PROGRESS IN MEDICINAL CHEMISTRY 18

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Preface

This volume contains six reviews, covering a wide range of topics in medicinal chemistry. Chapter 1 describes amantadine and its derivatives, compounds originally introduced in 1966 to combat influenza virus infections but later, through a chance observation, used in the treatment of Parkinson's disease. The basis of Chapter 2 resolves around the fact that both the tetrahydroisoquinoline and the tetrahydronaphthalene series contain members with sympathomimetic potencies of a similar order to those of the most active analogues of the natural catecholamine transmitters. Their most significant sympathomimetic effects have been identified as involving β-adrenergic receptors.

The mechanisms of the cytotoxicity of some nitroimidazoles are described in Chapter 3. These drugs are widely used in the treatment of several protozoal diseases such as trichomoniasis and intestinal infections. Reviews of a particular family of organic compounds are useful to medicinal chemists and a survey of recent work on 1,2-benzisothiazoles in Chapter 4 shows that a wide variety of biological activities are exhibited by these compounds.

The biological properties of tilorone and related bis-basic-substituted polycyclic aromatic and heterocyclic compounds are outlined in Chapter 5. These compounds were introduced as orally active anti-viral agents but, as so often happens in medicinal chemistry, other properties emerged and the compounds are now used as low molecular-weight immuno-modulating agents. Synthetic hypoglycaemic drugs were comparative new-comers on the therapeutic scene when they were reviewed in Volume 1 of this Series, and recent developments in this field are discussed in Chapter 6.

It is now twenty years since the first volume of this Series appeared and we hope that readers will welcome the cumulative indexes of all review titles and authors; these are included at the end of this volume.

Much effort has been involved in the preparation of these reviews and we are indebted to the authors for their diligence. We are also grateful to the owners of copyright material which has been reproduced in this volume. Finally, the staff of our publishers have, as usual, assisted us greatly and we acknowledge their contribution.

January 1981

G.P. Ellis
G.B. West

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1 Aminoadamantane Derivatives

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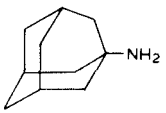
INTRODUCTION

Amantadine hydrochloride (1), 1-aminoadamantane hydrochloride, was introduced for clinical use in the U.S.A. in 1966 under the trade name Symmetrel^{R} (E.I. DuPont de Nemours and Company). Its initial use was restricted to prophylactic treatment for the prevention of respiratory illness due to susceptibe influenza A2-Asian viruses. It was the first orally active antiviral agent to be used in the U.S.A., and, in the intervening fifteen years since its introduction, has been the subject of, or included in, numerous review articles related to its chemistry [1-2] and biological activity [3-12].

Controversy over the clinical efficacy of amantadine against influenza [13-14] and the chance observation of its effect in Parkinson's disease [15] have contributed to a continuing interest in amantadine and the synthesis and evaluation of numerous derivatives. In the present review, recent developments in the clinical applications and mechanism of action of amantadine as well as of the two analogues, rimantadine (2) and memantine (DMAA) (3), which are of current interest as, respectivily, antiviral and anti-Parkinson's agents, will be dicussed. In the last section of the review, data on less extensively investigated compounds incorporating aminoadamantyl or related moieties which show significant biological activity are presented.



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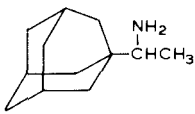

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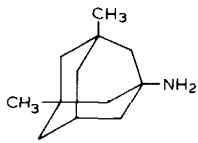

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ANTIVIRAL ACTIVITY OF AMANTADINE HYDROCHLORIDE

The majority of clinical trials with amantadine have been directed at establishing prophylactic or therapeutic efficacy against respiratory disease caused by influenza A group viruses. Other viruses, however, have been reported to be inhibited by amantadine in a variety of test systems such as tissue culture, embryonated eggs, animals and man.

SPECTRUM OF ACTIVITY

The cytotoxicity of amantadine and its activity against different viruses have

been reviewed by Hoffmann [4]. Although differing in sensitivity, all strains of influenza A group viruses were inhibited by amantadine with newer isolates more sensitive, in general, than the older ones [4]. More recently, a virus which was serologically related to the swine virus implicated in the 1918-1919 pandemic was associated with an outbreak of influenza in 1976, and, this virus, influenza A/New Jersey/8/76 (Hsw1N1), was found to be inhibited by the drug in experimental animals and in tissue culture [16-19]. Of particular importance has been the finding that group B influenza viruses and other respiratory pathogens such as rhinoviruses and respiratory syncytial and adenoviruses were resistant to amantadine. However, other viruses inclusing parainfluenza, pseudorabies, rubella, murine, and avian tumor viruses, arena viruses, lymphocytic choriomeningitis and junin virus were reported to be inhibited by amantadine in tissue culture or in experimental animals [4]. Very recently, Koff, Elm and Halstead [20] showed that amantadine decreased the titers of all four types of dengue viruses grown in LLC-MK2 cells by more than 90% at a concentration of 50μg/ml. It was also shown that the growth of dengue virus type 2 was reduced in both human and rhesus peripheral blood leukocytes by the drug. Although amantadine has shown activity against different viruses, its clinical use is currently limited to group A influenza viruses.

MECHANISM OF ACTION

The mode of action of amantadine against susceptible viruses has been the subject of numerous studies, and yet the exact mechanism responsible for its antiviral effect has not been clearly established. It does not inactivate the virus on contact nor is virus adsorption into or release from infected cells inhibited, but it must be present immediately after the absorption period for an antiviral effect to be achieved [4, 21-22]. Initial studies by the DuPont group suggested that amantadine inhibited virus penetration of infected cells [23-24], but a subsequent study with fowl plague virus [25] reported that it interfered with the uncoating of the virus but not with penetration. Later electron microscope studies also indicated that it did not inhibit the penetration of the chick chorioalantoic membrane by influenza A2 viruses [26] or the penetration of chick embryo fibroblasts by fowl plague virus [27].

In a study of the effect of amantadine on early events following influenza virus infection [28], it was suggested that the release of virus RNA into cells may consist of two steps, one of which is inhibited by amantadine: (a) uncoating of the virus envelope, and (b) release of RNA from the nucleocapsid. An analysis of fowl plague virus-specific RNA synthesis in chick embryo fibroblasts [27] indicated that the presence of amantadine from the time of virus

addition prevented expression of the virus genome and the synthesis of even the first detectable transcripts catalyzed by the polymerase of the infecting virus particles was prevented. The earlier report [29] of an inhibitory effect of the drug on the viral RNA-dependent RNA polymerase was not confirmed either *in vitro* or *in vivo* [27]. Addition of amantadine immediately after virus adsorption has been shown [18, 27] to inhibit virus-specific protein synthesis. At present, it is only possible to state that amantadine prevents some unknown post-adsorption stage of the virus replication cycle before virus RNA transcription occurs [11, 27].

The development of resistance to amantadine by influenza A virus has been demonstrated in experimental animals [30]. It has subsequently been shown that the infection of embryonated eggs, cell culture or mice with a mixture of amantadine-resistant and amantadine-sensitive strains of influenza virus resulted in the transfer of amantadine-resistance or sensitivity between strains [31]. It was further shown that the response of a recombinant virus to amantadine was not related to either of its surface antigens (hemagglutinin or neuraminidase) and that resistance to amantadine was transferred as an all-or-none character [31]. Additional studies with influenza virus recombinants have indicated that the gene coding for the M protein influences sensitivity or resistance to amantadine and this suggests a possible association of the M protein with the process inhibited by amantadine [32].

It is of interest to note that, with the exception of amantadine, compounds inhibiting penetration and/or viral uncoating are not clinically useful [33].

CURRENT USE AND RECOMMENDATIONS

Earlier reviews of amantadine have described the results obtained against experimental and naturally-acquired respiratory disease caused by influenza A virus strains [4, 8, 10-12, 34, 35]. The main conclusion drawn from numerous clinical studies was that amantadine had both prophylactic and therapeutic efficacy and was well tolerated by the vast majority of patients. More recently [36], amantadine was demonstrated to be effective in preventing illness and infection caused by Russian (H1N1) influenza.

Although fifteen years have elapsed since the introduction of amantadine in 1966 for prophylactic use against influenza A2/Asian infection and even though clinical efficacy has been amply demonstrated, the use of amantadine as an antiviral has remained controversial and limited in extent. It was only in 1976 that amantadine was cleared for use in the U.S.A. for the prevention and symptomatic management of infections caused by influenza A virus strains other than A2/Asian [8]. Chang and Butler [37] have analyzed several of the factors

responsible for the limited use of amantadine by physicians. These included (a) an unfavourable evaluation of the early clinical trails with amantadine [13-14], (b) the short duration of prevalence of influenza/Asian viruses and the lack of effect of amantadine against influenza B virus strains, (c) the difficulty of diagnosing sporadic cases of influenza, and (d) the degree of side effects of the drug. In addition, the belief that uncomplicated influenza usually requires no treatment has contributed to the reluctance of many physicians to use the drug. Opposed to this view are data from studies of pulmonary function in patients with uncomplicated influenza which commonly reveal peripheral airway dysfunction as well as gas-exchange abnormalities [38-42]. These abnormalities, suggesting involvement of the lower respiratory tract, may persist for weeks or even months. It has been shown that the use of amantadine in uncomplicated influenza improves the abnormalities in lung function [39-41] and when used as prophylaxis markedly reduces the occurrence of chest complications [42].

A Consensus Development Conference entitled, 'Amantadine: Does it Have a Role in the Prevention and Treatment of Influenza', was held at the National Institute of Health (U.S.A.) in the Fall of 1979. Sponsored by the National Institute of Allergy and Infectious Disease, a panel of experts from the U.S.A., United Kingdom and the Soviet Union met to consider four specific questions: (a) what are the potential benefits of the prophylactic and therapeutic uses of amantadine for influenza A infections, (b) who should take amantadine, and when should it be taken, (c) what are the risks associated with the use of amantadine and (d) is there a role for the use of amantadine in combination with vaccines [43]?

The panel of experts concluded that the use of amantadine, as a prophylactic agent as well as for the treatment of influenza A, had significant potential value in reducing the morbidity associated with this respiratory disease and its complications. Use of amantadine was judged to be of particular value among persons with cardiopulmonary disease – especially the elderly who are suffering from more severe, life-threatening forms of disease where pneumonic complications are more likely. It was felt that, under epidemic conditions, the use of amantadine in institutionalized aged persons would be of benefit to the patient population and the staff that care for them. A similar beneficial effect of amantadine would be expected in the case of vulnerable patients exposed to influenza A in hospitals. An earlier report had produced evidence for the prophylactic use of amantadine for high risk patients who had not been immunized or in whom a vaccine had not had sufficient time to produce a serologic response [44]. The panel suggested that other high-risk groups among whom beneficial results may be expected under epidemic conditions would include public ser-

vants such as firemen, policemen and military personnel, especially those without adequate influenza virus vaccine immunization [43].

When there is both epidemiologic and virologic evidence of an outbreak in the community or region, the panel designated the following groups with highest priorities for receipt of amantadine: (a) unvaccinated children and adults at high risk of serious morbidity and mortality because of underlying diseases (pulmonary, cardiovascular, metabolic, neuromuscular or immunodeficiency), (b) non-vaccinated adults whose activities are required for community function and (c) persons in semiclosed institutional environments – especially older persons who have not been adequately immunized. Since the teratogenic risk of amantadine in pregnant women is not well understood, the panel suggested that amantadine should be administered to pregnant women only after a comparative evaluation of the possible risks to the foetus and the benefits to the patient.

In considering the therapeutic effect of amantadine, the panel concluded that treatment should be administered as soon as possible and not later than 48 hours after onset of symptoms. Therapy with amantadine was suggested for the following groups: (a) high-risk patients as previously defined, (b) patients with life-threatening primary influenza pneumonia or influenza-associated croup and (c) persons whose functions are vital to the community.

In making an assessment of the risks of the use of amantadine for the prophylaxis and treatment of disease caused by influenza A viruses, the panel drew on the data from numerous clinical trials involving over 11,000 subjects. Approximately 7% of persons receiving amantadine (200 mg daily) had experienced transient nervous system symptoms (insomnia, light-headedness, nervousness, difficulty in concentration, or drowsiness). The experience gained with the use of amantadine in elderly patients with Parkinson's disease did not appear to present special toxicity problems. The panel concluded that the physician must determine when the possible mild impairment of intellectual acuteness and decreased motor function associated with the use of amantadine would present a special hazard to a patient in a particular job situation if prophylaxis with amantadine was to be given.

Because of the effect of amantadine on the nervous system, the panel also evaluated the potential for drug abuse. The use of amantadine for the specific purpose of altering the state of consciousness has not been reported, and it appears to have a low potential for abuse since it does not provide prominent analgesia or euphoria.

The panel also considered the possible selection of amantadine-resistant strains of influenza A virus as a consequence of extensive clinical use of the drug. *In vitro* studies have demonstrated the spontaneous development of amantadine

resistance among influenza A viruses, but such resistant variants have not been isolated from patients who have received the drug. The panel, however, cautioned that the overuse of amantadine may result in an increase in drug-resistant strains of influenza A virus in the population.

In evaluating the role for the combined use of amantadine and influenza immunization, the panel recommended that immunization be used as the primary method for prophylaxis against influenza. Amantadine should be given as adjunctive therapy and discontinued once a seriologic response to the immunization was to be expected. In a subject with a normal immune system who had previously been immunized with antigen related to the current epidemic strain, antibody response of protective quality is achieved about 10 days after administration of the vaccine. Treatment with amantadine is given for four to six weeks after immunization when the subject had not received an antigenically-similar vaccine in the past and when influenza persists in the community [43].

ANTIVIRAL ACTIVITY OF RIMANTADINE HYDROCHLORIDE

Rimantadine hydrochloride or 1-(1-aminoethyl)adamantane (2) is a congener of amantadine that was also developed by the DuPont Company. It has been reported to be active against influenza A, rubella, rubeola, respiratory syncytial and parainfluenza types 2 and 3 viruses [45]. Interest has generally been directed to its effect against influenza A viruses where activity has been shown *in vitro* [45, 46] and *in vivo* [47, 48]. The mechanism of the antiviral effect of rimantadine as well as its antiviral spectrum are similar to those of amantadine [46]. Koff and Knight [49, 50] have recently reported that rimantadine, as is the case with amantadine, acts at an early stage in the influenza A virus replicative cycle. Drug added late in the replicative cycle had no inhibitory effect and addition of rimantadine during the viral adsorption period and removal thereafter did not result in an antiviral effect [49]. It was further shown that rimantadine did not alter the structural integrity of influenza virus. When added after the viral adsorption period, rimantadine inhibited the stimulation of cellular DNA-dependent RNA polymerase II from virus-infected cells but had no effect on RNA polymerase II from uninfected cell nuclei. Rimantadine, present from 0–8 hours postinfection, was shown to inhibit the formation of viral RNA-dependent RNA polymerase activity in infected cells, but inhibition of enzyme activity was not observed *in vitro*. Rimantadine was also shown to inhibit viral and cellular protein synthesis but the inhibition was not considered to be great enough to be responsible for the antiviral effect found with rimantadine against influenza A virus [49]. In a separate study,

Koff and Knight [50] demonstrated that rimantadine treatment of influenza A/WSN virus-infected MDCK cells inhibited influenza virus uncoating. An earlier study by Kato and Eggers [25] had reported that amantadine inhibited the uncoating of fowl plague virus in chicken embryo cells. Taken together, the available data suggest that rimantadine, like amantadine, inhibits influenza virus replication by acting as a step following viral adsorption and preceding primary transcription of the influenza virus genome [49].

An *in vitro* effect of rimantadine on cellular immunity has recently been described [51]. Rimantadine inhibited the *in vitro* proliferative response of human peripheral blood lymphocytes to mitogenic and antigenic stimulation. An earlier report [52] described the inhibition of the mitogenic response of human lymphocytes stimulated with phytohemagglutinin by amantadine. The clinical significance of these observations is not defined at present but studies of experimental influenza virus infections in mice indicated that cell-mediated immune responses increased the severity of influenza pneumonia in mice [53-56].

Rimantadine has been reported in some studies to be more active than amantadine in cell culture [57] and in mice [58] while Tisdale and Bauer [59] concluded that there was no compound available with significantly greater activity than amantadine for the treatment of influenza virus infections in mice. Although the therapeutic efficacy of rimantadine in naturally occurring influenza A2 respiratory illness in man has been established [48], rimantadine has been used extensively only in Russia and has been reported to have fewer side effects than amantadine [10]. La Montagne and Galasso [35] have reviewed the recent comparative trials of amantadine and rimantadine in the U.S.A. The results of chemoprophylactic comparison of amantadine and rimantadine were characterized as not meaningful because of the low incidence of influenza in the study population but therapeutic studies indicated that both drugs exhibited significant antiviral activity when given orally. Side effects were not a major concern with either drug in these studies [35]. At present rimantadine is an investigative drug in the U.S.A.

AMANTADINE IN HUMAN NEUROLOGICAL DISORDERS

PARKINSON'S DISEASE

The first report of the utility of amantadine for the treatmenr of Parkinson's disease was made in 1969 when a 58 year old woman with moderately severe Parkinsonism noted a dramatic improvement in her symptoms while taking 200 mg of amantadine daily for flu prophylaxis. This result prompted an open trial

in a group of 163 patients during which 58% showed an improvement which was sustained over three to eight months [15]. Several follow-up studies confirmed the activity of amantadine either alone or in combination with anticholingeric agents in Parkinson's disease [60-63]. However, it was generally found to be less effective than L-dopa and in one study was claimed to be only slightly effective [61].

Since these initial investigations, the use of amantadine in Parkinson's disease has been reviewed several times [64-68]. The data indicate that the drug is safe in the doses normally prescribed (200-300 mg per day). The usual side effects are nervousness, hallucinations, tremor, insomnia and loss of appetite and disappear upon withdrawal of the drug. Amantadine is moderately effective in a majority of cases at least initially (vide infra) and can be used alone or in combination with an anticholinergic or L-dopa.

In a recent open trial, Savery [69], found amantadine to be of additional benefit to a group of 16 patients who were being maintained on a fixed combination of L-dopa and the dopa decarboxylase inhibitor, carbidopa. Only one patient regressed after six weeks of the triple combination. In another study [70] a tendency toward lowered scores on objective motor skill ratings and clinical disability rating was noted after withdrawal of amantadine in a group of six idiopathic Parkinson's patients who had been receiving both L-dopa and amantadine for a 12–24-week period. However, the observed changes did not reach the level of statistical significance.

Timberlake and Vance [71] reported that half of 94 patients improved with amantadine during an acute, double-blind trial. In a four-year follow-up, they found that amantadine either alone or in combination with L-dopa had its greatest effect during the first month and was of benefit to only a few patients longer than six months. They further indicated that the incidence of side effects was the same with 200 mg per day as with 300 mg per day, only appearing more rapidly at the higher dose. Finally, they suggested that the worsening of Parkinson's symptoms observed in many cases after withdrawal of amantadine may be due to withdrawal effects and is not necessarily a sign that the drug was effective.

Fahn and Isgreen [72] described a year long double-blind, triple cross-over study in which 23 Parkinson's patients received either placebo or amantadine (200 mg twice daily) for two weeks each in addition to any anticholingeric medication they had been receiving. During the first period, 70% of the patients improved on amantadine as compared with placebo. During the next five months, the responsive patients were maintained on amantadine and the others on placebo as L-dopa was added to the regime for all those in the study. All of the eighteen patients completing this phase of the study improved and L-dopa was judged to be significantly more effective than amantadine alone.

A second cross-over period of two weeks each on amantadine and placebo, while continuing L-dopa therapy, indicated that 9 of the 13 patients who had been receiving amantadine for five months continued to respond the drug. Furthermore, one of the five who initially had failed to respond now showed improvement when amantadine was added to the regimen. All of these amantadine-responsive patients were maintained on the combination with L-dopa for the next four months while the remainder was given placebo plus L-dopa.

During the twelfth month, a third double-blind cross-over was carried out. A total of nine of those who were responsive to amantadine during the second cross-over period completed the study and five of these remained responsive. Of the seven non-responders from the second cross-over period, three benefitted from amantadine during the third cross-over, one of whom had shown no improvement on amantadine in either of the first two trial periods.

Quantitative evaluation of those patients who responded to the combination of L-dopa and amantadine in the third cross-over period showed that these patients were more improved than those who were on L-dopa plus placebo for the last five months of the study. The authors conclude that amantadine should be added to L-dopa therapy if an additional effect is desired, even if the patient has failed to respond in a previous trial.

Pearce [66] recommends starting Parkinson's patients on amantadine (200 mg twice daily) if no improvement is observed after a trial of anticholinergic therapy. Fahn and Calne [67] recommend initial treatment with amantadine rather than L-dopa in order to delay the introduction of the latter drug because the beneficial effects of L-dopa are known to wear off with time. They suggest that this loss of effectiveness may be due to the development of a denervation or drug-induced super-sensitivity at terminals involved with psychiatric and diskinetic reactions. In support of this approach, data reported by Yahr [73] imply that the loss of effectiveness of L-dopa generally begins two to three years after initiation of therapy regardless of the disease state at which L-dopa was first introduced.

DRUG-INDUCED PARKINSONISM

Drug induced extrapyramidyl symptoms, sometimes referred to as drug-induced Parkinsonism, occur in up to 40% of psychiatric patients receiving neuroleptics. These movement disorders have been treated successfully with anticholinergics, but troublesome side effects due to blockade of central and peripheral cholinergic receptors such as dryness of the mouth, visual disturbances, loss of appetite, and reduced intestinal motility often intervene to complicate therapy. Several studies which have been summarized by Parkes [65] suggest that aman-

tadine has a beneficial effect on drug-induced Parkinsonism, but that patient variability is great.

More recently, amantadine has been compared with the anticholinergic agents benztropine, ethopropazine and trihexyphenidyl in several open and double-blind studies [74-79]. Amantadine was found to be as effective or slightly less effective than anticholinergic therapy and generally was associated with fewer side effects.

In a blind study carried out in a group of hospitalized schizophrenic patients demonstrating extrapyramidyl symptoms [80], an attempt was made to correlate the steady-state plasma levels of amantadine with therapeutic benefits. Patients receiving 100 mg of amantadine twice daily reached steady-state plasma concentrations of 0.12-1.12 μg/ml by the seventh day. Improvement in rigidity was significantly correlated with amantadine plasma levels at days 7 and 14 of the study, but the improvement scores for other extrapyramidyl symptoms were not significantly correlated. Also no association between plasma drug levels and schizophrenic manifestations were observed.

A closely related study was conducted in a group of 15 female patients whose neuroleptic induced extrapyramidyl symptoms were not adequately controlled by anti-cholingeric therapy [81]. Again plasma amantadine levels reached a steady-state concentration of 0.2-0.9 μg/ml after 4-7 days of treatment. In this case, a significant relationship was found between plasma drug levels and the degree of symptomatic improvement observed. Rigidity and tremor were most favorably affected although the hypokinesia and vegetative disturbances were also favourably influenced. In this study, as in others, there was considerable interpatient variability and the degree of improvement was not related to the severity of the symptoms. In all cases, the extrapyramidyl symptoms reemerged within 48-72 hours after withdrawal of the drug.

Gelenberg [82] has reported a study in which amantadine was administered to 14 schizophrenic patients with extrapyramidyl symptoms which were refractory to benztropine. Six patients improved markedly within two days and six others improved after the dose was increased from 200 mg to 300 mg daily. It would thus appear that amantadine may be useful in some cases of drug-induced Parkinsonism, particularly for those patients unresponsive to or unable to tolerate anti-cholingeric drugs.

TARDIVE DISKINESIA

Tardive diskinesia is an extrapyramidyl disorder which is clinically indistinguishable from Huntington's chorea. It is characterized by involuntary, rythmic movements of the tongue, lips, and facial muscles and may also affect the

extremities. The disease is caused by long term neuroleptic treatment and the symptoms tend to be masked by the neuroleptic so that the condition often is not diagnosed until drug withdrawal. Once present, it is usually not reversible. It has been hypothesized that tardive diskinesia results from a development of super-sensitivity of dopamine receptors in the striatum as a result of prolonged blockage by neuroleptic drugs [83]. The suggestion has been made that amantadine, by virture of its dopaminergic properties, may help to avert development of this syndrome when administered in combination with a neuroleptic agent [83].

Two clinical studies have been reported in which amantadine has been administered to patients suffering from tardive diskinesia [84, 85]. In both studies, symptomatic relief was obtained with 200-300 mg of amantadine daily for the duration of the study and the symptoms returned upon withdrawal. While these results seem encouraging, the studies included only eight patients.

REVERSAL OF NEUROLEPTIC-INDUCED CATATONIA

Parkes [65] has summarized a number of experiments in rodents in which amantadine was found to antagonize the cataleptic effects of the neuroleptics. It has now been reported by Gelenberg and Mandel [86] that amantadine has a similar effect in man. They describe a group of eight patients on haloperidol or fluphenazine who developed symptoms of catatonia including withdrawal, regression, rigidity, Parkinsonism, and waxy flexibility which responded only slowly to drug withdrawal or treatment with benztropine. Six of these responded rapidly when given amantadine either alone or in combination with benztropine.

CHOREA

In a preliminary clinical trial, amantadine (100-200 mg daily) was tried in 13 patients displaying choreiform movements, six of which had Huntington's chorea [87]. In three patients, one with Huntington's chorea and two with senile chorea, a marked reduction in involuntary movements was noted. Six others, four of which suffered from Huntington's chorea, one from senile chorea and one from choreaathetosis showed only a marginal improvement which nevertheless was sufficient to justify continued therapy for some.

In a group of six long-term Huntington's chorea patients, amantadine was compared with L-dopa plus a peripheral decarboxylase inhibitor, a combination of haloperidol with diazepam and a combination of reserpine with diazepam [88]. The patients were given each regimen for one week and were evaluated

by a panel of three neurologists on a blind basis. The haloperidol-diazepam therapy was of the most benefit in this study, while amantadine and the combination of reserpine with diazepam provided a significant, but considerably weaker effect and L-dopa was of no benefit.

CREUTZFELDT-JAKOB DISEASE

Creutzfeldt-Jakob disease is a virtually universally fatal brain disorder which may be viral in origin. Two reports describing the effect of amantadine on three patients with Creutzfeldt-Jakob disease have appeared. The first case involved a 69-year-old male who made a substantial improvement for two months on 200-800 mg of amantadine daily before he deteriorated and died [89]. In the second case, a 55-year-old female was treated with 100-200 mg of amantadine daily and has apparently made a full recovery. Amantadine was gradually withdrawn from this patient after one and one-half years [89] and the patient has remained well after a total of 30 months. The last case reported a male who improved markedly on 400 mg of amantadine daily. Although he did not return to normalacy, he maintained a stable condition for five years while on amantadine before suffering an accidental death [90].

The presence of Creutzfeldt-Jakob disease was confirmed histologically in both of the patients who died. It would appear that amantadine may have an important role in the treatment of this extremely rare disease state [65].

SENILE BRAIN DISEASE AND DEPRESSION

Amantadine was tried in a group of 20 elderly females showing signs of mental deterioration in a cross-over study versus placebo [91]. The drug reportedly caused a marked improvement in both clinical and psychometric variables during the 15-day period of treatment, having its most pronounced effect on the parameters of anxiety, depression, 'general activity', and attention.

A less positive conclusion can be drawn from a study of 18 patients displaying mild to moderate signs of senile dementia. Although seven of these patients showed a definite clinical improvement and two others improved marginally, only one patient was able to maintain his improved state without drug-induced complications [92].

Amantadine was given to four aged, depressed patients at the rate of 300 mg per day for two to three weeks [93]. Plasma amantadine levels were measured and found to be between 0.68-1.01 μg/ml which is within the usual therapeutic range. In these four patients, little indication of antidepressant activity was noted and all four developed signs of hostility and agressive behaviour.

Amantadine seems to be of questionable benefit to patients suffering from age-related deterioration of mental function and depression.

DOWN'S SYNDROME

A single report suggests that amantadine may be of benefit in treating the symptoms of mongolism. White [94] described a case of an infant with Down's syndrome who was given amantadine for viral prophylaxis. The child reached all of the mental-age milestones normally up to 8-9 months. On the days he did not get the drug a loss of muscle tone and drooling at the mouth were noted. The published report does not indicate the longer term development of this case.

EFFECT IN COUNTERING THE SIDE EFFECTS OF KETAMINE INDUCTION

Ketamine is a phencyclidine derivative which is useful as an inducing agent in anesthesia. Unfortunately, its use is associated with a number of undesirable side effects such as increased muscle tone, involuntary movements, salivation, emergence delirium and unpleasant dreaming [95]. In an open study of 59 patients undergoing minor surgical procedures, amantadine and diazepam were evaluated for their ability to suppress these signs. Amantadine, given at a dose of 150 mg i.v., 15 min after the end of anaesthesia was effective in reducing the incidence of involuntary movements, hypertonus, and space-time disorientation.

SPASMATIC TORTICOLLIS

Spasmatic torticollis is a disease characterized by an irregular, unnatural spasm of the neck muscles causing a turning movement of the head. In his review, Parkes [65] indicates amantadine is of little use in the treatment of this disorder. West [96] has recently reported on a double-blind cross-over study of amantadine versus placebo which further supports this conclusion. West also observed no improvement when amantadine was combined with haloperidol.

PHARMACOLOGY OF AMANTADINE

The pharmacology of amantadine has been reviewed [6, 65, 97, 98]. Amantadine has generally been considered to exert its pharmacological actions primarily through a dopaminergic effect, although the details of this effect have been

subject of considerable debate. In the following sections, we will emphasize results which have been obtained since Baily and Stone's comprehensive review [6].

PERIPHERAL EFFECTS

The peripheral effects of amantadine in animal models, as in man, are only of secondary importance to its central effects. A weak pressor response has been observed in the rabbit and rat which was blocked by phentolamine [99]. A pressor response has also been observed in the vagotomized dog with a bell-shaped dose-response curve having a maximum at 2 mg/kg i.v. After an accumulative dose of 17 mg/kg, a pronounced depression of blood pressure was recorded [100].

In the anaesthetized dog, a positive inotropic effect associated with a reduction in total peripheral resistance occurred after 1-3 mg/kg of amantadine [101]. This effect was ascribed to a drug-induced release of catecholamines. At higher dose in this model, amantadine significantly reduced cardiac contractility.

A marked potentiation of the pressor effects caused by accumulative doses of amantadine totalling 0.064-7.0 mg/kg in the vagotomized dof were obtained by prior loading with dopamine, but not norepinephrine [100]. Similar results have been reported from experiments in anaesthetized dogs [102] and rabbits [103]. An inhibitory action on catecholamine uptake [103, 104] or a facilitation of release [100, 102] have been proposed to account for these findings.

Pita and Perez [105] have provided direct evidence for a catecholamine-releasing activity of amantadine in the rat. The animals were given amantadine (2-30 mg/kg, i.p.) and after a time lapse of 30 min to 24 hours, they were sacrificed and their adrenal tissue was fixed within 30 sec of death. Electron microscopic examination of these adrenal tissue samples revealed that low doses (2-5 mg/kg) of amantadine caused a profound depletion of the norepinephrine-containing vesicles between 1 and 6 hours after dosing. Higher doses were progressively less effective at producing this response. Amphetamine caused an analogous degranulation, but the duration of the effect was much shorter.

BEHAVIOURAL EFFECTS

Bailey and Stone [6] have summarized numerous studies in which amantadine at relatively high doses (50-150 mg/kg) was found to cause an increase in spontaneous motor activity in mice and rats. The data cited in the review suggest that this locomotor stimulation was insensitive to reserpine pretreatment but was attentuated by pretreatment with α-methyl-*p*-tyrosine, implying that cate-

cholamine release was occurring from reserpine resistant 'extragranular' stores. The inability of amine-depleting agents to completely inhibit the amantadine-induced increases in motor activity was taken as evidence that it also exerts a direct action at catecholamine receptors although a non-catecholaminergic effect could also account for the observed results.

Very high doses of amantadine (150 mg/kg) have been reported to anagonize amphetamine-induced hyperactivity and hyperthermia in reserpinized mice [106]. It has further been shown that lower doses of amantadine, in the range of 2.5-5 mg/kg, significantly raise the ED_{50} of the locomotor affects of amphetamine while at the same time potentiating its anorexic activity in mice [107].

Senault [108] has reported that amantadine in doses of up to 50 mg/kg i.p. induces a very slight aggressive effect in rats while it inhibits the fighting behaviour induced by 1 mg/kg of apomorphine in a dose-dependent manner. In common with other dopamine agonists, amantadine caused an increase in the electroshock-induced fighting behaviour of normal rats [109].

Centrally-acting dopaminergic agents are capable of inducing stereotyped behaviour in rats and mice which is characterized by compulsive head and limb twitching, gnawing, sniffing, and licking. Amphetamine and apomorphine are capable of inducing very intense stereotype while amantadine is a much weaker agent, capable of inducing only a mild stereotypy even at nearly toxic doses [6, 110-113]. Like other indirectly acting drugs, amantadine antagonizes apomorphine-induced stereotyped behaviour [111-113].

Pretreatment with α-methyl-*p*-tyrosine has been reported to reduce the percentage of rats exhibiting stereotyped behaviour after 100 mg/kg of amantadine [113]. However, in another study, no effect of α-methyl-*p*-tyrosine pretreatment was observed on stereotyped behaviour induced by lower doses of amantadine although pretreatment with a combination of α-methyl-*p*-tyrosine and reserpine abolished it [112]. Amphetamine-induced stereotyped behaviour in the rat is inhibited by pretreatment with α-methyl-*p*-tyrosine while amantadine in a dose range of 20-70 mg/kg i.p. is able to restore it [114]. The finding that the degree of this restorative effect declined with time after the inhibition of dopamine synthesis suggests that amantadine acts to facilitate utilization of dopamine stores.

Amphetamine causes a potentiation of the choreiform movements and circling behaviour induced by β, β'-iminodipropionitrile. On the other hand, amantadine (50-80 mg/kg) produces a biphasic response consisting of an initial depression of this response during the first 30 min followed by a marked enhancement which lasts for 2 hours [115].

A similar biphasic response has been observed in the effect of amantadine on electrical self-stimulatory behaviour [97]. Furthermore, the effects of low

doses of amantadine and amphetamine on electrical self-stimulation were algerbraically additive. Herberg and Stephens [97] account for these results by hypothesizing that amantadine affects two separate dopamine pools. Amantadine could cause a dose-related, stimulus-independent dopamine loss from one pool mediating inhibitory effects while at the same time facilitating stimulus-evoked amantadine release from a second, stimulatory pool. Thus, the apparent amphetamine antagonistic effects of amantadine noted above [106, 107] could be due to the initial depressant effect of amantadine, particularly after the very large doses which have often been employed.

Intermittent high frequency electrical stimulation of the caudate nucleus of rats induces contralateral head turning [116]. Barnett and Goldstein found that the dopaminergic antiparkinson drugs L-dopa, apomorphine and amantadine all inhibit this response at dose levels which correlate well with those used clinically. The ED_{50} value for amantadine in this test was 1.5 mg/kg p.o. and 1.5 μg/rat after intracaudate administration implicating that the caudate nucleus is the site of drug action [116].

Although the ED_{50} value (head turning) for the anticholinergic drugs, benztropine and trihexyphenidyl, did not correlate as well with the usual clinical doses for Parkinson's disease, the model has been recommended for screening new potential antiparkinson's agents.

When unilateral lesions are introduced by the action of 6-hydroxydopamine on the nigrostriatal neuronal system of rats, a characteristic circling behaviour is seen after administration of dopaminergic drugs. Indirectly-acting agents such as amphetamine cause a stimulation only on the intact side, causing circling toward the lesioned side, whereas direct dopamine receptor agonists are thought to stimulate the supersensitive dopamine receptors on the denervated side, causing turning towards the intact side. Studies summarized by Bailey and Stone [6] indicate that amantadine displays an amphetamine-like indirect effect in this model.

Costall and Naylor [112] sound a cautionary note with regard to these studies. They point out that the striatum, from which circling behaviour is thought to originate, contains large amounts of 5-hydroxytryptamine as well as dopamine. The local application of 6-hydroxydopamine near the substantia nigra may cause concomitant damage to the ascending 5-hydroxytryptamine systems in the ventromedial part of the medial forebrain bundle as a consequence of its irritant effects. Amantadine caused bursts of circling in both directions following lesions in the ventromedial part of the forebrain bundle while amphetamine caused turning toward the lesioned side.

Costall, Naylor and Pycock [117] have reported that a more pronounced and consistent rotational behaviour is produced by unilateral lesions in the

nigrostriatal pathway in the area of the lateral hypothalamus. In this model both amantadine and amphetamine induced circling toward the lesioned side, as expected for indirectly acting agents.

Amantadine and apomorphine, but not amphetamine or tyramine, inhibit chemosenory discharges originating in the carotid body of the cat. The intracarotid ID_{50} value for amantadine is 340 times that of dopamine [118].

Amantadine is capable of reversing the catalepsy induced by neuroleptic agents such as haloperidol and spiroperidol [6, 65]. The 5-hydroxytryptamine antagonist, cyproheptadine, also significantly inhibited spiroperidol catalepsy in rats and the effects of amantadine and cyproheptadine were additive [119].

Pretreatment of mice with amantadine (100 mg/kg) delays the onset of ethanol narcosis, measured as the length of time required for the loss of righting reflex to occur after 5 g/kg of ethanol [120]. The duration of the effects of ethanol were also decreased. Amantadine had no effect on blood levels of ethanol, but did cause a slight decrease in whole brain levels of ethanol at the time of onset of narcosis and 30 min after onset of narcosis. Although a possible role for amantadine in treating ethanol intoxication in man has been suggested based on the above results [120], the doses used in these experiments are 50-fold those which can be safely used clinically.

In a study using normal rats, amantadine at 10 mg/kg produced a pronounced decrease in normal sleep time, with a lesser effect in diminishing barbitol-induced loss of righting reflex and mebubarbitaol narcosis [121].

The acquisition of conditioned avoidance responses in rats is facilitated by treatment with low doses of amantadine and other dopaminergic drugs [122]. Higher doses of these agents were inhibitory. Amantadine also reverses the inhibitory effects of the neuroleptics haloperidol and pimozide on the acquisition of avoidance responses [123].

Amantadine at 25-100 mg/kg i.p. causes a marked reduction of rectal temperature in mice and rats [6, 124]. Both rimantadine and amantadine when administered intracerebroventricurlarly in small doses produced the effect suggesting that it is central in origin. The hypothermic activities of apomorphine and piribedil are blocked by prior treatment with pimozide, but the effect of amantadine is not modified [124]. The conclusion drawn from these results is that the hypothermic effects of amantadine are central, but not dopaminergic in origin.

BIOCHEMICAL STUDIES

Little evidence exists for an effect of amantadine on dopamine synthesis or turnover. Brown and Redfern [125] have examined the dopamine and nore-

pinephine content of whole rat brain homogenates after doses of amantadine ranging from 10 to 160 mg/kg and found no significant drug effect on catecholamine levels. A similar lack of effect was observed after chronic treatment of the animals with 94 mg/kg/day of amantadine for nine days.

The rate of synthesis of dopamine in the corpus striatum of the rat has been measured by determining the amount of a standard i.v. dose of ^{3}H-labelled tyrosine which is incorporated into labelled dopamine. Treatment with 30 to 100 mg/kg of amantadine had no effect on the rate of tyrosine incorporation whereas both haloperidol and amphetamine caused an increase in the rate of dopamine synthesis and apomorphine depressed it [126]. The results obtained with haloperidol and apomorphine are consistent with previous findings which indicate that dopamine receptor blocking agents enhance and direct-acting dopamine agonists supress dopamine turnover, presumably through an effect on presynaptic dopamine receptors.

Levels of the dopamine metabolite homovanillic acid in the rat corpus striatum were determined fluorometrically from extracts of homogenized striatal tissue [126]. While amantadine at 30 mg/kg had no effect on homovanillic acid levels, after 100 mg/kg a 50% increase was observed. Apomorphine decreased homovanillic acid levels by one third and haloperidol caused a seven-fold increase. No increase in urinary catecholamine metabolites was noted in a group of Parkinson's disease patients being treated with amantadine [127].

Dopamine turnover in rats has also been investigated by determining the rate of decline of the whole brain [125, 128] and striatal [126] dopamine content after inhibition of dopamine synthesis with α-methyl-*p*tyrosin. In each set of experiments, amantadine was administered simultaneously with or 1-3 hours after a large dose (200-250 mg/kg i.p.) of α-methyl-*p*-tyrosine. After an interval of up to 3 hours, the brains were dissected and homogenized and the dopamine content of the homogenates was assayed fluorometrically. Amantadine had no effect on the rate of decline of dopamine levels whereas in each case apomorphine decreased and haloperidol increased this rate. These results could be due to feedback inhibition and stimulation respectively of dopamine release and suggest that amantadine lacks important direct effects at dopamine receptors.

Stone and Bailey [6] have summarized a body of data which indicated that amantadine is only a weak inhibitor of catecholamine uptake, particularly in the striatum. Further support for this conclusion is provided by Brown and Redfern [125] who have measured the effects of amantadine and amphetamine on the rate of uptake of [^{3}H] dopamine by synaptosomes isolated from rat corpus striatum. They found that amantadine is a weak, non-competitive uptake inhibitor with a K_i of 125 μM. Amphetamine, by way of contrast, was found to be a competitive inhibitor with a K_i of 1.94 μM.

Some of the pitfalls in the classification of drugs as catecholamine uptake inhibitors or releasing agents have been discussed [129]. From a study of the effects of amantadine on the accumulation of [^{3}H] dopamine in rat neostriatal slices in comparison with amphetamine and the pure uptake inhibitor cocaine, it was concluded that amantadine selectively affects dopamine release [129]. Similar data obtained with cerebral cortex slices indicate that in this tissue, amantadine is a very weak uptake inhibitor with little affect on release.

Brown and Redfern [125] were unable to observe a significant effect of amantadine at concentrations of up to 1 X 10^{-4} M on the release of acuumulated [^{3}H] dopamine from rat strial homogenates; in contrast, tyramine produced a significant effect at 1 X 10^{-5} M. In the artificially-perfused cerebroventricular system of anaesthesized cats, amphetamine provoked the release of [^{3}H]-dopamine at 1X 10^{-5} M. Amantadine was ineffective at 1 X 10^{-4} M, but did increase the efflux of [^{3}H]-dopamine at a concentration of 10^{-3} M [130].

Both amantidine and memantine (3) cause the release of accumulated dopamine and 5-hydroxytryptamine from nerve endings isolated from rat brain at concentrations between 5 X 10^{-5} – 5 X 10^{-4} M [131]. When the isolated nerve endings were incubated with subthreshold concentrations of amantadine and memantine (5 X 10^{-6} – 5 X 10^{-5} M), a significant potentiation of electrically induced transmitter release was observed.

Cunnane, Draper, and Refern [132] have found that amantadine promoted the release of norepinephrine from an isolated rat anoccygeus muscle preparation. In the absence of bretylium pretreatment, an effect was first observable at an amantadine concentration of 1.5 X 10^{-6} M, while after bretylium pretreatment, a 30-fold higher concentration of amantadine was required to obtain a simular effect. However, like amphetamine, a much lower concentration of amantadine, 0.5 X 10^{-6} M, was sufficient to reverse the effects of bretylium (1 μg/mL) on contraction induced by transmural electrical stimulation. The authors conclude that amantadine acts on the 'labile' or extragranular norepinephrine pool thought to be preferentially affected by bretylium [133]. A similar conclusion was reached by Enna and Shore [134] who found that amphetamine, but not amantadine, released the granule-bound amine metaraminol from rat heart slices.

Earlier work which has been summarized by Bailey and Stone [6] support the concept that an important effect of amantadine is its ability to selectively enhance dopamine release from a presynaptic site in the corpus striatum.

The impact of amantadine on dopaminergic pathways has also been investigated directly in single central neurons. Microiontophoretically-applied dopamine, amphetamine, and amantine all had a similar depressant effect on neuronal firing rate in single cells of both the cerebral cortex and the caudate nucleus of the rat [135, 136]. The failure of amantadine to potentiate the effect of dopamine was

regarded as further evidence that amantadine does not exert a physiologically important blockade of dopamine uptake. The response to dopamine was unaffected by pretreatment of the animals with resperpine or α-methyl-*p*-tyrosine, but the amphetamine and amantadine responses were markedly attenuated by catecholamine depletion.

In more recent studies, Stone [137, 138] has investigated the ability of the specific dopamine receptor antagonist α-flupenthixol to block the depressant effect of amantadine. Microiontophoretically-applied doses of α-flupenthixol which completely blocked the dopamine response reversibly attenuated, but did not abolish the response to amantadine. In these experiments, application of either propanol or phentolamine were without effect on the amantadine activity, while previously Stone [136] found that the relatively nonspecific dopamine antagonist, chlorpromazine, blocked both the dopamine and amantadine response but did not affect that to norepinephrine.

The conclusion drawn from the above work is that amantadine has a dopaminergic action, probably due to its ability to enhance release and also a depressant effect in the presence of α-flupenthixol. This could be due to activity at dopamine receptors not blocked by α-flupenthixol or a non-dopaminergic effect which is also independent of norepinephrine receptors.

Tang and Cotzia [139] have found that incubation with amantadine increases the level of cAMP within homogenates derived from the mouse caudate nucleus. Amantadine, in common with several other synthetic dopaminergic agents, displayed a bell-shaped dose-response relationship with a maximum effect at 10 μM. Higher concentrations of the synthetic agents, but not dopamine, led to a decreased positive response. Neuroleptic drugs reversed the effects of dopamine agonists and surprisingly, both atropine and acetylcholine, while having no activity alone, competitively inhibited the dopamine-induced activation of adenylate cyclase.

Remarkably similar results have been obtained from studies of the ability of dopamine, amantadine, memantine (3), and apomorphine to induce accumulation of cAMP in slices of rat striatum and cerebral cortex [140]. When tested on striatal slices, all four drugs induced a dose-dependent accumulation of cAMP with a peak effect at 10 μM. The cAMP content of cerebral cortex slices was not raised significantly by any of the four dopaminergic compounds although norepinephrine did induce a significant increase. The results could be due to a stimulatory action of the test substances on adenylate cyclase or an inhibitory effect on phosphodiesterase. The authors report that dopamine, amantadine, and apomorphine had no influence on phosphodiesterase activity in either of the brain structures investigated when assayed at full substrate saturation [140].

The same authors priviously described the characterization of two phosphos-

diesterases from rat striatum, one with a low K_m (2.9×10^{-6} M) and one with a higher K_m (2.05×10^{-4} M) [141]. They found that amantadine and apomorphine competitively inhibited the high affinity phosphodiesterase with K_i values estimated from Lineweaver and Burk plots of 94.4 ± 0.6 μM and 87.2 ± 0.4 μM respectively. Neither dopamine or amphetamine affected the hydrolysis kinetics of either phosphodiesterase. These results may not be of great physiological significance in light of the above findings.

There have also been two negative reports concerning the stimulation of cAMP accumulation by amantadine. Karobath [142] was unable to observe any effect with either amantadine or memantine (3) while working with rat striatal homogenates in an experimental design similar to that employed by Tang and Cotzias [139]. Schordiret [143] found an accumulation of cAMP in the intact rabbit retina after exposure to several dopamine agonists including dopamine and apomorphine. Both amantadine and amphetamine were ineffective in this test.

Amantadine at 50-100 mg/kg has been reported to induce dose-dependent increases in the cGMP content of the mouse cerebellum and medial forebrain with a more profound effect in the medial forebrain area [144]. The time course of the cGMP elevation corresponded to the onset and duration of the weak stereotyped behaviour seen after large doses of amantadine. Other drugs which were investigated, such as apomorphine, induced a larger effect on cGMP levels and also a more pronounced stereotype whose time course also corresponded to cGMP levels. The authors of this work did not observe any effects on cAMP levels, although they did not analyse discrete areas of the brain and specific localized effects could have been missed.

Evidence for an amantadine effect on 5-hydroxytryptamine mediated central pathways has been presented [131, 145–148]. Both amantadine and memantine (3) have been shown to non-competitively inhibit 5-hydroxytryptamine uptake by isolated synaptic vesicles obtained from rat brain [145]. At high concentrations (5 mM), both drugs also induced the release of 5-hydroxytryptamine and dopamine from this preparation. In analogy to the results previously cited, subthreshold concentrations (50 μM) of either compound enhanced the release of the two transmitter substances in response to electrical stimulation. In this experiment, memantine was more potent than amantadine, giving a significant effect at 5 μM.

