Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, Mitchell W, Olinger L, Tatusov RL, Zhao Q (1998) Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science 282:754–759
354. Kalman S, Mitchell W, Marathe R, Lammel C, Fan J, Hyman RW, Olinger L, Grimwood J, Davis RW, Stephens R (1999) Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*. Nat Genet 21:385–389

355. Carlson JH, Porcella SF, McClarty G, Caldwell HD (2005) Comparative genomic analysis of *Chlamydia trachomatis* oculotropic and genitotropic strains. Infect Immun 73:6407–6418
356. Thomson NR, Holden MT, Carder C, Lennard N, Lockey SJ, Marsh P, Skipp P, O'Connor CD, Goodhead I, Norbertzcak H (2008) *Chlamydia trachomatis*: genome sequence analysis of lymphogranuloma venereum isolates. Genome Res 18:161–171
357. Weiss E, Neptune E, Gaugler R (1968) Influence of gas environment on catabolic activities and on reoxidation of reduced nicotinamide adenine dinucleotide phosphate in Chlamydia. J Bacteriol 96:1567–1573
358. Weiss E (1967) Transaminase activity and other enzymatic reactions involving pyruvate and glutamate in Chlamydia (psittacosis-trachoma group). J Bacteriol 93:177–184
359. Cecchini G (2003) Function and structure of complex II of the respiratory chain. Annu Rev Biochem 72:77–109
360. Lancaster CRD (2013) The di-heme family of respiratory complex II enzymes. Biochim Biophys Acta Bioenerg 1827:679–687
361. Yao J, Abdelrahman YM, Robertson RM, Cox JV, Belland RJ, White SW, Rock CO (2014) Type II fatty acid synthesis is essential for the replication of *Chlamydia trachomatis*. J Biol Chem 289:22365–22376
362. Saka HA, Valdivia RH (2010) Acquisition of nutrients by Chlamydiae: unique challenges of living in an intracellular compartment. Curr Opin Microbiol 13:4–10
363. Sixt BS, Siegl A, Müller C, Watzka M, Wultsch A, Tziotis D, Montanaro J, Richter A, Schmitt-Kopplin P, Horn M (2013) Metabolic features of Protochlamydia amoebophila elementary bodies—a link between activity and infectivity in Chlamydiae. PLoS Pathog 9: e1003553
364. Haider S, Wagner M, Schmid MC, Sixt BS, Christian JG, Häcker G, Pichler P, Mechtler K, Müller A, Baranyi C (2010) Raman microspectroscopy reveals long-term extracellular activity of chlamydiae. Mol Microbiol 77:687–700
365. Collingro A, Tischler P, Weinmaier T, Penz T, Heinz E, Brunham RC, Read TD, Bavoil PM, Sachse K, Kahane S (2011) Unity in variety—the pan-genome of the Chlamydiae. Mol Biol Evol 28:3253–3270
366. Thomas N, Lusher M, Storey C, Clarke I (1997) Plasmid diversity in Chlamydia. Microbiology 143:1847–1854
367. Pickett MA, Everson JS, Pead PJ, Clarke IN (2005) The plasmids of *Chlamydia trachomatis* and *Chlamydophila pneumoniae* (N16): accurate determination of copy number and the paradoxical effect of plasmid-curing agents. Microbiology 151:893–903
368. Rockey DD (2011) Unraveling the basic biology and clinical significance of the chlamydial plasmid. J Exp Med 208:2159–2162
369. Seth-Smith HM, Harris SR, Persson K, Marsh P, Barron A, Bignell A, Bjartling C, Clark L, Cutcliffe LT, Lambden PR (2009) Co-evolution of genomes and plasmids within *Chlamydia trachomatis* and the emergence in Sweden of a new variant strain. BMC Genomics 10:239
370. Fredlund H, Falk L, Jurstrand M, Unemo M (2004) Molecular genetic methods for diagnosis and characterisation of *Chlamydia trachomatis* and Neisseria gonorrhoeae: impact on epidemiological surveillance and interventions. APMIS 112:771–784
371. Gaynor B, Chidambaram J, Cevallos V, Miao Y, Miller K, Jha H, Bhatta R, Chaudhary J, Holm SO, Whitcher J (2005) Topical ocular antibiotics induce bacterial resistance at extraocular sites. Br J Ophthalmol 89:1097–1099
372. Rasmussen SJ, Eckmann L, Quayle AJ, Shen L, Zhang Y-X, Anderson DJ, Fierer J, Stephens RS, Kagnoff MF (1997) Secretion of proinflammatory cytokines by epithelial cells in response to Chlamydia infection suggests a central role for epithelial cells in chlamydial pathogenesis. J Clin Investig 99:77
373. El-Asrar AMA, Tabbara KF, Al-Kharashi SA, Geboes K, Missotten L, Desmet V (1998) Immunopathogenesis of conjunctival scarring in trachoma. Eye 12:453–460