Incubation of blood platelets with a 5 mM solution of amantadine causes depletion of the platelet 5-hydroxytryptamine stores [146, 147]. Concanavalin A and thrombin cause the release of 5-hydroxytryptamine, adenine nucleotides, and α-mannosidase from washed humen platelets. At 1 mM, amantadine had no effect alone, but selectively potentiated the release of 5-hydroxytryptamine induced by either agent [147].

Cox and Tha [148] have determined that amantadine in a concentration range of 10-80 μg/mL had no effect on the spontaneous contractions of isolated rat fundus strips. However, the responses to added 5-hydroxytryptamine were potentiated in a dose dependent manner in the presence of amantadine. The potention observed was considerably greater than that achieved with maximally effective concentrations of the pure uptake inhibitor cocaine suggesting an explanation other than uptake inhibition for the observed effect.

Amantadine does not inhibit the effects of acetylcholine on guinea pig ileum [102] or chick oesophagus [103] and does not block the vasodepressor response of acetylcholine in the dog [102]. Unlike atropine, amantadine does not antagonize the tremorgenic action of oxotremorine on mice [102, 149].

Amantadine has, however, been shown to exert a marked antinicotinic effect in the chick oesophagus preparation [103] and to antagonize the effects of centrally administered nicotine in mice after either i.v. or intracerebralventricular administration [149]. It has also been reported to reduce the amplitude of the ponto-geniculo-occipital (PGO) waves induced by the benzoquinolizine derivative Ro 4-1284 in the lateral geniculate bodies of unanesthetized cats. An i.v. dose of 1-3 mg/kg gave a 50% reduction of the amplitude of the PGO waves [150]. The authors report that amantadine has antinicotic effects on autonomic ganglia and propose an inhibitory action at central nicotinic receptors to account for its effects on PGO wave amplitude [150].

In a partly curarized electrically-stimulated frog sartorius muscle-sciatic nerve preparation, amantadine (0.2-0.8 mM) caused a dose-dependent decrease in tension output [151, 152]. The intracellularly measured endplate potentials in single curarized pectoris muscle fibers were reduced by amantadine (50-150 μM) while resting potential was unaffected [151]. Amantadine was less potent at extrajunctional acetylcholine receptors than at junctional ones in the rat soleus muscle. Thus, a concentration which effectively attenuated the endplate potential did not affect extrajunctional acetylcholine sensitivity [152].

The mechanism of the antinicotinic effect of amantadine has been further defined in work with the electric organ of the electric ray *Torpedo ocellata* which is rich in nicotinic neuromuscular type junctions [152–154]. The authors of these papers propose that the postsynaptic effects of acetylcholine are regulated by two coupled proteins, the acetylcholine receptor and an ion conductance modulator (ICM). Drugs which affect postsynaptic neuromuscular events may thus act directly at the acetylcholine recepter (d-tubocurarine) or on the ICM (histrionicotoxin).

The binding of [^{3}H]-acetylcholine and [^{3}H]-perhydrohistrionicotoxin were studied in a membrane preparation derived from the electric organ of the electric ray *Torpedo ocellata* by equilibrium dialysis [153]. The binding of [^{3}H]-

perhydrohistrionicotoxin was saturable (K_d = 4 X 10^{-7} M) and specific, being blocked only by drugs which reduced the ionic conductance of the endplate. Amantadine was shown to significantly reduce the binding of perhydrohistrionicotoxin at concentrations which did not affect acetylcholine binding. On the other hand, acetylcholine, but not perhydrohistrionicotoxin binding was inhibited by d-tubocurarine. The results suggest that the antinicotinic effects of amantadine may be due to an effect on the ionic channel of the postsynaptic receptor. A similar effect on calcium ionic channels has been proposed to account for the oxytoxic effect of amantadine on isolated rat urerus [155].

In conclusion, the bulk of the evidence suggests that amantadine has a dopaminergic component of its activity which is probably due to a facilitory effect on dopamine release in response to stimulation. Recently, evidence has been accumulating that there are at least two classes of dopamine receptors, one of which is not coupled to adenylate cyclase [156, 157]. The specificity of amantadine at physiological doses could be due to a selective effect on inhibitory dopamine receptors. The single cell studies of Stone [135–138] imply that amantadine alsoo has a non-dopamineric component of iets activity. A considerable body of evidence suggests that amantadine is capable of exerting an antinicotinic effect which may be due to an interference with postsynaptic ionic conductance. The antinicotinic effect appears to be capable of central expression as well [149–150] and may contribute to the overall profile of amantadine in man.

TOXICITY OF AMANTADINE

In a previous section of this revieuw, several factors which may be responsible for the relatively minor use of amantadine by physicians for prophylaxis or symptomatic management of influenza A respiratory disease were discussed. The long list of adverse reactions which a prescribing physician would encounter in the Physicians' Desk Reference [158] would include those of a serious nature such as depression, congestive heart failure, orthostatic hypotensive episodes, psychosis, urinary retention, convulsions, leukopenia and neutropenia. Examples of adverse reactions of a less serious nature which have been observed are hallucinations, confusion, anxiety and irritability, anorexia, nausea, constipation, ataxia, dizziness, livedo reticularis, and peripheral oedema. Less frequently observed adverse reactions would include vomiting, dry mouth, headache, dyspnea, fatigue, insomnia, sense of weakness, skin rash, slurred speech and visual disturbances. Eczematoid dermatitis and oculogyrie episodes have been rarely seen [104]. If a physician considered amantadine to be of questionable value against influenza A respiratory disease which was thought to be self-limiting and of

minor consequence in an otherwise healthy individual, the potential for adverse reactions would certainly have influenced any decision on the use of amantadine.

It is interesting to note that when amantadine was administered to influenza patients at the recommended oral dosage of 100 mg twice daily, the occurrence of adverse reactions was uncommon [43, 104, 159–161]. In a recent study of the comparative effect of amantadine and rimantadine against influenza A disease, it was shown that side effects of amantadine treatment were generally mild and occurred within the first 48 hours and were not as substantial as those seen with the use of antihistamines for upper respiratory infections [35]. In addition, it was shown that, during the period of the trial, subjects receiving amantadine had higher test scores in academic studies that did subjects receiving placebo.

The adverse reactions observed in amantadine-treated patients are dose-related. In the case of Parkinson's disease, chronic use of amantadine (greater than 5 to 6 months) is well tolerated with adverse effects occuring more frequently at higher dosage levels (400 mg daily or more) [162, 163]. Livedo reticularis in the extremities and associated ankle oedema are common side effects [164] when the daily dosage exceeds 200 mg. When given at the recommended dosage the side effects of amantadine in patients with Parkinson's diseases are generally mild, often transient in nature and reversible in patients with normal renal function. Amantadine produces fewer side effects than either levodopa or the anticholinergic agents used in the treatment of Parkinson's disease but amantadine has been reporterd to enhance the side effects of the anticholinergic drugs when treatment with both substances is administered [162, 163]. Loss of visual acuity which returned to normal after cessation of therapy with amantadine [165] and visual hallucinations and delirium during treatment with amantadine have been observed in patients with Parkinson's disease [166]. Treatment with a combination of amantadine and an anticholinergic agent increased the probability of delirium by causing retention of urine [166].

Since amantadine is not metabolized in man and more than 90% of the ingested dose can be recovered unchanged in the urine [167], special caution must be exercised when administering the drug to patients with impaired renal function. In patients with normal renal function, a steady-state plasma concentration of 0.2 to 0.9 μg/ml of amantadine has been found when the recommended daily dose of 200 mg was administered [168, 169]. A variety of neuropsychiatric toxic effects of amantadine have been noted in patients with renal insufficiency and plasma levels of the drug > 1.0 μg/ml should be viewed with concern [168–173]. Physostigmine salicylate has been of value in the treatment of amantadine intoxication [174, 175].

Amantadine has been reported to be embryotoxic and teratogenic in rats at 50 mg/kg/day (12 times the recommended human dose) but not at 37 mg/kg/day. No effect was seen in rabbits which received up to 25 times the recommended human dose. Amantadine has not been studied in pregnant women and its use in women of child-bearing age must be balanced between the benefit to the patient and the risk to the foetus. The drug is secreted into the milk and should not be administered to nursing mothers [158].

PHARMACOKINETICS OF AMANTADINE

Following oral administration of amantadine (100 mg), the drug is completely absorbed and peak serum levels (0.2 mg/ml) are achieved within 1 to 8 hours and the average serum half-life is 20 hours [167]. About 90% of absorbed amantidine is excreted unchanged in the urine with the elimination half-life being approximately 20 hours [167, 176, 177]. The rate of excretion of amantadine has been shown to be dependent on urinary pH with greater excretion in acid urine [178]. Amantadine is found in cerebrospinal fluid, saliva, nasal secretions and breast milk. It has not been reported to significantly interact with a variety of compoounds including antibiotics, aspirin, antihistamines, and cardiovascular drugs [10] but pretreatment of rats with amantadine in single or multiple doses depressed the activity of drug metabolizing enzymes in the liver [179]. Since amantadine has been used in Parkinson's disease as part of a multiple drug therapy, a demonstration of a similar effect of amantadine on drug metabolizing enzymes in man could have important pharmacological implications [179].

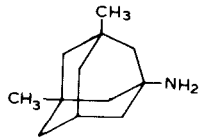
In a study of amantadine kinetics in healthy young subjects after long-term dosing, a relative bioavailability independent of dose over the range of 50 to 300 mg daily was demonstrated [177]. If lung is one of the tissues in man saturated at lower doses, it is possible that a dose of amantadine lower than that currently recommended for influenza might be efficacious and also reduce the incidence of adverse reactions to amantadine [177, 180].

MEMANTINE

Memantine chemically is 1-amino-3,5-dimethyladamantane (3) and is also referred to in the literature as D-145, DMAA and dimethylamantadine. This latter designation is unfortunate as it has caused some abstractors to misassign the structure as 1-(*N,N*-dimethylamino)adamantane.

Memantine is of interest primarily on account of its pharmacological profile, and we are not aware of any reference to its possible chemotheraputic potential.

Pharmacologically, it is similar to amantadine, but it is more potent. Memantine has been tested clinically in Parkinson's disease patients.



(3)

In behavioural studies, memantine was found to produce a more pronounced stimulation of motor activity than is achievable with amantadine. This effect has been noted in mice [181, 182] and rats after 5-40 mg/kg s.c. [183, 184] and is not abolished by pretreatment with reserpine, FLA-63, or α-methyl-*p*-tyrosine.

Menon and Clark [182] have reported that in mice, pretreatment with either of the gabaminergic drugs, muscimol or 4,5,6,7-tetrahydroisoxazolo[5,4-*c*] pyridin-3-ol, blocks the motor hyperactivity induced by 20 mg/kg of memantine but not that induced by amphetamine. Amino-oxyacetic acid causes an increase in brain GABA levels, Administration of 50 mg/kg to mice caused a sedation which was reversed by amphetamine, but not by memantine. Based on the above results, the authors propose a selective GABA antagonistic role for memantine in the basal ganglia structures. However, the close association between gabaminergic, dopaminergic and cholingenic neurons suggest that the observed results may be due to a memantane-induced presynaptic inhibition of dopamine release caused by the gabaminergic agents [156, 185, 186].

Like amantadine, memantine is capable of inducing only a low level of stereotyped behaviour in rats although the effect obtained with memantine is more consistent [183, 184]. When given to rats with unilateral lesions in the substantia nigra, memantine induced ipsilateral turning behaviour of an intensity comparable with that obtained with apomorphine [184]. In animals with lesions in the medial raphe nucleus, it induced contralateral circling only slightly more intense than that observed after amantadine [184].

Memantine, in dose up to 10 mg/kg, did not affect the flexor response in the spinal rat [181, 183]. Both amantadine at 10 mg/kg and amphetamine potentiated it [183].

Memantine antagonizes neuroleptic-induced catatonia [183] and like other dopaminergic drugs (apomorphine, piribedil, 2-bromo-α-ergocryptine), it potentiates withdrawal symptoms induced by naloxone in morphine dependent rats [187].

Catecholamine levels in the rat caudate-putamen and hypothalamic paraventricular nucleus were reduced 2-3 hours after administration of 20 mg/kg

of memantine [188]. The effect on the paraventricular nucleus was blocked by spiperone. The loss of norepinephrine from all areas of the rat brain after the dopamine β-hydroxylase inhibition was accelerated by memantine and apomorphine, an effect which is presumably secondary to dopaminergic stimulation [189]. Spiperone blocked the effects of apomorphine, but affected the activity of memantine only in the neocortex and not in the limbic system or thalamus plus hypothalamus.

As mentioned above during the discussion of amantadine biochemistry, memantine is capable of inducing the release of dopamine and 5-hydroxytryptamine from isolated nerve endings obtained from rat brain. Subthreshold concentrations enhance the transmitter release observed after electrical stimulation and memantine appears somewhat more potent than amantadine in this regard [31, 145].

A number of dopaminergic drugs were investigated for their direct dopaminergic presynaptic effects on isolated, perfused rabbit central ear arteries in the presence of yohimbine and cocain. Unlike dopamine and apomorphine, memantine was unable to inhibit metoclopramide-sensitive vasocontriction due to electrical sympathetic nerve stimulation [190]. The direct postsynaptic activities of the same drugs were evaluated by their haloperidol-reversible effects on renal blood flow and renal vascular resistance in anesthetized, phenoxybenzamine pretreated beagles. Memantine was more than an order of magnitude less active than apomorphine and dopamine in this test [190].

As discussed previously, memantine at a concentration of 10^{-5} M causes an accumulation of cAMP in rat striatal homogenates [140]. Memantine reduced the amplitude of Ro 4-1284 induced PGO waves in the lateral geniculated body [191]. Apomorphine was also inhibitory in some concentrations, but whereas the apomorphine effects were blocked by spiroperidol, those of memantine were not.

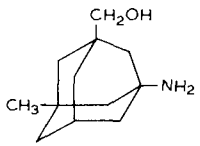
In vitro studies of the effects of memantine on rat sensory nerve bundles were carried out using the sucrose gap method. At 10^{-4} M in normal Locke solution, memantine decreased the action potential with no effect on resting potential. It increased membrane resistance due to an inhibitory effect on the conductance of sodium, potassium, and chloride ions and decreased the spike amplitude and depressed the repetitive discharges caused by rectangular pulses [192, 193]. The authors of these studies hypothesize that memantine also inhibits repetitive activity in the descending spinal pathways necessary for the maintenance of rigidity through a membrane effect.

The above results could account for the observation that memantine significantly reduces decerebrate muscle rigidity [194] and the activity of alpha motoneurons in the vibration reflex in decerebrate cats [195]. Memantine also reduces

the excitability of gamma motoneurons [196] and the reflex excitability of flexor muscles induced by fusimotor activity in spinal cats [197].

Memantine exerts effects similar to those evoked by dopaminergic agents. However, there is little evidence supporting a direct action on pre- or post-synaptic dopamine receptors. Furthermore, dopamine receptor antagonists are only partially effective in blocking the effects of memantine on the loss of norepinephrine from rat neocortex and on the amplitude of PGO waves. The activity of memantine is likely due in part to a selective indirect effect at dopaminergic and possibly 5-hydroxytryptaminergic neurons. Effects on gabaminergic neurons and direct effects on ionic conductance of neuronal membranes may also contribute to the overall profile of memantine.

The metabolism of memantine has been studied in rats and man [198]. Between 6 and 11% of a 50-100 mg/kg dose was excreted unchanged in rat urine with 4% accounted for as hydroxylated metabolites. After a single 10 mg oral dose in a volunteer, 1-amino-3-hydroxymethyl-5-methyladamantane (4) was isolated along with unchanged memantine from the urine.



(4)

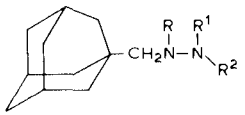
Memantine has been tested in an open trial in 12 Parkinson's patients [199]. A dose of 40 mg was administered by i.v. infusion and the patients were followed for 24 hours. The effects were qualitatively similar to those seen with amantidine therapy with the most pronounced effect on tremor. The authors indicate that memantine was better tolerated than amantadine. However, long-term controlled studies would be required to determine whether the drug truly has utility in Parkinson's disease or in other neurological disorders in which amantadine has been shown to be useful.

OTHER AMINOADAMANTANE DERIVATIVES

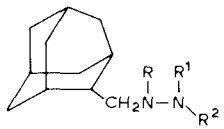
In addition to the three adamantane derivatives which have been reviewed in detail in the preceding sections, many other compounds containing the adamantyl moiety have been prepared during the course of structure activity studies in various therapeutic areas. Medicinal chemists have employed the adamantyl group to confer additional steric bulk and lipophilicity to lead compounds and

also have expended considerable effort to improve upon the anti-Parkinson's activity and, in particular, the antiviral activity of amantadine. A number of analogues with interesting antiviral activity have been included in earlier reviews [3, 7, 9, 200] and we will limit our comments to those compounds for which there is evidence of activity at least in tissue culture and which have not been previously reviewed. In the section which follows, we have attempted to cover developments in structure-activity studies involving the adamantyl moiety which have occurred between 1975 and the beginning of 1980.

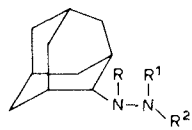
In two patents [201, 202], Israeli workers have presented *in vitro* data indicating that selected examples from a large number of 1- and 2-substituted adamantylhydrazine derivatives depicted by the general structures (5-7) (R, R^1, R^2 = alkyl or aryl) have significant activity against four strains of mycoplasma, protozoa (*Leishmania* and *Trypanosoma*), human fungi (*Candida albicans, Trichophton rubrum*), various plant fungi and herpes simplex virus. Individual compounds tended to be active in only one or two tests, making generalizations about structure-activity relationships difficult. However, the *in vitro* activities described are unusual for adamantyl derivatives.



(5)



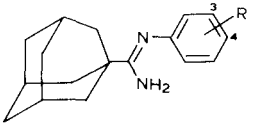
(6)



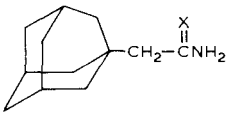
(7)

Members of a series of *N*-substituted-1-adamantylcarboxamidines were evaluated in tissue culture for activity against influenza A2, vaccinia, and herpes viruses [203, 204]. Compounds (9-13) effectively inhibited the growth of all three viruses while other analogues possessing electron withdrawing substituents on the aromatic ring, alkyl instead of aryl substitution on the amidine nitrogen, or substituents on both amidine nitrogen atoms were inactive. Interestingly, the 4-amino derivative (14), while inactive against the above three viruses, had some activity against polio virus *in vitro*.

The adamantylacetamidine (15) has been claimed to inhibit the growth of Newcastle disease virus (NDV) in chick embryo fibroblasts by greater than 60% at a concentration of 100 μg/ml and by greater than 95% at 250 μg/ml [205]. The corresponding thioacetamide (16) was reported by the same group to be active at 25 μg/ml and no signs of cytotoxicity was observed up to 250 μg/ml [206]. In contrast, amantadine and 1-adamantylthiocarboxamide had little effect on NDV at concentrations below 250 μg/ml.



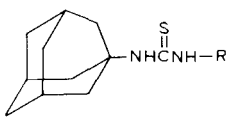
(8) R = H
(9) R = 4-OCH_3
(10) R = 4-OC_2H_5
(11) R = 4-OH
(12) R = 4-Cl
(13) R = 3-OCH_3
(14) R = 4-NH_2



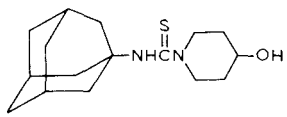
(15) X = NH_2
(16) X = S

Tilley, Levitan and Kramer [207] described a series of acylated adamantylthiourea derivatives. Two of these, (17) and (18) protected mice against influenza A2/Asian virus infection with protective $dose_{50}$ (PD_{50}) values of 16 and 158 mg/kg intraperitoneally (i.p.) repectively. Amantadine had a PD_{50} value of 6 mg/kg ip in this test. Neither analogue was active *in vivo* against herpes simplex, Semliki forest or Sendai viruses. The most interesting compound, the aminophenylsulphonylthiourea (17), also had therapeutic activity against influenza A2/Asian virus in mice when treatment was delayed up to 24 hours after infection. While the ratio of acute toxicities (LD_{50}) of amantadine and (17) in mice (245 mg/kg i.p. and 900 mg/kg i.p. respectively) were comparable with the ratio of their PD_{50}s, fewer CNS symptoms were observed with (17) at subtoxic doses.

The alkyl substituted thioureas (19–21) have been reported to be active in tissue culture against herpes, vaccinia, and adeno viruses although no details of the test procedures are availabel [208, 209]. Methyl adamantylpseudothioureas inhibitory to influenza A2 have also been claimed [210].



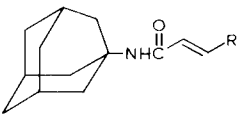
(17) R = SO_2-C$_6$H$_4$-NH_2
(18) R = $PO(OC_2H_5)_2$
(20) R = $CH(CH_3)_2$
(21) R = $CH_2CH(CH_3)_2$



(19)

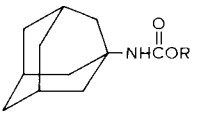
Kreutzberger and Schröders [211] identified the *N*-(1-adamantyl)cinnamide (22) as the most interesting of a series of amide derivatives of amantadine. Compound (22) provided significant protection to mice infected with influenza A/PR8 virus after i.p. administration of a dose of 0.25 mg/kg. It was also effective in tissue culture against Newcastle disease virus and both (22) and the corresponding furyl analogue (23) [212] had in vitro activity against herpes and vaccinia viruses although (22) was the more effective. Of the other derivatives tested, the 4-methoxyphenyl analogue of (22) was more than an order of magnitude less active than the parent against Newcastle disease virus.

Russian workers have reported that the propyl and butyl carbamates (24) and (25) have antifungal activity particularly against *Aspergillus niger* [213].



(22) R = Ph

(23) R = 



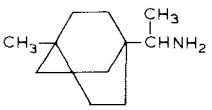
(24) R = $(CH_2)_2CH_3$

(25) R = $(CH_2)_3CH_3$

In addition to their efforts to modify the substituent groups on the adamantyl nucleus, medicinal chemists have investigated the effect on antiviral activity of substituting various polycycloalkanes for the adamantyl moiety and this work has been reviewed [200]. The aminomethylbicyclo[2,2,2]-octane (26) was one of the most active compounds described in that review; this substance was three times as potent as rimantadine against influenza virus in mice, but its CNS side effects limited its further development.

More recently, a Japanese group described a series of 4-homotwistane derivatives which were tested for activity against Newcastle disease virus in tissue culture. The amino- and aminomethyl-analogues (27) and (28) were 30–50 times as potent as amantadine and the minimum cytotoxic concentrations were 2–8 times the minimum inhibitory concentrations [214]. No data on activity against influenza viruses or *in vivo* activity were given.

The aminospirane (29) was effective in prolonging the survival of chick embryos infected with either type A (A2/Taiwan/64) or type B (B/Lee) influenza viruses and was also more effective than amantadine in protecting mice from otherwise lethal infections with influenza A2 virus [215]. Several analogues of (29) were active in vitro against influenza A2 and, additionally, the positional isomer (30) inhibited parainfluenza type 3 virus in tissue culture. The dialkylaminoalkyl analogues (31) and (32) were active against rhinovirus type 14 in HeLa cell culture; no data were given on their effect in vitro against influenza viruses.



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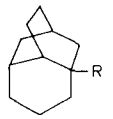
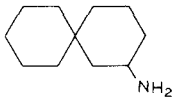


(27) R = NH_2

(28) R = CH_2NH_2



(29)

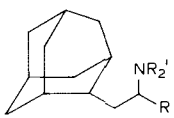
(30) R = NH

(31) R = $CH_2N(C_2H_5)_2$

(32) R = $(CH_2)_3N(C_2H_5)_2$

The amphetamine-like pharmacological profile of amantadine has prompted investigation of amphetamine analogues in which the adamantine ring replaces the aromatic ring. A group of Italian workers reported [216] that the 2-(2-aminoethyl)-adamantine (33) is about one-fourth as potent as amphetamine as an anoretic agent in mice [217]. The corresponding 2-(2-aminoethylidene analogue was also active in this test, but unlike amphetamine neither compound had a significant effect on spontaneous motor activity or the duration of barbiturate-induced sleep. Compounds (34) and (35) were synthesized in several routine steps from 2-adamantanone and both decreased spontaneous motor activity and exhibited an antinicotinic effect in mice, but were not active in reversing chlorpromazine-induced catalepsy or in reversing oxotremorine tremors [218].

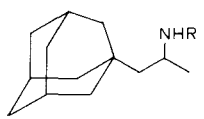
The analogues (36) and (37) were selected as the most interesting of a series of 1-(2-aminopropyl)adamantane derivatives; (36) is reported to have antiviral activity comparable to that of amantadine [219]. A follow-up pharmacological investigation of (36) and (37) revealed that both compounds moderately increased the spontaneous motor activity of mice at a dose of 5 mg/kg i.p. and decreased, although less potently than amphetamine, the metrazol LD_{50} value in mice. No significant effect on hexobarbital sleeping time or antifatigue activity as determined by the rotarod test was observed for either compound [220].



(33) R = H, R' = H

(34) R = CH_3, R' = H

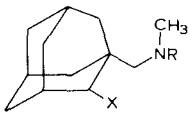
(35) R = CH_3, R' = CH_3



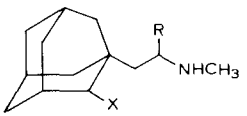
(36) R = H

(37) R = CH_3

A group at Eli Lilly using reversal of reserpine-induced catelepsy as an indication of anti-Parkinson's activity has identified a series of active 2-substituted-1-(aminoalkyl)-adamantanes [221, 222]. The aminomethyl compounds (38–40) completely or nearly completely reversed reserpine-induced catalepsy in rats although only (39) showed a marked effect in the mouse [221]. Amantadine in this test had a less pronounced effect in the rat and no effect in the mouse. The 1-aminoethyl anlogue (41) was of comparable activity with (39) and in contrast to the aminomethyl series in which the 2-phenyl derivative corresponding to (38) and (39) was inactive, compounds (42) and (43) were as active as (41) in the rat [222]. Both (41) and (42) also caused a marked antagonism of reserpine induced hypothermia in mice at a dose of 20 mg/kg po, while (43) was less active. Although (43) was selected for further development as an anti-Parkinson's agent [223–225] and a pharmacokinetic study was carried out in man, we are unaware of any published reports of human therapeutic trials.



(38) X = Cl, R = H
(39) X = Br, R = H
(40) X = Br, R = CH_3

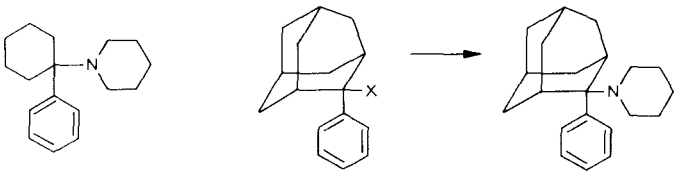


(41) X = Cl, R = H
(42) X = Phenyl, R = H
(43) X = Phenyl, R = CH_3

Israeli workers have examined the anticholinergic effects of the phencyclidine anlogues (46) and (47). Compound (46) was prepared from 2-phenyl-2-adamantanol [226] by reaction with hydrazoic acid in acidic chloroform followed by reduction of the resulting azide with Raney nickel. The further conversion to (47) was accomplished by reaction of (46) with 1,5-dibromopentane [227].

These workers sought to test the hypothesis that increasing the rigidity and capacity for hydrophobic binding of phencyclidine (44) by substitution of an adamantane ring for the cyclohexyl moiety would enhance the anticholinergic potency of the derivatives. Antimuscarinic activity was evaluated in a guinea pig preparation by measuring dose-dependent reversal of acetylcholine-induced contractions and in mouse brain by competition with [^{3}H]-*N*-methyl-4-piperidyl benzilate and [^{3}H]-acetylcholine binding [227]. In both preparations, (47) was a competitive inhibitor approximately two orders of magnitude more potent than phencyclidine with dissociation constants of $9.5 \pm 0.4 \times 10^{-8}$ M (guinea pig ileum) and $9.3 \pm 0.5 \pm 10^{-8}$ M (mouse brain) respectively. The antinicotinic activity of (44) and (47) was compared in the frog rectus abdominus muscle

[228]. While neither compound was a truly competitive inhibitor, both substances caused a 50%inhibition of the contraction induced by 2 X 10^{-6} M acetylcholine at a concentration of 1.2 X 10^{-6} M. Neither compound affected the binding of [^{125}I]-α-bungarotoxin to solubilized nicotinic receptors isolated from *Torpedo ocellata* at concentrations below 10^{-4} M. A similar pattern of anti-nicotinic activity was noted with amantadine [153]. The analogue (46), lacking the piperidine ring, was generally one-third as active as phencyclidine in these test systems.

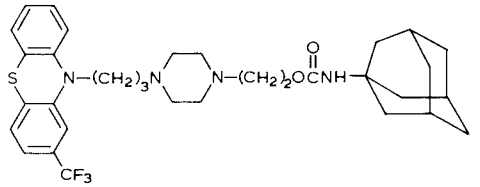


(44) (45) X = OH (47)

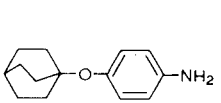
(46) X = NH_2

The carbamate (48) was prepared from the neuroleptic fluphenazine and 1-adamantylisocyanate in the hope that it would undergo hydrolysis *in vivo* to amantadine and fluphenazine. It was felt that the released amantadine would antagonize the extrapyramidyl side effects associated with the neuroleptic [229]. However, compound (48) was weaker and shorter acting than fluphenazine in antagonizing amphetamine-induced increased spontaneous motor activity and avoidance responses in rats. While (48) depressed spontaneous motor activity, in contrast to fluphenazine, it actually caused a stimulation of avoidance responses which lasted for seven days.

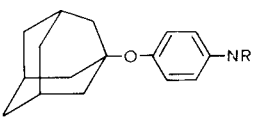
Based on earlier work which indicated that the bicyclo-octyloxyaniline (49) was one of the most interesting of a series of related hypobetalipoproteinemic agents [230], an Upjohn group has investigated the effect of substituting adamantyl for the bicyclo-octyl moiety of (49) [213]. The resulting compound (50) was more active than (49), causing a significant reduction in serum cholesterol, heparin precipitating lipoprotein (HPL), and the ratio of HPL to cholesterol, at a dose of 50 mg/kg in diet-induced hypercholesteremic rats. The activity was retained both when the amino group of (50) was substituted with lower alkyl and lower acyl groups and when the ether linkage was replaced with methylene or sulphur. The 2-adamantyl analogues (51) and (52) were also active. One of the most interesting compounds was the piperidine (53) which, in addition to the above activities, decreased the serum level of atherogenic low-density lipoproteins and increased the level of the possibly beneficial high density lipoproteins.



(48)

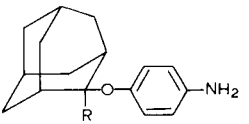


(49)



(50) R = H_2

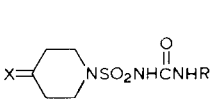
(53) R = $(CH_2)_5$



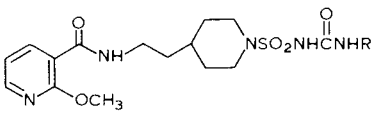
(51) R = H

(52) R = CH_3

From a 1965 study of hypoglycaemic sulphamoylureas carried out in the Pfizer laboratories, the 4,4-disubstituted piperidines (54), X = $(CH_3)_2$, $O(CH_2)_nO$, $(CH_2)_n$; R = cyclohexyl, or cycloheptyl were among the most active and were found to lower blood sugar in man [232]. More recently, workers in the same institution have found that substitution of an arylacylaminoethyl moiety in the 4-position of the piperidine ring of (54) markedly enhanced hypoglycaemic activity [233, 234]. In the 2-methoxynicotinyl series, compounds (55–57) showed significant hypoglycaemic activity in normal Charles River rats at 1 mg/kg i.p. In this series, the adamantyl derivative (55) produced a somewhat weaker response than the corresponding cyclohexyl and endo-bicycloheptenyl analogues (56) and (57). The corresponding exo-bicycloheptenyl and the saturated endo-bicycloheptanyl analogues were not active at 1 mg/kg and a similar structure activity relationship was observed with other arylacylaminoethyl groups. Compound (57) was selected for clinical trials.



(54)



(55) R = 1-adamantyl

(56) R = cyclohexyl

(57) R =

In the above work, we have seen examples of attempts to improve on the antiviral or central nervous system effects of amantadine through chemical modification as well as efforts to utilize the adamantyl moiety as the extreme of a bulky, lipophilic component in a veriety of structure-activity studies. To date, it would appear that, despite the synthesis of numerous active analogues, there has been only limited success in improving the efficacy of amantadine. The adamantyl groups remains an interesting tool for the medicinal chemist due to its unique physical properties and the commercial availability of numerous functionalized derivatives. Successful applications of this tool to increase biological activity have been realized in the areas of anticholinergic, hypobetaliproproteinemic, and hypoglycaemic agents.

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2 The β-Adrenomimetic Activity of Tetrahydroisoquinolines and Tetrahydronaphthalenes

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INTRODUCTION

Much of the early work aimed at exploring the active conformations of the natural transmitters adrenaline and noradrenaline, by the incorporation of the significant structural features in rigid or semirigid analogues, was hampered by the very low potency of the compounds thus derived [1-8]. It is doubtful whether any real significance attaches to the difference in potency between conformational imitators, where their absolute potency only attains the levels of flexible analogues with important functional groups missing. Both the tetrahydroisoquinoline and tetrahydronaphthalene series contain members with potencies of a similar order to those of the most potent analogues of the natural transmitters, so that there is a valid basis for comparison. At first sight, the two series appear to represent two of the major possible conformers of the flexible natural transmitters. Tetrahydroisoquinolines resemble the gauche conformer and tetrahydronaphthalenes, suitably substituted, the trans conformer, with severe conformational restrictions (*Figure 2.1*). These concepts will form the basis of much of the discussion. The terminology of conformation has been explained by Patil, Miller and Trendelenburg [9].

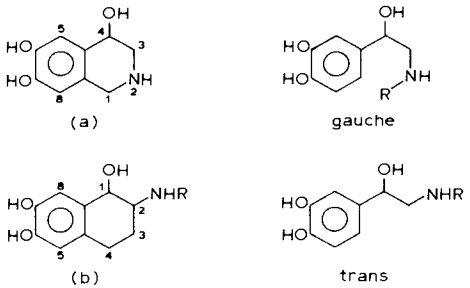


Figure 2.1. The resemblance between (a) suitably substituted 1,2,3,4-tetrahydroisoquinolines and the gauche adrenaline-noradrenaline conformer and (b) 1,2,3,4-tetrahydronaphthalenes and the trans conformer.

We have tried to resist the temptation to expand the scope of the review, for example, to include other types of sympathomimetic agent, to discuss the classification of α-adrenoreceptors, or to include compounds acting at dopamine receptors, many of which are close structural relatives of the compounds under discussion. However, since both the naphthalenes and isoquinolines have their most significant sympathomimetic actions on β-receptors, it is appropriate to consider the results of those actions and their clinical utility.

THE CLASSIFICATION OF ADRENORECEPTORS

Adrenoreceptors are those molecular constituents of the effector cells of a tissue with which molecules of the natural transmitters noradrenaline and adrenaline first interact in order to cause a tissue response. A third transmitter, dopamine, may exert some of its natural actions through adrenoreceptors.

The division of adrenoreceptors into α- and β-types on the basis of their relative sensitivities to natural and modified catecholamines [10] has proved to be of fundamental physiological significance because α- and β-receptors are now thought to mediate different biochemical events within the stimulated cell.

All β-adrenergic effects are associated with an elevation of intracellular levels of cyclic adenosine monophosphate (cAMP) produced by stimulation of adenyl cyclase [11-14]. cAMP activates protein kinases which phosphorylate a variety of substrates which in turn mediate the characteristic responses attributed to many drugs and hormones [12]. The early history has been reviewed [15] and more recent developments have been discussed [16].

The situation for α-adrenergic effects is less clear. Evidence has accumulated suggesting that α-effects may be associated with increased intracellular levels of cyclic guanosine monophosphate (cGMP) and a distinct class of cGMP-activated protein kinases has been described [13] and this stimulated the proposal of a model for α-effects analogous to that for β-effects [12]. However, attempts to demonstrate α-adrenergic or other hormonal regulation of guanyl cyclase were not successful [12].

More recently, a third class of receptor was defined [17, 18]. Dopaminergic receptors were found in certain brain regions and in the renal and peripheral vasculature [18].

The natural transmitters at adrenoreceptors possess different potencies at different types of receptor. α-Receptors are stimulated to a similar extent by noradrenaline and adrenaline and to a lesser extent by dopamine [18, 19]. β-Receptors are stimulated by adrenaline and to a much lesser extent by noradrenaline and dopamine [18, 20]. Dopaminergic receptors are stimulated by dopamine while noradrenaline and adrenaline are virtually inactive [18] and this suggests that the structural requirements for activation of dopaminergic receptors are more restricted than for activation of either α- or β-receptors. Dopamine also has an indirect action at both α- and β-receptors by stimulating the release of noradrenaline from post-ganglionic adrenergic nerve terminals [18, 19].

Noradrenaline is the predominant transmitter in the mixture released from post-ganglionic adrenergic nerve endings [21] although in some such nerves, with a limited distribution, dopamine is the predominant transmitter [17, 18]. Adrenaline is the main hormone released from the adrenal medullae [21].

THE SUB-CLASSIFICATION OF ADRENORECEPTORS

The effects of various agonists and antagonists on β-receptors in different tissues vary quantitatively to such an extent that subclassifications of β-receptors have been postulated. Selective stimulation is the best criterion for differentiating receptors [22] because all the functional groups of the receptor are likely to be involved with interactions with full agonists whereas antagonists usually interact with only part of the physiological receptor and have affinity for other, adjacent sites termed exo-receptor sites. Selectivity in an antagonist may reflect varying interactions with exo-receptors rather than differences between the physiological receptors themselves.

β-Receptors were sub-classified into two groups according to the responses of different organs to modified catecholamines [23]. When the ratio of the potencies of noradrenaline and adrenaline for the stimulation of β-receptors in different tissues was compared, a bimodal distribution was observed. The subgroups were termed β_1 and β_2, β_2-receptors being relatively more sensitive to adrenaline [23].

An updated version of this classification is given in *Table 2.1.*

Table 2.1. THE SUB-CLASSIFICATION OF β-RECEPTORS IN MAMMALIAN TISSUES [24]

Responses mediated by β_1-receptors	Responses mediated by β_2-receptors
Increased force and rate of contraction of cardiac muscle	Relaxation of smooth muscle in bronchi, uterus and arterioles in skeletal muscle
Dilation of coronary blood vessels	Decreased duration and tension of skeletal muscle twitch
Relaxation of smooth muscle in the alimentary tract	Decreased intra-ocular pressure [25]
Lipolysis *,+	Glycogenolysis
Insulin release	Glycolysis
Renin release [27]	Hypokalaemia

* There is a controversy over which β-receptor sub-type mediates certain metabolic effects [26].

\+ Species-dependent. Reported as β_2-receptor mediated in rat and mouse [28].

This sub-classification, though useful [29], is an oversimplification in that many responses cannot be classed categorically as β_1- or β_2-receptor mediated. Three sub-types were postulated to mediate cardiac, vascular and bronchiolar β-receptor effects [30]. A further sub-division of cardiac β_1-receptors into those mediating only chronotropic and those mediating only inotropic effects was

suggested [31] but others attributed the differentiation of cardiac chronotropic and inotropic effects of β-agonists to the existence of both β_1- and β_2-receptors in the heart. While β_1-receptors were the predominant sub-type in all regions, it was suggested that the $\beta_1 : \beta_2$ ratio was higher in the myocardium than in the sinus node [32].

It was shown that in a single species some receptors assigned to either the β_1- or β_2- sub-class exhibited significant quantitative differences in certain pharmacological characteristics and it was suggested that this was justification for considering multiple sub-types of β-receptor [15]. However, it is generally accepted as being practical and pharmacologically convenient to consider only two sub-classes of β-receptor, with the receptors in each sub-class having similar but not necessarily identical pharmacological characteristics. No agonist or antagonist has been found to show absolute specificity for either β_1- or β_2-receptors.

Until recently, within one species, all α-receptors were considered as a single type [15] but sub-classifications have now been proposed [33-35]. Similarly, sub-classifications of dopaminergic receptors were recently proposed [18, 36].

CLINICAL ASPECTS OF β-RECEPTORS

Drugs acting on β-receptors fall naturally into two groups, agonists and antagonists, and each is used in the treatment of different pathological conditions, obstructive lung diseases and cardiovascular disorders respectively.

Obstructive lung diseases

The pathological processes which increase airways resistance are well documented [37]. Asthma is an allergy characterized by breathlessness and wheezing which is caused by variable obstruction of the airways and is reversible by appropriate treatment. The process is initiated and maintained by the inhalation of foreign proteins by sensitive individuals (extrinsic asthma) or by bacterial or viral infection within the bronchi which causes a localised generation of foreign proteins (intrinsic asthma) [24]. The chemical mediators released in these reactions include acetylcholine, histamine, slow reacting substance of anaphylaxis (SRS-A), prostaglandin $F_{2\alpha}$, plasma kinins and other, less well defined spasmogens. On present evidence, SRS-A is regarded as the most significant mediator in asthma except during the first few minutes of a sudden challenge when a flood of histamine is predominant [38]. Both are released from mast cells.

The pathological manifestations of bronchitis are similar to those of asthma

but their cause is non-allergenic. In bronchitis, airways obstruction is more likely to be caused by nervous (*e.g.* vagal) constrictory impulses which fail to be balanced by sufficient sympathetic dilator impulses, whereas in asthma nervous mechanisms assume less significance compared to allergic mechanisms. In chronic bronchitis, anticholinergic drugs produce a greater degree of bronchodilatation than do β-agonists [39] whereas asthmatics generally respond poorly to anticholinergics and better to β-agonists [39, 40].

In addition to mediating bronchorelaxation, β_2-stimulation is thought to decrease the release of endogenous spasmogens and to increase mucociliary clearance, though the clinical significance of these effects has not been evaluated [37].

α-Receptors were identified as mediating bronchoconstriction in human bronchial muscle. *In vitro* α-receptor mediated bronchoconstriction is reinforced by bacterial endotoxins which partially explains why infections aggravate bronchopasm [37]. It was shown *in vitro* that α-antagonists, which possess no intrinsic bronchodilating activity, potentiate the effects of β-agonists [41]. α-Agonists decongest bronchial mucous membranes which is particularly effective in children where mucosal oedema has greater significance because of their small airways dimensions [37]. However, the use in asthma of agents possessing both α- and β-agonist properties is not generally recommended as any favourable mucus decongesting effect is likely to be more than forfeited by undesirable α-receptor bronchoconstricting effects.

In addition to removing any recognisable trigger mechanisms, the basic therapy for the treatment of asthma is generally the use of a suitable β-agonist to relieve bronchospasm [42].

Cardiovascular disorders

Initially, β-antagonists were conceived as a means of treating angina pectoris [43]. They decrease the sympathetic drive to the heart which reduces myocardial work and relieves pain. They are used to treat cardiac arrhythmias but their mode of action in this respect is uncertain [43]. Possibly the major use of β-antagonists is in the treatment of hypertension where several different modes of action have been postulated [44, 45], the most recent hypothesis involving a vascular site of action [44].

Paradoxically, some β-antagonists retain a degree of agonist activity. This intrinsic sympathomimetic activity, which is observed prior to the onset of blockade, was shown to have clinical significance in only isolated cases [43]. Some β-antagonists possess membrane-stabilising activity but this is manifest only at plasma levels much higher than those attained with normal clinical

doses and whereas β-antagonism is stereospecific, membrane-stabilising activity is not [43]. The anit-anginal, antiarrhythmic and antihypertensive effects of all β-antagonists, whether or not they possess intrinsic sympathomimetic activity or membrane-stabilising activity, depend only on β-antagonism.

Side effects and the use of selectively acting β-receptor agents

Selectivity, when considering β-agonists refers primarily to their selectivity for bronchial over cardiac adrenoreceptors. When considering β-antagonists, the term refers to their selectivity for cardiac over bronchial adrenoreceptors.

The side effects from the use of non-selective β-agonists generally reflect their action on β_1-receptors (see *Table 2.1*). Selective β_2-agonists, while causing fewer cardiovascular side effects, produce muscle tremor when administered systemically due to their action on skeletal muscle β_2-receptors. All known side effects of β-agonists result only from their actions on β-receptors. All β-antagonists can precipitate congestive heart failure when the heart is on the borderline of failure before treatment. The side effects of non-selective β-antagonists include Raynaud's phenomenon due to constriction of skeletal muscle blood vessels in the extremities, delayed recovery from hypoglycaemia and, in asthmatics, bronchoconstriction [43]. In non-asthmatic patients, bronchoconstriction does not occur because sympathetic drive to the bronchi is normally very low. Extreme caution must be employed when treating either diabetic or asthmatic patients, even with cardioselective β-antagonists, as they lose their apparent selectivity at high doses [43, 46]. The desirability of using selective β-antagonists in the treatment of hypertension is not clear, in view of the latest hypothesis proposing a major site of action to be vascular β_2-receptors [44].

Side effects of β-antagonists unassociated with β-blockade include the oculocutaneous syndrome which resulted in the withdrawal of practolol and carcinogenicity which resulted in the withdrawal of pronethalol and tolamolol [43].

METABOLISM OF CATECHOLAMINES

Released adrenergic neurotransmitters are rapidly removed from the area of the neuroeffector junction, mainly by active transport back into the pre-junctional nerve terminal where the transmitter is bound in storage vesicles or, if the storage and binding capacity of the amine is exceeded, metabolism by monoamine oxidase (MAO) [15]. This active transport into neuronal cells is termed uptake$_1$ [15, 9]. In some tissues, when the uptake$_1$ system becomes saturated, a second active transport system into surrounding non-neuronal cells, termed

uptake$_2$, occurs. Uptake$_2$ is thought to allow access of the amine to catechol O-methyl transferase (COMT) which is thought to be an intracellular enzyme in non-neuronal cells [15]. Both MAO and COMT are also present in the liver.

MAO catalyses the deamination of amines, particularly primary amines, to the corresponding aldehyde [47]. Secondary and tertiary amines are more resistant and, in the liver, are first dealkylated by transaminase enzymes. COMT catalyses the transfer of the methyl group from S-adenosylmethionine to one hydroxyl of a dihydric phenol [47]. For catecholamines, either the 3- or 4-methoxy metabolite can be formed but in the metabolism of natural transmitters, the 3-methoxy metabolite predominates [48]. Exogenous catecholamines are not effectively removed by neuronal uptake and inactivation by MAO is not a major metabolic pathway [15]. They are readily transported by the uptake$_2$ process and metabolised by COMT [24].

Orally administered catecholamines are efficiently metabolised to an ethereal sulphate by sulphokinases in the gut wall cells [24]. However, this enzyme has a limited capacity which can be overwhelmed by high doses of catecholamines or by the prior administration of a substrate which is preferentially sulphated, such as ascorbic acid [49]. In the normal range of doses though, catecholamines are inactive when given orally.

THE PHARMACOLOGICAL EVALUATION OF β-AGONISTS

There are no suitable methods in which experimental asthmatic reactions comparable with those in man can be reproduced in animals [50].

The main unwanted effects of adrenergic bronchodilators are cardiac stimulation and skeletal muscle tremor in the extremities [42]. Animal experiments should be designed to measure the relative extent to which a drug produces these effects and to determine what degree of selectivity it possesses for the β-receptors of bronchial muscle over those in other tissues.

Guinea-pig isolated tracheal smooth muscle preparations are most commonly used to assess the bronchodilating activity of β-stimulants [24]. They possess intrinsic tone when suitably prepared but bronchodilation is usually measured as the inhibition of a constant background of bronchoconstrictor tone induced by agents such as histamine, acetylcholine or potassium chloride [51]. Hence the dose of agonist necessary to produce a given response, usually measured as 50 per cent inhibition of bronchoconstriction, varies with the degree of background tone present. This is similar to the situation which applies in asthma where the effective bronchodilating dose depends on the severity of the asthma. However, this means that bronchodilator potency cannot be measured in absolute terms and new drugs are usually compared with a standard, often isoprenaline.

The same standard should be used when determining other parameters and a measure of the selectivity of the test compound can be gained in the form of a selectivity ratio compared with the standard bronchodilator for each tissue.

This method cannot measure absolute selectivity as it is unlikely that any standard would exhibit no selectivity. In cats, isoprenaline was reported to show a slight selectivity for bronchial β-receptors over those in skeletal muscle and for the latter over those in the heart [42]. Isolated right atrial preparations, which contain the sino-auricular node, beat spontaneously and are used to measure the direct chronotropic effects of β-stimulants.

The measurement of adrenomimetic-induced skeletal muscle tremor in animals is difficult but it is accepted that the action of adrenomimetics on the contractions of slow-contracting muscles, such as the soleus, of anaesthetized mammals provides a suitable model [24, 42]. β-Agonists produce a decrease in tension and an increase in the rate of relaxation of maximal twitches of the cat muscle.

When comparing the struture-activity relationships of a series of compounds, structural modifications to a compound may modify its potency in different ways in different species. Tissue selectivity comparisons should therefore only be made between tissues from the same species. The selectivity of β-stimulants for bronchial over cardiac β-receptors in experimental animals is generally most marked in the guinea-pig and least marked in the cat. Potential β-stimulant bronchodilators are often initially screened using guinea-pig isolated tracheal and atrial preparations and when favourable potency and selectivity are observed, their effects on anaesthetized cat cardiac, respiratory and skeletal muscle systems examined.

In many clinical trials in man, potential bronchodilating drugs are administered by aerosol inhalation, a route of administration which itself imparts a high degree of bronchial selectivity. In most studies on intact, anaesthetized animals, drugs are administered intravenously. It is thus difficult to compare the results of animal studies with those of clinical trials, a not uncommon problem. In intact animals, there is generally no control over the distribution and disposition patterns of systemically administered drugs and the response of a single organ or tissue could be modified by reflex mechanisms, giving misleading results [15]. However, results from the few studies in man which are directly comparable with results from animal studies indicate that the cat is the species of choice for the screening of potential β-adrenomimetic bronchodilators [42].

Some β-stimulants may also possess α-agonist properties which can be assessed using smooth muscle preparations containing only α-receptors, such as rat isolated seminal vesicle or vas deferens [22].

Recently, it was reported that two 1,2,3,4-tetrahydroisoquinoline derivatives

with agonist activity at both α- and β-receptors also exhibited agonist activity at muscarinic cholinergic receptors [52]. Such cholinomimetic activity, demonstrated using the chick isolated biventer cervicis preparation, clearly complicates the use of such agents as adrenomimetics. It may be worthwhile screening potential bronchodilators for similar activity, since in some tests the two effects are antagonistic.

STRUCTURE-ACTION RELATIONSHIPS IN SOME β-SYMPATHOMIMETIC PHENYLETHANOLAMINES

Before considering the effects of substituent variation in the compounds named in the title, it is appropriate to consider the effects of similar substitution in the flexible analogues. The following discussion is not intended to be exhaustive, but a selective assessment of what is considered to be significant in the present context. To avoid confusion, we will refer to the ethanolamine carbons as 1 and 2, rather than α and β (*Figure 2.2*).

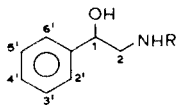


Figure 2.2. Phenylethanolamines

(a) Within a series of arylethanolamines containing the same aryl substitution, an increase in the bulk of the *N*-alkyl substituent increases β_2-potency and selectivity, the effect being maximal with *N*-*t*-butyl substitution. Potency decreases with larger *N*-alkyl groups but is maintained or enhanced by certain *N*-aralkyl substituents [24]. Disubstitution to give a tertiary amine abolishes activity [53].

(b) Removal of either the 1-hydroxy or of one or both phenolic functions causes a large decrease in β_2-potency though loss of the 3′-hydroxy causes a greater potency decrease than loss of the 4′-hydroxy [24].

(c) 2-Alkyl substitution gives a large decrease in β_2-potency and an even greater decrease in β_1-potency, thus giving improved β_2-selectivity. Potency and β_2-selectivity have been shown to be optimal for 2-ethyl substitution [24].

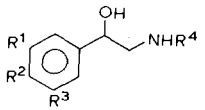
(d) The 3′-hydroxy can be replaced by a variety of functions, the structural requirements of which are not clearly defined, to give compounds with improved β_2-selectivity. However, replacement of the 4′-hydroxy with similar substituents leads to loss of activity [22].

(e) Replacement of the 4′-hydroxy with 5′-hydroxy, to give a resorcinol

instead of a catechol derivative, gives a large decrease in potency with little, if any, change in β_2-selectivity [24]. However, there is a clinical advantage in obtaining a longer-acting drug which can be given orally, since the resorcinol moiety, unlike the catechols, is not metabolised by either sulphatase enzymes in the gut wall or catechol *O*-methyltransferase.

These general observations are exemplified by data for a few selected compounds in *Table 2.2.*

Table 2.2. RELATIVE β_2-POTENCIES AND SELECTIVITIES OF REPRESENTATIVE ARYLETHANOLAMINES

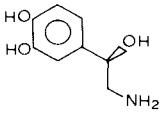


	R^1	R^2	R^3	R^4	β_2-potency	β_2/β_1	i.a. atrial test	Refs.
Isoprenaline	OH	OH	H	*i*-Pr	1.00	1.0	1.0	
	OH	OH	H	*t*-Bu	5.45	11.4	1.0	54
Salbutamol	CH_2OH	OH	H	t-Bu	0.65	58	0.7	55
	NH_2	OH	H	t-Bu	0.34	10.8	1.0	54
	NHMe	OH	H	t-Bu	71	191	1.0	54
Carbuterol	$NHCONH_2$	OH	H	t-Bu	0.37	71	0.5	54
Sulfonterol	CH_2SO_2Me	OH	H	*t*-Bu	0.42	3440	0.3	56
Soterenol	$NHSO_2Me$	OH	H	*i*-Pr	0.27	6.3	0.7	54
Terbutaline	OH	H	OH	*t*-Bu	0.06	5.8	0.8	54

All results were obtained from guinea pig isolated tracheal and atrial preparations. β_2 potency $= \frac{ED_{50} \text{ isoprenaline}}{ED_{50} \text{ test compound}}$ in tracheal test i.e. values greater than in indicate that the compound is more potent than isoprenaline. The i.a. (intrinsic activity) of all compounds in the tracheal test is 1 i.e. all full agonists. $\beta_2/\beta_1 = \frac{\beta_2 \text{ potency in tracheal test}}{\beta_1 \text{ potency in atrial test}}$ i.e. the larger the index, the more β_2-selective the compound.

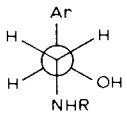
CONFIGURATIONAL AND CONFORMATIONAL REQUIREMENTS FOR SYMPATHOMIMETIC PHENYLETHANOLAMINES

The natural transmitters, adrenaline and noradrenaline and related arylethanolamines, the active isomers of which have all proved to be laevorotatory, possess the R absolute configuration [9].



(−)-R Noradrenaline

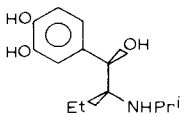
X-ray crystallography [57, 58, 59], NMR spectral data [60, 61] and molecular orbital calculations [62, 63] indicate that, in both the solid state and in aqueous solution, whether protonated or not, the preferred C_1-C_2 rotamer of the natural transmitters and their active derivatives can be represented by (1).



(1)

Newman *projection of the trans conformation of an arylethanolamine, R. configuration*

Such a conformation is clearly reasonable since it places the bulky aryl group between the two hydrogen atoms and may allow hydrogen bonding between oxygen and nitrogen (either OH...N or $\overset{+}{N}$H...O) to reduce steric repulsion. The most potent isomer of isoetharine [64] is the (−) erythro form (2).



(2)

(2) (−)-erythro isoetharine

From molecular models, it was suggested [65] that steric crowding would lead to restricted rotation of the ethanolamine side chain, with the adoption of two preferred conformations, both retaining the hydrogen bonding capability (*Figure 2.3*).

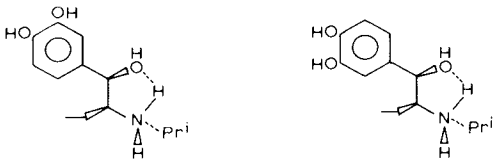
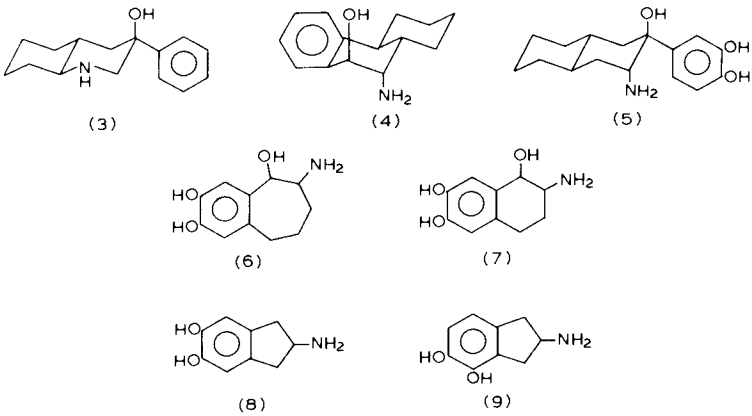


Figure 2.3. Alternative conformations of (−) erythro isoetharine.

These structures highlight the possible significance of the aryl rotamers, an issue which had been partly obscured in the early work on semi-rigid analogues. Thus, it might be possible to construct a semi-rigid analogue of the phenyl ethanolamines with the correct orientation of the hydroxy and amino functions to fit the receptor but find little or no activity when the catechol moiety were wrongly oriented. Much of the earlier work on rigid analogues, represented by compounds (3) to (5), concentrated on the cisoid-transoid arrangement of the hydroxy and amino groups and either did not incorporate the catechol function [1, 2] or allowed it to totate freely [3, 4]. However, both the bicyclic compounds (6) [6, 7] and (7) [66] can reproduce the preferred spatial relationship of the phenylethanolamine OH and NH functions, with a fixed catechol unit. Their low potency may be related to imitation of the wrong catechol rotamer (see later discussion). The related indane derivatives (8) and (9), in which both rotamers were imitated, showed low potency in a variety of tests [67]. In this case, low activity may be associated with their relatively poor ability to imitate the preferred phenylethanolamine conformations. Molecular models indicate that planar cyclopentene rings lead to eclipsing of substituents on the 1,2 bond, rather than the favoured staggered arrangement of the phenyl-



ethanolamines, as well as an increased distance between OH and NH in the *trans* isomer (see later), and that even distorted (envelope) conformations cannot imitate properly the *trans* phenethylamine relationship between the aromatic ring and amino group.

TETRAHYDROISOQUINOLINES

1-ALKYL AND 1-UNSUBSTITUTED TETRAHYDROISOQUINOLINES

The tetrahydroisoquinoline skeleton has been recognised as a rigid cisoid phenylethylamine [67]. In this context, a 4-hydroxy substituent is highly significant but very few 4-OH-THIQ have been prepared (see section on Synthesis for reasons).

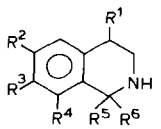
Sympathomimetic activity in the 6,7-dihydroxy analogue (10a) was very weak [67, 68, 69]. The 4-hydroxy derivative (10b) [70, 71] was inactive as an α-agonist [72] and was not tested for β-activity. The analogues (10c, d) of the gauche conformer of terbutaline were inactive as β-agonists [73]. These observations tend to support the belief that the cisoid aryl-amino arrangement is not a good fit to either α- or β-adrenoceptors. However, the 1-methyl derivative salsolinol (10e), possesses definite β-agonist properties [74, 52] along with some cholinomimetic activity [52]. Salsolinol has attracted a great deal of attention because of its formation *in vivo* from dopamine and acetaldehyde [75] and its possible role as a false neurotransmitter in alcohol intoxication [76, 77]. Such studies continue [78], but are not directly relevant in this context.

The 1,1-dimethyl THIQ (10f) which can be regarded as an isoprenaline analogue [67], possesses only weak activity at adrenoceptors [67, 52]. Its major effect *in vivo* and *in vitro* is as an anticholinesterase [52]. There may be some interesting structure-action comparisons with pempidine and norpempidine in this series, both the latter being potent ganglion-blocking agents.

In a series of 1-alkyl THIQs (10,$R^1 = R^4 = R^6 = H$, $R^2 = R^3 = OH$, R^5 = alkyl), the isopropyl derivative (10g) was shown to be the most potent bronchial relaxant in intact guinea-pigs [74]. Preliminary data indicated that tachycardia (β_1) occurred only at a concentration about one thousand times greater than that at which similar effects were shown by isoprenaline, but these authors do not appear to have published the complete pharmacological data. Later work using guinea-pig isolated trachea showed that (10g) was equiactive with adrenaline and about ten times less potent than isoprenaline [79]. *In vivo*, in the cat, however, it was much weaker and lacked selectivity [80].

If the catechol moiety of these compounds is fulfilling the same role as the

catechol moiety in the active phenylethanolamines, modification as in structures (10h, i, j) should produce effects on potency and β_1/β_2 selectivity comparable with those in *Table 2.2.* However, all three amino compounds (10h, i, j) showed a marked loss of activity compared with (10g). On bronchospasm in guinea pigs *in vivo* the 6-amino-7-hydroxy derivative possessed only one twelfth the activity of the 6,7-dihydroxy derivative (10g), and the two others (10i and 10j) were inactive [81]. This failure of structure-action relationships to translate from the phenylethanolamines to the tetrahydroisoquinolines is also a feature of the 1-benzyl-1,2,3,4-tetrahydroisoquinolines, which have received much greater attention.



(10)

(10)	R^1	R^2	R^3	R^4	R^5	R^6
a	H	OH	OH	H	H	H
b	OH	OH	OH	H	H	H
c	OH	OH	H	OH	H	H
d	OH	OH	H	OH	Me	Me
e	H	OH	OH	H	Me	H
f	H	OH	OH	H	Me	Me
g	H	OH	OH	H	Pr^i	H
h	H	NH_2	OH	H	Pr^i	H
i	H	NHMe	OH	H	Pr^i	H
j	H	OH	NH_2	H	Pr^i	H

1-BENZYL-1,2,3,4-TETRAHYDROISOQUINOLINES

Tetrahydropapaveroline (11a, THP) was first observed as an adrenomimetic agent in 1910 [82] and its pharmacology has since been extensively studied [83-85], as has its formation *in vivo* [75, 78]. It is approximately twentyfive times [79] and twenty times [84] less potent than isoprenaline on guinea pig isolated tracheal and atrial preparations respectively.

Since THP is a catecholamine it was natural to look for similarities in structure with the phenylethanolamines. There are two ways (A and B) that THP could resemble the flexible catecholamines (*Figure 2.4*).

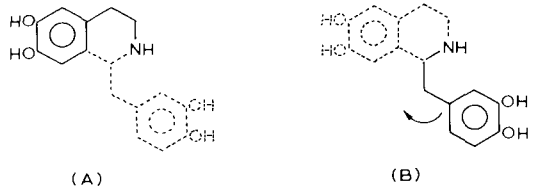


Figure 2.4. Alternative catecholamine moities in the tetrahydropapaveroline molecule.

The possibility remains that, in THP at least, the catechol moieties serve as alternative binding groups towards the same receptor function. Masking the hydroxy groups as ethylenedioxy on the tetrahydroisoquinoline was more unfavourable than masking those on the benzyl group [83], but on the other hand the parent compound (11b) was respectively fifty times and one hundred times less potent than isoprenaline on guinea pig isolated tracheal [79] and atrial [86] preparations.

Many analogues of tetrahydropapaveroline have been prepared in the hope of improving potency, but with one or two exceptions this has been unsuccessful. In many cases no quantitative pharmacological data have been reported. The list in *Table 2.3* includes most of the analogues which have been synthesised and found to be at least an order of magnitude less potent than isoprenaline, and which are not mentioned elsewhere in this review.

The most potent compound in *Table 2.3* is the 2-bromo-4,5-dihydroxy analogue [80]. This is 6′-bromotetrahydropapaveroline, which has recently been more widely investigated [90].

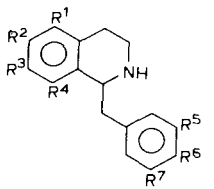
Table 2.3. ANALOGUES OF TETRAHYDROPAPAVEROLINE

Substituted-benzyl 6,7-dihydroxy tetrahydroisoquinolines (Substitution on benzyl group given
2-methoxy, 3-methoxy, 4-methoxy, 3,5-dimethoxy, 2,3,4-trimethoxy, 3,4,5-trihydroxy [87], 2-bromo-4,5-dimethoxy [88], 2-bromo-4,5-dihydroxy [80].

Other 1-substituted 6,7-dihydroxy tetrahydroisoquinolines (1-substituent given)
Phenyl [79, 83], 4-chlorophenyl, 4-pyridyl [79], 3,4-ethylenedioxyphenyl, 2-furyl, 2-thienyl, 4-piperidylmethyl, 3-piperdylmethyl, [79, 87], 2-pyridylmethyl, 2-piperidylmethyl [87], 2-phenethyl, 3-phenylpropyl [79, 83].

Other 6,7-dihydroxyisoquinolines
1-benzyl-3,4-dihydro, 1-benzyl-3-methyltetrahydro [83], 4-(3,4,5-trimethoxybenzyl) -tetrahydro [89] shows selectivity for β_2 over β_1 receptors.

No doubt interest in the series as bronchodilators would have waned, were it not for the observation of uniquely high potency in the 3,4,5-trimethoxybenzyl analogue (11c), now known as trimetoquinol (TMQ) (Inolin). In the initial studies, TMQ was eight times more potent on guinea pig isolated trachea [79] and five times less potent on guinea pig isolated atria [86] than isoprenaline, i.e. very potent and usefully selective. It is a directly acting β-agonist and as with all β-agonists was shown to increase cyclic AMP levels in guinea pig isolated trachea [91]. It is devoid of α-agonist activity [92] but exhibits slight α-antagonist effects [93]. Initial clinical trials in man, from which no side effects were reported other than 'palpitation of the heart' [94], were criticised for not being rigorously controlled [22]. It was later suggested that the cat was the best laboratory animal in which to test adrenomimetic bronchodilators and in the cat there was shown to be little difference between the doses of trimetoquinol necessary to affect pulmonary resistance, heart rate and contraction of the soleus muscle and the effective doses were not significantly different from those of isoprenaline [95]. Trimetoquinol has a long duration of action and it was suggested but not verified, that this may be due to a phosphodiesterase inhibitory action [95].



(11)

(11)	R^1	R^2	R^3	R^4	R^5	R^6	R^7
a	H	OH	OH	H	OH	OH	H
b	H	OH	OH	H	H	H	H
c	H	OH	OH	H			
d	H	OH	H	H			
e	H	H	H	OH			
f	H	OH	H	OH			
g	H	H	OH	OH			
h	H	H	OH	H	OMe	OMe	OMe
i	OH	H	OH	H			
j	OH	OH	OH	H			
k	H	OH	OH	OH			
l	H	CH_2OH	OH	H			
m	H	OH	CH_2OH	H			

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline is metabolised by COMT [96] but there is some dispute as to whether or not trimetoquinol is similarly metabolised [97, 98]. The contribution of the hydroxy groups to the bronchodilating action of trimetoquinol was recently investigated in cats [99]. The 6-hydroxy (11d) [86], 8-hydroxy (11e), 6,8-hydroxy (11f) and 7,8-dihydroxy (11g) derivatives [100] were reported to be only weak β-agonists. The more potent derivatives are included in *Table 2.4*.

Table 2.4. RELATIVE POTENCIES AND BRONCHOSELECTIVITIES OF 1-(3,4,5-TRIMETHOXYBENZYL)-1,2,3,4-TETRAHYDROISOQUINOLINES IN ANAESTHETISED CATS [99]

Substituent(s)		β_2-potency	β_2-selectivity
6,7-dihydroxy (11c)	(trimetoquinol)	0.38	1.9
7-hydroxy (11h)		0.028	9.3
5,7-dihydroxy (11i)		0.25	2.1
5,6,7-trihydroxy (11j)		0.13	2.2
6,7,8-trihydroxy (11k)		0.043	1.7
	isoprenaline	1	

β_2-potency and β_2-selectivity are as defined for Table 2.2.

COMPARISON OF THE STRUCTURE-ACTION RELATIONSHIPS OF TRIMETOQUINOL AND THE PHENYLETHANOLAMINES

Similarities:

(a) Trimetoquinol is a catecholamine with potent β-sympathomimetic activity. In the cat, it is roughly equipotent with isoprenaline and shows a similar lack of selectivity for β_1/β_2-receptors [95].

(b) Loss of one or other of the phenolic hydroxy groups drastically decreases potency [79, 99].

(c) *N*-methylation to give the tertiary base results in total loss of activity [79].

Differences

(a) Trimetoquinol lacks the β-hydroxy group of the phenylethanolamines and yet is highly potent. The possibility that NH fills the role of the missing OH [22] is discussed below.

(b) Replacement of either OH by CH_2OH, to give the salbutamol analogues (11 l,m) results in loss of activity, presumably total [101], although the full pharmacology appears not to have been published.

(c) The 5,7-dihydroxy analogue (11i), which might be regarded (see below) as similar to terbutaline (*Table 2.2*), retains most of the activity of TMQ [99], whereas terbutaline is roughly one hundred times less potent than N-t-butylnoradrenaline.

SPECIAL PROPERTIES OF THE 3,4,5-TRIMETHOXY GROUP

Trimetoquinol is by far the most potent of the sympathomimetic THIQs tested to date. In view of the very low potency of even close analogues, such as the 2,4,5-trimethoxy derivative [102], some kind of explanation is called for. It would be rewarding theoretically and possibly therapeutically useful if other potent THIQs could be found by changing the 3,4,5-trimethoxybenzyl unit.

The most promising approach appears to centre on the lack of coplanarity of the central methoxy with the benzene ring. There is evidence that aromatic methoxy substituents tend to orient so that there is maximum overlap of the oxygen lone pairs with the aromatic π bonds [103], with a resulting loss of basicity and ability to H-bond with water, so that aromatic and aliphatic methoxy groups make different contributions to partition coefficients [104].

In 1,2,3-trimethoxybenzene the central methoxy is forced out of plane by steric repulsion, so that it is much more capable of H-bonding to water, and its contribution to partitioning resembles that of aliphatic methoxy groups. This effect is expressed numerically in the observed [105] log partition coefficient for 1,2,3-trimethoxybenzene of 1.53, compared with the value of 2.13 calculated using normal aromatic methoxy fragmental constants [104]. It appears that the 'trimetoquinol receptor' requires a relatively large substituent in the 1-position of the THIQ, but without excessive accompanying hydrophobicity. We have calculated the hydrophobic fragmental constant for a selection of 1-substituents to illustrate this point (*Table 2.5*).

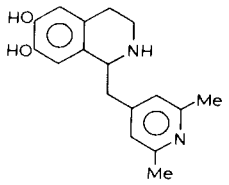
Table 2.5. BRONCHODILATOR POTENCY OF 6,7-DIHYDROXYL-1,2,3,4-TETRAHYDROISOQUINOLINES AND HYDROPHOBIC FRAGMENTAL CONSTANTS OF 1-SUBSTITUENTS

1-Substituent	Potency[+]	Ref	f*
Benzyl	0.017	79	2.4
3,4-Dihydroxybenzyl	0.037	79	1.3
3,4,5-Trimethoxybenzyl	8.5	79	1.9
4-Pyridylmethyl	0.2	79	1.1
3-Pyridylmethyl	0.045	79	1.1
1-Naphthylmethyl	0.03	88	3.7
2-Naphthylmethyl	0.008	88	3.7
Isopropyl	0.096	79	1.6
2,4,5-trimethoxybenzyl	0.054	102	2.5

[+] Calculated from $E_{max\ so}$ isoprenaline/$E_{max\ so}$ test compound for guinea-pig isolated trachea preparations.

* Calculated as far as possible according to Nys and Rekker [104], but using the experimental log P for 1,2,3-trimethoxybenzene [105].

In view of the potency differences between the pyridyl and naphthyl isomers it is clear that hydrophobicity and steric bulk are not the whole story. However, new compounds such as the collidine derivative (12) and 4′-aminobenzyl analogues may be worth investigation.



(12)

TETRAHYDROISOQUINOLINES AS MIMICS OF THE *TRANS* CONFORMER OF PHENYLETHANOLAMINES

From the previous discussion, it is clear that THIQ sympathomimetics are unlikely to be acting as mimics of the *cisoid* conformers of phenylethanolamines. However, it has been suggested [22] that if the NH of the THIQs fulfils the function of the β-hydroxy groups of the phenylethanolamines, then mimicry of the *trans* conformer is possible. There is some support for this argument. TMQ is asymmetric at C-1, and its favoured stereochemistry resembles that of the natural transmitters, (−)-*S*-trimetoquinol* and related THIQs [98, 106-108], which are active, possess the comparable configuration to the more potent (−)-R noradrenaline and adrenaline (*Figure 2.5*).

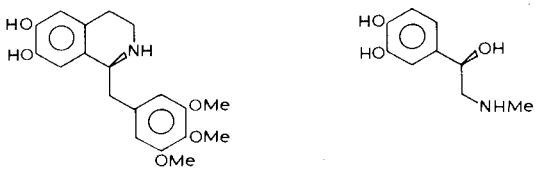
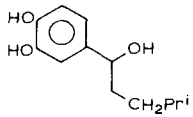


Figure 2.5. Comparison of the absolute configuration of (−)-S trimetoquinol and (−)-R adrenaline.

* We can find no direct reference to the determination of the absolute configuration of the TMQ isomers, although (−)-S trimetoquinol is widely referred to.

It would also be anticipated that the 5,7-dihydroxy analogue (11i) of TMQ, which would then relate to terbutaline, would be more potent than the 6,8-dihydroxy isomer, as is indeed the case (*Table 2.4*).

It will be noted that this approach requires the trimethoxyphenyl moiety of TMQ to replace the amino function of the phenylethanolamines. This appears unlikely, although the simple carbon isostere of isoprenaline (13) has been reported to exhibit slight β-agonist activity [109], and it has been suggested that it is the *N*-alkyl or aralkyl group of ethanolamines which binds to the receptor [63]. Furthermore, the diamine (14) has been reported to retain



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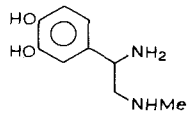
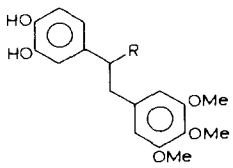


(14)

about one twentieth of the bronchodilating potency of adrenaline [110]. However, the fragmented trimetoquinol derivatives (15a, c) were virtually inactive as β-agonists: the analogue (15b) possessed only one thousandth the potency of TMQ, and the alcohol (15d) was also inactive [111].

The importance of the intact THIQ nucleus was underlined [92] by the relative lack of potency of the dopamine derivative (16), here drawn in an unlikely conformation to show the resemblance to TMQ.

Some support was given to the THIQ-transoid phenylethanolamine hypothesis by a claim for 'useful' levels of 'muscle relaxant' activity [112] in some members of a series of 1-aminoalkyl THIQs, the parent member of which is the noradrenaline analogue (17a).



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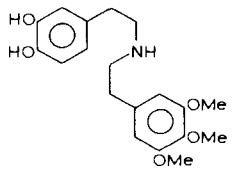

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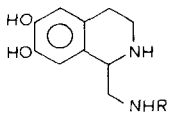


(17)

(15) a, R=NH_2; b, R=NHMe; c, R=NHEt; d, R=OH

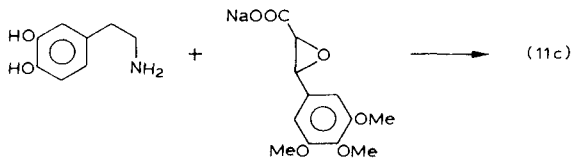
(17) a, R=H; b, R=Pr^i; c, R=Bu^t

In view of the theoretical significance of this series a more detailed study was made of the diamines (17b, c), representing analogues of isoprenaline and *N*-*t*-butylnoradrenaline, as well as the parent (17a). In each case, activity in the guinea-pig isolated trachea and atrium was negligible (less than one two-thousandth of (−)-isoprenaline) [81]. These findings appear finally to rule out the possibility of finding any systematic similarities between THIQs and phenylethanolamines as sympathomimetics. Those similarities which have been observed would appear to be due to chance, although this view is not shared by all workers in the field [113].

SYNTHESIS OF 1-SUBSTITUTED-1,2,3,4-TETRAHYDROISOQUINOLINES

(1) Bischler-Napieralski synthesis. The most commonly used synthesis of 1-alkyl and 1-arylmethyl-1,2,3,4-tetrahydroisoquinolines involves cyclisation of a suitably substituted and protected 2-arylethylamide in a Bischler-Napieralski reaction [114] to give a 3,4-dihydroisoquinoline, followed by reduction then deprotection. This method was not initially favoured for the synthesis [83] of trimetoquinol, possibly owing to concern that the central methoxy group of the 3,4,5-trimethoxybenzyl substituent might cleave under the acid cyclizing conditions [115]. However, trimetoquinol was more recently synthesized in good yield by this method [91].

(2) Pictet-Spengler synthesis. This reaction involves the acid catalysed condensation of a 2-arylethylamine with a carbonyl compound to yield a 1,2,3,4-tetrahydroisoquinoline directly [116] and generally does not require hydroxy groups to be protected. The first reported synthesis of trimetoquinol [83] involved reaction of dopamine hydrochloride with 3,4,5-trimethoxybenzaldehyde generated *in situ* from the corresponding sodium glycidate. These reaction conditions have more recently been improved to give trimetoquinol in 75% yield [117] (*Scheme 2.1*).

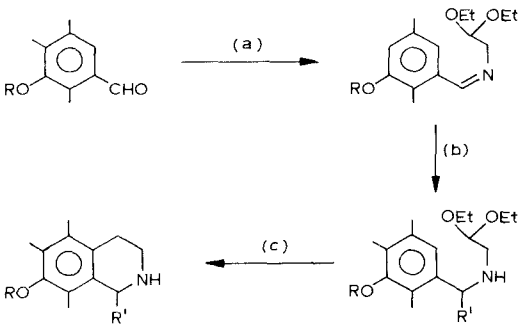


Scheme 2.1. Use of glycidate precursor in Pictet-Spengler synthesis of trimetoquinol, pH 2.3 for 115 h.

The synthesis of 1,2,3,4-tetrahydroisoquinolines lacking an activating function *para* to or bearing a bulky substituent *ortho* to the point of cyclisation is not readily achieved by either of these methods of synthesis.

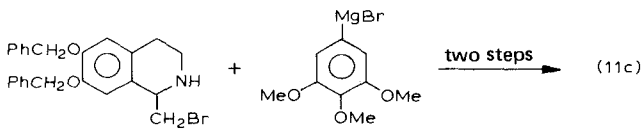
(3) The Bobbitt modification of the Pomeranz-Fritsch synthesis. The Pomeranz-Fritsch synthesis, which involves the acid catalysed cyclisation of the Schiff's base formed by the condensation of an arylaldehyde with an aminoacetaldehyde acetal to give an isoquinoline has not achieved widespread use due to erratic and poor yields and the general inability to prepare 1-substituted, other than 1-methyl, derivatives [118].

Bobbitt modified the cyclisation [119] by reducing the Schiff's base and cyclising the benzylaminoacetal formed using less rigorous cyclising conditions to give, initially, a 4-hydroxy-1,2,3,4-tetrahydroisoquinoline [120, 121] which was not usually isolable and readily dehydrated to a 1,2-dihydroisoquinoline which could then be reduced (*Scheme 2.2*).



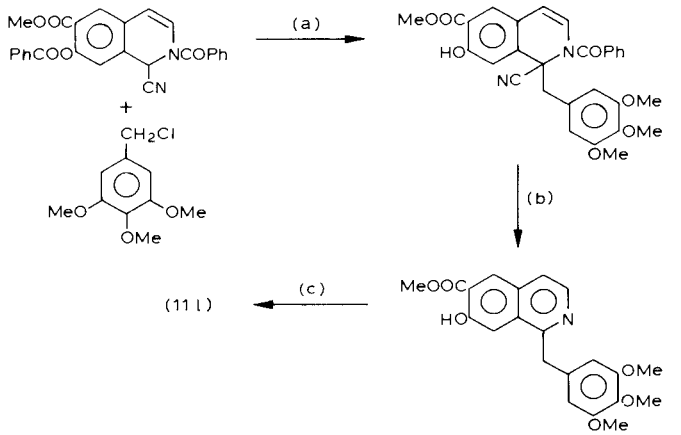
Scheme 2.2. Bobbitt synthesis of tetrahydroisoquinolines. Reagents (a) $H_2N.CH_2.CH(OEt)_2$ (b) H_2 and catalyst or Grignard (c) 6N HCl, H_2, Pd on C.

(4) Introduction of 1-substituents into a pre-formed heterocyclic nucleus. The reaction of Grignard reagents with 3,4-dihydroisoquinolines [122] and 3,4-dihydroisoquinolinium salts [52,123] has been reported. Alkoxybenzyl Grignards tend to be unstable, but trimetoquinol was synthesized by a related method (*Scheme 2.3*) which circumvented the problem [124].



Scheme 2.3. Synthesis of 1-benzylisoquinolines.

The introduction of a 1-substituent via Reissert compounds has been reported on several occasions [101, 125-127]. The saligenin analogue of trimetoquinol was synthesized by this method [101] (*Scheme 2.4*)



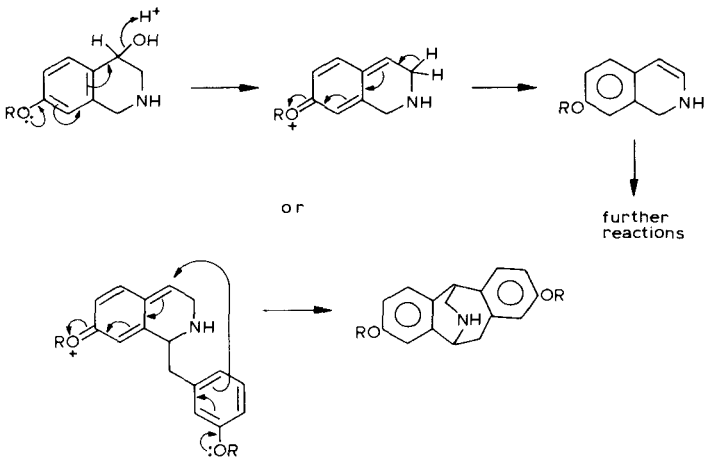
Scheme 2.4 The Reissert route to 1-substituted isoquinolines. Reagents (a) NaH-DMF (b) NaOMe then HCl (c) H_2PtO_2 then $LiAlH_4$.

4-Hydroxy-1,2,3,4-tetrahydroisoquinolines

On only a few occasions have the intermediate 4-hydroxy-1,2,3,4-tetrahydroisoquinolines in Bobbitt type cyclisations been isolated [70, 71, 120]. In only one instance has a 1-substituted derivative, 4-hydroxy-6,7-methylenedioxy-1-(2-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline, been isolated [128] although 1-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline was obtained in an impure state [70].

The source of the problem is the ease with which the 4-hydroxy substituent can be lost, particularly assisted by oxygen in the 7-position of the THIQ (*Scheme 2.5*). The next step can either be loss of a proton from the 3-position, giving a 1,2-dihydroisoquinoline which as an enamine will show characteristic high reactivity [129], or attack by a nucleophile at C-4. This is appropriately represented in the present context by intramolecular attack by a suitably activated 1-benzyl substituent to give an isopavine [121, 130].

Limited success in the preparation of 1-substituted-4-hydroxy THIQs has recently been reported using a Pictet-Spengler approach [73]. Several 4,6,8-trihydroxy derivatives were successfully synthesized, but it may be noted that these do not possess an oxygen in the critical 7 (or 5) position.



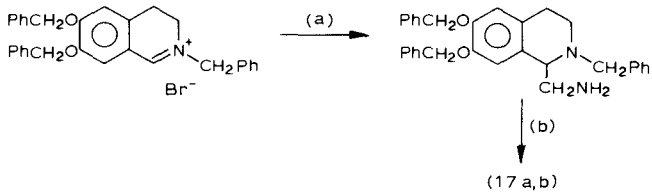
Scheme 2.5. Instability of 7-oxygenated 4-hydroxy-1,2,3,4-tetrahydroisoquinolines in acid

Attempts to prepare the corresponding 4,6,7-trihydroxy derivatives including the 4-hydroxy analogue of trimetoquinol were unsuccessful. The above 4,6,8-trihydroxy derivatives were inactive as β-agonists [73].

1-Aminomethyl-1,2,3,4-tetrahydroisoquinolines

1-Aminomethyl-1,2,3,4-tetrahydroisoquinolines were first successfully synthesized by the Bischler-Napieralski cyclisation of a suitable activated *N*-phenylethyl-2-phthalimidoacetamide followed by reduction to the 1,2,3,4-tetrahydroisoquinoline and hydrazinolysis of the phthalimido group [131].

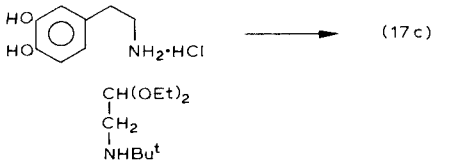
A more convenient synthesis of 1-aminomethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines involves the reduction of a suitably protected 1-cyano derivative with aluminium hydride [132, 81] (*Scheme 2.6*). The primary amino



Scheme 2.6. Reagents (a) KCN than AlH_3 (b) H_2, Pd-C or acetone $NaBH_3CN$ then H_2, Pd-C.

function can be derivatised by the reductive amination of carbonyl compounds with sodium cyanoborohydride [133]. The 1-isopropylaminomethyl derivative was prepared in this fashion by the reductive amination of acetone followed by deprotection by hydrogenolysis over Pd on charcoal [81].

The 1-*t*-butylaminomethyl derivative cannot be synthesized by this method and a Pictet-Spengler synthesis was employed (*Scheme 2.7*) [81]. The synthesis by this method of similar derivatives possessing less bulky amino substituents requires protection of the amino group of the *N*-substituted aminoacetal diethylacetal in order to prevent self-condensation of the aminoacetal. The 1-aminomethyl and 1-isopropylaminomethyl-6,7-dihydroxy derivatives, among many others, were synthesized using *N*-benzyloxycarbonyl protected aminoacetaldehyde diethylacetals [112].



Scheme 2.7. Synthesis of 1-aminomethylisoquinolines.

TETRAHYDRONAPHTHALENES

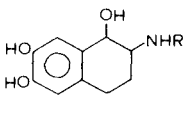
The most likely conformations of isoetharine (*Figure 2.3*) have been described [65]. It is apparent that incorporation of the side-chain ethyl group into a second ring will provide two tetrahydronaphthalenes (18) and (19).

2-AMINO-1-HYDROXYTETRAHYDRONAPHTHALENES

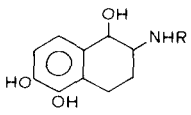
The 2-amino-1,6,7-trihydroxy-1,2,3,4-tetrahydronaphthalene derivatives (18, R=H, alkyl) possess little β-agonist activity [66] whereas the 1,5,6-trihydroxy derivatives (19, R=H, alkyl) are potent β-stimulants [134, 135] which suggests that the molecular geometry of the latter series corresponds closely to the 'active' conformation of the natural transmitters at β-receptors. Compound (19, R=H), a semi-rigid noradrenaline analogue, was reported to be fifty times and two and one-half times more potent than noradrenaline in its bronchodilating and positive chronotropic actions respectively [134]. This implies that fixation of the side chain of arylethanolamines into a suitably substituted tetrahydronaphthalene skeleton markedly enhances β_2-selectivity. Compound (19, R=H) was also reported to be devoid of α-agonist activity which suggests that noradrenaline may bind to α- and β-receptors with different conformations

and that the *N*-alkyl substituent which was considered an essential factor for differentiating between α- and β-activity [63], may be only of secondary importance [134]. Data concerning possible α-agonist activity of the corresponding 1,6,7-trihydroxy derivative (18, R=H) have not been published.

The *cis*- and *trans*-diastereoisomeric pairs of (19, R=methyl) were separated and the racemic *trans*-isomer was reported to be ten times more potent than the racemic *cis*-isomer [134].



(18)



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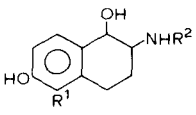


(20)

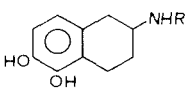
R^2 = Pr^i; a, R′ = CH_2OH; b, R′ = $NHSO_2Me$; c, R′ = $NHCONH_2$; d, R′ = NH_2; e, R′ = NMe

In an attempt to increase β_2-potency and selectivity, structural modifications analogous to those made in the arylethanolamine series were made to the 1,5,6-trihydroxy derivatives (19).

Increasing the bulk of the *N*-alkyl substituent increased β_2-potency up to R=isopropyl but had little effect on β_2-selectivity [136]. The *N*-*t*-butyl derivative was not prepared and the most potent member of the series was the cyclobutyl homologue (19, R=cyclobutyl).

Saligenin derivatives such as (20a) were reported to show decreased potency but increased β_2-selectivity over the corresponding catechols and were more potent and β_2-selective than the corresponding arylethanol amines [137-139] (compare *Table 2.6* with *Table 2.2*). In this saligenin series, the *N*-isopropyl derivative (20a) was reported to be more potent than the corresponding *N*-*t*-butyl derivative [138].

Diamino-dihydroxy-tetrahydronaphthalene derivatives (20 b-e) have been synthesized [140, 141]. The pharmacology of (20b) and (20c) which are analogues of soterenol and carbuterol (*Table 2.2*) respectively, has not been published. Compounds (20d) and (20e) were reported to be less potent but more β_2-selective than the corresponding catechol (19, R=isopropyl).



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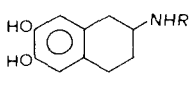


(22)

a, R = H; b, R = Me; c, R = Pr^i

Table 2.6. RELATIVE POTENCIES AND BRONCHOSELECTIVITIES OF TETRAHYDRONAPHTHALENE DERIVATIVES (20)[a]

R^1	R^2	C_1-C_2	β_2-potency	β_2-selectivity	i.a. atrial test	Refs.
OH	i-Pr	*trans*	6.6	16.7	1	136
OH	i-Pr	*cis*	0.06	13.2	1	136
OH	b	*trans*	20.4	10.8	1	136
OH	i-Pr	*cis*	0.15	10.3	0.9	136
CH_2OH	i-Pr	*trans*	1.1	75.5	1	137
NH_2	i-Pr	*trans*	0.52	69.1	0.9	140
NHMe	i-Pr	*trans*	0.91	104.6	0.75	141
		isoprenaline	1	1	1	

[a] The data were obtained using guinea-pig isolated tracheal and atrial preparations. The intrinsic activity (i.a.) for all compounds in the tracheal test is 1. β-Potency and selectivity are as defined in Table 2.2.

[b] Cyclobutyl.

2-AMINOTETRAHYDRONAPHTHALENES

When tested for direct α-agonist activity using rabbit isolated aortic preparations, (22a) and (22b) were respectively thirty and fifty times more potent than (21a) and (21b) [142] which suggests that the *trans*-B-rotamer rather than the *trans*-A-rotamer is the preferred conformation of dopamine for action at α-receptors (*Figure 2.6*). Dopamine was shown to be one-half as potent as noradrenaline and twelve times more potent than (22a) as an α-agonist [142].

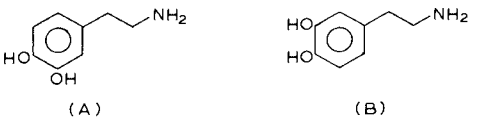


Figure 2.6. Trans rotamers of dopamine, here called A and B rather than α and β to avoid confusion with α and β receptors.

When tested for their ability to stimulate vascular β_2-receptors by measuring increased femoral blood flow in intact dogs after α-blockade, (21b) was found to be respectively five hundred times and three times more potent than (22b) and (21a) [69]; Isoprenaline was eighteen times more potent than (21b) and dopamine, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and (22a) were virtually inactive [69].

On guinea-pig isolated tracheal and atrial preparations, (21c) was found to be respectively thirtyfive times and two and one-half times more potent than *N*-

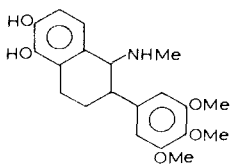
isopropyldopamine and isoprenaline as a β_2-agonist but possessed only two thousandths of the potency of isoprenaline as a β_1-agonist [143]. Thus for *N*-alkylated dopamine derivatives, the *trans*-A-rotamer appears to be the preferred conformation for activation of β_2-receptors. This is in agreement with studies using 1,5,6-trihydroxy-1,2,3,4-tetrahydronaphthalene derivatives (19). No conclusions can be drawn from these studies concerning conformational requirements for selective β_1-agonist activity.

The increased α- and β-agonist potency of flexible catecholamines with a β-hydroxyl compared with those without could be a reflection of their ease of adopting the 'active' conformation possibly aided by intramolecular hydrogen bonding between the β-hydroxyl and ammonium group (*Figure 2.3*).

A β-hydroxyl is not necessary, in itself, for α- or β-agonist activity. Dopamine possesses a moderate degree of α-agonist activity [20, 69] and the semi-rigid *N*-isopropyldopamine analogue (21c) has a similar β_2-potency (about three times more potent than isoprenaline [143]) to the corresponding semi-rigid isoprenaline analogue (19, R=isopropyl) (about seven times more potent than isoprenaline [136]) and exhibits much greater β_2-selectivity (1350 and 17, β_2-selectivity as defined in *Table 2.2*). The function of a β-hydroxyl or analogous group in flexible arylethanolamines may therefore be to aid formation of the preferred conformation for receptor interaction either by intramolecular hydrogen bonding or possibly by hydrogen bonding with a macromolecular site. In suitable semi-rigid derivatives this function might not assume such prime importance as the preferred conformation is approximated by the rigidity of the molecule.

TRIMETHOXYPHENYL-SUBSTITUTED TETRAHYDRONAPHTHALENES

A few compounds have been synthesized which attempt to bring together the tetrahydroisoquinoline and tetrahydronaphthalene groups of β-adrenomimetics. Structure (23) follows directly from the previous arguments concerning the possible interchangeability of NHR and trimethoxyphenyl groups. Not surprisingly, in view of the previously discussed structure-action relationships, both *cis*- and *trans*-isomers were very weak as β-agonists on guinea-pig isolated trachea, although the *trans*-isomer was ten times more potent than the *cis* [144].



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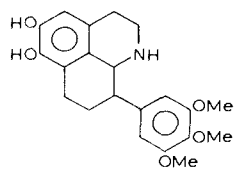


(24)

The more complex derivatives (24) have also been synthesized and separated into *cis*- and *trans*-isomers [145, 146].

The *trans*-isomer was reported to be eighty times more potent than the *cis* [145], but again both were much less potent than trimetoquinol (guinea pig isolated trachea), so that, on arguments previously expressed, no reliable conclusions can be drawn about the active conformation of TMQ. Equally, these data offer no support for the idea of a replacement role for the trimethoxyphenyl moiety.

SYNTHESIS OF 2-AMINO-1-HYDROXY-1,2,3,4-TETRAHYDRONAPHTHALENES

The key intermediates in the synthesis of the title compounds are suitably substituted 3,4-dihydronaphthalen-1(2H)-ones. 1,6,7-Trihydroxy derivatives, which lack β-agonist activity, were synthesized from 6,7-dimethoxy-3,4-dihydronaphthalen-1(2H)-one which was readily obtained from 1,2-dimethoxybenzene and succinic anhydride *via* a Haworth synthesis [66, 147].

The 1,5,6,-trihydroxy derivatives, which generally exhibit potent β-agonist activity, were synthesized from 5,6-dimethoxy-3,4-dihydronaphthalen-1(2H)-one (26) which is somewhat more difficult to obtain. Three routes from 2,3-dimethoxybenzaldehyde, all involving 4-(2,3-dimethoxyphenyl)butanoic acid (25) have been recorded:

(25) (26)

(i) 2,3-Dimethoxybenzaldehyde was reacted with malonic acid in a Knoevenagel reaction and the product reduced to give 3-(2,3-dimethoxyphenyl)propanoic acid which was converted into its higher homologue 4-(2,3-dimethoxyphenyl)butanoic acid (25) by an Arndt-Eistert reaction [148].