374. Ingalls RR, Rice PA, Qureshi N, Takayama K, Lin JS, Golenbock DT (1995) The inflammatory cytokine response to *Chlamydia trachomatis* infection is endotoxin mediated. Infect Immun 63:3125–3130
375. Prebeck S, Kirschning C, Dürr S, da Costa C, Donath B, Brand K, Redecke V, Wagner H, Miethke T (2001) Predominant role of toll-like receptor 2 versus 4 in *Chlamydia pneumoniae*-induced activation of dendritic cells. J Immunol 167:3316–3323
376. Prebeck S, Brade H, Kirschning CJ, da Costa CP, Dürr S, Wagner H, Miethke T (2003) The gram-negative bacterium *Chlamydia trachomatis* L 2 stimulates tumor necrosis factor secretion by innate immune cells independently of its endotoxin. Microbes Infect 5:463–470
377. Heine H, Müller-Loennies S, Brade L, Lindner B, Brade H (2003) Endotoxic activity and chemical structure of lipopolysaccharides from *Chlamydia trachomatis* serotypes E and L2 and *Chlamydophila psittaci* 6BC. Eur J Biochem 270:440–450
378. Kol A, Bourcier T, Lichtman AH, Libby P (1999) Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. J Clin Investig 103:571
379. Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA (2000) Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. J Immunol 164:13–17
380. Vabulas RM, Ahmad-Nejad P, da Costa C, Miethke T, Kirschning CJ, Häcker H, Wagner H (2001) Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. J Biol Chem 276:31332–31339
381. Bulut Y, Faure E, Thomas L, Karahashi H, Michelsen KS, Equils O, Morrison SG, Morrison RP, Arditi M (2002) Chlamydial heat shock protein 60 activates macrophages and endothelial cells through toll-like receptor 4 and MD2 in a MyD88-dependent pathway. J Immunol 168:1435–1440
382. Costa CPD, Kirschning CJ, Busch D, Dürr S, Jennen L, Heinzmann U, Prebeck S, Wagner H, Miethke T (2002) Role of chlamydial heat shock protein 60 in the stimulation of innate immune cells by *Chlamydia pneumoniae*. Eur J Immunol 32:2460–2470
383. Bulut Y, Shimada K, Wong MH, Chen S, Gray P, Alsabeh R, Doherty TM, Crother TR, Arditi M (2009) Chlamydial heat shock protein 60 induces acute pulmonary inflammation in mice via the toll-like receptor 4-and MyD88-dependent pathway. Infect Immun 77:2683–2690
384. Abdul-Sater AA, Koo E, Häcker G, Ojcius DM (2009) Inflammasome-dependent caspase-1 activation in cervical epithelial cells stimulates growth of the intracellular pathogen *Chlamydia trachomatis*. J Biol Chem 284:26789–26796
385. Boncompain G, Schneider B, Delevoye C, Kellermann O, Dautry-Varsat A, Subtil A (2010) Production of reactive oxygen species is turned on and rapidly shut down in epithelial cells infected with *Chlamydia trachomatis*. Infect Immun 78:80–87
386. D'Autréaux B, Toledano MB (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 8:813–824
387. Rank RG, Bowlin AK, Kelly KA (2000) Characterization of lymphocyte response in the female genital tract during ascending Chlamydial genital infection in the guinea pig model. Infect Immun 68:5293–5298
388. Rank RG, Whittimore J, Bowlin AK, Dessus-Babus S, Wyrick PB (2008) Chlamydiae and polymorphonuclear leukocytes: unlikely allies in the spread of chlamydial infection. FEMS Immunol Med Microbiol 54:104–113
389. Rank RG, Lacy HM, Goodwin A, Sikes J, Whittimore J, Wyrick PB, Nagarajan UM (2010) Host chemokine and cytokine response in the endocervix within the first developmental cycle of *Chlamydia muridarum*. Infect Immun 78:536–544
390. Tseng C-TK, Rank RG (1998) Role of NK cells in early host response to chlamydial genital infection. Infect Immun 66:5867–5875
391. Mabey DC, Solomon AW, Foster A (2003) Trachoma. Lancet 362:223–229