(ii) 2,3-Dimethoxyphenylacetaldehyde, prepared from the corresponding benzaldehyde by Darzens glycidic ester condensation with methyl chloroacetate followed by decarboxylation, reacted with malonic acid and the product reduced to give (25) [135].

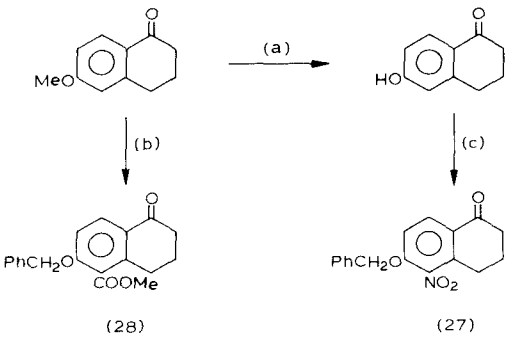
(iii) 2,3-Dimethoxyphenylacetaldehyde was treated with carboxymethyltriphenylphosphorane in a Wittig reaction followed by reduction of the product to give the methyl ester of (25) [135]. Alkaline hydrolysis gave the acid (25).

The derivative (25) was converted to the acid chloride and cyclised with tin

(IV) chloride to give 5,6-dimethoxy-3,4-dihydronaphthalen-1(2H)-one (26) in 92% yield [135]. The methyl ester of (25) was similarly cyclised to (26) in 70% yield by treatment with 85% sulphuric acid [148]. Polyphosphoric acid was a cyclising agent gave consistently low yields [135, 148].

2,5-Diamino-1,6-dihydroxy-1,2,3,4-tetrahydronaphthalenes were prepared from 6-benzyloxy-5-nitro-3,4-dihydronaphthalen-1(2H)-one (27) [140]. 6-Methoxy-3,4-dihydronaphthalen-1(2H)-one was demethylated and the hydroxy derivative nitrated to give a mixture of the 5- and 7-mononitro derivatives. The desired 5-nitro derivative was separated in 42% yield by fractional crystallisation from benzene [140]. The hydroxy group was reprotected with a benzyl function to give (27).

2-Amino-1,6-dihydroxy-5-hydroxymethyl-1,2,3,4-tetrahydronaphthalenes were synthesized from methyl 2-benzyloxy-5-oxo-5,6,7,8-tetrahydro-1-naphthoate (28) which was synthesized in eight steps from 6-methoxy-3,4-dihydronaphthalen-1(2H)-one [137].

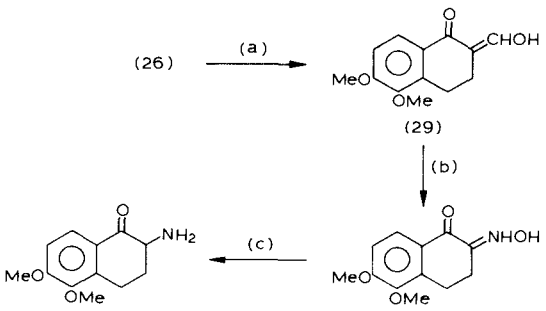


Scheme 2.8. Reagents (a) conc HBr or $AlCl_3$ (b) 8 steps (c) nitration, separation of isomers, benzylation.

There are several methods of converting 3,4-dihydronaphthalen-1(2H)-ones into 2-amino-1-hydroxy-1,2,3,4-tetrahydronaphthalenes:

Method 1. Nitrosation followed by reduction [135]

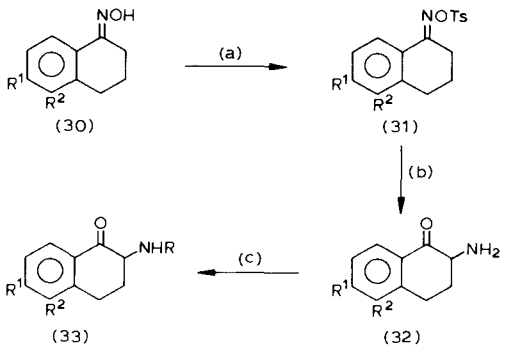
Although direct nitrosation of 6,7-dimethoxy-3,4-dihydronaphthalen-1(2H)-one gave a satisfactory yield of the corresponding α-oximino ketone [66], an unsatisfactory yield was reported for reaction with the 5,6-dimethoxy isomer [149]. However, good yields were obtained by proceeding via an α-hydroxymethylene derivative (29) [135].



Scheme 2.9. Reagents (a) HCOOEt, NaOMe (b) $NANO_2$ (c) H_2, Pd-C.

Method 2. Neber rearrangement of an oxime O-p-toluenesulphonate [135, 136, 141]

An oxime (30) prepared from a 3,4-dihydronaphthalen-1(2H)-one was treated with *p*-toluenesulphonyl chloride to give (31) which when subjected to a Neber rearrangement by treatment with potassium ethoxide in benzene gave (32).



((30) – (33))

Reagents: (a) tosyl chloride (b) EtOK (c) carbonyl compound with $NaBH_3CN$ or trifluorosulphonamide, MeI, hydrolysis.

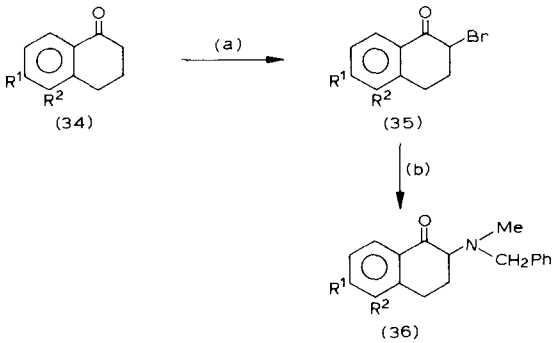
Oxime *O*-benzenesulphonates have been similarly untilised [137].

The 2-amino-3,4-dihydronaphthalen-1(2H)-ones (32) obtained by either method 1 or 2 can be *N*-alkylated at this stage by the reductive amination of carbonyl compounds using a cyanoborohydride reducing agent or, for *N*-methyl substitution, reaction of *N*-trifluoroacetamido derivative with methyl iodide

followed by hydrolysis of the trifluoroacetamido protecting group [135]. Synthesis via methods 1 and 2 does not allow the preparation of *N-t*-alkyl substituted derivatives.

Method 3. Formation of an α-bromoketone followed by reaction with an amine [135, 137, 140]

A 3,4-dihydronaphthalen-1(2H)-one (34) was treated with pyridinium hydrobromide perbromide to give an α-bromoketone (35) which was treated with *N*-methylbenzylamine to give (36).



(34) (35) (36)

Reagents: (a) pyridinium hydrobromide perbromide (b) *N*-methylbenzylamine.

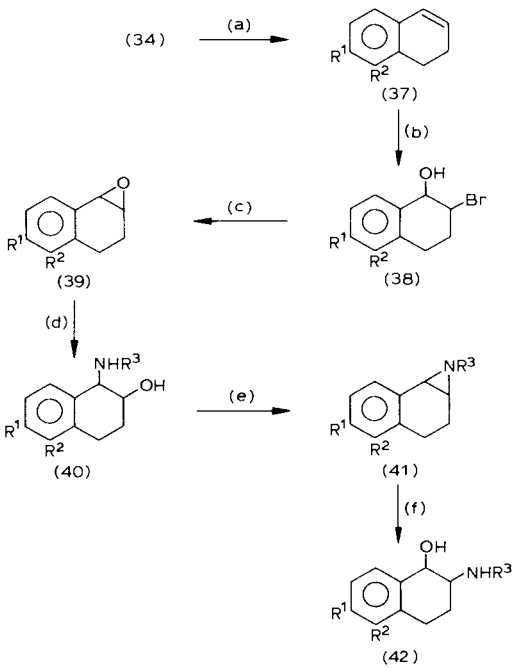
Reduction of derivatives (36) with sodium borohydride gave mixtures of *cis*- and *trans*-isomers which were separated by fractional crystallisation [135] or column chromatography [137, 140], a greater proportion of the *trans*-isomer always being isolated.

Reduction of (33) prepared either by method 1 or 2 to a 2-amino-1-hydroxy derivative (42) by catalytic hydrogenation is not stereospecific [135] whereas reduction of (33) with sodium borohydride favours formation of the *trans* isomer [136, 137, 140, 141]. Reduction with diborane gave mixtures of *cis* and *trans* isomers, the relative ratio depending on whether the amino function was substituted or not [137].

Method 4. Formation of 1-alkylamino-2-hydroxy-1,2,3,4-tetrahydronaphthalenes and transposition to the 2-alkylamino-1-hydroxy derivative via 1,2-aziridines [136, 138]

This method is stereoselective and enables the introduction of 2-*t*-alkyl-amino substituents. A 3,4-dihydronaphthalen-1(2H)-one (34) was reduced with sodium borohydride to the alcohol which was dehydrated with potassium

bisulphate in benzene to give a 1,2-dihydro derivative (37). A *trans*-α-bromohydrin (38) was prepared from (37) by reaction with *N*-bromosuccinimide in 'wet' dimethyl sulphoxide. Dehydrohalogenation with solid sodium hydroxide in benzene gave a *cis*-epoxide (39) which when reacted with amines, including *t*-alkylamines [138], gave a 1-amino-2-hydroxy derivative (40). Transposition was achieved by hydrolysis of an aziridine derivative (41) prepared from (40) by reaction with a mild sulphating agent, sulphur trioxide-triethylamine complex, followed by solid sodium hydroxide in benzene.



((42))

(a) $R^1=R^2=OH$, $R^3=H$
(b) ,, , $R^3=Me$
(c) ,, , $R^3=Pr^i$
(d) $R^1=OH$, $R^2=NHR$, $R^3=H$, alkyl
(e) $R^1=OH$, $R^2=CH_2OH$, $R^3=Bu^t$

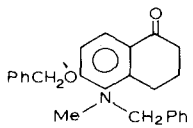
((37) – (42))

Reagents: (a) $NaBH_4$ then potassium bisulphate (b) N.B.S. in wet DMSO (c) NaOH (D) RNH_2 (e) SO_3-Et_3N (f) NaOH.

Hydrolysis of (41) to (42) using acetic acid gave the *trans*-isomers exclusively whereas sulphuric acid gave a mixture of both *cis*- and *trans*-isomers [138]. A detailed investigation of the stereoselective ring-opening of aziridines was reported [150].

The conformationally restricted noradrenaline, adrenaline and isoprenaline derivatives (42a, b and c respectively) were prepared from (34,R^1=R^2=OMe) prepared by method 1 [135]. Compound (32,R^1=R^2=OMe) was N-alkylated if necessary, demethylated by treatment with conc. hydrobromic acid and then catalytically reduced to give (42 a-c). 2,5-Diamino-2,6-dihydroxy-1,2,3,4-tetrahydronaphthalenes (42d) were synthesized from (32,R^1=OCH_2Ph,R^2=NO_2) prepared by method 2 [140].

Compound (32,R^1=OCH_2Ph,R^2=NO_2) was catalytically reduced to (42d) or, if a 2-substituted amino function was required, reduced to an aminoalcohol with sodium borohydride, *N*-alkylated then catalytically reduced to (42d) [140]. To obtain 5-substituted amino derivatives, the 5-amino function was formed, derivatised and, if necessary protected at the 3,4-dihydronaphthalen-1(2H)-one stage [141]. For example, 5-methylamino derivatives were prepared from (43) by method 2. Compound (43) was obtained from (27) by reduction of the nitro group with Raney nickelhydrazine hydrate, *N*-methylation by methyl iodide via the *N*-trifluoroacetamido derivative followed by *N*-protection with benzyl chloride [141].



(43)

Saligenin derivatives were obtained from the 2-amino-1-hydroxy derivatives (42,R^1=OCH_2Ph,R^2=COOMe,R^3=alkyl) which were obtained either by method 2 or method 4. Method 4 was employed to obtain the conformationally restricted analogue of salbutamol, (42e).

The hydroxymethyl function was obtained by reduction of the derivatives (42,R^1=OCH_2Ph,R^2=COOMe,R^3=alkyl) with lithium aluminium hydride. Debenzylation then gave the saligenin derivatives [137, 138].

CONCLUSIONS FROM THE STRUCTURE-ACTION RELATIONSHIPS OF THE TETRAHYDRONAPHTHALENES

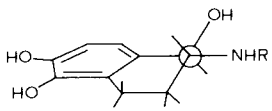
On the evidence presented to date, the tetrahydronaphthalene series represents a success for the restricted-rotation analogue approach to drug-receptor interactions. For this approach to be accepted, the analogues must be potent

and must show comparable quantitative and qualitative changes in activity, compared with the flexible molecules when substituents are varied. The tetrahydronaphthalenes broadly satisfy both these criteria, although it has first to be recognised that the presence of the $-CH_2-CH_2-$ group attached to the 2-position imposes a degree of selectivity, first for β over α receptors and then for β_2 over β_1, so that the effect of substituents which normally confer such specificity in the phenylethanolamines must be expected to be reduced. The increase in lipophilicity associated with the extra $-CH_2-CH_2-$ substituent may also account for maximum potency being observed where the *N*-substituent is isopropyl, rather than with *t*-butyl, as in the phenylethanolamines. The most striking difference between the two (semi-rigid and flexible) series is the much reduced influence of the side-chain OH on β_2 potency in the tetrahydronaphthalenes, where the absence only reduces potency by half. This may be a reflection of improved receptor fit of the rest of the molecule, although it should be noted that this OH group is still important for β_1 activity.

The slight decrease in potency and increase in selectivity which accompanies the substitution of 5-OH by CH_2OH is entirely in accord with the salbutamol experience, and substitution by NH_2 or NHMe has the anticipated effect on selectivity.

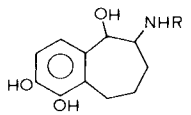
We can find no account, as yet, of the optical resolution of any member of this series. It will be surprising if activity is not found to reside mainly in the 1R,2S-isomer. If it is assumed that the preferred (and active) conformation has both OH and NHR in the pseudoequatorial position, the molecule has the 3-dimensional relationships depicted by (44), which closely resembles the preferred phenylethanolamine conformation.

If these assumptions are verified, the question of the preferred conformation of flexible agonists at β-receptors will largely have been solved.

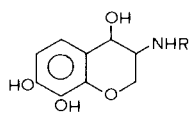


(44)

(1-R, 2-S), 2-alkylamino-1,5,6,-trihydroxy-1,2,3,4-tetrahydronaphthalene viewed along the 1,2 bond.



(45)

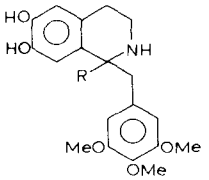


(46)

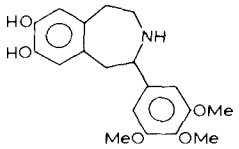
Miscellaneous observations on related compounds

Nothing appears to have been carried out with β-blockers in the tetrahydronaphthalene series. The analogues (45) and (46) have been examined for β-agonist activity, with some success [151, 152], although the full pharmacology of (45) has not been reported.

Some β-blocking activity has been discovered by accident in the THIQ series. Apart from the 1-aminomethyl compounds (17) mentioned earlier, which have weak β-blocking activity [81], the 1-substituted trimetoquinol derivatives (47) show selective blocking activity [153, 154].



(47)



(48)

R = Me, CH_2Ph

An intriguing recent discovery concerns the potent β_1 and β_2 agonist activity of the trimetoquinol isomer (48), which also shows β-blocking activity [155].

The trimethoxyphenyl group in (48) is much more restricted conformationally than the related benzyl group of trimetoquinol, but it is possible to partially superimpose the two structures, which may be a guide to the active conformation of trimetoquinol. It will be interesting to see if analogues of the benzazepine (48) show any structure-action correlations with the phenylethanolamines. The spatial relationships in molecular models of (48) bear some resemblance to those in crystals of trimetoquinol hydrochloride [145].

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3 Mechanisms of Cytotoxicity of Nitroimidazole Drugs

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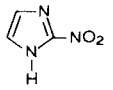
INTRODUCTION

Nitroimidazole drugs have been in use for over twenty years and their use is increasing not only as major antimicrobial drugs but also as sensitizers of hypoxic tumours in conjunction with radiotherapy. Such drugs have a wider spectrum of useful clinical activity than that of any antibiotic. It is timely to review the action of these drugs and attempt to explain the basis of their selective toxicity and their unique contribution to microbial and tumour chemotherapy.

The first 2-nitroimidazole to be discovered was azomycin (1) (2-nitroimidazole), an antibiotic isolated in Japan from a streptomycete in 1953 [1, 2]. Three years later in France, azomycin was rediscovered from a soil streptomycete and used as the starting point for a systematic search for a drug for trichomoniasis. This led to the synthesis of metronidazole (2) and dimetridazole (3) in 1957 and



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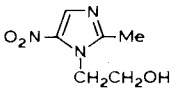

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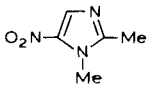

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the marketing of metronidazole in 1960. It may seem strange that a systematic search for an effective drug against trichomoniasis should be made, but in the early 1950's it was becoming apparent that the disease caused by the protozoan *Trichomonas vaginalis* was widespread in both males and females with an incidence ranging from 5% to 70% in certain ethnic groups [3]. Treatment was mostly ineffective and included vinegar douches to normalise vaginal pH and methyl violet-impregnated tampons. Until the advent of metronidazole, the eradication of *T. vaginalis* was an insoluble problem – not because the parasite possessed an effective defence mechanism, but because of the protection afforded to it by the host. Elimination from the vagina was a relatively simple task but, once sheltered within the confines of the glands of Skene or Bartholin, the trichomonad became inaccessible to topical agents. The market for an effective systemic agent was therefore established and was the spur for a considerable research effort within the pharmaceutical industry.

CLINICAL USES OF NITROIMIDAZOLES

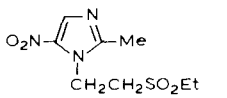
The major clinical uses of nitroimidazole drugs are for diseases caused by bacteria and protozoa. Since far more work has been published for metronidazole and because its clinical use far outstrips any other nitroimidazole, it will be regarded as the type example.

Metronidazole (2) (1-β-hydroxyethyl-2-methyl-5-nitroimidazole) is the cur-

rent drug of choice for the treatment of several protozoal diseases including trichomoniasis [4] and intestinal infections caused by *Giardia lamblia* [5], all forms of *Entamoeba histolytica* [6, 7] and Balantidium [8]. The drug has been tried in other protozoal diseases with conflicting results. In cutaneous leishmaniasis, a 78% cure rate has been claimed against *Leishmania mexicana* [9] but other workers find it not particularly effective against *L. brasiliensis* [10, 11]. A similar situation exists with Chagas's disease, some workers reporting useful activity and others no useful activity. Metronidazole is not in general use for these latter diseases but has widespread use against a variety of bacterial infections.

The first indication of the usefulness of the drug as an antibacterial came from the observation that a patient with Vincent's disease (an acute ulcerative gingivitis) was rapidly cured whilst receiving metronidazole therapy for trichomoniasis [12]. Subsequent controlled clinical trials confirmed its remarkable effectiveness for this condition [13] which is caused by a variety of anaerobic bacteria including fusiforms, Streptococcus and Bacteroides. Many controlled trials on the use of metronidazole in controlling post-operative surgical infection have now established its role as the drug of choice against anaerobic bacterial infections. [14, 15].

During the 20 years since the discovery of metronidazole, a number of other useful nitroimidazoles have emerged. Thus tinidazole (4) is particularly useful in amoebiasis and giardiasis [16–18] and ornidazole (5) which, in common with tinidazole, has a longer serum half-life than metronidazole, is in use as a single dose treatment for trichomoniasis [19], amoebiasis [20] and in an injectable form for bacterial meningitis [21]. Benznidazole (6) is the most valuable drug for *Trypanosoma cruzi* infections (Chagas's disease) at present [22, 23]. Nimorazole (7), carnidazole (8) and secnidazole (9) are also clinically available for the treatment of trichomoniasis [24–26] but show no sufficiently improved activity over metronidazole for this disease.



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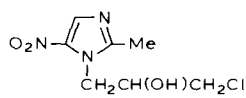

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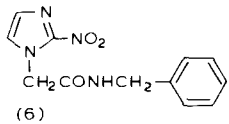

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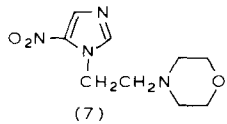

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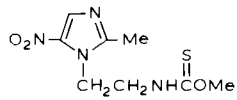

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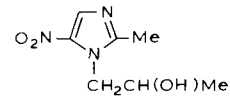

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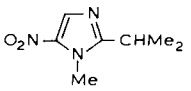
In the veterinary field, dimetridazole is used extensively against histomoniasis in turkeys [27] and together with ipronidazole (10) and ronidazole (11) is also used in the treatment of swine dysentery [28–29].

More recently, nitroimidazoles, notably metronidazole and misonidazole (12) have been investigated in the treatment of hypoxic tumours and have opened up the exciting prospect that these drugs may have a useful clinical potential as anticancer agents. This aspect of nitroimidazole action will be discussed later.



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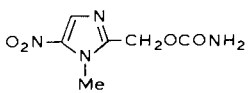

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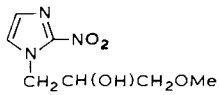

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EFFECTS OF NITROIMIDAZOLES ON MICRO-ORGANISMS

EFFECT ON RESPIRATION

Selective toxicity is an important concept in chemotherapy and the basis for the selective activity of many antimicrobial drugs is the interference of molecular mechanisms which are specific to certain taxonomic groups; for example, those drugs which exploit structural or biosynthetic differences between prokaryotic cells or those whose action centres upon a target which is unique to a particular group or taxon. The so-called spectrum of activity of antimicrobial drugs therefore reflects the taxonomic boundary beyond which the action of the drug is absent or minimal and clearly delineates them from disinfectants and antiseptics which have no selective toxicity.

In this respect, the nitroimidazole group of drugs is unique in that their action is selectively toxic but transcends major taxonomic boundaries. The spectrum of activity includes both Gram-positive and Gram-negative bacteria, protozoa and even a few nematode worms. No other drug encompasses this range of selective toxicity. Examination of the organisms susceptible to nitroimidazoles reveals that they all have a common factor; that susceptibility is a reflection of the growth conditions of the organisms which are anaerobic, *i.e.*, where the redox potential is lower or more negative than that encountered by aerobes.

This observation poses an obvious question: do nitroimidazoles inhibit a mechanism unique to anaerobic metabolism or structure? The answer is by no means a simple one. Early work showed that anaerobic metabolism differed in several important respects from aerobes. Both in typical anaerobic bacteria such as Clostridium and protozoa such as *Trichomonas vaginalis*, the end point of respiration is CO_2 and H_2. Unlike aerobes, O_2 does not act as a final electron acceptor and thus the classical electron transport system containing cytochromes is absent, as is a fully functional Krebs's cycle. Initial studies with metronidazole using Warburg respirometry revealed that the drug specifically inhibited H_2 evolution both in protozoa [30–32] and in bacteria [32].

Hydrogen evolution in micro-organisms is intimately linked with electron transport mechanisms [33, 34]. Although those for bacteria are well documented, those for protozoa are not [35, 36]. In *T. vaginalis* however, the mechanism of both CO_2 and H_2 evolution was established as the clostridial-type pyruvate phosphoroclastic mechanism which is shown in *Figure 3.1*. In this mechanism the overall reaction may be written as:

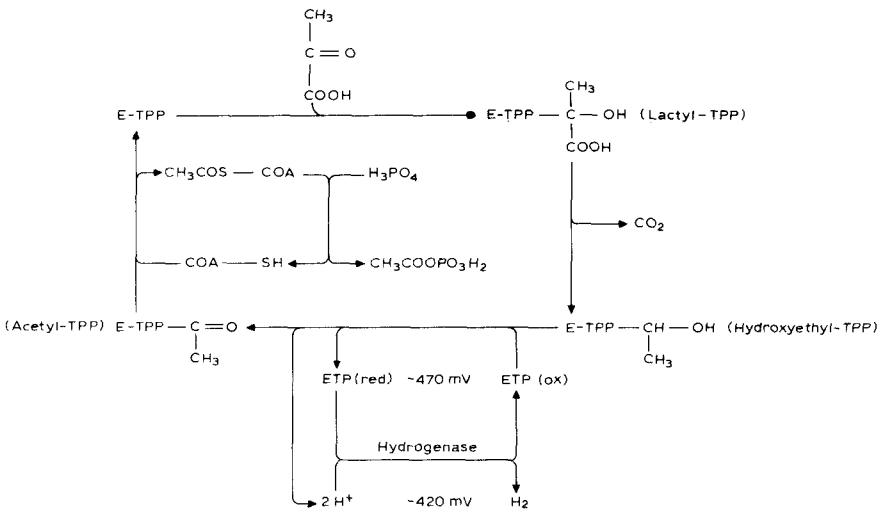


Figure 3.1. The pyruvate phosphoroclastic reaction [32, modified]. All reactions are carried out bound to an enzyme-thiamine pyrophosphate complex (E-TPP). ETP is an electron transfer protein which is normally ferredoxin in Clostridia and an uncharacterized ferredoxin-like protein in *Trichomonas vaginalis*. The redox potentials are given for ferredoxin (−470 mV) and the hydrogenase system (−420 mV).

Since the synthesis of acetyl phosphate is unaffected by nitroimidazoles [31] and CO_2 output is not a primary effect of the drugs but reflects the death of the cell, it follows that the site of nitroimidazole action must be in the cycle involving H_2 evolution. One of the clues as to the action of metronidazole in inhibiting H_2 evolution came from the observation that no drug was detected polarographically in susceptible cells. Polarography detects the nitro group of the drug and one interpretation is that the nitro group is reduced, i.e. acts as an electron acceptor. This was confirmed by showing that metronidazole inhibited anaerobic electron transfer to various dyes including methylene blue and 2,6-dichlorophenolindophenol [30]. In the phosphoroclastic system of Clostridia, electrons are donated to protons by an electron transfer protein, ferredoxin, which has a redox potential (470 mV) far lower than that of any redox system in aerobes. In aerobes, electron transfer is carried out by cytochromes. In anaerobes, these are replaced by ferredoxins—proteins which contain Fe linked to S-containing residues. The inference therefore is that nitroimidazoles inhibit respiration in anaerobes by accepting electrons from reduced ferrodoxin through the imidazole nitro group which is simultaneously reduced. (32) Such a mechanism of action is attractive in that it explains the selective toxicity of these drugs towards anaerobes insofar as no aerobe has redox mechanisms of such a negative potential to donate electrons to the drug. The role of nitroimidazoles in inhibiting electron transport in anaerobes is important but does not explain the microbicidal action of these drugs. O'Brien and Morris [37] showed that, once all available metronidazole had been reduced by *Clostridium acetobutylicum,* the phosphoroclastic reaction recovered. Similar results were achieved in protozoa [38]. Inhibition of respiration by nitroimidazoles was therefore a transitory effect and could not account for the biochemical lesion resulting in cell death. It seemed likely that susceptible cells were killed as a consequence of drug reduction, *i.e.* by a reduced derivative at a site in the cell which was removed from the phosphoroclastic complex.

EFFECT ON MORPHOLOGY

Few studies have been undertaken to establish the effect of nitroimidazoles on the ultrastructure of susceptible organisms but those which have are equivocal.

All the experiments have concentrated on protozoa rather than on bacteria because of the relative ease of electron microscopy. The major studies all involve metronidazole and the first one [39] using fluorescence microscopy, electron microscopy and autoradiography showed that the drug was distributed evenly both in the cytoplasm and the nucleus of *Trichomonas vaginalis*, causing extensive vacuolization of the cell which was interpreted as a secondary effect of the drug. The major effects were significant changes in the structure of the nuclear membrane and those of the endoplasmic reticulum with no obvious changes in nuclear material. These changes were interpreted as consistent with a hypothesis of inhibition of DNA synthesis and correlated with those obtained from a study of morphological changes of trophozoites of *Entamoeba histolytica* [40].

Prior to the above study, two previous reports showed that the major cytotoxic effects of metronidazole centred on the microgranular systems and the fundamental plasma of the organism [41] or on degenerative lesions of the nucleus [42]. Confusion in interpretation arose as a difficulty in assigning a role to the 'chromatic granules' and the differing nomenclature used by different research groups. For example, some regard the chromatic granules as mitochondria [43–45], some that they occur with mitochondria [46–50], and others that, since mitochondria are absent in anaerobes, the granules must be a distinct organelle [51–55]. The distinction is an important one because it is upon these chromatic granules that nitroimidazoles appear to exert their major ultrastructural effect. The most convincing evidence for the nature of these organelles come from reports [54, 55] which show that they contain a clostridial-type phosphoroclastic system responsible for H_2 evolution, being now called hydrogenosomes which are distinct from lysozomes or peroxisomes. It is very relevant therefore that the most recent morphological study showed effects on these hydrogenosomes, with breakdown of the nuclear membrane with metronidazole and nitrofurans and condensation of nuclear material with nitrofurans [56]. The common interpretation of these events is that nitroimidazoles cause disruption of cytoplasmic hydrogenosomes as a consequence of interrupted electron flow in the phosphoroclastic system and the reduced drug then initiates significant disruptive events in the nucleus of the cell.

EFFECTS OF NITROIMIDAZOLES ON PHOTOSYNTHESIS

Ferredoxin occurs not only as an electron transport protein in anaerobic microorganisms but also in plants where two interlinked electron transport systems (photosystems 1 and 2) occur. The overall scheme, shown in *Figure 3.2* indicates that photosystem 2 corresponds experimentally to the Hill reaction in which

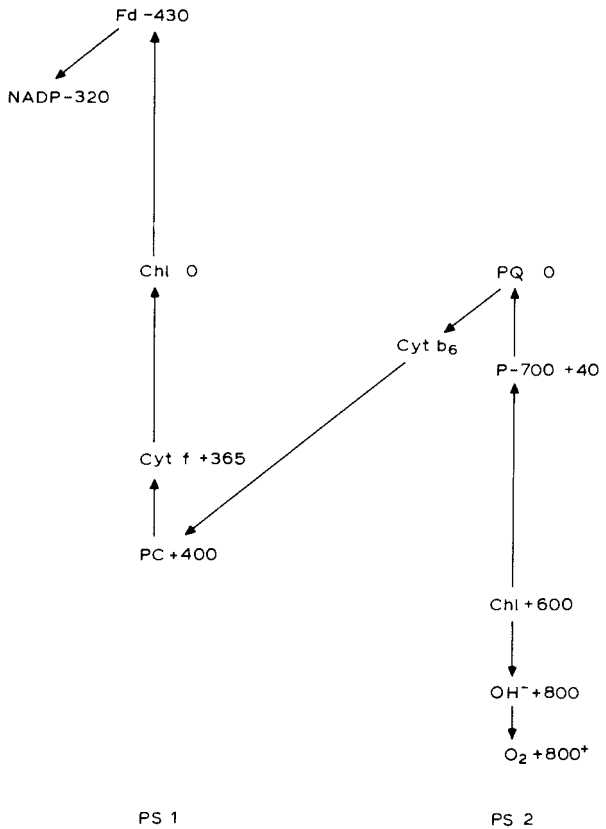


Figure 3.2. Photosynthetic electron transport. PS1 and PS2 refer to photosystems 1 and 2 respectively. Chl is chlorophyll, PQ is plastoquinone, PC is plastocyanin, Cyt is cytochrome and Fd is ferredoxin. The numbers indicate the redox potential in mV.

electron flow is experimentally measured as the decolourisation of 2,6-dichlorophenolindophenol in broken chloroplast preparations. Studies with metronidazole [57] show that the drug has no effect on photosystem 2, but inhibits photosystem 1 [58]. Only photosystem 1 involves ferredoxin and the inference is that inhibition occurs at this level since it can be shown that the drug is reduced in photosystem 1 only (where redox levels of the drug and ferredoxin are compatible) and that it can replace ferredoxin as an autoxidisable electron acceptor [57]. The overall effects of the drug in leaf discs is to inhibit sugar synthesis and potentiates the degradation of chlorophyll in the light [56], as a result of interrupted electron flow from ferredoxin to NADP.

This effect of metronidazole (and that of other nitroimidazoles) as an intermediate electron acceptor or electron sink in photosynthesis may be used to select organisms which are mutant in photosystem 1. Such mutants are useful in the studies of chloroplast component function. Metronidazole has been shown to be effective in the selective enrichment of mutants defective in photosystem 1 which are potentially useful for the identification of structural genes for chloroplast polypeptides or regulatory genes which affect chloroplast biogenesis [59]. The metronidazole-enrichment technique is superior to those methods utilizing aresenate or diquat [59].

Rhodopseudomonas acidophila is a bacterium which can grow chemoorganotrophically in the dark or photo-organotrophically in the light. Under photo-organotrophic conditions, the organism utilises ferredoxin-linked photosynthetic pathways whereas in the absence of light it does not. Photo-organotrophically *R. acidophila* is killed by metronidazole at concentrations of 25 μg/ml, producing elongated cells. These effects are related to the metronidazole concentration and at 1000 μg/ml (lmg/ml) cells fifteen times the normal length are produced, reflecting an inhibition of the normal septation process [57]. In contrast, the drug produces no effect on the bacterium when grown chemo-organotrophically up to drug concentrations of 1 mg/ml. Significantly, only when grown photo-organotrophically is the organism killed and concomitant reduction of the drug occurs, reflecting further interaction between the nitroimidazole and ferredoxin-linked reactions [57].

EFFECTS OF NITROIMIDAZOLES ON DNA

Since reduction of nitroimidazoles in pyruvate phosphoroclastic type reactions are transient and last until all drug is reduced, it is tacitly assumed that cell death is due to a nitroimidazole reduction product. To this effect it has been postulated that nucleic acid is a possible site of action on the evidence that metronidazole inhibits uptake of ^{14}C-labelled thymine and uridine into *T. vaginalis* [60] and that radiation induces binding of metronidazole to DNA under anoxic or anaerobic conditions only [61]. An extended study showed that DNA synthesis was not only inhibited by a variety of nitroimidazole drugs including metronidazole and tinidazole but, using different techniques it was demonstrated that existing DNA was degraded in *Clostridium bifermentans* [62] without affecting RNA synthesis or stability. This effect supported the observation that DNA could not be prepared from metronidazole-treated cells because its viscosity was insufficient to 'spool out' during the preparation.

The evidence thus far indicates that nitroimidazoles act as electron sinks in

anaerobic reactions which have redox potentials compatible with that of the drug. In so doing they transiently interrupt normal electron flow, becoming reduced in the process, and inhibit DNA function as at least one major target. This enables a more detailed study of the drug-target interaction to be made since both may be prepared in high purity for *in vitro* studies. The first of these [63] used metronidazole chemically reduced by sodium dithionite in the presence of T_2-phage or calf-thymus DNA. After dialysing the drug and reacted dithionite from DNA, the latter was then analysed by melting profiles and sucrose gradient sedimentation centrifugation at drug nucleotide ratios of 0.3 to 1.0. The results showed that metronidazole decreased the temperature of the midpoint of the helix-coil transition (the T_m value) and increased the range over which 90% of the transition occurred but had no effect on the cooling profile of DNA. This may be interpreted as the drug destabilising the DNA by a non-intercalative and non-cross-linking process since intercalators generally tend to raise the T_m value and cross-linking agents affect the cooling profile of DNA. The increase in melting range, however, indicates that the population of DNA molecules has become less homogeneous or more polydisperse, indicating a strand-breakage effect. Such an action of metronidazole was subsequently demonstrated, metronidazole causing both single and double strand breaks in DNA as adduced by alkaline and neutral sucrose density sedimentation respectively [63]. Significantly, the above effects occurred only under anaerobic or anoxic conditions.

Such a reduction system is, however, open to criticism because dithionite itself may cause strand breakage of DNA [64] making unambiguous interpretation difficult, and some workers using viscosity measurements have been unable to detect DNA damage using dithionite-reduced metronidazole [65]. To overcome these problems, an electrolytic technique was developed [69, 70] in which the drugs are reduced at a constant voltage which is related to their redox potential. To achieve this, each drug's redox characteristics are established polarographically and the half-wave potential (that is, the point at which the drug is 50% reduced) or $E_{½}$, is regarded as a close approximation to the redox potential. The voltage chosen for electrolytic reduction is lower (that is, more negative) than the $E_{½}$ because, during the process in unbuffered medium, the pH rises and the $E_{½}$ decreases. These electrochemical characteristics are summarized in *Table 3.1*. In these experiments, the reduction potential does not exceed −1.1 V because degradative interactions occur between the electrodes and DNA [66, 67]. Control experiments show that, with a variety of nitroimidazole drugs, damage to DNA occurs only when reduction occurs anaerobically or anoxically, the unreduced drugs having no effect. For reference, the activity of nitrofurantoin (14) is included in the table.

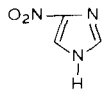
Table 3.1. ELECTROCHEMICAL CHARACTERISTICS OF NITROHETEROCYCLIC DRUGS

Drug (formula)	$E_{1/2}$ (pH7.0) (mV)[a]	pH dependence (V)[b]	E^1_7 (mV)[c]
Nitrofurantoin (17)	−190	$E_{1/2} = 0.022 - 0.06$ pH	
Benznidazole (6)	−200	$E_{1/2} = 0.325 - 0.07$ pH	−380
Misonidazole (12)	−272	$E_{1/2} = 0.22 - 0.07$ pH	−389
Tinidazole (4)	−340	$E_{1/2} = 0.08 - 0.06$ pH	−464
Ornidazole (8)	−345	$E_{1/2} = 0.11 - 0.065$pH	−467
Nimorazole (7)	−345	$E_{1/2} = 0.04 - 0.054$pH	−457
Azomycin (1)	−375	$E_{1/2} = 0.20 - 0.082$pH	−418
Dimetridazole (3)	−385	$E_{1/2} = 0.06 - 0.064$pH	−475
Metronidazole (2)	−385	$E_{1/2} = 0.07 - 0.065$pH	−486
8609RP (14)	−475	$E_{1/2} = 0.02 - 0.065$pH	
M&B 4998 (15)	−500	$E_{1/2} = 0.08 - 0.06$ pH	
4(5)-nitroimidazole (13)	−545	$E_{1/2} = 0.05 - 0.07$ pH	

[a] The half-wave potential is that at pH 7.0 with a Hg cathode and Ag/AgCl anode.
[b] The equation is linear over the pH range 2.5 to 9.0. Values outside this range have not been determined.
[c] The one-electron redox potential determined by pulse radiolysis.

NATURE OF NITROIMIDAZOLE-INDUCED DNA DAMAGE

Recent evidence shows that reduced drugs cause different damage to DNA, when elicited by different techniques. In general, there is a marked decrease in DNA viscosity indicating damage caused by strand breakage or strand separation but not helix 'bending' [68]. The latter possibilities may be dismissed on the grounds that the extent of decreased viscosity is below that of single stranded DNA [69], indicating that strand breakage is the most likely action. This interpretation is supported by the effects of nitroimidazoles on the thermal hypochromicity and renaturation of DNA [69–72) which show that metronidazole, misonidazole, 4(5)-nitroimidazole (13), 8609 RP (14), and M & B 4998 (15) decrease the



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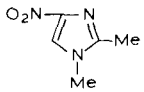

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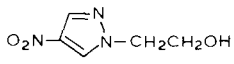

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thermal hyperchromicity of DNA and inhibit renaturation of random coil DNA to the double helix form. When thermal renaturation is controlled and measurements taken from the Tm value, the Rowley equation [71] enables a direct calculation to be made of the amount of intact helix in any DNA preparation. The

loss of intact helix caused by nitroimidazole damage can be as high as 70%, indicating extensive strand nicking or single strand scission of the phosphodiester backbone.

Further corroboration of strand breakage of DNA caused by nitroimidazoles comes from alkaline and neutral sucrose density gradient sedimentation centrifugation, hydroxyapatite chromatography and agarose gel electrophoresis. Metronidazole, misonidazole, 8606 RP, 4(5)-nitroimidazole and M and B 4998 all cause single and double strand breakage [71, 72] as adduced by sucrose gradient sedimentation but to differing extents. Hydroxyapatite chromatography quantitatively separates single from double stranded DNA [73] and may be used as an index of strand breakage induced by nitroimidazoles [71, 72]. Agarose gel electrophoresis in which the intercalating drug, ethidium bromide or acridine, is incorporated into the gel enables separation and visualization of double stranded DNA under UV light. Strand breakage of DNA and the concomitant loss of helix integrity results in a decreased amount of the intercalator binding to an intact DNA helix [75–78] as indicated by a decreased fluorescence under UV light, and an increase in the migration distance. This latter effect arises because under the conditions of electrophoresis there are no charge effects and separation occurs on the basis of molecular weight alone – thus low molecular weight average DNA migrates faster (and thus further) than high molecular weight DNA [74–78]. Nitroimidazole drugs typically cause an increase in migration distance of DNA and a decrease in fluorescence intensity, indicating double strand breakage and loss of intact helix [71, 72].

The variety of techniques which may be used to detect DNA damage all indicate that reduced nitroimidazole drugs exert their effect by causing single and double strand breaks in DNA. However, the extent of DNA damage varies with the drug and more significantly with the source of DNA, indicating that strand breakage may not be random but may result from the drugs acting on a specific target in DNA.

THE TARGET OF NITROIMIDAZOLE ACTION IN DNA

Recent evidence indicates that misonidazole damage to DNA varies with the base composition of the DNA [79–81]. In such experiments, irrespective of the techniques used to measure DNA damage, there is a linear relationship between the degree of DNA damage and the adenine + thymine (A + T) content of DNA over the range 29% A + T to 72% A + T. More damage occurs with DNAs of high A + T content (for example, from *Clostridium perfringens* 71% A + T) than from those of low content, (for example, *Micrococcus lysodeciticus* 28% A + T), indicating A or T as possible targets in DNA [79, 80].

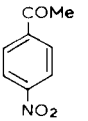
Even at drug-nucleotide ratios as low as 1:10, damage to DNA is sufficiently extensive that degradation products may be released and separated by dialysis from higher molecular weight DNA, enabling their separation, purification and characterization. Such studies using Sephadex chromatography and subsequent analysis by UV absorption, radioactivity and absorption spectra show that the only products of nitroimidazole-induced damage are thymine-derived [81]. Thus, misonidazole liberates from *Escherichia coli* DNA (49.5% A + T) about 5% of its total thymine content as thymine (3%), deoxyribose(d)-thymidine (18%), d-thymidine-3-phosphate (32%), d-thymidine-5-phosphate (22%) and d-thymidine-3,5-diphosphate (15%). The value for d-thymidine-3-phosphate is probably artificially high and that for the diphosphate low because the diphosphate tends to hydrolyse to yield the 3-phosphate. The results are a clear indication that nitroimidazoles react with thymine in DNA causing scission of the sugar-phosphate bonds around the base. The basis of such a specificity is at present unknown but such degradative effects are absent in poly([dG–dC]) and apyrimidinic acid but present in RNA (Knox R.J. and Edwards D.I., unpublished), indicating a specific requirement for the pyrimidine ring. The specificity and degree of damage varies with the redox potential of nitroheterocycles, nitrofurans, for example, liberating as much as 25% of the total thymine of DNAs irrespective of their base composition, whereas the less electron-affinic nitroimidazoles liberate less thymine-derived products.

These nuclease-like effects of nitroimidazoles on DNA, not unlike that of phleomycin and bleomycin [82], have important implications both for their radiosensitizing and antimicrobial action.

NITROIMIDAZOLES AS HYPOXIC TUMOUR CELL RADIOSENSITIZERS

Hypoxic cells occur in human tumours when the tumour size exceeds 0.5–1.0 cm and result from the tumour growth outpacing its blood supply and thus its oxygen requirements. Such cells are important because they usually occur around the area of tumour necrosis and are relatively resistant to radiation [83]. These hypoxic cells may be sensitized to the killing effects of radiation by various chemicals, the largest and most important group being the electron-affinic agents in which the sensitizing efficiency is directly related to their electron affinity or redox potential [84]. The concept of electron affinity [85] led to a systematic search for compounds with radiosensitizing properties not only in bacteria (a convenient experimental system) but also in mammalian tumour cell lines. One of the most effective early compounds was *p*-nitroacetophenone (16) which did not sensitize aerobic cells, only hypoxic or anoxic

(anaerobic) cells [86, 87]. Its use was limited by its solubility, but soon after, the nitrofurans were discovered to be effective radiosensitizers of hypoxic cells [87] but not suitable clinically because of toxicity at the doses required for sensitization. At about this time, metronidazole (2) was discovered to be a good radiosensitizer [88, 89] and generated much interest because of its known lack of clinical toxicity and early reports of useful activity against human gliomas [90].



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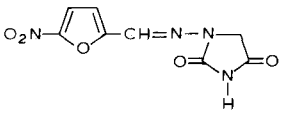

(17)

The 2-nitroimidazoles are more electron-affinic than 5-nitroimidazoles and thus are expected to be better radiosensitizers. Misonidazole was synthesized as a specific hypoxic cell radiosensitizer and its efficiency proved *in vitro* and *in vivo* [91, 92]. More recent studies have now revealed that both misonidazole (12) and metronidazole (2) are cytotoxic to hypoxic mammalian cells in the absence of radiation, that is, the overall effect of the drugs as radiosensitizers include a cytotoxic effect [93]. This raises the interesting question – is the mechanism of hypoxic cytotoxicity similar to that in anaerobic micro-organisms?

The radiosensitization effect is fast (ca 50 msec) and the cytotoxic one relatively slow (850–1000 msec); radiosensitization is temperature-independant whereas cytotoxicity is not. Both effects, however, have one feature in common – they are inhibited by aminothiols which are known radical scavengers or radioprotectors whose effects are also slow (ca 850 msec). Should the mechanism of cytotoxicity in both hypoxic and anaerobic cells be similar, the drugs are reduced *via* the nitro group since this is an essential prerequisite for activity. The evidence points to this occurring since radiation itself can reduce nitroimidazoles but, significantly, only under anoxic or anaerobic conditions [94], and reduced misonidazole has been detected in tumour cells [95]. These observations, taken together with *in vitro* studies on electrochemically-reduced nitroimidazoles suggest that the cytotoxic effect in hypoxic cells is identical to that in anaerobes. The effects of radiosensitization are also similar to those of cytotoxicity since radiosensitizers cause strand breakage [96–98] which is dependent on the generation of free radicals [97]. Thus the damage caused to DNA by the radiation process is radical-induced strand breakage and is shown diagrammatically in *Figure 3.3*. The bases most susceptible to radical attack whether radiation or drug-induced appear to be thymine and to a lesser extent adenine [99, 100]. The similarities of both processes are such that both mechanisms must be closely interlinked. *Figure 3.4* shows a possible mechanism of cytotoxicity and its relation to the radiosensitization mechanism. In this scheme, it is envisaged that

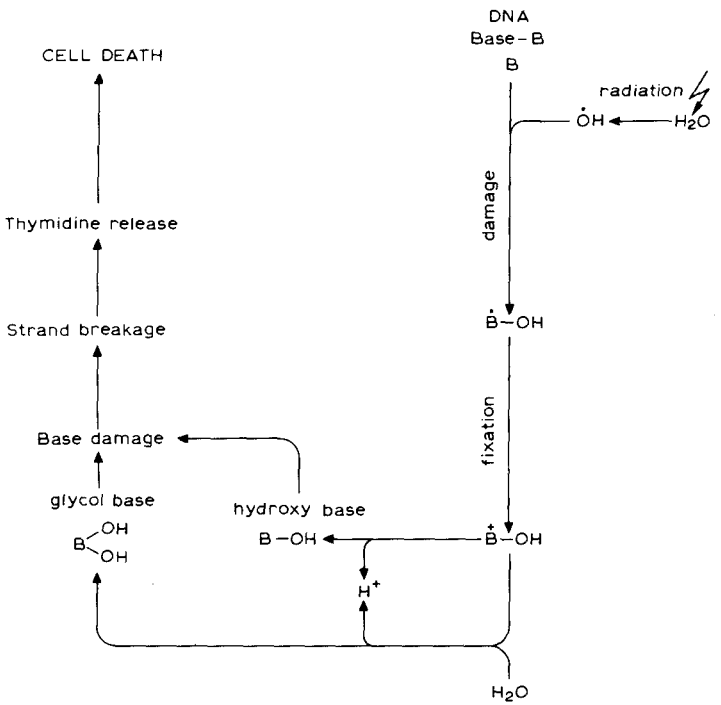

Figure 3.3. Mechanism of radiosensitization-induced damage to DNA. B refers to a base in DNA, the most sensitive of which appears to be thymine.

the major function of an electron-affinic nitroimidazole radiosensitizer is to enhance or potentiate the damage fixation step, becoming reduced in the process to the one electron radical anion ($RNO_2^{\cdot -}$) which is responsible for cytotoxicity or to a further reduction product yet to be characterized. Cytotoxicity then is either caused by or induced by the nitro anion radical. This model also explains why combinations of electron-affinic radiosensitizers (for example nitroimidazoles and O_2) do not show increased enhancement ratios since both compete for the same step by acting as electron sinks at the damage fixation stage [101]. The logical deduction is that both O_2 and nitroimidazoles have an identical sensitization mechanism and site and there is recent evidence in favour of this [101A]. But why should this process be selectively toxic to anaerobes or hypoxic cells? In aerobes, any nitro radical formed will be immediately scavenged by O_2 to form the superoxide radical which in the presence of superoxide dismutase is detoxicated to hydrogen peroxide and water. In cells containing superoxide dismutase, therefore, the lifetime of the nitro radical anion is insufficiently

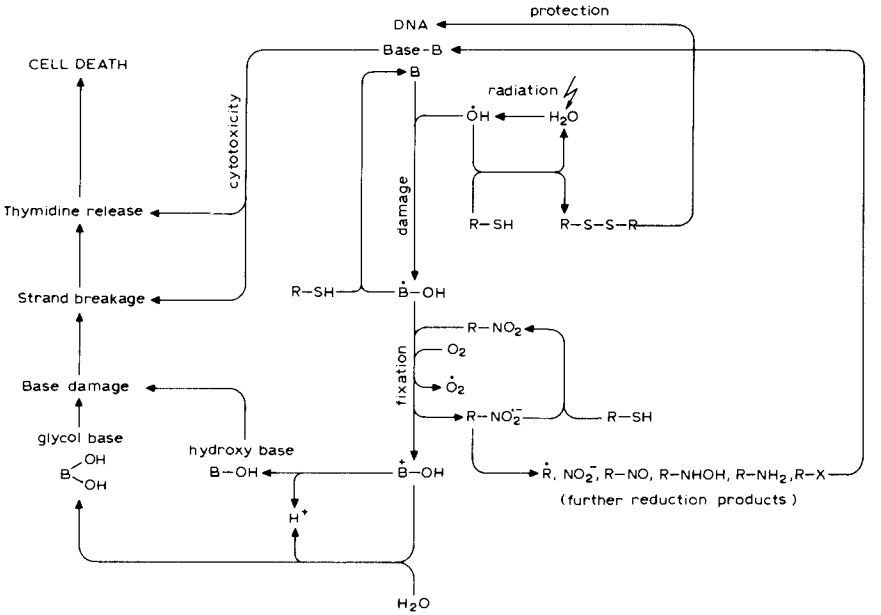

Figure 3.4. Mechanisms of nitroimidazole cytotoxicity and its relation to radiosensitization.

long to diffuse and cause damage before reaction with the enzyme. In cells without the enzyme, damage may be caused by the superoxide radical or by a further disproportionation (the Haber-Weiss reaction) to form singlet oxygen, a hydroxyl radical and hydroxide ion. These reactions have been postulated to occur as the mechanism of action of the nitrofuran, nifurtimox, which is one of the most effective drugs at present in use for the treatment of trypanosomiasis [102–105]. These reactions which are shown in *Figure 3.5* are also relevant to hypoxic tumour cells where in the presence of O_2 partial radical reactions have been observed *in vivo* [106] in mammalian cells.

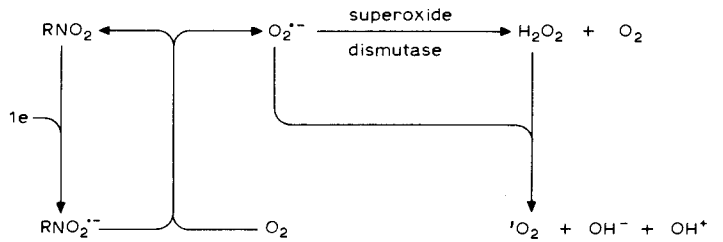

Figure 3.5. Interaction between nitroimidazoles and superoxide formation in aerobic cells.

PROTECTION OF CYTOTOXICITY BY AMINOTHIOLS

Aminothiols such as glutathione, cysteine and its decarboxylation product, cysteamine, are known to inhibit both the radiosensitizing and the anaerobic cytotoxic effects of nitroimidazoles [107]. Such compounds are generally regarded as being radical scavengers and radioprotectors. Several theories have been proposed to explain their mechanism of action including protecting against radical-induced DNA damage by binding to DNA [108, 109]. A more recent and widely accepted theory is that the SH group is involved in repairing the damage caused by radicals [110] (*Equation 1*).

$$\dot{X} + RSH \longrightarrow XH + R\dot{S} \qquad (1)$$

Here, $\dot{X}$ is a damaged biomolecule, for example, DNA and RSH repairs the damage by H-atom transfer forming a sulphydryl radical which may undergo further reactions. The inhibition of cytotoxicity observed with aminothiols may thus be due to a reaction between the nitro radical anion, $RNO_2^{\cdot -}$, of the drug and the thiol, R'SH [111] (*Equation 2*).

$$RNO_2^{\cdot -} + R'SH \longrightarrow RNO + R'\dot{S} \quad OH^- \qquad (2)$$

The above mechanism does not explain, however, the nature of protection of DNA damage by cysteamine in the electrolytic reduction system. Protection may be measured by any of those techniques used to measure DNA damage [69–72], and with cysteamine the degree of protection varies with the half-wave potential of the drug as shown in *Figure 3.6*. This effect suggests that aminothiol protection is related to the difference between the redox potential of the drug and aminothiol couple and further indicates that the aminothiol is acting as an electron acceptor as shown in *Figure 3.7*.

The precise mechanisms of the protection of cytotoxicity by aminothiols have yet to be resolved but has an important bearing on the selective toxicity of such drugs since cells containing relatively high levels of aminothiols will be less susceptible to damage.

RESISTANCE AND INACTIVATION OF NITROIMIDAZOLES

The development of resistance to any widely-used drug is of particular importance with nitroimidazoles in view of their important role in the chemotherapy

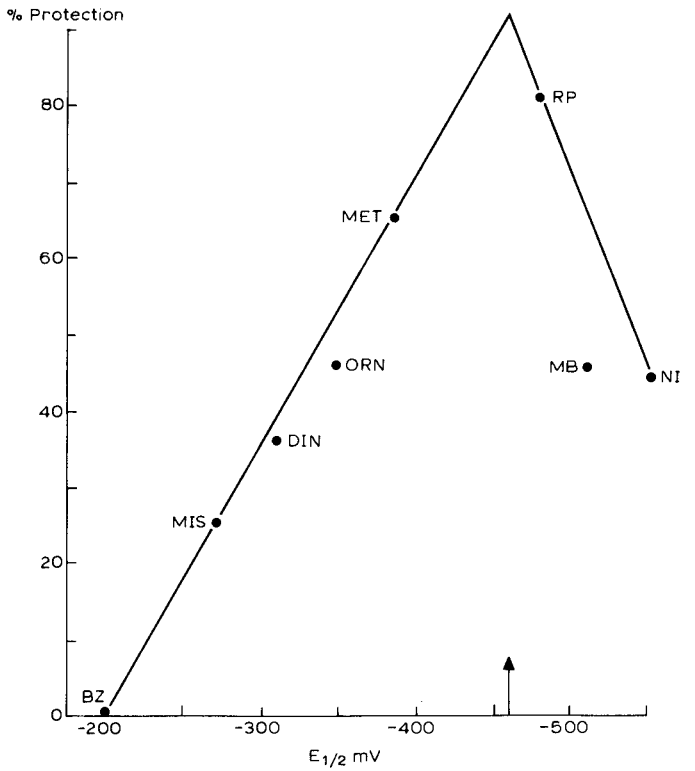

Figure 3.6. Relationship between the degree of cysteamine protection to DNA by reduced nitroimidazoles and their half wave potentials. Protection was measured as the percentage decrease in relative viscosity of DNA at a reduced drug-nucleotide ratio of 0.75. $E_{1/2}$ is the half-wave potential, pH 7.0 with a Ag/AgCl reference electrode. The arrow indicates the $E_{1/2}$ of cysteamine. The drugs are benznidazole (BZ), misonidazole (MIS), 2,5-dinitroimidazole (DIN), ornidazole (ORN) metronidazole (MET), 8609 RP (RP), M&B 4998 (MB) and 4(5)-nitroimidazole (NI).

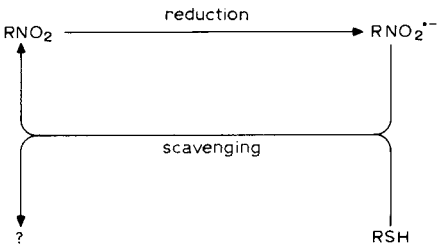

Figure 3.7. Interaction between aminothiols and nitroimidazoles.

of anaerobes. It is surprising therefore that in the 20 years of clinical use of metronidazole and other nitroimidazoles, very few cases of resistance have been reported. Treatment of trichomonal vaginitis with nitroimidazoles occasionally results in clinical failure [112–114] but the protozoan is usually fully sensitive to the drug *in vitro*. In such cases the vaginal flora includes many bacteria including *Streptococcus faecalis, Escherichia coli,* [115] and occasionally Proteus, Klebsiella and Mimae [114, 115]. Often the apparent resistance can be overcome by doubling the normal drug-dose, suggesting that organisms present in the vagina are capable of absorbing or binding the drug, thus reducing the drug concentration below therapeutic levels. Ralph and Clarke [116] noted that metronidazole was inactivated by anaerobes which were killed, the disappearance of drug (metronidazole) from the medium being related to the death curves. With aerobes, however, there was no significant loss of viability but significant loss of drug, suggesting an inactivation mechanism. More recently it has been shown *in vitro* that metronidazole and misonidazole are bound by a variety of organisms without significant viability loss, for example, by *E. coli* alone or in combination with *Streptococcus faecalis* and *K. aerogenes* [117]. In contrast, Proteus and Candida which are frequent inhabitants of the vagina are not capable of absorbing or inactivating metronidazole or misonidazole [117].

Further interest has been aroused in this aspect of nitroimidazole action by the observation that metronidazole exerts a significant bacteriocidal effect on facultative anaerobes under enhanced anaerobic conditions using glucose and ascorbate as supplements to normal media [118, 119]. Glucose increases pyruvate levels which would enhance the rate of drug reduction by a pyruvate dehydrogenase or phosphoroclastic reaction whereas ascorbate lowers the redox level of the culture also to facilitate drug reduction [120]. The relevance of these findings *in vivo* is a matter of speculation at present, but it highlights the important point that the sensitivity of an anaerobe or facultative anaerobe varies with the redox potential of the medium. It is this factor which has not been taken into account in many reports of metronidazole-resistant *T. vaginalis* which are the result primarily of faulty anaerobic technique [121–124]. The precise redox levels in the vagina and other relevant clinical environments, for example, pus, need to be established before the relevance of the *in vitro* data can be assessed and experiments to establish this are in progress in our laboratory.

The situation is somewhat clearer with the anaerobic bacterium *Bacteroides fragilis* which can grow only under enhanced anaerobic conditions. The first report in 1977 was the account of resistance to metronidazole in *B. fragilis* peritonitis following an appendicectomy but no *in vitro* levels of resistance were determined [125]. However, a resistant strain of this organism was isolated from a patient who had received over 2 kg of metronidazole over 3.5 years of

continuous therapy [126, 127]. The strain had a minimum inhibitory concentration of 32 μg/ml. This resistant strain had no extrachromosomal DNA and metronidazole uptake was markedly less than that of a strain susceptible to the drug [128]. However, since uptake is dependent upon the intracellular rate of reduction of the nitro group, these findings do not indicate an altered permeability mechanism in the resistant organism but an altered reduction mechanism [128]. Such a mechanism has been shown to occur in metronidazole-resistant strains of *B. fragilis* which have been mutated by powerful alkylating agents [129]. In this study, mutant strains and Ingham's resistant strain [126] showed depressed levels of pyruvate dehydrogenase and altered end-products of glucose metabolism [129]. Similar results have been obtained in our laboratory (Rowley D.A. and Edwards D.I., unpublished), indicating that the inability to utilize pyruvate properly could be a major resistance mechanism amongst anaerobic bacteria.

In *T. vaginalis,* true resistance does exist [130] but to date no data are available concerning the biochemical mechanisms involved. Of the strains isolated in the USA, it appears that resistance does not involve pyruvate utilization since all the strains are susceptible anaerobically to metronidazole but involve increased resistance to the normal inhibitory effect of oxygen [130]. However, no *T. vaginalis* strain yet isolated is resistant to nitroimidazole concentrations in excess of those obtained clinically.

MUTAGENICITY AND CARCINOGENICITY OF NITROIMIDAZOLES

Since a major target of nitroimidazoles is DNA, it might be expected that such drugs may show mutagenic and carcinogenic toxicity related to the mechanism of action in susceptible anaerobic and hypoxic cells. There is no doubt that mutagenicity of many nitroimidazoles may be demonstrated but often the results have been misinterpreted when relating them to drug use in chemotherapy. The standard and commonly used method for mutagenicity testing is that developed by Ames [see 131, 132] in which strains of histidine-requiring *Salmonella typhimurium* are exposed to the chemical under test and the numbers of colonies which have reverted to the wild-type measured. The significance of the test and, more particularly, its relevance to potential carcinogenicity is the subject of much discussion and in the case of nitroimidazoles is compounded by many workers failing to take account of the mechanism of action of the drugs [133].

Of the clinically relevant nitroimidazoles tested in this way, those found to give a positive Ames test are metronidazole [134–139] (which also gives positive mutagenicity results with other systems [140]), dimetridazole [134, 135, 137]

tinidazole [137, 138, 141, 142], ipronidazole [142], ronidazole [135, 143], nimorazole [135, 137], ornidazole [142], benznidazole [144] and misonidazole [138]. In addition, metronidazole has been found after therapeutic dosing to be mutagenic in the urine of mice using the Ames test [145, 146] and marginally in the host-mediated assay in mice [145]. In those Ames tests which did not include a microsomal liver fraction, mutation occurs generally only under anaerobic conditions and occurs both aerobically and anaerobically with the microsomal fraction included. It is not generally appreciated that the microsomal preparation added to convert drugs to possible mutagenic metabolites has a high respiratory quotient and utilizes large amounts of available O_2 rapidly making the environment anaerobic. It is possible therefore that nitroimidazoles become reduced under these conditions and the mutagenicity observed is that of the reduced drug and not the parent compound. Lindmark and Muller [137] have demonstrated unequivocally that mutagenesis in the Ames test only occurs under anaerobic conditions or when the nitroimidazoles are reduced. Since reduction occurs only under conditions of low redox potential which is unattainable in human cells, the relevance of Ames-type mutagenicity testing to potential human toxicity is questionable and may well be irrelevant.

Reports of carcinogenicity of nitroimidazoles are conflicting. The first report [147] indicated that metronidazole caused lung tumours at high dose levels in Swiss mice and also lympho-reticular neoplasms in females. Later studies have shown lung tumours in male mice only [148] or in both sexes [148] while in the rat (females only) one study was negative [149] while the other gave a non-significant increase in mammary tumours [148]. In hamsters, two studies have shown negative carcinogenicity [150–152]. No data on carcinogenicity studies of other nitroimidazoles have been published to date. Thus, as Roe [153] points out, increased incidences of lung tumours have been seen in three experiments in mice but two in hamsters and two in mice have given negative results. The important observation on these results is that the type of tumour developing is typical of those which arise by overfeeding leading to increased mammary tumour incidence [154–155], thereby indicating that metronidazole is not carcinogenic. In the only report in humans, a ten-year study showed that metronidazole did not increase the risk of cancer in women [156].

The present position therefore is that metronidazole and other nitroimidazoles are mutagenic in bacteria as a consequence of nitro group reduction but appear not to be mutagenic in human cells, according to methods involving the dominant lethal test in rats [157], the micronucleus test in mice [158], *in vitro* studies of unscheduled DNA synthesis in human lymphocytes and fibroblasts [157, 159], and chromosome aberration frequency tests and mitotic indices in human lymphocytes [160].

THE BIOCHEMICAL BASIS OF SELECTIVE CYTOTOXICITY OF NITROIMIDAZOLES

In the discussion thus far, it is evident that a major mechanism of action of nitroimidazoles is to damage DNA by specific excision of deoxythymine and d-thymine nucleosides and nucleotides. In this respect, the drugs have an action not unlike bleomycin which also exhibits a thymine-specific nuclease activity [161, 162]. This mechanism of action prompts the question: what relevance does the molecular action of nitroimidazoles have to their antimicrobial and antitumour activity?

It is logical to assume that microbes would be more susceptible to nitroimidazoles if they possessed a DNA of high A + T content. *Table 3.2* shows

Table 3.2. RELATIONSHIP BETWEEN DNA BASE COMPOSITION OF MICRO-ORGANISMS AND THEIR SUSCEPTIBILITY TO METRONIDAZOLE. MIC IS THE MIMIMUM INHIBITORY (MICROBICIDAL) CONCENTRATION.

Organism	MIC ($\mu g/cm^3$)	% A+T
Entamoeba histolytica	0.5 – 5.0	73–78
Clostridium bifermentans	0.1 – 2.0	73
Trichomonas vaginalis	0.5 – 5.0	71
Fusobacterium fusiformis	0.1	68.5
Veillonella parvula	1.6	63.5
Bacteroides ovatus	0.5	60
Bacteroides fragilis	1.0	58
Bacteroides distasonis	2.0	56
Bifidobacterium bifidum	3 – 16	42
Rhodopseudomonas acidophila	25	35
Actinomyces SPP.	32	30

for a variety of organisms that there is a trend which demonstrates that organisms of high A + T content are more susceptible to metronidazole, at least as evidenced by MIC determination. Moreover, it is germane that those organisms against which metronidazole is clinically most effective (trichomoniasis, amoebiasis and Bacteroides infections) are those with DNA of particularly high A + T contents and the lowest MIC values. One factor, therefore, which contributes to the selective toxicity of nitroimidazoles in anaerobic micro-organisms is the relative base composition of its DNA. This feature may not be so significant for nitroimidazole action in hypoxic tumour cells since both normal and tumour cells have similar base composition (*ca* 50% A + T), but it may be important in the future treatment of hypoxic tumours if a suitable drug combination can be logically developed to attack different targets in DNA. In this respect, it has

been shown *in vivo* that there is a strong enhanced interaction between nitroimidazoles and alkylating agents in experimental tumours [163].

DNA damage is caused solely by reduced nitroimidazoles and consequently the second factor in their selective toxicity is the relative ease or difficulty of reduction of the nitro group. This factor is governed by the electron affinity of the nitro group which in turn is related to the redox potential. More electron-affinic compounds (that is, those which are more easily reduced) have more positive redox potentials and in general the order of decreasing electron affinity is 5-nitrofurans, 2-nitroimidazoles, 5-nitroimidazoles and 4-nitroimidazoles. For example, 5-nitrofurans have a range of about −200 mV to −340 mV. An aerobic cell has many redox reactions within this range, the most negative being NAD-NADH (about −350 mV), thus nitrofurans will be reduced in aerobic as well as anaerobic cells and consequently are not selectively toxic to anaerobes. In contrast, 5-nitroimidazoles range from about −370 mV to −540 mV and so cannot be reduced in aerobic cells. They are thus selectively toxic to anaerobes.

The redox potentials of hypoxic cells in tumours are not known but will be lower than the corresponding oxic cells. Reduction of the nitro group of nitroimidazoles does occur in the absence of radiation since the 5-amino derivative of misonidazole has been detected in the urine of patients receiving the drug [95]. Reduction during radiotherapy may be potentiated because radiation itself will reduce nitroimidazoles but not under oxic conditions [94].

The rate of nitro group reduction in susceptible cells is also important since the reduction process generates a concentration gradient which facilitates entry of the drug into the cell [60]. As mentioned previously, the level of a pyruvate phosphoroclastic or dehydrogenase complex in a cell will determine its level of susceptibility to nitroimidazoles [129], as will the level of intracellular aminothiols which may protect against the fixation of the drug-induced lesion.

The cytotoxic effect of nitroimidazoles is caused by a short-lived reduced compound which as yet is uncharacterized. Studies on electrolytically-reduced metronidazole and misonidazole indicate the formation of at least seven reduction products from misonidazole and six from metronidazole. None show any cytotoxic activity towards typically sensitive anaerobes which may suggest inactivity *per se* or an inability to penetrate the cell because of their relatively increased polarity compared with the parent drug [164]. A new approach to elucidating the identity of the active intermediate uses the techniques of coulometry in which the number of electrons added to each drug molecule may be measured during electrolytic reduction. Such measurements may reveal valuable insights into the probable nature of the reduction intermediates of any nitro-compound since a possible six electrons may be involved in a full reduction (*Equation 3*).

$$RNO_2 \xrightarrow{1e} RN\dot{O}_2^- \xrightarrow{1e} RNO \xrightarrow{2e} RNHOH \xrightarrow{2e} RNH_2 \qquad (3)$$

Nitro radical anion — Nitroso — Hydroxylamine — Amine

Misonidazole on electrolytic reduction shows an electron requirement of 4.01, indicating the probable formation of the hydroxylamine derivative. In the presence of DNA, however, this value changes to 3.88 (the decrease is a significant one). A similar situation exists with 4(5)-nitroimidazole which requires 6.08 electrons (indicating possible amine formation) which decreases to 5.5 in the presence of DNA. These results [164] may be interpreted as the drug abstracting electrons from DNA causing strand breakage and the reduction product either decomposing or undergoing further reduction. Similar reduction values have been obtained by other workers for a variety of nitroimidazoles [165, 166].

In contrast to the above reduction data, there are recent reports of a different reduction pathway leading tot the formation of a nitrite ion NO_2^-; and an imidazole radical [167] (*Equation 4*).

$$RNO_2 \xrightarrow{1e} RN\dot{O}_2^- \longrightarrow \dot{R} + NO_2^- \longrightarrow \qquad (4)$$

The imidazole radical may undergo further reactions and together with other reduction intermediates shown above may be involved in the cytotoxic mechanism of nitroimidazoles. Further data will be necessary in order to elucidate the molecular basis of selective toxicity of nitroimidazoles and to discover the reduction species responsible for damage at the biological target.

NITROIMIDAZOLES AND THE QSAR APPROACH

Quantitative structure-activity relationships (QSAR) have assumed great importance in drug design since the work of Hansch [168, 169]. In this approach the physico-chemical characteristics or properties of a molecule are identified, quantified and correlated with a known important parameter of biological activity. The properties of nitroimidazoles appear only after reduction and thus a useful parameter is the relative electron affinity of the nitro group which can be measured as the 1-electron redox potential and a suitable biological parameter such as MIC, radiosensitization efficiency or cytotoxicity.

It has been clearly shown that electron affinity can be correlated with radiosensitization efficiency or cytotoxicity for a wide variety of 2-, 4-, and 5-nitro-

imidazoles and 5-nitrofurans [170–172]. That the correlation is not an exact one is probably due to factors which influence drug behaviour, for example, lipid solubility and charge, both of which influence the rate of entry into the cell and the nature of any side chains present which may sterically hinder entry or binding of the drug to essential cellular targets. The available data indicate that drugs of higher electron effinity (that is those having more positive redox potentials) are more efficient radiosensitizers and are more cytotoxic [170–172]. Using different techniques, however, Reynolds [173] has shown that the activity of 19 nitro compounds against *Bacteroides fragilis* (an anaerobe) may be correlated with electron affinity but significantly in the reverse direction than reported previously for radiosensitizing efficiency [172], cytotoxicity [174] and mutagenicity [175].

What appears to be needed to reconcile these two conflicting viewpoints is the determinations of the relative stability of the nitro radical anion and its correlation with suitable biological activity parameters, because it is the nitro radical anion which is responsible directly or indirectly for biological damage. The QSAR approach, however, is a valuable one and may ultimately lead to the development of nitroimidazoles with enhanced activity and less toxicity.

ACKNOWLEDGEMENTS

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4 Biologically Active 1,2-Benzisothiazole Derivatives

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INTRODUCTION

Benzisothiazoles are bicyclic compounds consisting of a benzene and an isothiazole ring. The two different modes of fusion of the homocyclic and the heterocyclic rings lead to two isomeric systems, viz., 1,2-benzisothiazole (1) and 2,1-benzisothiazole (2). Comprehensive reviews [1, 2] of the chemistry of both of them have been published. The more recent of the two reviews also provides a

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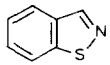

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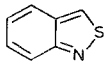

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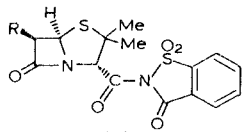
synopsis of their biological activities, reported in the literature up to 1970 [2]. In the present review, biological activities of 1,2-benzisothiazoles, reported between 1971 and 1979 are surveyed. Numerous examples of novel syntheses of 1,2-benzisothiazole and their conversion to other heterocyclic systems [3], many of which are no doubt of great interest from a purely chemical view point, have also been reported in the literature. They have been reviewed, from time to time, in various Specialist Reviews [4] and will not be dealt with here. Synthesis of a particular compound or a series of compounds will be briefly described, only when claims have been made about their biological activities. However, a high proportion of the recent literature of 1,2-benzisothiazoles consists of patents which furnish little or no worthwhile biological information.

1,2-BENZISOTHIAZOLES AS ANTIBACTERIALS

Among the 1,2-benzisothiazoles (or their 1,1-dioxides), substituted on the nitrogen, penicillin saccharamides of general formula (3) showed promising ac-



(3)

(4) R = $PhCH_2CONH$
(5) R = $PhOCH_2CONH$
(6) R = NCO
(7) R = PhMeNNHCONH
(8) R = BuNHCONH
(9) R = $PhCH_2CMe_2NMeCONH$
(10) R = $NHCONH(CH_2)_3N(CH_2)_4O$

tivity against Gram positive bacteria. These compounds were developed [5] with the idea of preventing their unduly rapid excretion from the host system and prolonging their action by virtue of their insoluble nature. They were prepared [6] by reacting saccharin with either penicillanic acid or its potassium salt carrying a suitable substituent in the 6-position. In a comparative study [5, 7] with benzathine penicillin V (DBED penicillin), Yurchenco, Hopper, Vince and Warren

demonstrated the superiority of (4) and (5) over the benzathine in treating infections caused by *Streptococcus pyogenes* C 203, which has a great capacity to invade tissues. These compounds did not, however, show any particular advantage over benzathine penicillin V in their effectiveness against *Staphylococcus aureus* Smith. In mice infected with either of these micro-organisms, (4) and (5) were equally effective, when administered four days prior to infection. However, (4) proved to be twice as active when given eight, twelve or sixteen days prior to the challenge. It was suggested [6] that the enhanced therapeutic effectiveness of (4) is due to its greater ability to release free penicillin G and to mobilize the leukocytes. When the 6-isocyanato derivative (6) was treated with amines or substituted hydrazines, ureido or semicarbazido derivatives were obtained [8]. Of these, compounds (7), (8), (9) and (10) have shown activities against *S. aureus* Smith at concentrations less than 10 or 50 μg/ml [9].

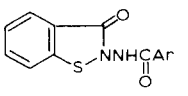
Aromatic acid hydrazides carrying appropriate substituents on the benzene ring, on base-catalyzed condensation with the acid chloride of 2′-carboxyphenylsulphenyl chloride gave compounds of the general formula (11) [10] which are active against *Mycobacterium tuberculosis*, *Diplococcus pneumonia*, *Mycoplasma gallisepticum* and Hella cell.

Acid-catalysed condensation of *N*-hydroxymethyl-3,5-dibromosalicylamide with saccharin in boiling ethanol [11] led to the amidomethylated product (12). This compound among others was reported to have been tested on various Gram positive and Gram negative bacteria including *Staphylococcus aureus* and *Streptococcus pyogenes*, but the authors have not supplied any data on the result of these tests for this particular member of the series.

Claims have also been made [12] about the bactericidal and fungicidal properties of the N-substituted 1,2-benzisothiazolinones (13–17), obtained by the action of the appropriate sulphenyl chloride on the sodium salt of 1,2-benzisothiazolin-3-one.

Among the bactericidal 1,2-benzisothiazole derivatives with a substituent in the 3-position, compound (18) was obtained in high yield, when saccharin was refluxed with an organic sulphonyl chloride in the presence of a base [13]. The synthesis obviously involves conversion of one molecule of saccharin into its 3-chloro derivative; the chlorine atom of the latter undergoes nucleophilic displacement by the hydrogen on the 2-position of another molecule of saccharin. Nucleophilic displacement of the same chlorine atom by allyloxy [14] and phenoxy [15] groups has also been claimed to give compounds with antimicrobial properties.

Chinese workers have reported [16] the mutagenicity of the 3-substituted 1,2-benzisothiazole using *Bacillus subtilis* and *Salmonella typhimurium* as test micro-organisms, and they found that the compound was mutagenic towards both.



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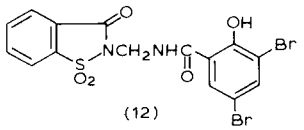

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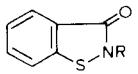
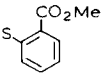
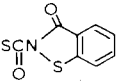
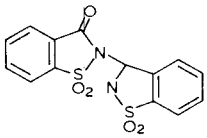


(13) R = $SCHMe_2$ (14) R = SPh

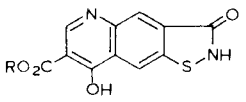
(15) R =  (16) R = $S(CH_2)_6SH$

(17) R = 



(18)

Benzisothiazoloquinolones (19) and (20) have been claimed to have bactericidal properties [17], but no specification has been made about the type of bacteria which are sensitive to them.



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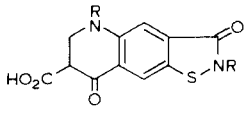

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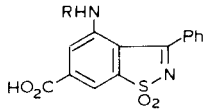
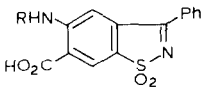


(21)

1,2-BENZISOTHIAZOLES AS DIURETICS

Several 1,2-benzisothiazole derivates have been reported to possess both diuretic and saluretic properties, which means that they are able to increase the volume of urine and at the same time facilitate the excretion of sodium ion from the system. Danish workers [18, 19] have reported the synthesis of a series of 3-phenyl-1,2-benzisothiazole-6-carboxylic acid 1,1-dioxides with an amino or substituted amino function in the 4- or 5-position (21, 22) as well as the dihydro compound (23). The free acids and their sodium salts were screened in dogs and

their efficiencies were evaluated on the basis of the total volume of urine per kilogram of body weight and the mole equivalent of Na^+ ion excreted. Their activities were compared with those of known sulphonamide diuretics like *N*-(2-furylmethyl)-4-chloro-5-sulphamylanthranilic acid (furosemide) [20] or 3-*n*-butylamino-4-phenoxy-5-sulphamylbenzoic acid (bumetanide) [21].



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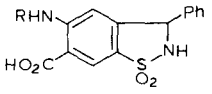

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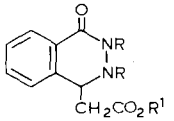
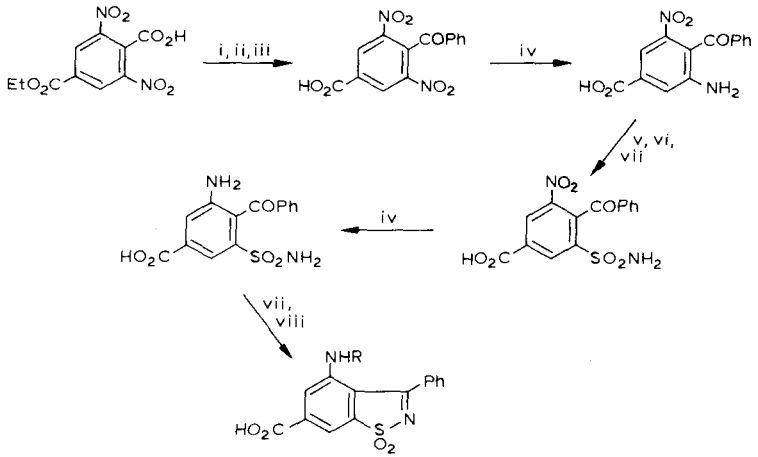


(24)

Of all the compounds tested, 5-(2-furylmethyl)-3-phenyl-1,2-benzisothiazole-6-carboxylic acid 1,1-dioxide proved to be the most potent, with a diuretic profile similar to that of bumetanide and furosemide. The volumes of urine (in ml/kg) per 3 hours, after the administration of 1, 0.1 and 0.01 mg/kg of this compound were 44, 26 and 10 with corresponding mole equivalents of Na^+ ions as 4.8, 2.7 and 1.0, respectively. Furosemide and bumetanide when given in doses of 0.01 mg/kg result in the excretion, respectively of 8 and 10 ml of urine (per kg, per 3 hours) while the Na^+ ion concentration was 1.0 mol equivalent in each case.

Synthesis of both (21) and (22) involves ring closure of an appropriately ortho-substituted sulphamoyl benzoic acid. *Scheme 4.1* shows the synthesis of



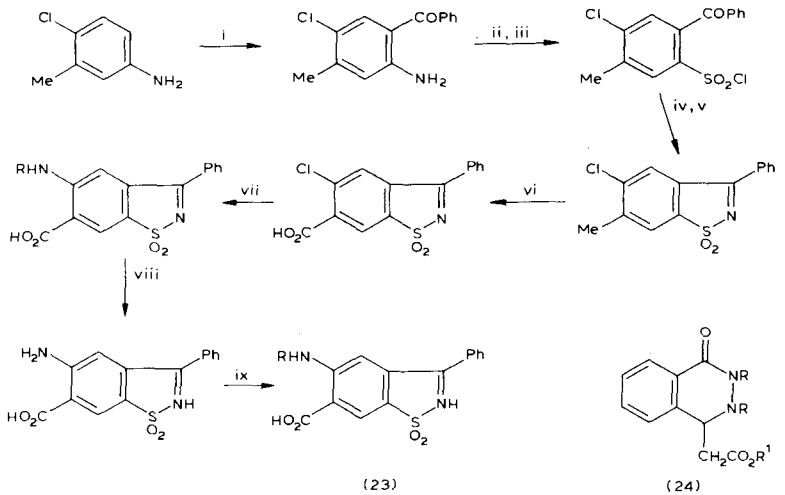
Scheme 4.1

i, $SOCl_2$; ii, C_6H_6, $AlCl_3$; iii, NaOH; iv, $Na_2S_2O_4$; v, $NaNO_2$, HCl; vi, SO_2, $CuCl_2$; vii, NH_3; viii, RCl.

(21) from 4-ethoxycarbonyl-2,6-dinitrobenzoic acid. Compounds of the series (22) have been synthesized *via Scheme 4.2*. The saturated compound (23, R=H) was obtained when the 5-benzylamino derivative (22, R=$PhCH_2$) was debenzylated with hydrogen and palladium charcoal. The amino group in the 5-position could be realkylated to give the corresponding substituted amino derivative.

It was suggested that the isothiazole ring may be cleaved under physiological conditions and the potent diuretic effect manifested by these compounds may be due to the interaction of the corresponding benzoylsulphamoylanthranilic acids with the receptor. The cyclic and acyclic systems exist in a dynamic equilibrium in the plasma. Infrared spectra of the aqueous solutions of these compounds, however, indicate the presence of only traces of the ring-cleaved structure.

Another group has reported a large number of *N*-substituted and *N, N'*-disubstituted phthalazines [22, 23] of the general structure (24) where R^1=H or alkyl, and R is an aromatic or heteroaromatic substituent including 1,2-benzisothiazole substituted in the 5- or 6-position. These compounds are claimed to be potent diuretics and saluretics. The screening was carried out in the same way as described above and the compounds were administered orally, rectally or parenterally.



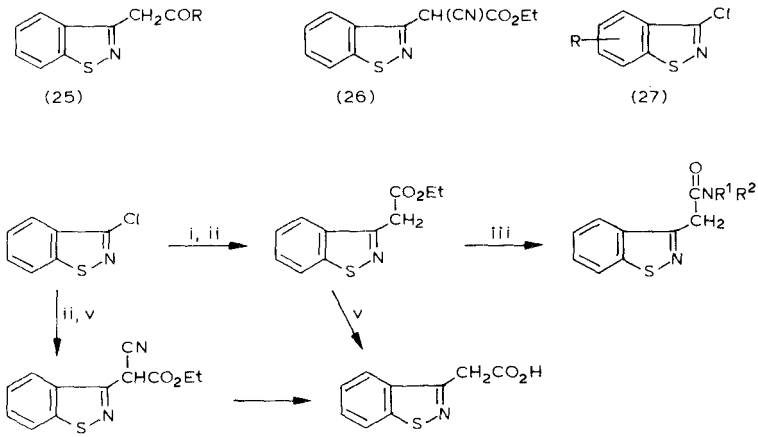
Scheme 4.2

i,$PhCOCl,ZnCl_2$; ii, $NaNO_2$,HCl; iii, SO_2,$CuCl_2$; iv, NH_4OH; v,NH_3; vi, $KMnO_4$; vii, RBr; viii, Pd-C; ix, RCl.

1,2-BENZISOTHIAZOLES AS SYNTHETIC AUXINS

A number of 3-substituted 1,2-benzisothiazole derivatives have shown auxin-like properties and have been used as herbicides. While the level of natural auxin, indol-3-ylacetic acid (IAA), a plant growth hormone, is maintained in the plant body by IAA oxidase, its synthetic analogues escape such control. The result is severe morphological aberration such as stem elongation, epinasty, stunting of young leaves and ultimate death [24]. Depending on its selectivity, a synthetic auxin can be used as a phytotoxic agent in weed control.

Italian workers [25–27] have reported the synthesis, chemical characteristics and phytotoxic activity of several 1,2-benzisothiazol-3-ylacetic acid derivatives (25, R=OEt, OH, NR^1R^2) and (26). These compounds have been synthesized from 3-chloro-1,2-benzisothiazole (27, R=H) as shown in *Scheme 4.3.*



Scheme 4.3

i, $CH_2(CO_2Et)_2$; ii, NaOET; iii, NHR^1R^2; iv, NaOH; v, $CH_2(CN)CO_2Et$;

Phytotoxic activity has also been claimed for certain 4-, 5- and 7-substituted 3-chloro-1,2-benzisothiazoles (27, R=4-Cl, 5-Cl, 5-MeO, 5-NO_2 and 7-Me) which are obtained in good yield by chlorinating the corresponding 1,2-benzisothiazol-3-one [28].

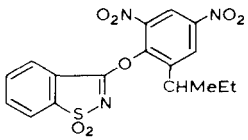
The weeds generally controlled by these compounds are: *Amaranthus retroflexus, Artemisia vulgaris, Chenopodium album, Convolvulus sepium, Echinocloa crus-galli, Setaria glauca* and *Vicia sativa* while crops like oats, beans, peas, wheat and corn are left relatively unharmed. The compounds have been claimed to show greater selectivity compared with other known synthetic auxins like indol-

3-ylbutyric acid or 2,4-dichlorophenoxyacetic acid (2,4-D). They are equally effective in pre- or post-emergence application and in relatively small amounts (0.5 to 8 kg obligue hectare).

Structure-activity relationships in these compounds have been discussed [29] and an even number of carbon atoms in the side chain has been shown to be essential for auxin-like properties. The effect of these compounds on the growth of pea-tissues and Jerusalem artichoke tubers has also been described [29, 30]. An increase in both length and fresh weight of pea internodes and inhibiton in the development of roots was reported. Increase in the production of ethylene was also noticed and acids were more active than esters and amides in inducing this effect. Cell multiplication and hydration in Jerusalem artichoke tubers were stimulated by these compounds which showed a degree of activity comparable with that of IAA.

The Italian workers have also reported the auxin-like properties of 3-acylamino- [31] and 3-tetrazolylmethyl- [32] 1,2-benzisothiazoles. The former was also tested for Hill reaction inhibition, a property generally shown by herbicides that act by interfering with photosynthesis [24]. The highest inhibition of the Hill reaction was shown by 1-chloro-3-*n*-propylamino-1,2-benzisothiazole. However, no correlation was observed between inhibition of the Hill reaction and auxin-like activities.

3-(2-s-Butyl-4,6-dinitrophenoxy)-1,2-benzisothiazole 1,1-dioxide (28), on pre-emergence application, has been reported to have completely controlled crabgrass, pigweed, wild black wheat, smart weed and ryegrass, with little phytotoxicity to cotton, corn, wheat and soybean. Another 1,2-benzisothiazole 1,1-dioxide that has been reported to have controlled broadleaf plants [34] was *O*-(1,2-benzisothiazol-3-yl-2,6-dichlorobenzaldoxime 1,1-dioxide (29).



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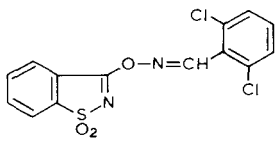


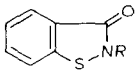
(29)

1,2-BENZISOTHIAZOLES AS FUNGICIDES

The fungicidal properties of several 1,2-benzisothiazole derivatives reported over the last decade make them useful as agro-chemicals. Japanese workers paid particular attention to the use of these compounds in controlling rice blast diseases caused by *Piricularia oryzae, Xanthomonas oryzae* or *Pseudomonas*

glumae [32–45]. Development of a resistant strain of the crop remains, however, the best way to combat these diseases.

Direct substitution in the 2-position of 1,2-benzisothiazolin-3-one 1,1-dioxide led to the compounds of the series (30) where R is 1,3-bis(carbamoylthio)-2-dimethylaminopropyl [35], 1,2-benzisothiazole-1,1-dioxo-3-yl [36], or benzylcarbamoyl [41]. All these compounds have been claimed to have inhibited *Piricularia oryzae*. Equally effective [41] against *Piricularia oryzae* and *Xanthomonas oryzae* were the compounds (31) where R is alkyl, alkoxyl or phenyl and



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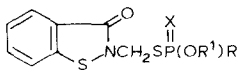
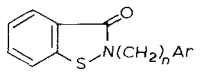


(31)

R^1 is alkyl; X is either sulphur or oxygen. Among the 1,2-benzisothiazole 1,1-dioxides with a substituent in the 3-position, 3-(*N*-cyano-*N*-methylamino)-1,2-benzisothiazole 1,1-dioxide completely controlled rice blast [36]. A large number of 3-alkoxy, -allyloxy, -cyano-alkoxy or benzyloxy derivatives also proved effective. One of the compounds which received particular attention was the 3-allyloxy derivative, marketed under the name probenazole. It gave best results when sprayed just before inoculation [43, 46]. Ideal field conditions consist of application with cupric hydroxide and nickel dimethyldithiocarbamate during August and September [45–48]. However, probenazole proved to be more effective in *in vivo* systems than in *in vitro* tests [49]. Studies have been carried out on the toxicity of probenazole in rats [50] and fresh water organisms [51]. When the compounds were administered intraperitoneally, LD_{50} values for male and female rats were 880 and 850 mg/kg respectively, corresponding values for oral administration being 2160 and 2350 mg/kg respectively. When tested in mice, wight loss, decrease in resistance towards infection, and histopathologic changes in liver at high doses were noticed. 3-Alkoxy-1,2-benzisothiazole 1,1-dioxides also proved effective against *Phytophthora infestans* infection in tomato [50], and *Rhizoctonia solani* in cucumber seedlings were prevented by 2-aryl-1,2-benzisothiazole and its 1,1-dioxide [54].

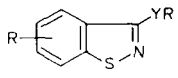
The 2-substituted 1,2-benzisothiazolin-3-one (32) [52, 53] and the 3-substituted 1,2-benzisothiazoles (33–35) [55–59] proved effective against *Candida albicans* and *Tricophyton mentogrophytes*. The phenyl ring in the benzyl group in (32) contained a 4-alkoxy group whose size influenced the antimycotic behaviour of the compound. These compounds were synthesized *via* cyclization of various 2-carboxyphenyl disulphides. Nucleophilic displacement of the chlorine atom in 3-chloro-1,2-benzisothiazole with phenol or thiophenol led to the compounds of the series (33). Their fungicidal activities were tested by adding them

to the nutrients of the pathogenic fungi (minimal inhibitory concentrations 4 to 20 μg/ml) as well as by local application on infected animals. Compounds of type (34) were obtained from 3-acylimino-3H-1,2-benzodithioles by treating with hydroxylamine, followed by mild acidic hydrolysis. *In vitro* tests carried out both in the presence and absence of serum showed that the minimal inhibitory concentration varies between 4 and 100 μg/ml. The 3-(γ-iodopropargyloxy) derivatives (35) were effective at 0.3-1 μg/ml level.



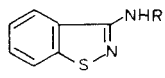
n = 0,1

(32)



Y = O, S

(33)



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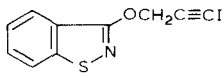


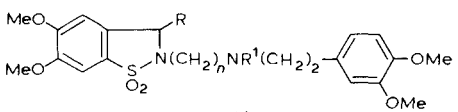
(35)

3-Oxo-1,2-benzisothiazolin-2-carboxamide 1,1-dioxides [60, 61] and 3-haloalkyl-1,2-benzisothiazole [62] have also been claimed to posesses antifungal properties.

Recently, the fungicidal properties of 1,2-benzisothiazole derivatives have found a useful application in the paint industry. When added to vinyl acetate or acrylic paints, 1,2-benzisothiazolin-3-one, either on its own [63] or together with 2-benzimidazolecarbamic acid [64], prevented the growth of *Aspergillus niger*, *Penicillium citrinum*, *Trichoderma* and *Alternaria*.

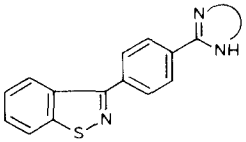
1,2-BENZISOTHIAZOLES IN HEART DISORDERS

2-[(3,4-Dimethoxyphenethyl)amino]alkyl-1,2-benzisothiazoline 1,1-dioxides (36, R=H, Ph; R^1=H, Me, Pr; n=2,3) have been shown to decrease the heart rate of animals [65]. They were obtained by reacting the corresponding *N*-3-chloropropyl-1,2-benzisothiazole 1,1-dioxide with 3,4-dimethoxyphenylethylamine. In guinea pigs, under narcosis induced by urethane, doses of 0.5 to 20 mg/kg of this compound reduced heart rate by 9.8 to 53.9 per cent.



(36)

Several 3-aryl-1,2-benzisothiazoles as well as compounds of the series (37) obtained from 3-p-tolyl derivatives *via* photochlorination and subsequent reaction with a diamine, have also been reported to possess powerful anti-arrhythmic properties. They were tested in rats under narcosis induced by thiobutabarbitone. One hour after treating the animals with the compounds under investigation, aconitine was given as an arrhythmogen. The effectiveness of the compound under investigation was estimated from the length of the arrhythmia. The compounds were found to be more than five times as active as procainamide [66, 67].



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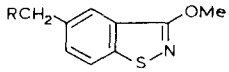

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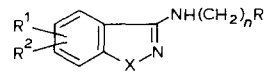

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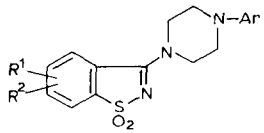

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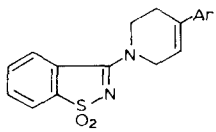

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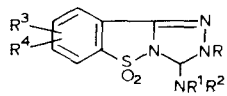


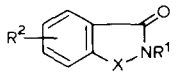
(42)

1,2-BENZISOTHIAZOLES SHOWING ANTI-INFLAMMATORY ACTIVITY

Several 1,2-benzisothiazoles and their 1,1-dioxides have shown anti-inflammatory activities. For example, 5-substituted-3-methoxy-1,2-benzisothiazoles (38) [68] have been tested for anti-inflammatory activity in the rat paw oedema test elicited by carrageenan. The 5-hydroxamic acid derivative (38, R=CONHOH) was the most promising and equal in activity to oxyphenbutazone. The 5-acetic acid derivative showed some activity while the corresponding amide and ethyl esters were inactive. These compounds also possessed analgesic properties.