392. Fan T, Lu H, Hu H, Shi L, McClarty GA, Nance DM, Greenberg AH, Zhong G (1998) Inhibition of apoptosis in chlamydia-infected cells: blockade of mitochondrial cytochrome c release and caspase activation. J Exp Med 187:487–496
393. Jendro MC, Fingerle F, Deutsch T, Liese A, Köhler L, Kuipers JG, Raum E, Martin M, Zeidler H (2004) *Chlamydia trachomatis*-infected macrophages induce apoptosis of activated T cells by secretion of tumor necrosis factor-α *in vitro*. Med Microbiol Immunol 193:45–52
394. Zhong G, Liu L, Fan T, Fan P, Ji H (2000) Degradation of transcription factor Rfx5 during the inhibition of both constitutive and interferon γ–inducible major histocompatibility complex class I expression in chlamydia-infected cells. J Exp Med 191:1525–1534
395. WHO (2015) Trachoma grading card (side 1). World Health Organization, Geneva. http://www.who.int/blindness/publications/trachoma_english.jpg?ua=1. Accessed December 2015
396. WHO (2015) Trachoma grading card (side 2). World Health Organization, Geneva. http://www.who.int/blindness/publications/trachoma_english1.jpg?ua=1. Accessed December 2015
397. Reacher MH, Muñoz B, Alghassany A, Daar AS, Elbualy M, Taylor HR (1992) A controlled trial of surgery for trachomatous trichiasis of the upper lid. Arch Ophthalmol 110:667–674
398. WHO (2006) Blinding trachoma: progress towards global elimination by 2020. World Health Organization, Geneva. http://www.who.int/mediacentre/news/notes/2006/np09/en/.Accessed December 2015
399. Rajak SN, Collin JRO, Burton MJ (2012) Trachomatous trichiasis and its management in endemic countries. Surv Ophthalmol 57:105–135
400. Burton M, Bowman R, Faal H, Aryee E, Ikumapayi U, Alexander N, Adegbola R, West S, Mabey D, Foster A (2005) Long term outcome of trichiasis surgery in the Gambia. Br J Ophthalmol 89:575–579
401. WHO (1998) Global elimination of blinding trachoma: 51st World Health Assembly: Resolution 51.11.1998. World Health Organization, Geneva
402. Prüss A, Mariotti SP (2000) Preventing trachoma through environmental sanitation: a review of the evidence base. Bull World Health Organ 78:267–273
403. Emerson PM, Lindsay SW, Walraven GE, Faal H, Bøgh C, Lowe K, Bailey RL (1999) Effect of fly control on trachoma and diarrhoea. Lancet 353:1401–1403
404. Thylefors B (1985) Development of trachoma control programs and the involvement of national resources. Rev Infect Dis 7:774–776
405. Schachter J (1983) Rifampin in chlamydial infections. Rev Infect Dis 5:S562–S564
406. Darougar S, Jones B, Viswalingam N, Poirier R, Allami J, Houshmand A, Farahmandian M, Gibson J (1980) Family-based suppressive intermittent therapy of hyperendemic trachoma with topical oxytetracycline or oral doxycycline. Br J Ophthalmol 64:291–295
407. Hoshiwara I, Ostler HB, Hanna L, Cignetti F, Coleman VR, Jawetz E (1973) Doxycycline treatment of chronic trachoma. JAMA 224:220–223
408. Tabbara KF, Summanen P, Taylor PB, Burd EM, Al Omar O (1988) Minocycline effects in patients with active trachoma. Int Ophthalmol 12:59–63
409. Tabbara KF (2001) Blinding trachoma: the forgotten problem. Br J Ophthalmol 85:1397–1399
410. Bailey R, Arullendran P, Mabey D, Whittle H (1993) Randomised controlled trial of single-dose azithromycin in treatment of trachoma. Lancet 342:453–456
411. Cochereau I, Goldschmidt P, Goepogui A, Afghani T, Delval L, Pouliquen P, Bourcier T, Robert P-Y (2007) Efficacy and safety of short duration azithromycin eye drops versus azithromycin single oral dose for the treatment of trachoma in children: a randomised, controlled, double-masked clinical trial. Br J Ophthalmol 91:667–672
412. Dawson C, Schachter J, Sallam S, Sheta A, Rubinstein R, Washton H (1997) A comparison of oral azithromycin with topical oxytetracycline/polymyxin for the treatment of trachoma in children. Clin Infect Dis 24:363–368
413. Nelson ML (2002) The chemistry and biology of the tetracyclines. Annu Rep Med Chem 37:105–114