The synthesis and anti-inflammatory activities of a number of 3-amino-1,2-benzisothiazoles and their 1,1-dioxides (39) (X=S, SO_2), (40) and (41) [69–72] as well as the triazolo derivative (42) are claimed in patents [73]. In all three cases the homocycle may carry alkyl or nitro group as substituent. No pharmacological data are given.

Compounds of the series (43) (X=S, SO, SO_2; R^1=$CHMeCO_2H$, CH_2CONHQ, R^2=H) have been developed as analogues of indoprofen [74] but they did not show any promising anti-inflammatory activity.



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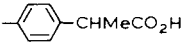
Q = 

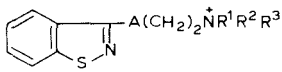
(44) $R^1 = (CH_2)_nNR^3R^4$, X = S
(45) X = S

1,2-BENZISOTHIAZOLES AS ANTITHROMBIC AGENTS

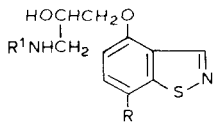
Several 2-substituted 1,2-benzisothiazolin-3-one derivatives of the general formula (44) have shown promising activities as inhibitors of platelet aggregation when administered in combination with non-steroidal anti-inflammatory agents, for example, aspirin or indomethacin [75–78]. These compounds have been synthesized [76, 78] from thiosalicyclic acid chloride dimer, by treatment with chlorine, followed by ring closure. Among the list of compounds examined for their antithrombic properties were (44) (R^2=H, halogen, nitro, amino, alkyl, alkoxy or alkanamido; n=1-12 and R^3 and R^4 may be alkyl, aryl or heteroaryl). A dose of 0.08 to 0.15 mmol/kg on oral administration inhibited collagen- and ADP-induced platelet aggregation.

MISCELLANEOUS BIOLOGICAL ACTIVITIES

The controversy regarding the carcinogenic properties [79] of saccharin still continues. Several 2-substituted 1,2-benzisothiazolin-3-ones (45) have been tested for possible teratogenic activity [80]. Of all the compounds studied, compound (45, R^1=CH_2CONH_2, R^2=H) showed an increased incidence of malformations but no accompanying elevation of foetal loss.



(46) A = O, $R^1 = R^2$
(47) A = CO_2, R^1 = Me



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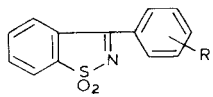


(49)

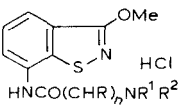
Quaternary salts of several 3-*O*-alminoalkyloxy derivates of 1,2-benzisothiazole (46, R^1=R^2=lower alkyl or -$(CH_2)_4$-) and the amino esters (47) showed antagonist properties against acetylcholine [81] and succinoylcholine [82] in different nerve-muscle preparations.

The synthesis of 4-aminoalkoxy-1,2-benzisothiazoles (48, R=H, NO_2, NH_2 or acylamino; R^1=alkyl, alkylthio or dialkylamino) from the corresponding 4-methoxy derivative by demethylation, treatment with epibromohydrin, and ammonolysis has been described [83]. The compounds showed potent β_1 and β_2 sympatholytic activities in rats (ED_{50} values 1.9 μg/kg and 4.2 μg/kg, respectively).

2,3-Disubstituted 1,2-benzisothiazole 1,1-dioxides (49, R=halogen, nitrile, alkyl, alkoxy or trifluormethyl) have been claimed [84] to possess hypotensive properties without being hypoglycaemic.

Among the twntyfive 3-methoxy-7-(alkylaminoacyl)-1,2-benzisothiazoles (50), two compounds, namely, (50, R=R^1=H; R^2=Et; n=1) and (50, R=H, R^1=R^2=Et; n=1) possessed local anaesthetic activity comparable with that of lidocaine hydrochloride [85].

N-[2-(Cyclohexylamino)ethyl]-1,2-benzisothiazole-3-carboxamide hydrochloride substituted at C-6 (51, R=Me, Cl) showed spasmolytic activity [86]. In *in vitro* tests on human myometrium strips, these compounds antagonised the contractile action of histamine.



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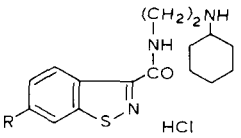

(51)

CONCLUSION

During the last decade, several kinds of biological activities have been claimed for 1,2-benzisothiazoles. The herbicidal and antifungal properties of these compounds have opened up the possibility of their potential use as agrochemicals and as veterinary medicines. However, other biological activities, that have also been claimed, are no less significant. Unfortunately, these claims in the published reports are not always accompanied by supporting data.

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5 Tilorone and Related Bis-basic Substituted Polycyclic Aromatic and Heteroaromatic Compounds

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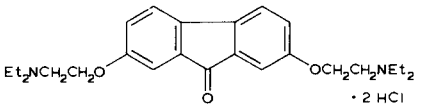
INTRODUCTION

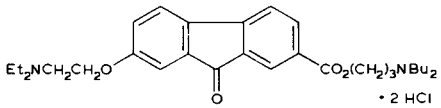
Tilorone (I, compound 262 in *Table 5.2)* was first reported in the scientific literature in 1970 in two papers [1, 2] which described the synthetic compound 2,7-bis[2-(diethylamino)ethoxy] fluoren-9-one as a broad-spectrum, orally active antiviral agent effective in mice against at least nine viruses of both RNA and DNA groups. Further, it was reported that an antiviral serum component fouund in mice treated with tilorone hydrochloride fulfills sufficient biological criteria to be classified as an 'interferon'. In the same year, a review article on antiviral agents [3] stated 'Perhaps the greatest excitement of the year was the disclosure of the interferon-inducing diamine, tilorone.'

In the ensuing decade, the mystique of interferon and interferon induction has resulted in an 'identity crisis' for the tilorone family of immuno-modulating chemicals – and has most probably set back their development as immunopharmacologic agents having therapeutic usefulness in man.

In the late 1960's, Krueger and Mayer at the Merrell Research Center (in Cincinnati, OH, U.S.A.) set up an *in vivo* screen to test chemicals for antiviral activity. Subsequently, random screening led to the demonstration that bis(3-dibutylaminopropyl)-9-oxofluorene-2,7-dicarboxylate dihydrochloride (II, compound 230 in *Table 5.2)* effective in protecting mice against lethal encephalomyocarditis infections.



(I)



(II)

Classical molecular modification of compound II resulted in the development of tilorone (I) and its analogues and their subsequent characterization as a new class of small-molecule immuno-modulating agents, many of which are orally active.

CHEMISTRY

A series of publications has presented most of the original chemistry embodied in the tilorone project. However, some of the 21 classes of bis-basic substituted polycyclic molecules have appeared only in the patent literature. Thus, this article presents for the first time a comprehensive listing of the tilorone analogues and congenors synthesized at the Merrell-National Laboratories. Further, primary screening data are included for this group of approximately 350 compounds. In

addition, some independent synthetic chemistry publications will be commented on briefly.

Bis-basic substituted polycyclic aromatic and heteroaromatic compounds are represented by generic formula III. The nuclei

$$R_2N\text{-alkyl-A- Nucleus -A-alkyl-}NR_2$$
III

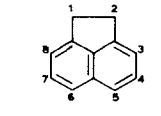
discussed in this review are presented in *Figure 5.1* and listed in *Tables 5.1 and 5.2.* Fragment A of the generic formula represents species that connect the amino-alkyl side chain to the nucleus listed in *Tables 5.1 and 5.2.* The alkyl and amino groups are listed in *Table 5.2.* The functional groups listed in the tables include esters, thioesters, amides, sulfonamides, ethers, ketones, oximes, ketooximes, alcohols, ethylenes and alkanes.

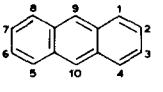
The general method of synthesis of the bis-basic substituted polycyclics is outlined in *Scheme 5.1.* References to the original literature are listed in *Table 5.1* in order of nucleus followed by side chain substituents for each class of compounds.

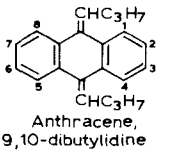
Bis-basic esters, thioesters and amides were prepared by allowing the appropriate dicarbonyl chloride to react with an aminoalkanol, aminoalkylthiol or an aminoalkylamine, respectively [7, 9*, 12, 15, 21, 23, 25]. Alternatively, bis-basic esters were prepared directly from the dicarboxylic acid by allowing it to react with an aminoalkyl chloride in the presence of catalytic amounts of benzyltrimethylammonium chloride [7, 10]. The intermediate dicarboxylic acids were prepared routinely by the Friedel-Crafts acetylation reaction followed by NaOCl oxidation. Diacids also were obtained *via* halogenation, cyanide displacement and hydrolysis.

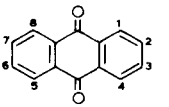
The Williamson ether synthesis was employed for the preparation of the bis-basic ethers [6, 7, 10, 12, 17, 21, 22, 25]. In situations where the dihydroxy intermediate was unstable (*i.e.*, *N*-ethylcarbazole-3,6-diol), the bis-basic ethers were derived from the corresponding diacetoxy intermediate [10]. Di-*N*-oxides of bis-basic ethers were prepared by peracid (*m*-chloroperbenzoic acid) oxidation of the immediate precursor [20]. Bis-basic thioethers were synthesized via a multi-step reaction sequence involving the rearrangement of the dimethylthiocarbamoyl derivative of the dihydroxy intermediate followed by hydrolysis and alkylation with an aminoalkylchloride [17, 25].

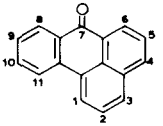
*Patent references are made to the first patent issued in order to cite an abstract as per editorial policy.

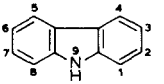

Acenaphthene

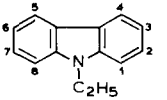

Anthracene

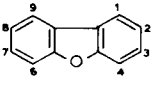

Anthracene, 9,10-dibutylidine

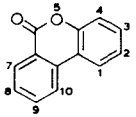

Anthraquinone

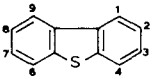

Benzanthracene-7-one

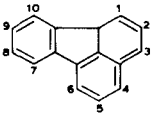

Carbazole

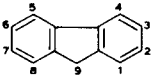

Carbazole,N-ethyl-,

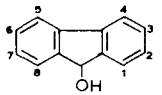

Dibenzofuran

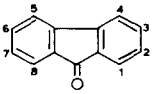

Dibenzopyran-6-one

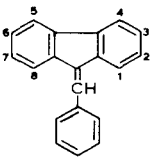

Dibenzothiophene

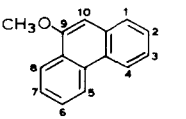

Fluoranthene

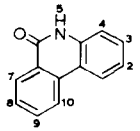

Fluorene

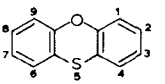

Fluoren-9-ol

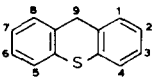

Fluoren-9-one

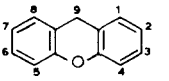

Fluoren, 9-benzylidine

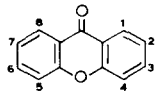

9-Methoxyphenanthrene


Phenanthridin-6-one


Phenoxathiin


Thioxanthene


Xanthene


Xanthen-9-one

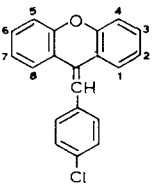

Xanthen, 9-benzylidine

Figure 5.1. Aromatic and heteroaromatic nuclei.

Table 5.1. REFERENCES TO SYNTHESIS OF BIS-BASIC SUBSTITUTED POLYCYCLICS

Chemical class	*Reference*	*Chemical class*	*Reference*
Acenaphthene		Fluoren-9-ol	
Ketones	4	Esters	15
		Amides	16
Anthracene		Ethers	17
Ketones	5	Alkanes	19
Anthracene-9,10-dialkylidene		Fluoren-9-one	
Ethers	6	Esters	15
		Thioesters	15
Anthraquinone		Amides	16
Esters	7	Ethers	17
Sulfonamides	8	Ethers-*N*-oxide	20
Ethers	7	Thioethers	17
		Ketones	18
Benzanthracene-7-one		Ketones-*N*-oxide	14
Esters	9	Alkanes	18
Carbazole		Fluoren-9-ylidene	
Esters	10	Esters	21
Ethers	10	Ethers	21
Ketones	10		
Alkanes	10	Phenanthrene-9-methoxy-,	
		Ethers	22
Dibenzofuran			
Esters	10	Phenanthridin-6-one	
Amides	10	Esters	23
Ethers	10		
Ketones	10	Phenoxathiin	
		Ketones	24
Dibenzopyran-9-one			
Esters	11	Thioxanthene	
		Ketones	25
Dibenzothiophene			
Esters	10	Xanthene	
Ethers	10	Esters	25
Ketones	10	Ethers	25
		Ketones	25
Fluoranthene		Alcohols	25
Esters	12	Ethylenes	25
Thioesters	13		
Ethers	12	Xanthen-9-one	
Ketones	12	Esters	25
Ketones-*N*-oxide	14	Amides	25
Alkanes	12	Ethers	25
		Ethers-*N*-oxide	20

Table 5.1. (continued)

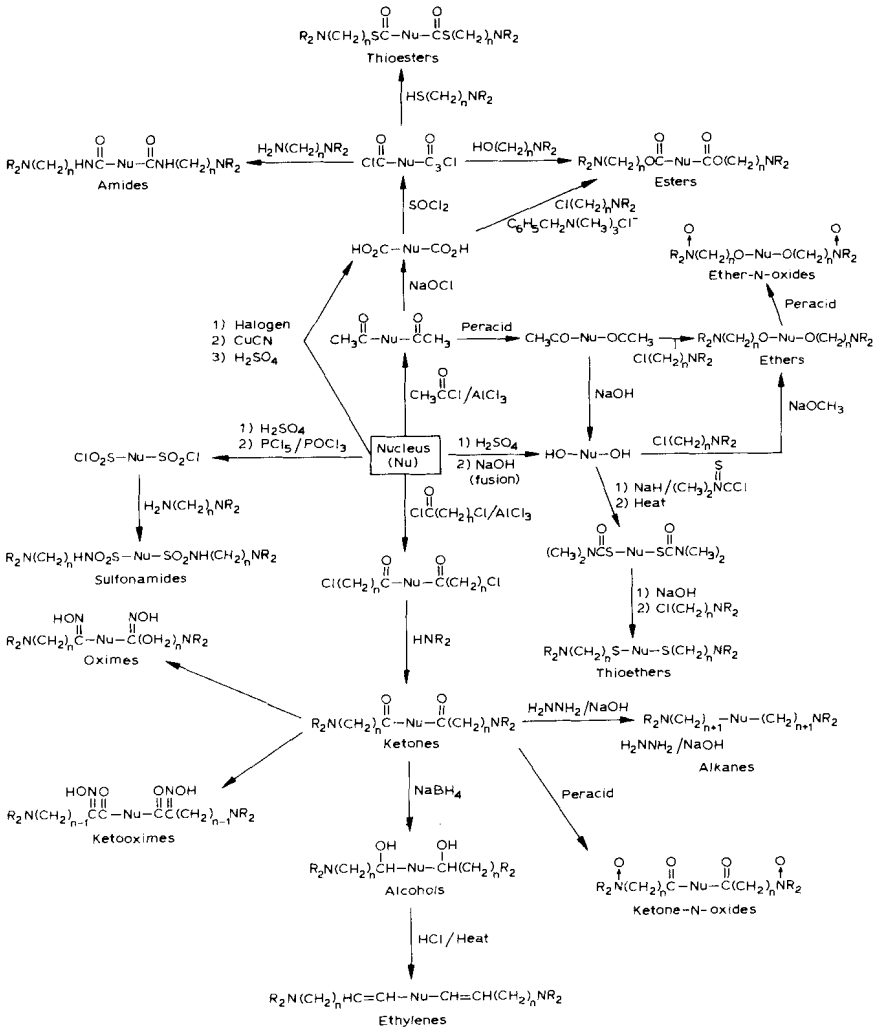
Chemical class	*Reference*	*Chemical class*	*Reference*
Fluorene		Xanthen-9-one	
Esters	15	Thioethers	25
Amides	16	Ketones	25
Ethers	17		
Ketones	18	Xanthen-9-benzylidene	
Ketones-*N*-oxide	14	Ethers	25
Oximes	18	Thioethers	25
Keto-oximes	19		
Alcohols	18		
Alkanes	18		

Bis-basic sulphonamides were prepared by amination of the disulphonyl chloride with the appropriate diamine [8]. The disulphonyl chloride was derived from the dipotassium salt of the disulphonic acid by treatment with phosphorus pentachloride-phosphorus oxychloride.

Nuclear acylation under Friedel-Crafts conditions utilizing ω-chloroalkanoyl chlorides yielded the di(ω-chloroalkanoyl) intermediates. Subsequent amination produced the bis-basic ketones [4, 5, 10, 12, 18, 24, 25]. A variety of bis basic derivatives were prepared from the bis-basic ketones including oximes [18], alcohols [18, 25], ethylenes [25], alkanes [12, 18, 19], and di-*N*-oxides [14].

Burke and Joullie [26] reported a novel synthesis for tilorone in which the intermediate 2,7-dihydroxyfluoren-9-one was obtained by sulphuric acid hydrolysis of the tetrazonium fluoroborate salt. The tetrazonium salt was also decomposed by boiling in acetic acid-acetic anhydride to yield the diacetate ester [27]. The ester was directly alkylated to give tilorone or hydrolyzed to the dihydroxy intermediate and alkylated. These authors also prepared the bis-basic ethers of benzophenone and dibenzothiophene-5,5-dioxide and reported on the antiviral and interferon properties of these compounds.

A series of carbonyl derivatives of tilorone was prepared and evaluated for antitumour activity [28]. These derivatives consisted mainly of substituted hydrazines and hydrazides. The synthesis and antiviral activity of a group of 'open model' analogues of tilorone was reported [29]. These 'open model' analogues included a group of bis-basic ethers of penten-1,5-diol and diethylene glycol as well as bis-basic esters and amides of aliphatic diacids (C_7–C_{13}). These compounds were synthesized by conventional methods.



Scheme 5.I. Synthetic routes for bis-basic substituted polycyclics.

PRIMARY SCREENING

Although the initial discovery was based on antiviral screening, the linkage with interferon led to a prompt probing of the immunological response elicited by tilorone. In the first report of immuno-modulating activity [30] it was shown that a single dose of tilorone in mice enhanced the primary immune response to sheep red blood cells and also increased serum hemolysin titres. Thus there was early recognition of the broader therapeutic potential of the tilorone family of compounds. Accordingly, the primary screening of new compounds was organized to cover two broad biological areas, as follows:

(A). *Anti-inflammatory-Immuno modulation*

(1) Passive arthus (rat).

(2) Complement inhibition (*in vitro*).

(3) Experimental allergic encephalomyelitis (EAE) in the rat.

(B). *Antiviral*

(1) Encephalomyocarditis (EMC) virus; a fatal infection (mouse) using an RNA virus.

(2) Vaccinia virus, mouse tail lesion: an *in vivo* DNA virus test system.

Table 5.2 B presents the condensed five-part screening data available on the bis-basic substituted tilorone compounds described in *Table 5.2 A*. The data are uniformly generated and interpreted. Therefore, the table provides a comprehensive overview of structure-activity relationships (SARs) for threehundred and fortyeight biologically active compounds. However, experienced medicinal chemists will know that SARs will change with different assays and many other variables and that more extensive biological testing often yields results at variance with the original primary screen.

Table 5.2A. BIS-BASIC SUBSTITUTED POLYCYCLIC AROMATIC AND HETEROAROMATIC COMPOUNDS: *CHEMICAL DESCRIPTION*

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
1	Acenaphthene	3,6	C=0	CH_2	NMe_2
2	Acenaphthene	3,6	C=0	CH_2	NEt_2
3	Acenaphthene	3,6	C=0	CH_2	c-NC_5H_{10}
4	Anthracene	1,5	C=0	CH_2	NEt_2
5	Anthracene	1,5	C=0	$(CH_2)_3$	NEt_2
6	Anthracene	1,5	C=0	$(CH_2)_4$	NEt_2
7	Anthracene-9,10-dibutylidine	2,6	0	$(CH_2)_2$	NEt_2
8	Anthraquinone	2,6	CO_2	$(CH_2)_3$	NEt_2
9	Anthraquinone	1,5	CO_2	$(CH_2)_3$	NBu_2

Table 5.2A. (Continued)

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
10	Anthraquinone	1,8	CO_2	$(CH_2)_3$	NBu_2
11	Anthraquinone	2,6	CO_2	$(CH_2)_3$	NBu_2
12	Anthraquinone	1,5	SO_2NH	$(CH_2)_3$	NBu_2
13	Anthraquinone	1,6	SO_2NH	$(CH_2)_3$	NBu_2
14	Anthraquinone	1,8	SO_2NH	$(CH_2)_3$	NBu_2
15	Anthraquinone	2,6	SO_2NH	$(CH_2)_2$	NEt_2
16	Anthraquinone	2,6	SO_2NH	$(CH_2)_2$	NBu_2
17	Anthraquinone	2,6	SO_2NH	$(CH_2)_3$	NEt_2
18	Anthraquinone	2,6	SO_2NH	$(CH_2)_3$	NPr_2
19	Anthraquinone	2,6	SO_2NH	$(CH_2)_3$	NBu_2
20	Anthraquinone	2,6	SO_2NH	$(CH_2)_3$	$N(C_5H_{11}\text{-}n)_2$
21	Anthraquinone	2,6	SO_2NH	$(CH_2)_3$	$N[(CH_2)_2CHMe_2]_2$
22	Anthraquinone	2,6	SO_2NH	$(CH_2)_3$	$N(CH_2CH\,CH_2)_2$
23	Anthraquinone	2,6	SO_2NH	$(CH_2)_4$	NEt_2
24	Anthraquinone	2,6	SO_2NH	$(CH_2)_4$	NBu_2
25	Anthraquinone	2,6	SO_2N	$(CH_2CH_2)_2$	$[NEt_2]_2$
26	Anthraquinone	2,7	SO_2NH	$(CH_2)_2$	NEt_2
27	Anthraquinone	2,7	SO_2NH	$(CH_2)_3$	NEt_2
28	Anthraquinone	2,7	SO_2NH	$(CH_2)_3$	NBu_2
29	Anthraquinone	2,6	O	$(CH_2)_2$	NMe_2
30	Anthraquinone	2,6	O	$(CH_2)_2$	NEt_2
31	Anthraquinone	2,6	O	$(CH_2)_2$	$c\text{-}N(C_2H_5)_2O$
32	Anthraquinone	2,6	O	$(CH_2)_2$	$N(CHMe_2)_2$
33	Anthraquinone	2,6	O	$(CH_2)_2$	$c\text{-}NC_4H_8$
34	Anthraquinone	2,6	O	$(CH_2)_2$	$c\text{-}NC_5H_{10}$
35	Anthraquinone	2,6	O	$(CH_2)_3$	NMe_2
36	Anthraquinone	2,6	O	$(CH_2)_3$	NEt_2
37	Anthraquinone	2,7	O	$(CH_2)_2$	NMe_2
38	Anthraquinone	2,7	O	$(CH_2)_2$	NEt_2
39	Anthraquinone	2,7	O	$(CH_2)_2$	$c\text{-}NC_5H_{10}$
40	Benzanthracene--7-one	3,9	O	$(CH_2)_3$	NBu_2
41	Carbazole	3,6	CO_2	$(CH_2)_3$	NEt_2
42	Carbazole	3,6	C=O	$(CH_2)_3$	$c\text{-}NC_5H_{10}$
43	Carbazole-*N*-ethyl	3,6	CO_2	$(CH_2)_3$	NEt_2
44	Carbazole-*N*-ethyl	3,6	O	$(CH_2)_2$	NEt_2
45	Carbazole-*N*-ethyl	3,6	O	$(CH_2)_2$	$c\text{-}NC_5H_{10}$
46	Carbazole-*N*-ethyl	3,6	O	$(CH_2)_2$	$c\text{-}N(CH_2CH_2)_2O$
47	Carbazole-*N*-ethyl	3,6	O	$(CH_2)_3$	NEt_2
48	Carbazole-*N*-ethyl	3,6	C=O	$(CH_2)_3$	NMe_2
49	Carbazole-*N*-ethyl	3,6	C=O	$(CH_2)_3$	$c\text{-}NC_5H_{10}$
50	Carbazole-*N*-ethyl	3,6	–	$(CH_2)_4$	NEt_2
51	Carbazole-*N*-ethyl	3,6	–	$(CH_2)_4$	$c\text{-}NC_5H_{10}$
52	Dibenzofuran	2,8	CO_2	$(CH_2)_3$	NMe_2

Table 5.2A. (Continued)

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
53	Dibenzofuran	2,8	CO_2	$(CH_2)_3$	NEt_2
54	Dibenzofuran	2,8	CO_2	$(CH_2)_3$	NPr_2
55	Dibenzofuran	2,8	CO_2	$(CH_2)_3$	NBu_2
56	Dibenzofuran	2,8	CO_2	$(CH_2)_3$	$N[(CH_2)_2CHMe_2]_2$
57	Dibenzofuran	2,8	CO_2	$(CH_2)_3$	c-NC_5H_{10}
58	Dibenzofuran	2,8	CO_2	$(CH_2)_3$	$N(CH_2CH\,CH_2)_2$
59	Dibenzofuran	2,8	CONH	$(CH_2)_3$	NBu_2
60	Dibenzofuran	2,8	O	$(CH_2)_2$	NEt_2
61	Dibenzofuran	2,8	O	$(CH_2)_2$	Ni-Pr_2
62	Dibenzofuran	2,8	O	$(CH_2)_2$	c-NC_4H_8
63	Dibenzofuran	2,8	O	$(CH_2)_2$	c-NC_5H_{10}
64	Dibenzofuran	2,8	O	$CH\,MeCH_2$	NMe_2
65	Dibenzofuran	2,8	O	$(CH_2)_3$	NMe_2
66	Dibenzofuran	2,8	O	$(CH_2)_3$	NEt_2
67	Dibenzofuran	2,8	O	$(CH_2)_3$	c-NC_5H_{10}
68	Dibenzofuran	2,8	O	$CH_2CHMeCH_2$	NMe_2
69	Dibenzofuran	2,8	C=O	CH_2	NMe_2
70	Dibenzofuran	2,8	C=O	CH_2	NEt_2
71	Dibenzofuran	2,8	C=O	CH_2	c-NC_5H_{10}
72	Dibenzofuran	2,8	C=O	$(CH_2)_3$	c-NC_5H_{10}
73	Dibenzofuran	2,8	C=O	$(CH_2)_3$	c-NC_5H_9-4-Me
74	Dibenzofuran	2,8	C=O	$(CH_2)_3$	c-NC_5H_9-4-CH_2Ph
75	Dibenzofuran	2,8	C=O	$(CH_2)_3$	c-$N(CH_2CH_2)_2O$
76	Dibenzopyran-9-one	3,8	CO_2	$(CH_2)_3$	NEt_2
77	Dibenzothiophene	2,6 2,8	CO_2	$(CH_2)_2$	NEt_2
78	Dibenzothiophene	2,8	CO_2	$(CH_2)_3$	NEt_2
79	Dibenzothiophene	2,6 2,8	CO_2	$(CH_2)_3$	NBu_2
80	Dibenzothiophene	2,6 2,8	CO_2	$(CH_2)_3$	$N[(CH_2)_2CH]_2Me_2$
81	Dibenzothiophene	2,6 2,8	CO_2	$(CH_2)_3$	c-NC_5H_{10}
82	Dibenzothiophene	2,6 2,8	CO_2	$CH_2CMe_2(CH_2)_3$	NMe_2
83	Dibenzothiophene	2,8	O	$(CH_2)_3$	NMe_2
84	Dibenzothiophene	2,8	O	$(CH_2)_2$	NEt_2
85	Dibenzothiophene	2,8	O	$(CH_2)_2$	Ni-Pr_2
86	Dibenzothiophene	2,8	O	$(CH_2)_2$	c-NC_5H_{10}
87	Dibenzothiophene	2,8	O	$(CH_2)_3$	NBu_2
88	Dibenzothiophene	2,8	O	$(CH_2)_3$	c-NC_5H_{10}
89	Dibenzothiophene	2,8	O	$CH_2CHMeCH_2$	NMe_2
90	Dibenzothiophene	2,8	C=O	CH_2	NMe_2
91	Dibenzothiophene	2,6 2,8	C=O	CH_2	NEt_2
92	Dibenzothiophene	2,8	C=O	CH_2	NEt_2
93	Dibenzothiophene	2,8	C=O	$(CH_2)_3$	NMe_2
94	Dibenzothiophene	2,6	C=O	$(CH_2)_3$	c-NC_5H_{10}
95	Dibenzothiophene	2,8	C=O	$(CH_2)_3$	c-NC_5H_{10}
96	Dibenzothiophene	2,8	C=O	$(CH_2)_3$	c-NC_5H_9-4-Me

Table 5.2A. (Continued)

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
97	Dibenzothiophene	2,6	C=O	$(CH_2)_3$	*c*-$N(CH_2CH_2)_2O$
98	Fluoranthene	3,9	CO_2	$(CH_2)_2$	NMe_2
99	Fluoranthene	3,9	CO_2	$(CH_2)_2$	NEt_2
100	Fluoranthene	3,9	CO_2	$CH(CH_3)CH_2$	NMe_2
101	Fluoranthene	3,9	CO_2	$(CH_2)_3$	NMe_2
102	Fluoranthene	3,8	CO_2	$(CH_2)_3$	NEt_2
103	Fluoranthene	3,9	CO_2	$(CH_2)_3$	NEt_2
104	Fluoranthene	3,9	CO_2	$(CH_2)_3$	NPr_2
105	Fluoranthene	3,8	CO_2	$(CH_2)_3$	NBu_2
106	Fluoranthene	3,9	CO_2	$(CH_2)_3$	NBu_2
107	Fluoranthene	3,9	CO_2	$(CH_2)_3$	$N(C_5H_{11}$-*n*$)_2$
108	Fluoranthene	3,8	CO_2	$(CH_2)_3$	$N[(CH_2)_2CH]_2$
109	Fluoranthene	3,9	CO_2	$(CH_2)_3$	$N[(CH_2)CHMe]_2$
110	Fluoranthene	3,8	CO_2	$(CH_2)_3$	$N(CH_2)CHMe]_2$
111	Fluoranthene	3,9	CO_2	$(CH_2)_3$	$N(CH_2CH{=}CH_2)_2$
112	Fluoranthene	3,8	CO_2	$(CH_2)_3$	*c*-NC_5H_{10}
113	Fluoranthene	3,9	CO_2	$(CH_2)_3$	*c*-NC_5H_{10}
114	Fluoranthene	3,9	CO_2	*c*-$CH(CH_2CH_2)_2$	NMe
115	Fluoranthene	3,9	CO_2	$CH(Me)(CH_2)_2$	NEt_2
116	Fluoranthene	3,9	CO_2	$CH_2CMe_2CH_2$	NEt_2
117	Fluoranthene	3,9	CO_2	CH(Me)CHMe	NEt_2
118	Fluoranthene	3,9	CO_2	$(CH_2)_4$	NEt_2
119	Fluoranthene	3,9	CO_2	$CH_2CMe_2(CH_2)_3$	NMe_2
120	Fluoranthene	3,9	COS	$(CH_2)_2$	NEt_2
121	Fluoranthene	3,9	O	$(CH_2)_2$	NEt_2
122	Fluoranthene	3,9	O	$(CH_2)_2$	*c*-NC_5H_{10}
123	Fluoranthene	3,9	O	$(CH_2)_2$	*c*-$N(CH_2CH_2)_2O$
124	Fluoranthene	3,9	O	$CHMeCH_2$	NMe_2
125	Fluoranthene	3,9	O	$CHMeCH_2$	NEt_2
126	Fluoranthene	3,9	O	$(CH_2)_3$	NMe_2
127	Fluoranthene	3,9	O	$(CH_2)_3$	NEt_2
128	Fluoranthene	3,9	O	$(CH_2)_3$	NBu_2
129	Fluoranthene	3,9	O	$(CH_2)_3$	*c*-NC_5H_{10}
130	Fluoranthene	3,9	C=O	CH_2	NMe_2
131	Fluoranthene	3,9	C=O	CH_2	NEt_2
132	Fluoranthene	3,9	C=O	CH_2	*c*-NC_5H_{10}
133	Fluoranthene	3,9	C=O	(CH_2)	*c*-NC_5H_9-4-Me
134	Fluoranthene	3,9	C=O	$(CH_2)_2$	*c*-NC_5H_{10}
135	Fluoranthene	3,9	C=O	$(CH_2)_3$	NMe_2
136	Fluoranthene	3,9	C=O	$(CH_2)_3$	NEt_2
137	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-NC_4H_8
138	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-NC_5H_{10}
139	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-NC_5H_{10}-*N*-oxide
140	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-Me

Table 5.2A. (Continued)

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
141	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-Pr
142	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-*t*-Bu
143	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-Ph
144	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-$(CH_2)_3$Ph
145	Fluoranthene	3,9	C=O	$(CH_2)_3$	1,2,5,6-H_4 pyrid-1-yl
146	Fluoranthene	3,9	C=O	$(CH_2)_3$	*c*-$N(CH_2CH_2)_2O$
147	Fluoranthene	3,9	C=O	$(CH_2)_3$	*a*
148	Fluoranthene	3,9	C=O	$(CH_2)_4$	NMe_2
149	Fluoranthene	3,9	C=O	$(CH_2)_4$	NEt_2
150	Fluoranthene	3,9	C=O	$(CH_2)_4$	NBu_2
151	Fluoranthene	3,9	C=O	$(CH_2)_4$	*c*-NC_5H_{10}
152	Fluoranthene	3,9	C=O	$(CH_2)_4$	*c*-NC_5H_9-4-Me
153	Fluoranthene	3,9	C=O	$(CH_2)_4$	*c*-NC_5H_9-4-Pr
154	Fluoranthene	3,9	C=O	$(CH_2)_4$	*c*-NC_5H_9-4-CH_2Ph
155	Fluoranthene	3,9	C=O	$(CH_2)_4$	1,2,5,6-H_4 pyrid-1-yl
156	Fluoranthene	3,9	C=O	$(CH_2)_4$	*a*
157	Fluoranthene	3,9	–	$(CH_2)_4$	*c*-NC_5H_{10}
158	Fluoranthene	3,9	–	$(CH_2)_4$	*c*-$N(CH_2CH_2)H_2O$
159	Fluoranthene	3,9	–	$(CH_2)_5$	*c*-NC_5H_9-4-Pr
160	Fluorene	2,7	CO_2	$(CH_2)_2$	NMe*c*-C_6H_{11}
161	Fluorene	1,7	CO_2	$(CH_2)_3$	NEt_2
162	Fluorene	2,5	CO_2	$(CH_2)_3$	NEt_2
163	Fluorene	2,7	CO_2	$(CH_2)_3$	NEt_2
164	Fluorene	2,7	CO_2	$(CH_2)_3$	NBu_2
165	Fluorene	2,7	CO_2	$(CH_2)_3$	$N[(CH_2)_2CHMe_2]_2$
166	Fluorene	2,7	CO_2	$CH_2CMe_2(CH_2)_3$	NMe_2
167	Fluorene	2,7	CO_2	$(CH_2)_6$	NEt_2
168	Fluorene	2,7	CO_2	CH_2	*N*-Me-piperid-3-yl
169	Fluorene	2,7	CONH	$(CH_2)_2$	Ni-Pr_2
170	Fluorene	2,7	CONH	$(CH_2)_3$	NEt_2
171	Fluorene	2,7	CONH	$(CH_2)_3$	$N[(CH_2)_2CHMe]_2$
172	Fluorene	2,7	CONH	$(CH_2)_6$	NEt_2
173	Fluorene	2,7	O	$(CH_2)_2$	NEt_2
174	Fluorene	2,7	O	$(CH_2)_3$	NEt_2
175	Fluorene	2,7	O	$(CH_2)_3$	NBu_2
176	Fluorene	2,7	C=O	CH_2	NMe_2
177	Fluorene	2,7	C=O	CH_2	NEt_2
178	Fluorene	2,7	C=O	CH_2	*c*-NC_5H_{10}
179	Fluorene	2,7	C=O	$(CH_2)_2$	*c*-NC_5H_{10}
180	Fluorene	2,7	C=O	$(CH_2)_3$	NEt_2
181	Fluorene	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_{10}
182	Fluorene	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_{10}-*N*-oxide
183	Fluorene	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-Me
184	Fluorene	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-Ph

Table 5.2A. (Continued)

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
185	Fluorene	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-CH_2Ph
186	Fluorene	2,7	C=O	$(CH_2)_3$	*c*-$N(CH_2CH_2)_2O$
187	Fluorene	2,7	C=O	$(CH_2)_4$	NMe_2
188	Fluorene	2,7	C=O	$(CH_2)_4$	NEt_2
189	Fluorene	2,7	C=O	$(CH_2)_4$	NBu_2
190	Fluorene	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_{10}
191	Fluorene	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_9-4-Me
192	Fluorene	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_9-4-CH_2Ph
193	Fluorene	2,7	C=O	$(CH_2)_4$	*c*-$N(CH_2CH_2)_2O$
194	Fluorene	2,7	C=NOH	$(CH_2)_3$	*c*-NC_5H_{10}
195	Fluorene	2,7	O NOH ‖ ‖ C-C	$(CH_2)_2$	*c*-NC_5H_{10}
196	Fluorene	2,7	O NOH ‖ ‖ C-C	$(CH_2)_2$	*c*-$N(CH_2CH_2)_2O$
197	Fluorene	2,7	O NOH ‖ ‖ C-C	$(CH_2)_3$	*c*-$N(CH_2CH_2)_2O$
198	Fluorene	2,7	CHOH	CH_2	NEt_2
199	Fluorene	2,7	CHOH	$(CH_2)_3$	*c*-NC_5H_{10}
200	Fluorene	2,7	–	CH_2	*c*-NC_5H_{10}
201	Fluorene	2,7	–	$(CH_2)_4$	*c*-NC_5H_{10}
202	Fluoren-9-ol	2,7	CO_2	$(CH_2)_2$	NMe-*c*-C_6H_{11}
203	Fluoren-9-ol	2,7	CO_2	$(CH_2)_3$	NEt_2
204	Fluoren-9-ol	2,7	CO_2	$(CH_2)_3$	NBU_2
205	Fluoren-9-ol	2,7	CO_2	$(CH_2)_3$	$N[(CH_2)_2CHMe_2]_2$
206	Fluoren-9-ol	2,7	CO_2	$(CH_2)_3$	$N(CH_2CH{=}CH_2)_2$
207	Fluoren-9-ol	2,7	CO_2	$(CH_2)_6$	NEt_2
208	Fluoren-9-ol	2,7	CONH	$(CH_2)_2$	$Ni\text{-}Pr_2$
209	Fluoren-9-ol	2,7	CONH	$(CH_2)_3$	NEt_2
210	Fluoren-9-ol	2,7	CONH	$(CH_2)_3$	$N[(CH_2)_2CHMe_2]_2$
211	Fluoren-9-ol	2,7	CONH	$(CH_2)_6$	NEt_2
212	Fluoren-9-ol	2,7	O	$(CH_2)_2$	NMe_2
213	Fluoren-9-ol	2,7	O	$(CH_2)_2$	NMeEt
214	Fluoren-9-ol	2,7	O	$(CH_2)_2$	NEt_2
215	Fluoren-9-ol	2,7	–	CH_2	Net_2
216	Fluoren-9-ol	2,7	–	CH_2	*c*-NC_5H_{10}
217	Fluoren-9-ol	2,7	–	CH_2	$N(CH_2CH{=}CH_2)_2$
218	Fluoren-9-one	2,7	CO_2	$(CH_2)_2$	Me_2
219	Fluoren-9-one	2,7	CO_2	$(CH_2)_2$	NEt_2
220	Fluoren-9-one	2,7	CO_2	$(CH_2)_2$	$Ni\text{-}Pr_2$
221	Fluoren-9-one	2,7	CO_2	$(CH_2)_2$	Bu_2
222	Fluoren-9-one	2,7	CO_2	$(CH_2)_2$	$N(C_6H_{13}\text{-}n)_2$
223	Fluoren-9-one	2,7	CO_2	$(CH_2)_2$	NMe-*c*-C_6H_{11}
224	Fluoren-9-one	2,7	CO_2	$(CH_2)_2$	*c*-$N(CH_2CH_2)_2O$

Table 5.2A. (Continued)

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
225	Fluoren-9-one	2,7	CO_2	$(CH_2)_2$	NEt_2
226	Fluoren-9-one	2,7	CO_2	$(CH_2)_3$	NMe_2
227	Fluoren-9-one	2,5	CO_2	$(CH_2)_3$	NEt_2
228	Fluoren-9-one	2,7	CO_2	$(CH_2)_3$	NEt_2
229	Fluoren-9-one	2,7	CO_2	$(CH_2)_3$	NPr_2
230	Fluoren-9-one	2,7	CO_2	$(CH_2)_3$	NBu_2
231	Fluoren-9-one	2,7	CO_2	$(CH_2)_3$	$N(C_5H_{11}\text{-}n)_2$
232	Fluoren-9-one	2,7	CO_2	$(CH_2)_3$	$N[(CH_2)_2CHMe_2]_2$
233	Fluoren-9-one	2,7	CO_2	$(CH_2)_3$	$c\text{-}NC_5H_{10}$
234	Fluoren-9-one	2,7	CO_2	$(CH_2)_3$	$N(CH_2CH{:}CH_2)_2$
235	Fluoren-9-one	2,7	CO_2	$c\text{-}CH(CH_2CH_2)_2NMe$	
236	Fluoren-9-one	2,7	CO_2	CH	N-Me-piperid-3-yl
237	Fluoren-9-one	2,7	CO_2	$(CH_2)_4$	NEt_2
238	Fluoren-9-one	2,7	CO_2	$CH(CH_3)(CH_2)_3$	NEt_2
239	Fluoren-9-one	2,7	CO_2	$(CH_2)_5$	NEt_2
240	Fluoren-9-one	2,7	CO_2	$CH_2CMe_2(CH_2)_3$	NH_2
241	Fluoren-9-one	2,7	CO_2	$CH_2CMe_2(CH_2)_3$	NMe_2
242	Fluoren-9-one	2,7	CO_2	$(CH_2)_6$	NEt_2
243	Fluoren-9-one	2,7	COS	$(CH_2)_2$	NEt_2
244	Fluoren-9-one	2,7	CONH	$(CH_2)_2$	NMe_2
245	Fluoren-9-one	2,7	CONH	$(CH_2)_2$	NEt_2
246	Fluoren-9-one	2,7	CONH	$(CH_2)_2$	$Ni\text{-}Pr_2$
247	Fluoren-9-one	2,7	CONH	$(CH_2)_2$	$c\text{-}N(CH_2CH_2)_2O$
248	Fluoren-9-one	2,7	CONH	$(CH_2)_3$	NMe_2
249	Fluoren-9-one	2,7	CONH	$(CH_2)_3$	NEt_2
250	Fluoren-9-one	2,7	CONH	$(CH_2)_3$	NBu_2
251	Fluoren-9-one	2,7	CONH	$(CH_2)_3$	$N[(CH_2)_2CHMe_2]_2$
252	Fluoren-9-one	2,7	CONH	$(CH_2)_3$	$N(CH_2CH{:}CH_2)_2$
253	Fluoren-9-one	2,7	CONH	$(CH_2)_6$	NEt_2
254	Fluoren-9-one	2,7	CONMe	$(CH_2)_2$	NEt_2
255	Fluoren-9-one	2,7	CONMe	$(CH_2)_3$	NEt_2
256	Fluoren-9-one	2,7	O	$(CH_2)_2$	NMe
257	Fluoren-9-one	2,7	O	$(CH_2)_2$	NMe_2N-oxide
258	Fluoren-9-one	2,7	O	$(CH_2)_2$	NMeEt
259	Fluoren-9-one	2,7	O	$(CH_2)_2$	NMeBu
260	Fluoren-9-one	2,5	O	$(CH_2)_2$	NEt_2
261	Fluoren-9-one	2,6	O	$(CH_2)_2$	NEt_2
262	Fluoren-9-one	2,7	O	$(CH_2)_2$	NEt_2
263	Fluoren-9-one	2,7	O	$(CH_2)_2$	NEt_2N-oxide
264	Fluoren-9-one	2,7	O	$(CH_2)_2$	NPr_2
265	Fluoren-9-one	2,7	O	$(CH_2)_2$	$Ni\text{-}Pr_2$
266	Fluoren-9-one	2,7	O	$(CH_2)_2$	NBu_2
267	Fluoren-9-one	2,7	O	$(CH_2)_2$	$c\text{-}NC_4H_8$
268	Fluoren-9-one	2,7	O	$(CH_2)_2$	$c\text{-}NC_5H_{10}$

Table 5.2A. (Continued)

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
269	Fluoren-9-one	2,7	O	$(CH_2)_3$	*c*-$N(CH_2CH_2)_2O$
270	Fluoren-9-one	2,7	O	$(CH_2)_3$	NMe_2
271	Fluoren-9-one	2,7	O	$(CH_2)_3$	NEt_2
272	Fluoren-9-one	2,7	O	$(CH_2)_3$	NBu_2
273	Fluoren-9-one	2,7	O	$(CH_2)_3$	*c*-NC_5H_{10}
274	Fluoren-9-one	2,7	O	$CH_2CHMeCH_2$	NMe_2
275	Fluoren-9-one	2,7	S	$(CH_2)_2$	NMe_2
276	Fluoren-9-one	2,7	S	$(CH_2)_2$	NEt_2
277	Fluoren-9-one	2,7	C=O	CH_2	NEt_2
278	Fluoren-9-one	2,7	C=O	$(CH_2)_3$	NEt_2
279	Fluoren-9-one	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_{10}
280	Fluoren-9-one	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_{10}-*N*-oxide
281	Fluoren-9-one	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-Me
282	Fluoren-9-one	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_9-4-CH_2Ph
283	Fluoren-9-one	2,7	C=O	$(CH_2)_3$	*c*-$N(CH_2CH_2)_2O$
284	Fluoren-9-one	2,7	C=O	$(CH_2)_4$	NMe_2
285	Fluoren-9-one	2,7	C=O	$(CH_2)_4$	NEt_2
286	Fluoren-9-one	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_9-4-Me
287	Fluoren-9-one	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_9-4-CH_2Ph
288	Fluoren-9-one	2,7	C=O	$(CH_2)_4$	*c*-$N(CH_2CH_2)_2O$
289	Fluoren-9-one	2,7	–	CH_2	NEt_2
290	Fluoren-9-one	2,7	–	CH_2	*c*-NC_5H_{10}
291	Fluoren-9-one	2,7	–	CH_2	$N(CH_2CH{=}CH_2)_2$
292	Fluoren-9-one	2,7	–	$(CH_2)_3$	*c*-NC_5H_9-4-Me
293	Fluoren-9-benzylidine	2,7	CO_2	$(CH_2)_3$	NEt_2
294	Fluoren-9-benzylidine	2,7	CO_2	$(CH_2)_3$	NBu_2
295	Fluoren-9-benzylidine	2,7	O	$(CH_2)_2$	NEt_2
296	9-Methoxyphenanthrene	2,7	O	$(CH_2)_2$	NEt_2
297	9-Methoxyphenanthrene	2,7	O	$(CH_2)_3$	*c*-NC_5H_{10}
298	Phenanthridin-6-one	3,8	CO_2	$(CH_2)_3$	NBu_2
299	Phenoxathiin	2,7	C=O	CH_2	NEt_2
300	Phenoxathiin	2,7	C=O	$(CH_2)_2$	NEt_2
301	Phenoxathiin	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_{10}
302	Phenoxathiin	2,8	C=O	$(CH_2)_3$	*c*-NC_5H_{10}
303	Phenoxathiin	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_{10}
304	Phenoxathiin	2,8	C=O	$(CH_2)_4$	*c*-NC_5H_{10}
305	Thioxanthene	2,7	C=O	CH_2	NEt_2
306	Thioxanthene	2,7	C=O	$(CH_2)_2$	NEt_2
307	Thioxanthene	2,7	C=O	$(CH_2)_3$	NEt_2
308	Thioxanthene	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_{10}
309	Thioxanthene	2,7	C=O	$(CH_2)_3$	*c*-$N(CH_2CH_2)_2O$
310	Thioxanthene	2,7	C=O	$(CH_2)_4$	NMe_2
311	Thioxanthene	2,7	C=O	$(CH_2)_4$	NEt_2
312	Thioxanthene	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_{10}

Table 5.2A. (Continued)

Compound No.	*Nucleus*	*Position of Substituent*	*A*	*Alkyl*	*NR_2*
313	Thioxanthene	2,7	C=O	$(CH_2)_4$	$N(CH_2CH{=}CH_2)_2$
314	Xanthene	2,7	CO_2	$(CH_2)_3$	NBu_2
315	Xanthene	2,7	O	$(CH_2)_2$	NMe_2
316	Xanthene	2,7	C=O	CH_2	NMe_2
317	Xanthene	2,7	C=O	CH_2	NMe-*c*-C_6H_{11}
318	Xanthene	2,7	C=O	CH_2	NEt_2
319	Xanthene	2,7	C=O	CH_2	*c*-NC_5H_{10}
320	Xanthene	2,7	C=O	CH_2	*c*-$N(CH_2CH_2)_2O$
321	Xanthene	2,7	C=O	$(CH_2)_2$	NEt_2
322	Xanthene	2,7	C=O	$(CH_2)_3$	NEt_2
323	Xanthene	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_{10}
324	Xanthene	2,7	C=O	$(CH_2)_3$	*c*-$N(CH_2CH_2)_2O$
325	Xanthene	2,7	C=O	$(CH_2)_4$	NMe_2
326	Xanthene	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_{10}
327	Xanthene	2,7	C=O	$(CH_2)_4$	$N(CH_2CH{=}CH_2)2$
328	Xanthene	2,7	CHOH	CH_2	*c*-$N(CH_2CH_2)_2O$
329	Xanthene	2,7	CHOH	$(CH_2)_3$	NEt_2
330	Xanthene	2,7	CHOH	$(CH_2)_3$	*c*-NC_5H_{10}
331	Xanthene	2,7	CH=CH	$(CH_2)_2$	*c*-NC_5H_{10}
332	Xanthen-9-one	2,7	CO_2	$(CH_2)_3$	NEt_2
333	Xanthen-9-one	2,7	CO_2	$(CH_2)_3$	NBu_2
334	Xanthen-9-one	2,7	CONH	$(CH_2)_3$	NBu_2
335	Xanthen-9-one	3,6	O	$(CH_2)_2$	NMe_2
336	Xanthen-9-one	3,6	O	$(CH_2)_2$	NMe_2N-oxide
337	Xanthen-9-one	3,6	O	$(CH_2)_2$	NEt_2
338	Xanthen-9-one	3,6	O	$(CH_2)_2$	Ni-Pr_2
339	Xanthen-9-one	3,6	O	$(CH_2)_2$	*c*-NC_5H_{10}
340	Xanthen-9-one	3,6	O	$(CH_2)_2$	*c*-$N(CH_2CH_2)_2O$
341	Xanthen-9-one	3,6	O	$(CH_2)_3$	NMe_2
342	Xanthen-9-one	3,6	S	$(CH_2)_2$	NEt_2
343	Xanthen-9-one	2,7	C=O	CH_2	NEt_2
344	Xanthen-9-one	2,7	C=O	$(CH_2)_3$	*c*-NC_5H_{10}
345	Xanthen-9-one	2,7	C=O	$(CH_2)_4$	*c*-NC_5H_{10}
346	Xanthen-9-benzylidine	3,6	O	$(CH_2)_2$	NMe_2
347	Xanthen-9-benzylidine	3,6	O	$(CH_2)_2$	NEt_2
348	Xanthen-9-benzylidine	3,6	S	$(CH_2)_2$	NEt_2

a = 3-Azabicyclo[3.2.2]nonan-3-yl.