414. Genilloud O, Vicente F (2013) Tetracycline antibiotics and novel analogs. Springer, Berlin
415. Chopra I, Hawkey P, Hinton M (1992) Tetracyclines, molecular and clinical aspects. J Antimicrob Chemother 29:245–277
416. Nikaido H, Thanassi D (1993) Penetration of lipophilic agents with multiple protonation sites into bacterial cells: tetracyclines and fluoroquinolones as examples. Antimicrob Agents Chemother 37:1393
417. Schnappinger D, Hillen W (1996) Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch Microbiol 165:359–369
418. Goldman RA, Hasan T, Hall CC, Strycharz WA, Cooperman BS (1983) Photoincorporation of tetracycline into *Escherichia coli* ribosomes. Identification of the major proteins photolabeled by native tetracycline and tetracycline photoproducts and implications for the inhibitory action of tetracycline on protein synthesis. Biochemistry 22:359–368
419. Zakeri B, Wright GD (2008) Chemical biology of tetracycline antibiotics. Biochem Cell Biol 86:124–136
420. Chiu LM, Amsden GW (2002) Current trachoma treatment methodologies. Drugs 62:2573–2579
421. Baltussen RM, Sylla M, Frick KD, Mariotti SP (2005) Cost-effectiveness of trachoma control in seven world regions. Ophthalmic Epidemiol 12:91–101
422. Hoepelman I, Schneider M (1995) Azithromycin: the first of the tissue-selective azalides. Int J Antimicrob Agents 5:145–167
423. Greenwood D (2008) Antimicrobial drugs: chronicle of a twentieth century medical triumph. Oxford University Press, New York
424. Hirsch R, Deng H, Laohachai M (2012) Azithromycin in periodontal treatment: more than an antibiotic. J Periodontal Res 47:137–148
425. Mao JC, Robishaw EE (1972) Erythromycin, a peptidyltransferase effector. Biochemistry 11:4864–4872
426. Gaynor M, Mankin AS (2003) Macrolide antibiotics: binding site, mechanism of action, resistance. Curr Top Med Chem 3:949–960
427. Garza-Ramos G, Xiong L, Zhong P, Mankin A (2001) Binding site of macrolide antibiotics on the ribosome: new resistance mutation identifies a specific interaction of ketolides with rRNA. J Bacteriol 183:6898–6907
428. Sugie M, Asakura E, Zhao YL, Torita S, Nadai M, Baba K, Kitaichi K, Takagi K, Takagi K, Hasegawa T (2004) Possible involvement of the drug transporters P glycoprotein and multidrug resistance-associated protein Mrp2 in disposition of azithromycin. Antimicrob Agents Chemother 48:809–814
429. Ballow CH, Amsden GW, Highet VS, Forrest A (1998) Pharmacokinetics of oral azithromycin in serum, urine, polymorphonuclear leucocytes and inflammatory vs non-inflammatory skin blisters in healthy volunteers. Clin Drug Investig 15:159–167
430. Amacher D, Schomaker S, Retsema J (1991) Comparison of the effects of the new azalide antibiotic, azithromycin, and erythromycin estolate on rat liver cytochrome P-450. Antimicrob Agents Chemother 35:1186–1190
431. Drugs.com (2015) Azithromycin side effects. http://www.drugs.com/sfx/azithromycin-side-effects.html. Accessed December 2015
432. Dawson CR, Daghfous T, Hoshiwara I, Ramdhane K, Kamoun M, Yoneda C, Schachter J (1982) Trachoma therapy with topical tetracycline and oral erythromycin: a comparative trial. Bull World Health Organ 60:347
433. Suchland R, Sandoz K, Jeffrey B, Stamm W, Rockey D (2009) Horizontal transfer of tetracycline resistance among Chlamydia spp. *in vitro*. Antimicrob Agents Chemother 53:4604–4611
434. West SK, Moncada J, Munoz B, Mkocha H, Storey P, Hardick J, Gaydos CA, Quinn TC, Schachter J (2014) Is there evidence for resistance of ocular *Chlamydia trachomatis* to azithromycin after mass treatment for trachoma control? J Infect Dis 210:65–71