Table 5.2B. BIS-BASIC SUBSTITUTED POLYCYCLIC AROMATIC AND HETEROAROMATIC COMPOUNDS: *PRIMARY SCREENING RESULTS*

Anti-Inflammatory[a]		*Complement Inhibition*[b]		*EAE*[c]		*EMC*[d]		*Vaccinia*[e]		*Compound No.*
Dose/Rt. (mg/kg)	*Paw Vol. (% C)*	*Dose (μg/ml)*	*Lysis (%C)*	*Dose/Rt. (mg/kg)*	*Paralysis (%)*	*Dose/Rt. (mg/kg)*	*Surv. time (%C)*	*Dose/Rt. (mg/kg)*	*Lesion score (%C)*	
20/s.c.	87	460	73	100/s.c.	20		NT[f]		NT	1
20/s.c.	88	480	87	50/s.c.	40	250/p.o.	196	250/p.o.	13	2
20/s.c.	87	500	99	50/s.c.	20		NT		NT	3
	NT		NT		NT	50/p.o.	165	250/p.o.	14	4
	NT		NT		NT	50/s.c.	143		NT	5
	NT		NT		NT	250/s.c.	110		NT	6
	NT		NT		NT	50/s.c.	171	100/s.c.	69	7
	NT		NT	100/s.c.	100	50/s.c.	114		NT	8
	NT		NT		NT	50/s.c.	114		NT	9
	NT		NT		NT	50/s.c.	114		NT	10
20/s.c.	53	72	85	100/s.c.	100	50/s.c.	126		NT	11
	NT		NT		NT	50/s.c.	111		NT	12
	NT		NT		NT	50/s.c.	121		NT	13
	NT		NT		NT	50/s.c.	95		NT	14
	NT		NT		NT	50/s.c.	102		NT	15
	NT		NT		NT	50/s.c.	100		NT	16
	NT		NT		NT	10/s.c.	98		NT	17
	NT		NT		NT	10/s.c.	109		NT	18
20/s.c.	23	78	110	100/s.c.	70	50/s.c.	207	50/s.c.	45	19
	NT		NT		NT	50/s.c.	116		NT	20
	NT		NT		NT	50/s.c.	120		NT	21
	NT		NT		NT	50/s.c.	168		NT	22
	NT		NT		NT	10/s.c.	73		NT	23

Table 5.2B. (Continued)

Anti-Inflammatory[a]		*Complement Inhibition*[b]		*EAE*[c]		*EMC*[d]		*Vaccinia*[e]		*Compound No.*
Dose/Rt. (mg/kg)	*Paw Vol. (%C)*	*Dose (μg/ml)*	*Lysis (%C)*	*Dose/Rt. (mg/kg)*	*Paralysis (%)*	*Dose/Rt. (mg/kg)*	*Surv. time (%C)*	*Dose/Rt. (mg/kg)*	*Lesion score (%C)*	
	NT		NT		NT	50/s.c.	129		NT	24
	NT		NT		NT	50/s.c.	107		NT	25
	NT		NT		NT	50/s.c.	70		NT	26
	NT		NT		NT	10/s.c.	109		NT	27
	NT		NT		NT	50/s.c.	140	50/s.c.	23	28
	NT		NT	100/s.c.	40	50/p.o.	117	50/p.o.	48	29
20/s.c.	73	511	85	100/p.o.	90	250/p.o.	220	50/p.o.	62	30
	NT		NT	100/p.o.	60		NT		NT	31
	NT		NT	100/s.c.	70	250/p.o.	166		NT	32
	NT		NT		NT	50/p.o.	139		NT	33
	NT		NT	100/p.o.	40	50/p.o.	202		NT	34
20/s.c.	75	483	31		NT	250/p.o.	177		NT	35
	NT		NT		NT	50/p.o.	132		NT	36
	NT		NT		NT	50/p.o.	183		NT	37
	NT		NT	50/s.c.	70	50/p.o.	178		NT	38
	NT		NT		NT	50/p.o.	153		NT	39
20/s.c.	21	73	89		NT	50/s.c.	202		NT	40
	NT		NT		NT	50/s.c.	169		NT	41
	NT		NT		NT	50/s.c.	96		NT	42
20/s.c.	68	582	5		NT	50/s.c.	152	50/s.c.	108	43
20/s.c.	62	499	32		NT	50/s.c.	88		NT	44
	NT		NT		NT	50/s.c.	132		NT	45
	NT		NT		NT	50/s.c.	108		NT	46
	NT		NT	100/s.c.	0	50/s.c.	110		NT	47
	NT		NT		NT	50/s.c.	163		NT	48
20/s.c.	74	584	10	100/s.c.	80	50/s.c.	158		NT	49

	NT		NT		NT	50/s.c.	122	NT	50
20/s.c.	14	547	14		NT	50/s.c.	145	NT	51
	NT		NT		NT	50/s.c.	126	NT	52
20/s.c.		556	16		NT	50/s.c.	116	NT	53
	NT		NT	100/s.c.	60	50/s.c.	121	NT	54
	NT		NT		NT	50/s.c.	140	NT	55
	NT		NT		NT	50/s.c.	107	NT	56
	NT		NT		NT	50/s.c.	122	NT	57
	NT		NT		NT	50/s.c.	120	NT	58
	NT		NT		NT	50/s.c.	124	NT	59
	NT		NT		NT	50/s.c.	130	NT	60
	NT		NT		NT	50/s.c.	124	NT	61
	NT		NT		NT	50/s.c.	104	NT	62
	NT		NT		NT	50/s.c.	125	NT	63
	NT		NT		NT	50/s.c.	108	NT	64
	NT		NT	50/s.c.	80	50/s.c.	124	NT	65
	NT		NT		NT	50/s.c.	88	NT	66
40/s.c.	2		NT	10/s.c.	80	50/s.c.	144	NT	67
	NT		NT	50/s.c.	60	50/s.c.	114	NT	68
	NT	447	10	100/p.o.	40	50/p.o.	180	100/s.c. 19	69
	NT		NT		NT	250/p.o.	165	100/s.c. 86	70
40/s.c.	46		NT		NT	250/s.c.	220	NT	71
	NT		NT		NT	50/s.c.	149	NT	72
	NT		NT		NT	50/s.c.	213	NT	73
	NT		NT		NT	50/s.c.	170	NT	74
	NT		NT		NT	50/s.c.	139	NT	75
	NT		NT		NT	50/s.c.	166	NT	76
	NT		NT		NT	50/s.c.	104	NT	77
	NT		NT		NT	50/s.c.	177	10/s.c. 31	78
	NT		NT		NT	50/s.c.	148	NT	79
	NT		NT		NT	50/s.c.	98	NT	80
	NT		NT		NT	50/s.c.	136	NT	81
	NT		NT	50/s.c.	80	50/s.c.	142	NT	82
	NT		NT	50/s.c.	70	50/s.c.	113	NT	83
	NT		NT		NT	50/s.c.	112	NT	84

Table 5.2B. (Continued)

Anti-Inflammatory[a]		*Complement Inhibition*[b]		*EAE*[c]		*EMC*[d]		*Vaccinia*[e]		*Compound No.*
Dose/Rt. (mg/kg)	*Paw Vol. (%C)*	*Dose (μg/ml)*	*Lysis (%C)*	*Dose/Rt. (mg/kg)*	*Paralysis (%)*	*Dose/Rt. (mg/kg)*	*Surv. time (%C)*	*Dose/Rt. (mg/kg)*	*Lesion score (%C)*	
	NT		NT		NT	50/s.c.	130		NT	85
	NT		NT		NT	50/s.c.	157		NT	86
	NT		NT		NT	50/s.c.	114		NT	87
	NT		NT		NT	50/s.c.	194		NT	88
	NT		NT	100/p.o.	20	50/s.c.	108		NT	89
	NT	445	78	100/p.o.	40	50/p.o.	175	100/s.c.	34	90
	NT		NT		NT	250/p.o.	190		NT	91
	NT		NT		NT	250/p.o.	222		NT	92
	NT		NT		NT	50/s.c.	166		NT	93
	NT		NT		NT	50/s.c.	171		NT	94
	NT		NT		NT	50/s.c.	190		NT	95
	NT		NT		NT	50/s.c.	184		NT	96
	NT		NT		NT	50/s.c.	133		NT	97
20/s.c.	87	510	46		NT		NT		NT	98
	NT		NT		NT	50/s.c.	138		NT	99
50/s.c.	80		NT		NT		NT		NT	100
40/s.c.	65	53	42		NT	50/s.c.	161		NT	101
20/s.c.	71	590	3	100/s.c.	40	50/s.c.	188		NT	102
50/s.c.	21	22	46	25/s.c.	20	50/s.c.	182	50/s.c.	50	103
40/s.c.	42	65	107		NT	50/s.c.	167		NT	104
40/s.c.	60	70	98		NT	50/s.c.	159		NT	105
40/s.c.	112	63	109		NT	50/s.c.	120		NT	106
40/s.c.	99	68	67	100/s.c.	90	50/s.c.	124		NT	107
40/s.c.	79	76	101		NT	50/s.c.	113		NT	108
40/s.c.	77	76	83		NT	50/s.c.	116		NT	109

40/s.c.	64	64	105		NT	50/s.c.	123	NT	110
40/s.c.	98	56	63	100/s.c.	80	50/s.c.	111	NT	111
40/s.c.	79	614	3		NT	50/s.c.	206	NT	112
40/s.c.	39	61	50		NT	50/s.c.	202	NT	113
40/s.c.	67	48	114		NT	50/s.c.	180	NT	114
50/s.c.	60	54	77		NT		NT	NT	115
50/s.c.	92		NT		NT		NT	NT	116
100/s.c.	81		NT		NT		NT	NT	117
40/s.c.	18	64	30		NT	50/s.c.	163	NT	118
40/s.c.	9	646	59		NT	50/s.c.	218	NT	119
20/s.c.	41	594	55		NT	50/s.c.	174	NT	120
100/p.o.	88	51	119	50/s.c.	30	50/s.c.	189	NT	121
40/s.c.	69	46	113		NT	50/s.c.	160	NT	122
20/s.c.	15		NT		NT	50/s.c.	195	NT	123
	NT		NT		NT	50/s.c.	118	NT	124
	NT		NT		NT	50/s.c.	173	NT	125
	NT		NT		NT	250/s.c.	184	NT	126
40/s.c.	29	534	9	50/s.c.	70	50/s.c.	216	NT	127
40/s.c.	44	65	111		NT	50/s.c.	171	NT	128
20/s.c.	15		NT		NT	50/s.c.	195	NT	129
100/p.o.	60	48	70	100/p.o.	70	50/p.o.	189	100/s.c. 6	130
100/p.o.	71	54	95		NT	50/p.o.	138	NT	131
100/p.o.	49	60	114		NT	50/s.c.	224	100/s.c. 7	132
	NT		NT		NT	50/s.c.	133	NT	133
40/s.c.	48	572	72		NT	250/p.o.	135	NT	134
	NT		NT		NT	50/s.c.	181	NT	135
	NT		NT		NT	50/s.c.	200	NT	136
40/s.c.	30	572	1		NT	50/s.c.	146	NT	137
40/s.c.	23	582	2	25/s.c.	20	50/s.c.	202	50/s.c. 63	138
	NT		NT		NT		NT	NT	139
40/s.c.	11	63	80		NT	50/s.c.	216	NT	140
40/s.c.	75		NT		NT	10/s.c.	188	NT	141
40/s.c.	83		NT	100/s.c.	90	50/s.c.	117	NT	142
40/s.c.	105		NT		NT	50/s.c.	109	NT	143
40/s.c.	76	75	161		NT	50/s.c.	100	NT	144
40/s.c.	37	62	64		NT	50/s.c.	219	NT	145

Table 5.2B. (Continued)

Anti-Inflammatory[a]		*Complement Inhibition*[b]		*EAE*[c]		*EMC*[d]		*Vaccinia*[e]		*Compound No.*
Dose/Rt. (mg/kg)	*Paw Vol. (%C)*	*Dose (μg/ml)*	*Lysis (%C)*	*Dose/Rt. (mg/kg)*	*Paralysis (%)*	*Dose/Rt. (mg/kg)*	*Surv. time (%C)*	*Dose/Rt. (mg/kg)*	*Lesion score (%C)*	
40/s.c.	23	60	103		NT	50/s.c.	161		NT	146
40/s.c.	78		NT	100/p.o.	20	50/s.c.	180		NT	147
40/s.c.	38	46	71		NT	10/s.c.	124		NT	148
40/s.c.	39	51	102		NT	50/s.c.	211		NT	149
20/s.c.	4	7	105	30/s.c.	20	50/s.c.	212	100/s.c.	0	150
	NT		NT		NT	10/s.c.	191		NT	151
40/s.c.	77	56	117		NT	50/s.c.	159		NT	152
40/s.c.	65		NT		NT	250/s.c.	196	100/s.c.	33	153
40/s.c.	64		NT	100/s.c.	80	50/s.c.	147		NT	154
40/s.c.	14	61	38		NT	50/s.c.	214		NT	155
40/s.c.	82		NT	50/p.o.	40	50/s.c.	177		NT	156
20/s.c.	11	554	98		NT	50/s.c.	220		NT	157
40/s.c.	67	56	103		NT	50/s.c.	131		NT	158
40/s.c.	52	67	137		NT	50/s.c.	155		NT	159
	NT		NT		NT	50/s.c.	135		NT	160
	NT		NT		NT	50/s.c.	129		NT	161
	NT		NT		NT	50/s.c.	114		NT	162
	NT		NT		NT	50/s.c.	109		NT	163
	NT		NT		NT	50/s.c.	129		NT	164
	NT		NT		NT	50/s.c.	110		NT	165
	NT		NT		NT	50/s.c.	167		NT	166
	NT		NT		NT	50/s.c.	125		NT	167
	NT		NT	100/s.c.	80	50/s.c.	127		NT	168
	NT		NT		NT	50/s.c.	157		NT	169
	NT		NT	10/s.c.	60	10/s.c.	112		NT	170

	NT		NT		NT	50/s.c.	119		NT	171
	NT		NT		NT	2/s.c.	86		NT	172
40/s.c.	56	469	107		NT	50/s.c.	105		NT	173
40/s.c.	60		NT		NT	50/s.c.	142		NT	174
	NT		NT	100/s.c.	90	50/s.c.	115		NT	175
	NT		NT		NT	50/s.c.	200		NT	176
20/s.c.	98	519	89	100/p.o.	50	250/p.o.	206	250/p.o.	76	177
	NT		NT		NT	50/p.o.	176		NT	178
	NT		NT		NT	50/s.c.	127		NT	179
	NT		NT		NT	50/s.c.	181		NT	180
	NT		NT	100/p.o.	90	50/s.c.	231	50/p.o.	24	181
	NT		NT		NT		NT		NT	182
	NT		NT		NT	50/s.c.	171		NT	183
	NT		NT		NT	50/s.c.	115		NT	184
	NT		NT		NT	50/s.c.	144		NT	185
	NT		NT		NT	50/s.c.	120		NT	186
20/s.c.	73	421	40		NT	50/s.c.	148		NT	187
20/s.c.	68	477	47		NT	50/s.c.	152		NT	188
20/s.c.	27	59	103		NT	50/s.c.	124		NT	189
	NT		NT		NT	50/s.c.	154		NT	190
	NT		NT		NT	50/s.c.	167		NT	191
	NT		NT		NT	10/s.c.	123		NT	192
	NT		NT		NT	50/s.c.	110		NT	193
	NT		NT		NT	50/s.c.	140		NT	194
	NT		NT		NT	50/s.c.	215		NT	195
	NT		NT		NT	50/s.c.	98	100/s.c.	91	196
	NT		NT		NT	50/s.c.	148		NT	197
20/s.c.	86	397	54	100/p.o.	20	50/s.c.	107		NT	198
	NT		NT		NT	50/s.c.	106		NT	199
	NT		NT	100/p.o.	40	50/s.c.	119		NT	200
	NT	445	88		NT	50/s.c.	118		NT	201
	NT		NT		NT	50/s.c.	117		NT	202
	NT		NT		NT	50/s.c.	123		NT	203
	NT		NT		NT	50/s.c.	133		NT	204
	NT		NT		NT	50/s.c.	129		NT	205

Table 5.2B. (Continued)

Anti-Inflammatory[a]		*Complement Inhibition*[b]		*EAE*[c]		*EMC*[d]		*Vaccinia*[e]		*Compound No.*
Dose/Rt. (mg/kg)	*Paw Vol. (%C)*	*Dose (μg/ml)*	*Lysis (%C)*	*Dose/Rt. (mg/kg)*	*Paralysis (%)*	*Dose/Rt. (mg/kg)*	*Surv. time (%C)*	*Dose/Rt. (mg/kg)*	*Lesion score (%C)*	
	NT		NT		NT	50/s.c.	143		NT	206
	NT		NT		NT	50/s.c.	133		NT	207
	NT		NT	50/s.c.	80	2/s.c.	98		NT	208
	NT	495	100		NT	2/s.c.	96		NT	209
	NT		NT	100/p.o.	60	50/s.c.	74		NT	210
	NT		NT		NT	2/s.c.	95		NT	211
	NT		NT		NT	50/s.c.	87		NT	212
	NT		NT		NT	10/s.c.	94		NT	213
	NT		NT	50/s.c.	60	50/s.c.	98		NT	214
	NT		NT	100/p.o.	60	50/s.c.	109		NT	215
	NT		NT		NT	50/s.c.	106		NT	216
	NT		NT		NT	50/s.c.	109		NT	217
	NT		NT		NT	50/s.c.	129		NT	218
	NT		NT		NT	50/s.c.	118		NT	219
	NT		NT		NT	50/s.c.	138		NT	220
	NT		NT		NT	50/s.c.	109		NT	221
	NT		NT		NT	50/s.c.	109		NT	222
	NT		NT		NT	50/s.c.	127		NT	223
	NT		NT		NT	50/s.c.	100		NT	224
	NT		NT		NT	50/s.c.	128		NT	225
40/s.c.	33		NT		NT	50/s.c.	213	50/s.c.	131	226
	NT		NT		NT	50/s.c.	120		NT	227
	NT		NT	100/s.c.	80	50/s.c.	163		NT	228
	NT		NT		NT	50/s.c.	130		NT	229
	NT		NT	100/s.c.	70	50/s.c.	138	100/s.c.	37	230

	NT		NT		NT	50/s.c.	117		NT	231
	NT		NT	100/s.c.	90	50/s.c.	107		NT	232
	NT		NT		NT	50/s.c.	157		NT	233
	NT		NT		NT	50/s.c.	119		NT	234
	NT		NT		NT	50/s.c.	208		NT	235
	NT		NT		NT	50/s.c.	150		NT	236
100/p.o.	75	596	8		NT	50/s.c.	140		NT	237
	NT		NT		NT	50/s.c.	139		NT	238
	NT		NT		NT	50/s.c.	153		NT	239
	NT		NT		NT	50/s.c.	155		NT	240
	NT		NT		NT	50/s.c.	129		NT	241
	NT		NT	100/s.c.	70	50/s.c.	157		NT	242
20/s.c.	84	57	98		NT	50/s.c.	113		NT	243
	NT		NT		NT	50/s.c.	107		NT	244
	NT		NT		NT	10/s.c.	102		NT	245
	NT		NT		NT	10/s.c.	125		NT	246
	NT		NT		NT	50/s.c.	109		NT	247
	NT		NT		NT	2/s.c.	100		NT	248
	NT		NT		NT	2/s.c.	102		NT	249
	NT		NT		NT	50/s.c.	161	10/s.c.	44	250
	NT		NT		NT	10/s.c.	100		NT	251
	NT		NT		NT	50/s.c.	138		NT	252
	NT		NT		NT	2/s.c.	98		NT	253
	NT		NT		NT	10/s.c.	112		NT	254
	NT		NT		NT	2/s.c.	96		NT	255
	NT	430	100	50/p.o.	0	250/p.o.	204	250/p.o.	41	256
	NT		NT		NT		NT		NT	257
	NT		NT		NT	250/p.o.	254		NT	258
	NT		NT	50/s.c.	70	50/s.c.	104		NT	259
	NT		NT		NT	50/p.o.	183		NT	260
	NT		NT		NT	50/p.o.	141		NT	261
100/p.o.	53	483	103	50/p.o.	10	50/p.o.	184	100/p.o.	44	262
100/p.o.	44		NT	100/p.o	20	250/p.o.	165		NT	263
	NT		NT		NT	50/s.c.	126		NT	264
	NT		NT		NT	50/p.o.	166		NT	265

Table 5.2B. (Continued)

Anti-Inflammatory[a]		*Complement Inhibition*[b]		*EAE*[c]		*EMC*[d]		*Vaccinia*[e]		*Compound No.*
Dose/Rt. (mg/kg)	*Paw Vol. (%C)*	*Dose (μg/ml)*	*Lysis (%C)*	*Dose/Rt. (mg/kg)*	*Paralysis (%)*	*Dose/Rt. (mg/kg)*	*Surv. time (%C)*	*Dose/Rt. (mg/kg)*	*Lesion score (%C)*	
	NT		NT		NT	50/s.c.	98		NT	266
	NT		NT		NT	50/p.o.	235		NT	267
	NT		NT		NT	10/p.o.	120		NT	268
	NT		NT		NT	50/s.c.	113		NT	269
40/s.c.	55		NT		NT	50/s.c.	193		NT	270
	NT		NT		NT	50/s.c.	189	50/s.c.	36	271
	NT		NT		NT	50/s.c.	193		NT	272
	NT		NT		NT	50/s.c.	155		NT	273
	NT		NT		NT	50/s.c.	125		NT	274
	NT		NT	100/p.o.	80	50/s.c.	141	100/s.c.	74	275
20/s.c.	72	516	55	100/p.o.	80	50/s.c.	104	100/s.c.	103	276
	NT		NT		NT	50/s.c.	114		NT	277
	NT		NT		NT	50/s.c.	187		NT	278
	NT		NT	100/s.c.	20	50/s.c.	219	250/p.o.	33	279
	NT		NT		NT		NT		NT	280
	NT		NT	100/p.o.	100	50/s.c.	190	100/s.c.	59	281
	NT		NT		NT	50/s.c.	152		NT	282
	NT		NT		NT	50/s.c.	169		NT	283
40/s.c.	61	43	76		NT	50/s.c.	179		NT	284
40/s.c.	80	491	56		NT	50/s.c.	126		NT	285
40/s.c.	34	5.4	91		NT	50/s.c.	210		NT	286
40/s.c.	29		NT		NT	50/s.c.	161		NT	287
40/s.c.	58		NT	100/s.c.	90	50/s.c.	159		NT	288
	NT		NT	100/p.o.	60	50/s.c.	130		NT	289
	NT	447	84	100/p.o.	30	50/s.c.	159		NT	290

	NT		NT		NT	50/s.c.	96		NT	291
	NT		NT		NT	50/s.c.	172		NT	192
	NT		NT		NT	50/s.c.	185		NT	293
	NT		NT		NT	50/s.c.	132		NT	294
	NT		NT	50/s.c.	20	50/s.c.	136		NT	295
	NT		NT		NT	250/s.c.	166		NT	296
	NT		NT		NT	50/s.c.	207		NT	297
	NT		NT		NT	50/s.c.	148		NT	298
	NT		NT		NT	50/s.c.	107		NT	299
	NT		NT		NT	10/s.c.	102		NT	300
20/s.c.	49	51	79		NT	50/s.c.	209	100/s.c.	77	301
20/s.c.	50	507	90	100/s.c.	60	50/s.c.	139	20/s.c.	69	302
20/s.c.	37	53	73		NT	50/s.c.	187		NT	303
20/s.c.	34	53	78		NT	50/s.c.	213		NT	304
20/s.c.	60	579	32		NT	50/s.c.	105		NT	305
	NT		NT	50/p.o.	80	50/s.c.	109		NT	306
100/p.o.	76		NT	50/s.c.	70	50/s.c.	220		NT	307
	NT		NT		NT	50/s.c.	213		NT	308
100/p.o.	93		NT	50/p.o.	80	50/s.c.	124	250/s.c.	80	309
	NT		NT		NT	10/s.c.	170		NT	310
20/s.c.	10	582	3	100/p.o.	40	50/s.c.	181		NT	311
	NT		NT	50/p.o.	100	10/s.c.	176		NT	312
	NT		NT		NT	50/s.c.	147	250/s.c.	92	313
	NT		NT	100/s.c.	80	50/s.c.	182	50/s.c.	47	314
	NT		NT		NT	50/s.c.	102		NT	315
100/p.o.	81	425	85	100/p.o.	80	50/p.o.	176	100/s.c.	53	316
	NT		NT		NT	50/s.c.	139		NT	317
	NT		NT		NT	250/p.o.	216		NT	318
	NT		NT		NT	50/s.c.	137		NT	319
	NT		NT		NT	50/s.c.	111	100/s.c.	78	320
	NT		NT	50/s.c.	80	50/s.c.	114		NT	321
	NT		NT		NT	50/s.c.	169		NT	322
	NT		NT		NT	50/s.c.	133		NT	323
	NT		NT		NT	50/s.c.	133		NT	324
	NT		NT		NT	50/s.c.	159		NT	325

Table 5.2B. (Continued)

Anti-Inflammatory[a]		*Complement Inhibition*[b]		*EAE*[c]		*EMC*[d]		*Vaccinia*[e]		*Compound No.*
Dose/Rt. (mg/kg)	*Paw Vol. (%C)*	*Dose (μg/ml)*	*Lysis (%C)*	*Dose/Rt. (mg/kg)*	*Paralysis (%)*	*Dose/Rt. (mg/kg)*	*Surv. time (%C)*	*Dose/Rt. (mg/kg)*	*Lesion score (%C)*	
	NT		NT		NT	50/s.c.	228		NT	326
	NT		NT	50/p.o.	100	50/s.c.	146		NT	327
100/p.o.	100		NT		NT	50/s.c.	112	250/s.c.	79	328
	NT		NT		NT	50/s.c.	98	50/s.c.	86	329
	NT		NT		NT	50/s.c.	131		NT	330
	NT		NT		NT	50/s.c.	229		NT	331
	NT		NT		NT	250/s.c.	181	50/s.c.	69	332
	NT		NT	100/s.c.	90	50/s.c.	136	50/s.c.	123	333
	NT		NT		NT	50/s.c.	178	50/s.c.	108	334
	NT	440	93	100/p.o.	30	50/p.o.	168	250/p.o.	17	335
	NT		NT		NT		NT		NT	336
20/s.c.	78	427	26		NT	50/s.c.	122		NT	337
	NT		NT		NT	50/s.c.	127		NT	338
	NT		NT		NT	50/s.c.	145	100/s.c.	93	339
	NT		NT		NT	50/s.c.	93		NT	340
20/s.c.	81	399	28		NT	50/s.c.	141	100/s.c.	98	341
	NT		NT	100/s.c.	40	50/s.c.	122		NT	342
	NT		NT		NT	50/s.c.	124		NT	343
	NT		NT		NT	50/s.c.	150		NT	344
20/s.c.	62	531	30	50/s.c.	90	50/s.c.	156		NT	345
	NT		NT		NT	50/s.c.	179		NT	346
	NT		NT		NT		NT		NT	347
20/s.c.	11	64	81		NT	50/s.c.	170		NT	348

BIOLOGICAL ACTIVITY OF THE TILORONES; SELECTED TOPICS

It is not possible in this article to present a complete summary of the published literature on tilorone and its analogs*. In the most recent review [35] Mayer and Krueger continue to link interferon with the various biochemical/biological activities of the tilorones and to assert that it has been proven 'that interferon induction is a major factor in the antiviral activity of tilorone and related compounds' [36].

The present authors take a contrary position. They believe that the activities of tilorone and its congeners result from variations on the theme of immuno modulation.

*A bibliography containing over 380 literature references on tilorone and analogues is available on request from the senior author.

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[a] *Direct passive arthus reaction* – A test designed to detect activity against immunologically-induced paw oedema in rat as potentially useful anti-arthitic agents. See reference [30] for experimental details. Activity is expressed as % of control (%C) where % C of > 75 is considered inactive, 61-75 is considered low activity, 40-60 is considered medium activity, < 40 is considered high activity. Typical response of the reference substance, cobra venom factor, is 49% C at 200-400 units/kg, i.v.

[b] *Anti-inflammatory, complement inhibition* – A test designed to detect anti-complement activity as measured by the compound's ability to inhibit lysis of sensitized sheep red blood cells by complement *in vitro*. See reference [31] for experimental data. Activity is expressed as % of control where % C of > 50 is considered inactive, 31-50 is considered low activity, 16-30 is considered medium activity, 0-15 is considered high activity. Typical response of the reference compound, maleopimaric acid, is 18% C at 401 μg/ml (10^{-3} M).

[c] *Experimental allergic encephalomyelitis (EAE)* – A test designed to detect compounds that are potentially immunosuppressive to delayed hypersensitivity or cell-mediated immunity. See reference [32] for experimental details. Activity is expressed as % of paralysis (% Paral.) in treated group where % Paral. of > 80% is considered inactive, 70-80 is considered low activity, 50-69 is considered medium activity, 0-49 is considered high activity. Typical response of the reference compound, hydrocortisone, is 10% Paral. at 25 mg/kg, s.c.

[d] *Encephalomyocarditis (EMC) virus, fatal infection* – A test designed to detect antiviral activity in mice infected with a fatal infection with an RNA virus. See reference [17] for experimental details. Activity is expressed as % control in the increase in survival time where % C of < 90 indicates early death, 90-109 is considered inactive, 110-119 is considered low activity, 120-129 is considered medium activity, > 130 is considered high activity. Typical response of the reference compound, Poly I:C is 172% C at 1.5 mg/kg, s.c.

[e] *Vaccinia virus, mouse tail lesion* – A test designed to detect antiviral activity against a DNA virus *in vivo*. See reference [33] for experimental details. Activity is expressed as % control where % C of > 69 is considered inactive, 50-69 is considered low activity, 30-49 is considered medium activity, 0-29 is considered high activity [34]. Typical response of the reference compound, *N*-methylisatin-β-thiosemicarbazone, is 22% C at 250 mg/kg, s.c.

[f] NT = Not Tested.

ANTIVIRAL ACTIVITY

INTERFERON INDUCTION AND ANTIVIRAL ACTIVITY

Turning first to data from the Merrel Research Center, nine compounds were highlighted in the recent review article by Mayer and Krueger [37]. The highest serum interferon levels of treated mice are reproduced in *Table 5.3*. The compounds are identified by RMI number and, in parentheses, the compound numbers from *Table 5.2* of this paper. The selected compounds are all highly active in the mouse against EMC virus. Concomitantly, all induce high levels of interferon. On inspection, it is evident that the efficacy values in the EMC virus screen (*Tabel 5.2*) are consistent with serum interferon levels as reproduced in *Table 5.3* for this RNA virus. Structures of compounds discussed in *Table 5.3* are presented in *Figure 5.2* for reference purposes.

However, if we examine the antiviral screening results reported for the DNA vaccinia virus, there is no discernible correlation with interferon induction. The widest swing in the screening data is represented by RMI 11,002 (compound

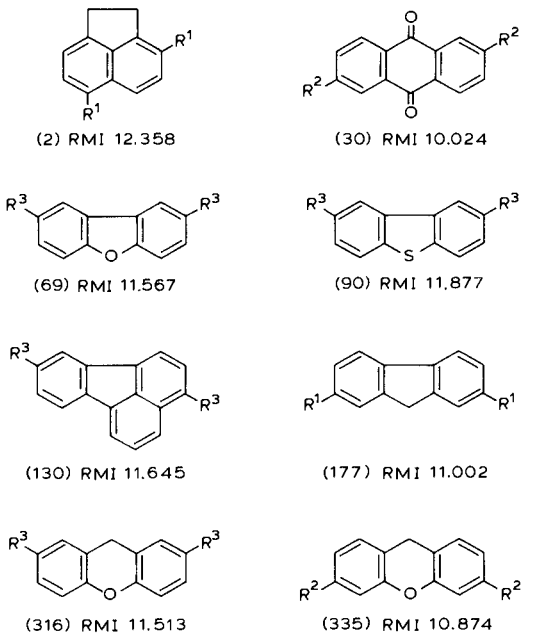

Figure 5.2. Structures of compounds listed in *Table 5.2*. R^1 = $COCH_2NEt_2 \cdot HCl$; R^2 = $OCH_2NEt_2 \cdot HCl$; R^3 = $COCH_2NMe_2 \cdot HCl$.

Table 5.3. COMPARISON OF INTERFERON INDUCTION AND *IN VIVO* (MOUSE) ANTIVIRAL ACTIVITIES SELECTED TILORONE ANALOGUES

No.	*RMI*	*Highest serum interferon titers*		*RNA virus EMC*	*DNA virus Vaccinia*				
		(100 mg/kg s.c.)	*(250 mg/kg p.o.)*	*Survival time*	*%*		*Lesion score (% of control)*		
				Dosage	*of control*	*Rating*[a]	*%*	*Rating*[a]	*Dosage*
2	12,358	800	3,200	250 mg/kg p.o.	196	H	13	H	250 mg/kg p.o.
30	10,024	12,800	12,800	250 mg/kg p.o.	220	H	62	L	50 mg/kg p.o.
69	11,567	6,400	25,600	50 mg/kg p.o	180	M	19	H	100 mg/kg s.c.
90	11,877	12,800	6,400	50 mg/kg p.o.	175	M	34	M	100 mg/kg s.c.
130	11,645	3,200	3,200	50 mg/kg p.o.	189	H	6	H	100 mg/kg s.c.
177	11,002	3,200	3,200	250 mg/kg p.o.	206	H	76	Inact.	250 mg/kg p.o.
262	Tilorone	12,800	6,400	50 mg/kg p.o.	184	M	44	M	100 mg/kg p.o.
316	11,513	3,200	6.400	50 mg/kg p.o.	176	M	53	L	100 mg/kg s.c.
335	10,874	1,600	6,400	50 mg/kg p.o.	168	M	17	H	250 mg/kg p.o.

a, H = high activity; M = medium activity; L = low activity; Inact = inactive.

177**). In both screens the compound was tested at an oral dose of 250 mg/kg. The EMC screening result of 206% survival time is in the high activity range; whereas the vaccinia virus tail lesion control score of 76% is rated as *Inactive.*

VACCINIA VIRUS STUDIES

A closer look at the effect of these tilorone congeners on the severity of vaccinia virus-induced tail lesions of mice further highlights the diverse non-parallel results versus interferon induction in the same animal [38].

RMI 12,358 (compound 2): Severity of tail lesions reduced by 87% with multiple subcutaneous doses of 250 mg/kg. A dose of 50 mg/kg was ineffective.

RMI 10,024 (compound 30): Tail lesion scores reduced by 71% and 38% with multiple oral doses of 250 mg/kg and 50 mg/kg, respectively.

RMI 11,567 (compound 69): A single oral dose of 250 mg/kg gave a 40%reduction in the tail lesion score; and multiple oral doses caused a 66% reduction. Using a multiple subcutaneous dosage regiment of 20 mg/kg resulted in a 55% reduction in lesion severity; and at 100 mg/kg an 81% reduction was achieved.

RMI 11,877 (compound 90): Multiple oral doses of 250 mg/kg reduced tail lesion severity by over 50% with either prophylactic or therapeutic regimens. However, a single prophylactic dose of 250 mg/kg had no effect. This result contrasts with compound 90's minimum effective dose of 21 mg/kg against Semliki Forest virus.

RMI 11,645 (compound 130): Multiple oral doses of 250 mg/kg did not reduce the severity of vaccinia virus induced tail lesions. Yet this compound was the most potent interferon inducer (among these nine compounds) at 50 mg/kg orally.

RMI 11,002 (compound 177): Multiple oral and subcutaneous doses of 250 mg/kg did not significantly reduce lesions. To again illustrate marked variability in virus susceptibility, *Table 5.4* presents the beautiful dose response obtained with the oral administration of this compound to mice 24 hours prior to inoculation with a fatal challenge of Semliki Forest virus.

Tilorone (compound 262): It required multiple oral doses of 250 mg/kg to achieve a 71% lesion reduction.

RMI 11,513 (compound 316): Multiple oral doses of 250 mg/kg did not significantly reduce the severity of vaccinia virus-induced tail lesions.

RMI 10,874 (compound 335): Multiple oral doses of 250 mg/kg and 50 mg/kg reduced tail lesion severity by 83% and 38%, respectively.

**The number in parentheses refers to compound number in *Tables 5.2A and 5.2B* of this paper.

Table 5.4. ORAL DOSE RESPONSE WITH RMI 11,002DA ADMINISTERED 24 HOURS PRIOR TO INOCULATION OF SEMLIKI FOREST VIRUS (17-24LD_{50}) IN 20-GRAM MICE

Treatment 24 hours before inoculation (mg/kg)	*Percent survivors*
0	17
10	30
50	40
100	70
250	100

INFLUENZA VIRUS STUDIES

Activity against the clinically important influenza viruses and herpes viruses was studied in some detail [38]. Practically all of the work was done in rodents. In general tilorone and its congeners were found to be less active against the influenza viruses than against the other RNA species, encephalomyocarditis virus and Semliki Forest virus. Tilorone demonstrated oral activity against the following five influenza strains: A (NWS); A_2 (Jap/305): B (Lee); B (Massachusetts); and A/Equine-2 (New Mexico). It was marginally active against A/Equine-1, and not active against A/Swine and A_0 (PR8). However, it required probing of experimental conditions and usually multiple dosing to achieve good activity against these respiratory viruses. Influenza A_2 (Jap/305) was used as the principal test virus in part to enable *in vivo* comparisons with amantadine. Thus, if the virus was administered to mice by aerosol treatment instead of nasal instillation, tilorone was more active than amantadine. In most other experiments, amantadine had greater activity. Tilorone's antiviral activity against influenza A_2 (Jap/305) is also additive with suboptimal doses of other agents and vaccines. In one illustrative experiment, tilorone-treated mice (using a multiple dose at 200 mg/kg) gave 30% survivors; a second group of mice given 10 mg/kg of amantadine resulted in 10% survivors; and inoculated, untreated controls resulted in 15% survivors. When the tilorone and amatadine regimens were combined, mice were completely protected from an intranasal instillation challenge of influenza A_2 (Jap/305) [39].

Tilorone congeners were tested mostly against influenza strains A_2 (Jap/305) and A_0 (PR8). Here, too, the authors [40] state that the order of anti-influenza activity does not follow the order of potency for interferon induction [41].

RMI 11,002DA (compound 177) was administered orally and subcutaneously

to mice at 28, 22, and 2 hours before as well as 2 hours after influenza A_2 (Jap/305) inoculation. The dosages were 50 and 250 mg/kg. No activity was demonstrated against a fatal challenge of this respiratory virus [42].

RMI 11,877DA (compound 90) was evaluated against influenza A_2 (Jap/305) virus in mice by the oral, subcutaneous, and intranasal routes. Significant activity, as judged by increases in the numbers of survivors and survival time, was obtained via the oral route with 250 mg/kg given in multiple doses 28, 22, and 2 hours before infection and 2 hours after infection. The compound was inactive when administered to mice by the subcutaneous or intranasal routes.

Activity against influenza A_0 (PR8) virus was obtained with a single oral dose of 250 mg/kg given to mice 24 hours before viral inoculation by aerosolization. Lower doses of 100, 50, or 25 mg/kg were not significantly active [43].

RMI 11,567DA (compound 69) was evaluated against two strains of influenza virus, influenza A_2 (Jap/305) and influenza A_0 (PR8). Oral administration was most effective against influenza (PR8) virus when the compound was given 24 hours before virus inoculation. Oral administration of 250 or 50 mg/kg to mice 28, 22, and 2 hours before and 2 hours after infection provided no significant protection against influenza A_2 (Jap/305) virus. Intranasal administration of single doses of 10 to 100 mg/kg given 24 hours before infection was not significantly active. No significant activity against influenza A_2 (Jap/305) was demonstrated with 20 to 100 mg/kg given subcutaneously 2 hours before, and 4 and 24 hours after virus infection [44].

HERPES VIRUS STUDIES

Evaluation of the same group of tilorone analogs against herpes virus hominis resulted in yet a different spectrum of activities [35, 45]. Tilorone itself was active orally against fatal challenges of herpes virus administered intraperitoneally to mice; but not when the virus was given intracranially. The results of experiments in which eye lesions were induced by direct trephine inoculation of herpes virus into rabbit eyes are summarized as follows: Pretreatment with a single oral 100 mg/kg dose of tilorone was compared with untreated controls and a single intravenous dose (at 400 mcg/kg) of poly I:C used as a reference standard. Six days after virus inoculation, untreated controls gave a mean lesion score of 7; whereas the tilorone and poly I:C scores were 1.4 and 0.9, respectively. On the other hand, tilorone was inactive on topical administration as well as oral administration 72 hours after inoculation.

A single oral prophylactic administration of 50 mg/kg suppressed herpes virus-induced mouse tail lesions. However, an exacerbation of lesions occurred with a multiple prophylactic dose regimen of 50 mg/kg; but at 250 mg/kg there

was no consistent influence on the severity of the lesions. 'Zoster-like' lesions induced in hairless mice were not affected by oral tilorone treatment. When tilorone was used topically at an 0.1% concentration, there was an exacerbation of the tail lesions [39].

The development of zoster-like lesions provoked by cutaneous inoculation of herpes virus hominis onto the backs of hairless mice was not prevented by prophylactic systemic administration of tilorone or analogs.

Nevertheless, the hairless mouse was found to be a useful model for evaluating topical antiherpetic activity. The lower back of the mouse was scarified and then inoculated with virus by application with a cotton swab. A viral-induced lesion develops unilaterally or bilaterally reaching maximum within 6 to 8 days. RMI 11,002 (compound 177); RMI 11.567 (compound 69); RMI 11,877 (compound 90) and tilorone all demonstrated antiherpetic activity in this topical treatment model. The other congeners were not studied. A buffered 5% solution of the 3 congeners was not irritating to the mouse skin; but tilorone itself was somewhat irritating and less active.

In a somewhat similar set of experiments, 5% solutions of the above tilorone congeners (compounds 177, 69, and 90) were tested topically afainst herpes virus hominis infections of the eye in rabbits. The right eye of each rabbit was challenged with 2300 tissue culture doses of virus by trephination. The left eye of each rabbit received treatment comparable with the right eye but minus trephination and infection. In order to meet subsequent clinical requirements, the protocol called for treatment to start after eye lesions were established and recognized. This severe four-day test resulted in only RMI 11,877 (compound 90) showing significant antiviral activity. After eight topical applications over a two-day period, RMI 11,877 effectively slowed the progress of herpes-induced lesions of the rabbit eye; whereas RMI 11,002 and RMI 11,567 were no better than the untreated inoculated control group. On the other hand, multiple 250 mg/kg oral doses of RMI 11,877 were evaluated against herpes vires hominis Types 1 and 2 in both mice and rats with inconclusive results.

The above tilorone congeners plus RMI 11,513 (compound 316), RMI 11,645 (compound 130), RMI 10,874 (compound 335), and RMI 10,024 (compound 30) all show consistent activity against herpes virus hominis in monkey vero cell culture screens. These results again support the concept that the tilorone family of compounds has antiviral actions that are not interferon-mediated.

The preceding encouraging experience with control of cutaneous herpes virus infections prompted further evaluation of RMI 11,567 (compound 69). In mice it is a potent an antiviral agent as tilorone but with again a different activity spectrum. Referring to *Table 5.3,* note that at a dosage of 250 mg/kg RMI 11,567 (compound 69) induced the highest levels of interferon recorded for the tilorone

series. Yet at 50 mg/kg the resulting interferon levels were only a minimal 50 units. However, RMI 11,567 (compound 69) has an oral ED_{50} value of 15 mg/kg against Semliki Forest virus in the same murine species. Oral doses of 250 mg/kg were ineffective in reproducibly influencing herpes virus hominis Types 1 and 2 cutaneous lesions in mice and rats. Mouse interferon induced by RMI 11,567 had no effect on HVH Type 1 cells in tissue cultures but *in vitro* RMI 11,567 inactivates herpes virus directly.

Accordingly, a pharmaceutically-acceptable topical formulation was prepared and found to be tolerated by hairless mice and Rhesus monkeys when administered twice a day for three weeks. This preparation of RMI 11,567, when applied to skin infected with herpes virus Type 1 at the base of the tail in Swiss white mice or in the lumbosacral region in hairless mice at concentrations of 3 or 5%, produces a marked reduction in the severity of the cutaneous lesions and largely prevents the ascending infection, myelitis, and paralysis which subsequently occur [46].