435. Leach AJ, Shelby-James TM, Mayo M, Gratten M, Laming AC, Currie BJ, Mathews JD (1997) A prospective study of the impact of community-based azithromycin treatment of trachoma on carriage and resistance of *Streptococcus pneumoniae*. Clin Infect Dis 24:356–362
436. Keenan JD, Sahlu I, McGee L, Cevallos V, Vidal JE, Chochua S, Hawkins P, Gebre T, Tadesse Z, Emerson PM et al (2015) Nasopharyngeal pneumococcal serotypes before and after mass azithromycin distributions for trachoma. J Pediatr Infect Dis Soc. doi:10.1093/jpids/piu1143
437. Kuper H, Solomon AW, Buchan J, Zondervan M, Foster A, Mabey D (2003) A critical review of the SAFE strategy for the prevention of blinding trachoma. Lancet Infect Dis 3:372–381
438. Beagley KW, Timms P (2000) *Chlamydia trachomatis* infection: incidence, health costs and prospects for vaccine development. J Reprod Immunol 48:47–68
439. Kim S-K, DeMars R (2001) Epitope clusters in the major outer membrane protein of *Chlamydia trachomatis*. Curr Opin Immunol 13:429–436
440. Stephens RS, Wagar EA, Schoolnik G (1988) High-resolution mapping of serovar-specific and common antigenic determinants of the major outer membrane protein of *Chlamydia trachomatis*. J Exp Med 167:817–831
441. Baehr W, Zhang Y-X, Joseph T, Su H, Nano FE, Everett K, Caldwell HD (1988) Mapping antigenic domains expressed by *Chlamydia trachomatis* major outer membrane protein genes. Proc Natl Acad Sci 85:4000–4004
442. Campos M, Pal S, O'Brien TP, Taylor HR, Prendergast RA, Whittum-Hudson JA (1995) A chlamydial major outer membrane protein extract as a trachoma vaccine candidate. Investig Ophthalmol Vis Sci 36:1477
443. Taylor HR, Whittum-Hudson J, Schachter J, Caldwell H, Prendergast R (1988) Oral immunization with chlamydial major outer membrane protein (MOMP). Investig Ophthalmol Vis Sci 29:1847–1853
444. Zhang D-J, Yang X, Berry J, Shen C, McClarty G, Brunham RC (1997) DNA vaccination with the major outer-membrane protein gene induces acquired immunity to *Chlamydia trachomatis* (mouse pneumonitis) infection. J Infect Dis 176:1035–1040
445. Kari L, Whitmire WM, Crane DD, Reveneau N, Carlson JH, Goheen MM, Peterson EM, Pal S, Luis M, Caldwell HD (2009) *Chlamydia trachomatis* native major outer membrane protein induces partial protection in nonhuman primates: implication for a trachoma transmission-blocking vaccine. J Immunol 182:8063–8070
446. Swanson KA, Crane DD, Caldwell HD (2007) *Chlamydia trachomatis* species-specific induction of ezrin tyrosine phosphorylation functions in pathogen entry. Infect Immun 75:5669–5677
447. Wehrl W, Brinkmann V, Jungblut PR, Meyer TF, Szczepek AJ (2004) From the inside out–processing of the Chlamydial autotransporter PmpD and its role in bacterial adhesion and activation of human host cells. Mol Microbiol 51:319–334
448. Crane DD, Carlson JH, Fischer ER, Bavoil P, Hsia R-C, Tan C, Kuo C-C, Caldwell HD (2006) *Chlamydia trachomatis* polymorphic membrane protein D is a species-common pan-neutralizing antigen. Proc Natl Acad Sci USA 103:1894–1899
449. Liu MA, Wahren B, Hedestam GBK (2006) DNA vaccines: recent developments and future possibilities. Hum Gene Ther 17:1051–1061
450. Zhang D, Yang X, Shen C, Brunham R (1999) Characterization of immune responses following intramuscular DNA immunization with the MOMP gene of *Chlamydia trachomatis* mouse pneumonitis strain. Immunology 96:314–321
451. Penttilä T, Vuola JM, Puurula V, Anttila M, Sarvas M, Rautonen N, Mäkelä PH, Puolakkainen M (2000) Immunity to *Chlamydia pneumoniae* induced by vaccination with DNA vectors expressing a cytoplasmic protein (Hsp60) or outer membrane proteins (MOMP and Omp2). Vaccine 19:1256–1265
452. Ramshaw IA, Ramsay AJ (2000) The prime-boost strategy: exciting prospects for improved vaccination. Immunol Today 21:163–165
453. Hajek R, Butch A (2000) Dendritic cell biology and the application of dendritic cells to immunotherapy of multiple myeloma. Med Oncol 17:2–15

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Athina Geronikaki, Phaedra Eleftheriou, and Vladimir Poroikov

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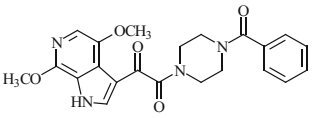
The chemical structure BMS-488043 of this chapter was displayed incorrectly. The correct form of the structure should be displayed as follows:



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A. Geronikaki (✉)
Department of Medicinal Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece
e-mail: geronik@pharm.auth.gr

P. Eleftheriou
Department of Medical Laboratory Studies, School of Health and Medical Care, Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece

V. Poroikov
Institute of Biomedical Chemistry, Moscow, Russia

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