HYPOREACTIVITY AND ANTIVIRAL ACTIVITY

Hyporeactivity, a condition whereby an initial dose of a compound interferes with the response of the host to a subsequent dose, is common to all interferon inducers and is regarded as a fundamental problem in their potential clinical usefulness [47]. With tilorone and its congeners the phenomenon has been demonstrated only in rodents. In this group of compounds, hyporeactivity, as measured by interferon response, correlates directly with activity against EMC virus. Nevertheless, incorporation of tilorone into feed protected mice from EMC viral challenge one week later. Moreover, a tilorone analog essentially inactive as an interferon inducer did establish hyporeactivity to tilorone [35]. Separately, with serum collected from tilorone-treated mice during the period of maximum hyporeactivity, the Merrell group could not: (a) prevent tilorone from inducing interferon *in vivo* when such serum was mixed with tilorone and aministered orally or subcutaneously to mice; (b) suppress the *in vitro* acitivity of serum interferon derived from tilorone-treated mice; and (c) suppress stimulation of interferon in mouse L929 cells by poly I:C in the presence of DEAE-dextran. Mayer and Krueger also reported that the incorporation of low levels of tilorone into drinking water one week before using mice for other experimental studies protected the mice from the lethal effects of an apparently transmissable infection of unknown etiology [48]. Independently, Glaz and Talas reported [49] that tilorone incorporated into drinking water of mice maintained the mice on a continuous low level of serum interferon.

Stringfellow has pointed to a role for the prostaglandins in interferon inducer

hyporeactivity [50]. However, for tilorone the picture is confusing, even in the rodent. It seems reasonable to conclude that hyporeactivity is not likely to be demonstrated in higher animal species and therefore will not be a negative factor in developing therapeutic applications for tilorone and its congeners.

ANTI-VENEZUELAN EQUINE ENCEPHALOMYELITIS (VEE) ACTIVITY IN MONKEYS

Except for one Russian team [51–53], investigators have not been able to detect levels of serum interferon in primates (including man) after administration of tilorone or any of its analogs [54–56]. Therefore, the Merrell group found it necessary to evaluate other animal models which could provide unequivocal evidence of antiviral activity. VEE was selected as a virus which could be administered to both monkeys and man in a suitable viral challenge protocol [57]. An attenuated live VEE virus vaccine commercially used in veterinary medicine is approved safe for use in man but has had only minimal human exposure. Vaccinated humans develop viremia and good immunity; a high proportion of the vaccinated subjects develop a brief illness with influenza-like symptoms. The advantages of using this VEE vaccine thus include: (a) objective laboratory meaurements are readily determined; (b) mild clinical symptoms can be utilized for subjective appraisal (in man) and (c) a large pool of susceptible human volunteers should be available because of the improbability of prior exposure to this virus. A supply of commercial VEE (TC-83) vaccine virus was obtained from the former Jensen-Salisbery Veterinary Division of Richardson-Merrell Inc., and determined to be biologically active in tissue culture systems, in mice and in Rhesus monkeys.

Administered parentarally, the live VEE vaccine used in these studies caused neither paralysis nor lethality in mice or monkeys, but a viremia and an antibody response to the live antigen could be readily demonstrated. Both responses depend on viral replication within the host as they were not measurable with comparable antigenic masses that were formalin-inactivated. Viremia and serum-neutralizing antibody were completely prevented in tilorone-treated mice. These responses were markedly evident in untreated mice.

Three tilorone analogues, RMI 11,002 (compound, 177), RMI 11,567 (compound 69) and RMI 11,877 (compound 90), were selected for Rhesus monkey studies [57]. However, they were first studied in the mouse. As determined by plaque neutralization, tilorone and the 3 congeners prevented the appearance of VEE virus antibody within 11 days when 1000 tissue cultures doses of vaccine were given 22 hours after oral treatment with 250 mg/kg of compound.

As evident in *Table 5.5,* the lack of serum antibody from mice treated with these compounds is presumed to be due to inhibition of virus. Some virus neu-

Table 5.5 PREVENTION OF VEE VIRUS ANTIBODY IN MICE TREATED ORALLY WITH TILORONE OR RELATED COMPOUNDS

Compound No. (Table 5.2)	*RMI number*	*% Virus neutralization by serum*
	None	98
262	Tilorone	0
177	RMI 11,002DA	35
69	RMI 11,567DA	2
90	RMI 11,877DA	0

tralization (35%) is seen with RMI 11,002DA but the most dramatic antiviral responses are seen with tilorone, RMI 11,877DA and RMI 11,567.

In the next experiment, 9 groups of 4 Rhesus monkeys were treated orally with single doses of 200 mg/kg of the same compounds; RMI 11,002, RMI 11,567 and RMI 11,877. One group of 4 monkeys was left untreated to serve as controls. Twenty-two hours after dosing with the compounds, all monkeys were challenged subcutaneously with 100,000 tissue culture doses of the live VEE vaccine. The monkeys were bled on days 4, 8, 11, and 14 post challlenge. The sera were diluted logarithmically and applied to confluent vero cell monolayers. The presence of virus was determined by cytopathology within 72 hours. *Figure 5.3* records viremia determinations in all monkeys. Virus was detectable only in the samples taken on day 4. All 4 control monkeys had at least 2 logs of virus in the blood. Complete suppression of viremia was achieved in all monkeys treated with RMI 11,002. Three of the 4 animals given RMI 11,567 had no viremia. Although only 2 of the 4 animals given RMI 11,877 had a suppressed viremia, the amount of virus in the 2 that did show viremia was considerably less.

Figure 5.4 shows the antibody response in these monkeys 11 days after virus challenge. The antibody response was generally less in treated monkeys than in the untreated monkeys. Although the correlation between virus and antibody is not absolute in these preliminary studies, there is no question that RMI 11,002, RMI 11,567 and RMI 11,877 demonstrate antiviral activity in the Rhesus monkey. And note that no interferon could be detected in the serum of Rhesus monkeys treated with a 200 mg/kg single oral dose of these compounds or with tilorone.

Other investigators have also reported on the lack of correlation between interferon induction and antiviral activity with tilorone, its congeners [58–61] and other inducers [47, 62, 63]. Giron, Schmidt and Pindak [58] studied the protection elicited by differend doses of tilorone given intraperitoneally against Mengo Meningitis virus infection in mice. At the same time, they determined the amount

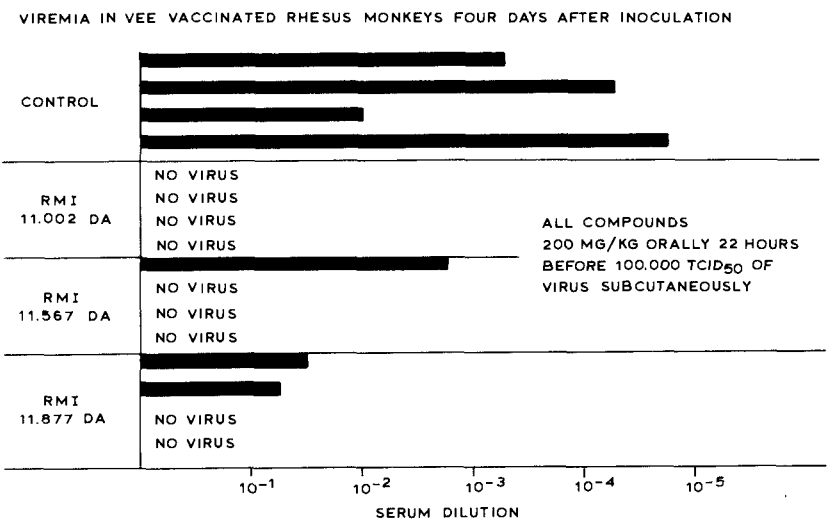

Figure 5.3. Viremia in vee vaccinated rhesus monkeys four days after inoculation (Modified from Ref. 57).

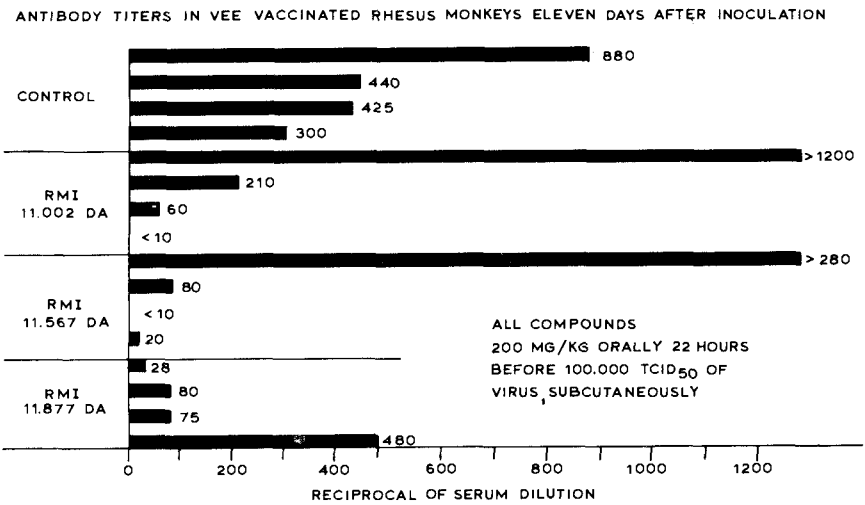

Figure 5.4. Antibody titres in vee vaccinated rhesus monkeys eleven days after inoculation (Modified from Ref. 57).

of circulating interferon induced by each dose of tilorone. Their data show that a tilorone dose of 7.5 mg/kg was fully protective, but circulating interferon could not be detected with tilorone doses of less than 150 mg/kg.

The preceding sections of this report clearly demonstrate that three tilorone congeners are able to act as antiviral agents in the Rhesus monkey, and that even in rodents, antiviral effects are measurable in the absence of serum interferon (as assayed by well-standardized procedures). However, the Russian workers, in their comparison of the interferon-inducing and antiviral effects of tobacco mosaic virus, sodium nucleinate (yeast RNA) and tilorone, used smaller doses of tilorone in their studies. In the mouse, their dosage regimen was 5 mg/kg to 30 mg/kg given orally to the animals on three consecutive days. The mice were infected one day later. Newcastle Disease Virus, strain H, was used as a reference standard for interferon induction. They regard 50 – 75 IU/ml as a fairly high interferon titer. In a mouse experiment, the oral administration of 10 mg/kg of tilorone produced a peak interferon titer of 80 IU/ml in 24 hours; and at 72 hours it was still at 40 IU/ml. The NDV reference standard gave a titer of 60 IU/ml. In monkeys, 25 mg/kg administered orally twice a day for two days generated the highest interferon titer of 128 IU/ml. After six days a significant interferon titer of 16 IU/ml was measured. The authors report that the tilorone was well tolerated by both the mice and the monkeys [52, 53].

These reports raise further questions regarding the role of (induced) interferon in the antiviral activity of tilorone and its congeners: (a) interferon assay methodology may not be completely standardized; (b) even trace amounts of interferon can exert an antiviral effect; or (c) other mechanisms may be involved in the observed protection of animals against viral challenge. This brings us to a brief discussion of the lymphokine-cytokine system.

LYMPHOKINES, MONOKINES AND CYTOKINES

Interferons are included in a large class of glycoproteins originally designated as lymphokines because they are produced by lymphocytes activated by either mitogens or specific antigens. Cohen and Yoshida [64] have suggested that lymphokines which are regarded as non-antibody mediators may be classified into three major groups: (1) factors that affect inflammatory cells, (2) factors that induce cell proliferation and (3) factors that destroy or exert toxic effects on cells. Monokines are similar molecules produced by macrophages, and the more general term of cytokines has been introduced to include mediators produced by other types of cells. According to B. Waksman [65], more than 100 lymphokine-cytokine factors have been reported since the most commonly

known lymphokine, macrophage migration inhibitory factor (MIF) was described 15 years ago. Each lymphokine is defined and assayed in terms of a specific biological activity in a target cell, in most cases under *in vitro* conditions. Progress has been slow indeed in characterizing (in a biochemical-chemical sense) this plethora of subcellular products.

Figure 5.5 schematically depicts lymphokine-cytokine formation by immune stimulation and names some of the commonly accepted factors.

In a series of elegant studies [61, 66, 71], a group of investigators from the Institute of Microbiology and Experimental Therapy, Academy of Sciences of the GDR, Jena, have documented the coincidental appearance of several other lymphokines-cytokines along with interferon in the serum of mice following oral dosing with 250 mg/kg of tilorone. The specific tilorone-induced factors are designated migration inhibitory cytokine and bacterial inhibiting cytokine. Their serum kinetics were differentiated from tilorone-induced interferon and also from other lymphocyte-derived cytokines. Tilorone can activate macrophages [72–74] to enhance phagocytosis [75]. For this and other reasons, it was suggested that the newly characterized cytokines are in fact produced by such tilorone-activated macrophages. The authors, Zschiesche, Fahlbusch, Schumann and Tonew [66] conclude that cytokine production (other than interferon) may be responsible for the immunoregulatory and antiviral effects of tilorone.

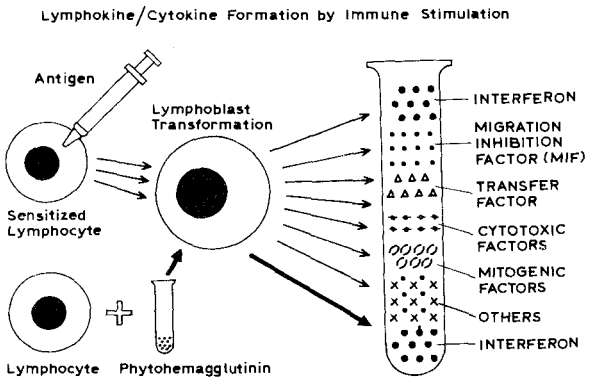

Figure 5.5. Lymphokine/cytokine formation by immune stimulation. Sensitized lymphocytes in the presence of specific antigen and phytothemagglutinin are known to undergo blast transformation and to release many biologically active substances thought to be important in specific cellular immunity. Reprinted with author's and publisher's permission from M. Ho, *Resident and Staff Physician*. October 1973, p. 52.

IMMUNOMODULATION

It is clear that many (perhaps most) of the biological activities of tilorone and its congeners are effected via intervention of host defence mechanisms; and that, broadly speaking, the lymphokine-cytokine system mediates these actions. Agents which alter a host's immune process have been variously labelled immuno-suppressants or immuno-stimulants. However, it is now universally acknowledged that immuno-stimulants can be made immuno-suppressive, given certain circumstances, and vice versa. Also there are drugs that modify the immune response, and there are other drugs that alter immune reactions without modifying the immun response. Participants in a 1977 meeting on 'Perspectives in Inflammation' urged that we should use the more general term 'immunomodulators'. Levamisole and cyclophosphamide were used as illustrative examples of compounds that act as immunostimulants or immunodepressants depending on the experimental circumstances [76].

The fact that levamisole has a beneficial action on host-defence mechanisms emerged (as a surprise) following retrospective analysis of a number of early animal and human studies of its parent drug, tetramisole, as an anthelmintic agent [77]. Levamisole was then reported to be an immunostimulant [68, 79], and more recently even an interferon inducer [80]. Recognition of the immunomodulatory role of tilorone was a surprise outgrowth of its broad spectrum antiviral activities [81]. We shall see later that while levamisole and tilorone are different in many of their biologic actions, they may have overlapping immunoregulatory effects in rheumatoid arthritis, but with a distinctly different pattern of side-effects.

In the early 1970's scientific dogma held that all immuno-suppressive agents were inherently 'of carcinogenic potential'; and, as recorded by one of us [82] the FDA used this concept to severely restrict early clinical pharmacology/efficacy studies of tilorone and its analogs in the areas of inflammation and viral diseases.

The overall immunodulatory responses of tilorone and selected analogs have been reviewed elsewhere by Megel [83], and other Merrell scientists [84–86]. Mayer and Krueger have provided additional information on the nine compounds highlighted in their recent publication [87]. These compounds did not uniformly effect humoral immunity as measured by stimulation of IgG and IgM antibody production in mice. They also show considerable variation in their ability to reduce the incidence of paralysis in the Lewis rat experimental allergic encephalomyelitis (EAE) model used as a measure of cell-mediated immunity. *Table 5.6* records the screening results of these selected compounds, plus their effect in IgG and IgM antibody production.

Table 5.6. IMMUNOMODULATORY SCREENING RESULTS SELECTED TILORONE ANALOGUES

Compond Number		*Arthus (Rat)*	*Humoral immunity stimulation*		*Cellular mediated immunity suppression*
Table 2	*RMI No.*		*IgG*	*IgM*	*EAE (Rat)*
2	12,358	Inactive 20 mg/kg, s.c.	Yes	Yes	High activity 20 mg/kg, s.c.
30	10,024	Low activity 20 mg/kg, s.c.	Yes	No	Inactive 100 mg/kg, p.o.
69	11,567	Not tested	Yes	Yes	High activity 100 mg/kg, p.o.
90	11,877	Not tested	Yes	No	High activity 50/mg/kg, p.o.
130	11,645	Moderate activity 100 mg/kg, p.o.	Yes	No	Moderate activity 100 mg/kg, p.o.
177	11,002	Inactive 20 mg/kg, s.c.	Yes	No	Moderate activity 100/mg/kg, p.o.
262	Tilorone	Moderate activity 100 mg/kg, p.o.	Yes	No	High activity 50 mg/kg, p.o.
316	11,513	Inactive 100 mg/kg, p.o.	Yes	No	Inactive 100 mg/kg, p.o.
335	10,874	Not tested	Yes	Yes	High activity 100 mg/kg, p.o.

Other investigators have reported a variety of experiments which they regard as demonstrating stimulation of the cell-mediated immune system. Tilorone augments the antigen-induced mitogenic response of human lymphocytes [88], and stimulates human lymphocytes to release a soluble factor which enhances mobility of human PMN cells [89].

In rabbits, an oral dose of 50 mg/kg of tilorone given three times over five days to animals with established Brown-Pearce carcinoma functions as an immunostimulant to normalize the functional capacity of hepatocytes and reticuloendothelial cells of the liver [90]. In guinea pigs, a tilorone dose of 15 mg/kg every other day for two weeks produced a slight increase in spleen weight, number of cells recovered, and T-cell percentage; by contrast, cyclophosphamide and azathioprine caused significant reductions in all three parameters of immune activity [91].

Two separate studies [92, 93] reported on the radioprotective effectiveness of tilorone in mice, and provided evidence that it was effected by mechanisms other than interferon induction. It was suggested that tilorone's actions (including protection from radiation produced chromosomal damage) could be related to RES enhancement.

Finally, a recently reported modified humoral immune assay for hemolysin determination clearly showed that tilorone stimulates humoral immunity, cyclophosphamide has a marked immunosuppressive action, and Bleomycin A2 has no immune effect [94].

The foregoing tilorone discussion, plus analogous reports on levamisole [95, 96], muramyldipeptides [97], glucans [98, 99], poly I:C [100], etc. [101], clearly establish the pleiotropic nature of immuno-modulators. Accordingly, the general characterization of such substances as 'immunostimulant' or 'immunosuppressive' must now be regarded as unscientific.

TRANSPLANTATION

Interplay of the various facets of immunomodulation is nowhere more critical than in the management of organ transplants. Here, too, the role of tilorone is still unfolding. Based on its immunopharmacologic profile, tilorone, given continuously in the drinking water, was shown to prolong mouse tail skin allografts [102]. Given for three consecutive days, tilorone suppressed a graft-versus-host (GVH) reaction in recipient rats [103]. Prolongation of skin and heart allografts in tilorone-treated rats was reported by Wildstein, Stevens and Hashim [104]. More importantly, the same group reported that tilorone prologed the mean survival time of canine renal allografts [105, 106]. The drug was given as a single agent to recipient animals at 35 mg/kg s.c. on days–1, 0, and then every other day until rejection. It is important to note that interferon could not be detected in tilorone-treated dogs [107, 108]. In concurrent experiments, recipient animals receiving low-dose methylprednisolone and azathiprine as immunosuppressants had an intermediate survival time between the tilorone group and the control group. The combination of tilorone plus low-dose immuno-suppressants was the most effective regimen, and all three drugs, tilorone, azathioprine and methylprednisolone were quite compatible.

In clinical practice, azathioprine-prednisone has been standard therapy for renal transplantation. Antilymphocyte globulin (ALG) is often added to this combination because it selectively depresses T-cells. As summarized in *Table 5.7,* tilorone and ALG share a number of immune responses, but one very important point has been overlooked. Whereas ALG causes T-cell depletion and lympho-

Table 5.7. EFFECT OF TILORONE AND ALG ON CELL-MEDIATED AND OTHER IMMUNE RESPONSES

Immune response	*Tilorone*	*ALG*
EAE	↓	↓
Adjuvant arthritis	↓	↓
Tuberculin skin reactions	↓	↓
GVH	↓	↓
Transplant rejection	↓	↓
Antibody responses	Increases	Increase or no effect
Spleen and lymph node	Transient T-cell deplation	T-cell depletion
WBC	Transient lymphopenia	Lymphopenia

↓ Suppresses

H. Megel, personal communication; editorial, Br. Med. J., 1 (1975) 644.

penia, with tilorone these two immune responses are *transient*. Thus, in the canine renal transplant study circulating lymphocytes were reduced by greater than 50% 24 hours after tilorone administration, but 48 hours after surgery lymphopenia could not be demonstrated in any tilorone-treated animal [106]. Similarly, using tilorone, 1000 mg/weekly, orally, in an ongoing study of 7 patients with acute leukemia in relapse, 5 patients recorded a *transient* decrease in percent of T-cells at three weeks which had returned to normal levels by the sixth week of drug administration [109].

Renal transplant epidemiology data [110] indicate that bone marrow suppression, increased risk of virus infection, and cancer are the major problems. It has recently been suggested [111] that the failure of interferon to act prophylactically against CMW and HSV viral infections in renal transplant patients may be due to cumulative immuno-suppressive effects of interferon and the standard azathioprine-prednisone maintenance therapy.

In a recent review [112] the following criteria are proposed in searching for an ideal agent for treating transplant patients: (a) depresses T-cell function, (b) has antiviral activity, and (c) inhibits cancer cell growth. Tilorone fulfills these criteria, plus providing the added advantage of not causing bone marrow suppression in animals or humans [109, 113, 114]. A recent Russian study [115] entitled 'Tilorone, a synthetic immunostimulant with antitumour activity', reported that the cyclic use of tilorone (in rats and mice) in combination with other antitumour medication (*i.e.*, thiophosphamide) inhibited tumour growth without provoking significant leukopenia. In fact, tilorone itself, when administered intraperitoneally or orally at doses of 20–30 mg/kg in therapeutic courses of 10 – 15 daily applications, increased the total leukocytes by 27%.

Again, it would appear that tilorone's image as a 'rodent only' interferon in-

ducer has mitigated against its controlled evaluation in the long-term maintenance of transplant patients – an area in critical need of improved drug therapy.

INFLAMMATION

Inflammation is the second most common reason (after infection) that causes people to seek medical help. And biomedical scientists are in broad agreement that immuno-modulatory treatment modalities offer the best promise of arresting and curing diseases such as rheumatoid arthritis. Yet, even in this area, where much progress is evident, a symposium chairman in his closing remarks said: 'Thus, we can pose a further question, do we suppress or enhance the immune response in the treatment of rheumatoid arthritis [116]'?

The potential of tilorone as an antirheumatoid arthritic agent has been adequately developed and reviewed by Megel [83]. The compound is active orally as an antiphlogistic agent in models of inflammation induced by immunologic (direct passive Arthus reaction, adjuvant arthritis) as well as by non-immunologic (carrageenan) methods. In many of the standard test systems, tilorone has been compared with the agents currently classified as Slow Acting Anti-reumatic Drugs (SAARD's), such as D-penicillamine, gold salts, azathioprine, chloroquine, and levamisole [117]. The Merrell group has found varying ranges of comparative activity among these agents, including tilorone and its analogues, except that levamisole was not active [118]. The Janssen Pharmaceutica group have also reported [119] that levamisole is not active in standard anti-inflammatory test systems. It was of interest to note in a recently published modification of the 18-hour anti-arthritic and carageenan oedema tests in rats that levamisole was shown to be a fully effective inhibitor of oedema formation in both experimental animal models but only following high oral doses. Tilorone was one of the more active compounds in these tests [120].

Tilorone also has an antipyretic effect in the cat [121] and other activities which seem to point to a role for cyclic AMP [122] and not prostaglandins [123] which remains to be elucidated. Even more exciting is the information that a copper (Cu^{2+}) complex of tilorone is up to 10 times more active than tilorone hydrochloride in several standard anti-inflammatory assays [124].

The question of interferon induction has naturally impinged on consideration of tilorone as a clinically useful anti-inflammatory agent. Tilorone is effective both prophylactically and therapeutically in treating adjuvent arthritis, a disorder that is uniquely inducible in rats and resembles human rheumatoid arthritis [125, 126]. Interferon is also effective in this test system. The broader role of viruses in the etiology of arthritis and other so-called auto-immune-slow virus

diseases is still under extensive debate [127]. Perhaps the recent report of chronic arthritis in goats caused by a retrovirus would provide a suitable model to test out the interferon question [128].

Finally, at the clinical level, tilorone was administered intra-articularly to three seriously afflicted rheumatoid arthritis patients in whom all other medication had failed. Tilorone demonstrated activity in 1 of 3 subjects when the dose reached 100 mg injected into the knee. In the best effect, there was dramatic improvement (lasting for six months) in the treated knee; but not in the other control knee. In two patients there was a temporary flare up of the injected knee, reminiscent of clinical experience with levamisole [129].

TOXICITY/DRUG SAFETY

Various preclinical drug safety studies, including six week sub-acute administration to rats and rhesus monkeys, have established reasonable no-effect dosage levels for tilorone, RMI 11,002 (compound 177), RMI 11,567 (compound 69), and RMI 11,877 (compound 90), to warrant cautious administration to humans with severe and life-threatening diseases [129]. In fact, Investigative New Drug applications (INDs) were filed with the U.S. Food and Drug Administration on these four tilorone compounds, and three compounds have been given to a limited number of healthy human volunteers and sick people.

Tilorone has been the most studied. In a 1976 letter (which is part of the FDA files), the Merrell Laboratories medical monitor stated: 'Approximately 350 patients and normal volunteers have received tilorone without any major adverse side effects' [129].

Human experience with tilorone was summarized in a 1975 symposium presentation at Stanford University [109]. Tilorone side effects consisting primarily of GI and CNS symptoms were reported to be dose-related. The gastrointestinal side effects of nausea, vomiting, and diarrhea occurred at single doses above 15 mg/kg and at daily doses over 8 mg/kg. Paradoxic effects have been observed in the CNS. Some subjects have complained of drowsiness, whereas others complained of stimulation and insomnia.

Corneal changes were seen in four patients on prolonged therapy. These changes developed after a total dose of at least 25 grams of tilorone. The patients complained of seeing halos around lights at night. Examination of the corneas showed haziness and superficial epithelial changes resembling those seen in some patients taking chloroquine or phenothiazines. These changes have been reversible after discontinuing the drug. More recently Weiss, Weinberg, and Regelson reported [130] that the corneal opacities are due to the deposition of tilorone it-

self, and seem related to the total amount of drug received. These authors confirmed that visual acuity was not effected and that the eye changes were slowly reversible after cessation of therapy.

In this connection, the possible use of the di-*N*-oxide of Tilorone, RMI 13,661 (compound 263) is being considered. RMI 13,661 is a fully active metabolite of tilorone which is considerably less toxic than the parent compound in oral and subcutaneous LD_{50} determinations in mice [129]. In preliminary metabolism studies, reduced and reduced-deethylated forms of RMI 13,661, corresponding to tilorone and deethylated tilorone, are found in liver following oral administration of RMI 13,661 to mice. It is thought that the pattern of migration to epithelial tissue may be different.

Long term experience of treating Stage IV cancer patients has been summarized by G.C. Mayer and R.F. Krueger [35]. In the area of viral diseases, a renal transplant patient diagnosed as having progressive multifocal leukoencephalopathy (PML) was satisfactorily maintained on a regimen of tilorone (2.5 mg/kg twice weekly) and prednisone for 31 months [131]. The use of tilorone to treat subacute sclerosing panencephalitis (SSPE) in scattered uncontrolled studies has given results similar to those reported for Isoprinosine in SSPE [132]. Where longer term administration of tilorone to SSPE patients has been possible (up to 20 mg/kg in single weekly oral doses), no significant side effects were noted. One young SSPE patient in Cincinnati, Ohio has currently been treated for over 36 months at a weekly dose of 500 mg administered as 250 mg each day on the first two days of the week [133].

Recent reviews [134, 135] have stressed the adverse effects of tilorone in animal studies. The cited reports deal with rodent experiments for the most part and fail to consider the important species differences with regard to interferon induction. Further, exogenously administered interferon produces the same toxicity symptoms (such as accelerated mortality in young mice [136, 137]). The adverse effect of T-cell depletion has been discussed. But the tilorone literature describes the transient nature of leukopenia in various animal species following multiple dosing with tilorone (in the 10-60 mg/kg range).

Even in rats and mice, the transient morphologic changes which occur after tilorone administration have been noted with great interest. Twenty-four hours after an initial dose of tilorone, a peripheral lymphopenia is observed with depletion of periarteriolar lymphocytes in the spleen and lymph nodes, areas thought to be populated by T-cells. In the bone marrow lymphocyte stimulation is observed, as manifested by an increase in lymphoblastic transformation and mitotic figures. Within 48 hours the peripheral lymphocyte count returns to normal and the previously depleted areas in the spleen and lymph nodes have also returned to normal. The bone marrow lymphocytes, however, continue to show

evidence of hyperactivity for up to 10 days. These changes are not thought to be due to lymphocytotoxicity. *In vitro* studies on human lymphocytes have not shown lymphocytolysis or a decrease in rosette-forming cells [109].

More importantly, in man the evidence to date is that tilorone functions primarily as a T-cell normalizer [109, 132]. Further, its lack of clinical bone marrow depression [35, 109, 113, 114] and possible stimulation of platelet production create a great incentive for the use of tilorone as part of cancer chemotherapy regimens and in the treatment of arthritis and other autoimmune diseases. The need now is for tilorone to be studied at lower dosage levels and in protocols utilizing alternate day or bi-weekly drug administration. It is rare indeed to find a drug which has tilorone's spectrum of immunomodulatory effects but does not seem to cause agranulocytosis in man.

The tilorone congener RMI 11,002 (compound 77) has been given to about 100 subjects. The side effects of RMI 11,002 are similar to those of tilorone with two important differences. First, no corneal changes have been observed, and second, rash seems to be significant side effect. Five of 44 subjects (11%) developed a rash twelve to fourteen days after daily oral intake of the drug. The rash was maculopapular in type and disappeared about seven days after discontinuing the drug. RMI 11,002 is well tolerated in single doses up to 10 mg/kg [109]. A stage IV cancer study involved 32 subjects who were treated daily with 1.6 to 25 mg/kg of RMI 11,002 for up to 91 days [138].

RMI 11,002 was chosen for phase II trial in patients with severe rhumatoid arthritis unresponsive to other forms of therapy [129]. The selection was based on its laboratory superiority to other anti-inflammatory agents (phenylbutazone, indomethacin, hydrocortisone) in suppressing the immunologically induced, complement-dependent paw edema in the direct passive Arthus model in rats [131]. The clinical protocol called for initial use of RMI 11,002 orally at a dose of 500 mg once a week for two weeks, followed by 1000 mg once a week for two weeks; and then 1500 mg once a week for the balance of the study. Seven of nine patients completed the first two weeks of therapy in the initial open phase of the planned double blind study. Two subjects completed only the first week on the drug. In the third week on the drug, a patient death due to cardiac disease (and determined to be not drug-related), plus a high incidence of worsening of the disease/side effects caused the study to be terminated. At that time four patients had completed at least the second two weeks of therapy taking 1000 mg of RMI 11,002. Two of these four subjects showed marked improvement of their arthritis and a third had improved slightly.

The clinical report records that six of the nine patients entered into this study demonstrated initial exacerbation of their disease. This includes two of the four subjects who showed improvement after 4 weeks on RMI 11,002. Retro-

spectively, it may be postulated that the immunomodulatory action of RMI 11,002 caused a transient exacerbation of the rheumatoid arthritis in a majority of the subjects. It is also reasonable to suggest (based on current criteria for evaluating slow-acting antirheumatic drugs) that the protocol should have called for administering RMI 11,002 at a dosage of 125 mg twice weekly or 250 mg once weekly for a periode of 3–6 months, rather than going rapidly to a higher dose.

The phenomenon of flare up or transient exacerbation of various diseases during initiation of immunomodulatory therapy deserves more careful study by clinicians. To cite but one of many recent literature reports related to this subject, Lindstrom and Dau [139] noted that in 50% of MG patients there is a transient exacerbation of weakness during the first month of prednisone therapy.

Given the toxic manifestations of drugs now in use or under investigation (including the interferons), the therapeutic potential of tilorone and its congeners in treating inflammation, cancer and viral diseases, and other immune abberations cannot be overlooked.

CONCLUSIONS

1. Tilorone and its congeners are immuno-modulatory agents demonstrating either immunostimulation or immunosuppression depending on the experiment, dosage, timing, etc.
2. In common with all other immuno-modulatory agents adequately studied, tilorone is pleiotropic and often displays a biphasic activity pattern.
3. The immunomodulatory effects of tilorone in man and all animal species other than rodents are produced in the absence of any detectable interferon induction.
4. Even in the rodent, many of tilorone's antiviral and anticancer activities do not correlate with concurrently measured interferon serum levels.
5. Hyporeactivity is not evident in any of the immunomodulatory effects of tilorone and its congeners. Hyporeactivity is associated only with interferon induction.
6. In various animal systems, tilorone demonstrates broad-spectrum anti-inflammatory activity. This includes the immunologically induced complement-dependent direct passive Arthus model, as well as classic anti-phlogistic models of inflammation.
7. The antiviral activity of tilorone and its congeners provides added theoretical support for their use as Slow Acting Antirheumatic Drugs (SAARD's) based on the viral etiology theory of rheumatoid arthritis and related autoimmune diseases.

8. A three-patient study of intra-articular tilorone in severe rheumatoid arthritis resulted in beneficial effects in two of the patients. Improvement in the injected knee was preceded by transient exacerbation of the arthritis; as is often observed in treatment of rheumatoid arthritis with levamisole.
9. In both animals and man, multiple dose administration of tilorone causes only a transient leukopenia, with lymphocyte levels returning to normal within days or a few weeks.
10. Based on good animal data and limited human experience, tilorone would seem to meet the criteria for a unique transplant maintenance drug as follows: a) control of T-cells, b) antiviral and antitumor activity, plus c) no evidence of bone marrow suppresion.
11. Tilorone congeners exhibit a diverse pattern of selective immunoregulatory effects which merit exploration at the clinical level.

FUTURE PERSPECTIVES

In the rapidly emerging field of clinical immuno-modulation, there is presently very little correlation between effects in animals and effects in man. The vacuum could be filled by increased collaboration between government, academic, disease foundation, and pharmaceutical industry laboratories – perhaps with major public funding.

A better understanding of the role of immuno-modulation in support of the body's host-defense mechanisms will be achieved if interferon is downgraded to its proper position as one member of the lymphokine-cytokine system.

Transient exacerbation or flare up as a manifestation of immuno-regulatory activity in drug therapy should be fully studied in a variety of clinical situations including viral diseases and cancer.

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6 Hypoglycaemic Drugs

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INTRODUCTION

Diabetes mellitus is a prevalent disease of great medical and economic importance. In the United States, an estimated 5% of the population is believed to have diabetes mellitus, although in 1977 only 4.8 million (2.3% of the population) had been diagnosed as diabetics [1, 2]. Of these, approximately 0.4 million are classified as having insulindependent diabetes mellitus, the others having varying degrees of diabetes ranging from non-insulin dependent diabetes mellitus to impaired glucose tolerance. These recently adopted criteria for classification and diagnosis of diabetes mellitus [1, 3] are listed in *Table 6.1*, together with the older classifications of diabetes, which may help the reader to put the various nomenclatures into proper perspective.

Table 6.1. CATEGORIES OF DIABETES

New names	*Old names*	*Characteristics*	*Clinical signs*	*Etiology/coexisting conditions*
Type I or IDDM (Insulin dependent Diabetes mellitus)	Juvenile Diabetes Juvenile Onset Diabetes (JOD) Ketosis Prone Diabetes Brittle Diabetes	Little or no endogenous insulin. Patients usually young	Polydipsia Polyuria	Immune abnormality plus viral infection
Type II or NIDDM (Non-Insulin dependent Diabetes mellitus)	Adult Onset Diabetes Maturity Onset Diabetes (MOD) Ketosis Resistant Diabetes Maturity Onset Diabetes in the Young (MODY) Insulin Independent Diabetes (IID)	Relative lack of insulin. Insulin resistance (abnormal receptors) Patients usually over 40, patients often obese		Genetic predisposition Obesity or environmental factors can trigger the disease
Diabetes Mellitus associated with other conditions or syndromes	Secondary Diabetes		Fasting plasma glucose >140 mg/dl Post-prandial plasma glucose >200 mg/dl	Pancreatic Disease Hormonal Disease Drug or chemical induced Abnormal insulin receptors Genetic syndromes

IGT (Impaired Glucose)	Asymptomatic Diabetes Chemical Diabetes	May progress to IDDM or NIDDM in a few patients	Fasting plasma glucose < 140 mg/dl	
(Tolerance)	Subclinical Diabetes Borderline Diabetes Latent Diabetes		Oral glucose tolerance test: plasma glucose >200 mg/dl (1 h) >140 mg/dl (2 h)	
GDM (Gestational Diabetes)	Gestational Diabetes	Diabetes begins or is recognized during pregnancy		Pregnancy
PrevAGT (Previous Abnormality of Glucose Tolerance)	Latent Diabetes Prediabetes	Patients who have had IGT or GDM	Plasma glucose normal: fasting plasma glucose < 115 mg/dl,	
PotAGT (Potential Abnormality of Glucose Tolerance)	Potential Diabetes Prediabetes	Increased Risk (Immune paramaters of IDDM, close relative of patients with NIDDM)	Oral Glucose Tolerance Test: Plasma Glucose <200 mg/gl (1 h) <140 mg/dl (2 h)	

The most serious consequences of diabetes mellitus are, apart from rare cases of life-threatening keto-acidosis, the chronic complications which occur many years after the onset of the disease: atherosclerosis, nephropathy, retinopathy, neuropathy, and cataracts [4]. These complications have been linked to chronic excessive excursions of blood glucose levels, and a major goal of antidiabetic therapy is therefore strict blood glucose control, which approximates normal fasting levels and normal elevations of postprandial blood glucose [5–10].

Depending on the severity of the disease, elevated blood glucose can belowered by changes of dietary composition, weight reduction (expecially in obese diabetics), oral hypoglycaemic agents and insulin. In the aftermath of the University Group Diabetes Program (UGDP) controversy (see below), therapeutic approaches in non-insulin dependent diabetics emphasize diet and weight reduction as the first line of treatment; oral agents, and ultimately insulin, are used only if these measures fail. An example of such a modern treatment strategy is summarized in *Figure 6.1;* it should be kept in mind that biguanides are no longer generally available in certain countries because of their tendency to cause potentially fatal lactic acidosis (see below). Several studies suggest that oral agents or insulin, as normally used, do not provide near normal blood glucose control, and they do not prevent the development of chronic complications [7, 8, 11–14].

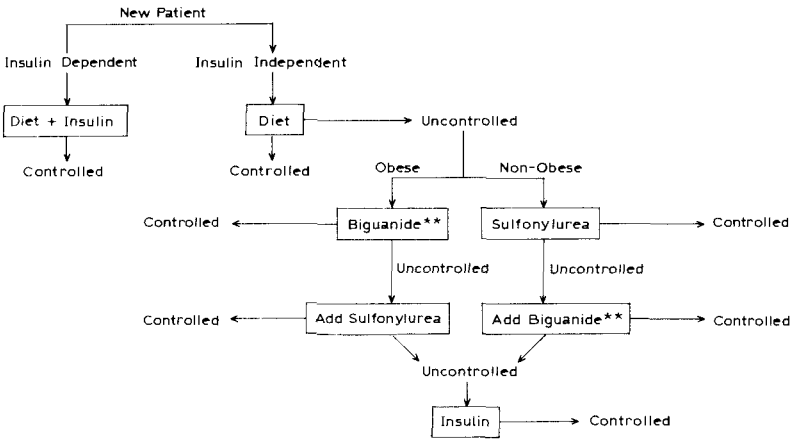


Figure 1. *Flow Chart of Treatment Strategy with Oral Hypoglycemic Drugs**

* Adapted from L.J. Borthwick and J.M. Stowers, Practitioner, 222 (1979) 358.

** In certain countries biguanides have been withdrawn from the market due to a low incidence of potentially fatal lactic acidosis.

When oral hypoglycaemic drugs were reviewed in this series almost 20 years ago by Slater [15], sulphonylureas and biguanides were the only clinically useful drug classes. This is still the situation today, although several structurally modified agents have been added in both categories, Despite the fact that a plethora of hypoglycaemic compounds has been reported in the literature or claimed in patents, no novel structural entities have been introduced into diabetic therapy in two decades. This may reflect the difficulty of finding efficacious agents which are sufficiently non-toxic for chronic use. Part of this difficulty may be caused by lack of appropriate animal models of the human disease (usual test system for oral hypoglycaemic agents measure glucose lowering in fasted rats, mice or guinea pigs, or improvement of glucose tolerance after a glucose load) and by the complexity of the metabolic control mechanisms of blood glucose levels.

In this review, agents presently in clinical use will be considered in some detail. In addition, selected newer research entities which have been examined in man or which appear to have therapeutic potential will be discussed. Insulin and newer developments in this area such as mono-component insulin will not be examined, since excellent reviews on that topic are available [16–18].

SULPHONYLUREAS AND RELATED AGENTS

MARKETED DRUGS

Drugs which are being marketed in 38 major non-communist countries for use in diabetes are described in this section in order of their economic importance, as measured by their total sales volumes in 1978. These sales figures are listed in *Tabel 6.2* [19]. Although commercial success is probably in small part based on the skill of the marketing arm of drug companies and on the length of time these drugs have been on the market, the inherent qualities of a drug are the primary factors responsible for the sales volume. The extent of clinical use is not necessarily proportional to the sales volume since price differences between daily doses of these drugs have been disregarded in this compilation.

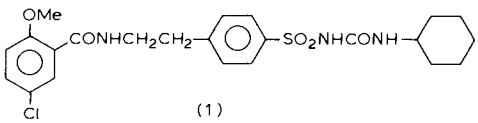
Glibenclamide (1, gliburide, glybiride, glybenzyclamide, HB–419) is a high potency drug which emerged from a large synthetic chemical effort some 10 years after the original hypoglycaemic sulphonylureas carbutamide (9) and tolbutamide (3) [20]. In animals [21] and in man [22] glibenclamide is about 250 times more potent on a weight basis than tolbutamide (3), from which it differs mainly in the attachment of a 5-chloro-2-methoxybenzamido alkyl side chain. Structure activity relationships in this sulphonylurea series [23] and the effect

Table 6.2. ORAL HYPOGLYCEMIC AGENTS

World-wide sales (1978, in million US dollars)*

Gilbenclamide (1)	113	Glisepoxide (11)	5
Chlorpropamide (2)	71	Phenformin (28)	5
Tolbutamide (3)	47	Buformin (29)	4
Tolazamide (4)	18	Glymidine (12)	3
Metformin (27)	15	Gliquidone (13)	3
Gliclazide (5)	14	Glycopyramide (14)	0.6
Acetohexamide (6)	9	Glybuzole (15)	0.3
Glibornuride (8)	8	Glypentide (17)	0.2
Carbutamide (9)	6	Metahexamide (18)	0.1
Glipizide (10)	5	Tolcyclamide (19)	0.1

* From audits of the pharmaceutical markets in 38 major non-communist countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Central America, Chile, Columbia, Denmark, Ecuador, Egypt, Finland, France, Greece, India, Indonesia, Iran, Ireland, Italy, Japan, Mexico, Netherlands, New Zealand, Pakistan, Peru, Phillipines, Portugal, Puerto Rico, Saudi Arabia, South Africa, Spain, Sweden, Switzerland, United Kingdom, United States of America, Venezuela, West Germany).



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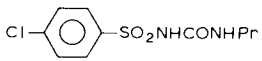

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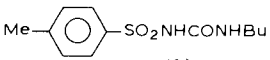

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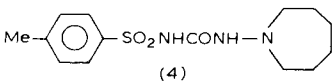

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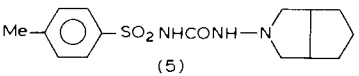

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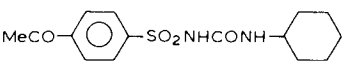
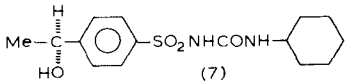


(6)

of attaching this 'high potency' side chain to sulphonylaminopyrimidines [24] and to sulphonylsemicarbazides [25] have been published.

After intravenous dosing, glibenclamide exhibited in man an insulin release profile which differed from that seen after tolbutamide (3); it caused a slower and shallower rise in the insulin levels, no tachyphylaxis after repeated administration 1 hour apart, and a more synergistic effect with glucose on the amount of insulin released [26]. Another group of researchers confirmed this difference between glibenclamide and tolbutamide (3), glibornuride (8), glisoxepide (11) or gliquidone (13) after intravenous dosing, with the exception of the glucose synergism, but they found that the insulin release profiles of all these sulphonylureas are superimposable after oral dosing and concluded that there is no practical difference between glibenclamide and the other sulphonylureas [27–30]. Although some clinicians claimed superior efficacy in the clinical management of diabetes with glibenclamide over older sulphonylureas such as tolbutamide (3),



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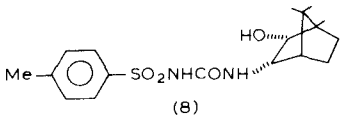

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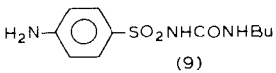

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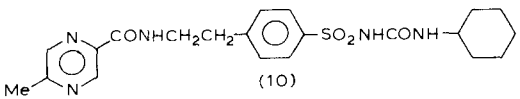

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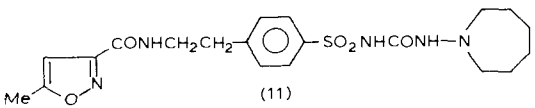

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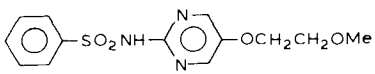

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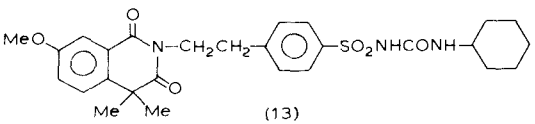


(13)

carbutamide (9), or glycodiazine (12) [31, 32], others reported no superiority over other sulphonylureas, such as chlorpropamide (2), in well-controlled long term studies with flexible dosing [33, 34]. Patients who are secondary failures, i.e. patients who are no longer controlled with other sulphonylureas (e.g., tolbutamide (3), carbutamide (9), glycodiazine (12)), may respond to glibenclamide [35]. The incidence of secondary failures was reported to be lower with glibenclamide than with older sulphonylureas [32] but this finding was not supported by that of another study [33]. One clear advantage of glibenclamide over less potent sulphonylureas, such as chlorpropamide (2) or tolbutamide (3), is the absence of adverse interactions with alcohol: glibenclamide does not cause the 'Antabuse Syndrome' of chlorpropamide (2) and other sulphonylureas [36].

In any event, glibenclamide has become an outstanding commercial success in the last few years and the principle of attaching an amidoalkyl function to the para position of arylsulphonylureas to obtain high potency agents has found many imitators.

In the clinic, glibenclamide is active at 1.25 to 20 mg although the usual daily clinical dose is 2.5 to 15 mg [22, 37]. In man, glibenclamide is absorbed to the extent of about 50% after oral administration. After intravenous dosing, the plasma half-life is biphasic, with values of 0.38 and 6.6 hours; after oral dosing, the half-life increases with time. The metabolism of glibenclamide in man involves mainly hydroxylation in the cyclohexyl ring; the major product is the 4-trans-hydroxy derivative, the minor one the 3-cis-hydroxy metabolite [38, 39].

Chlorpropamide (2, P-607) is one of the early, clinically successful sulphonylureas [40] which is distinguished by its long plasma half-life of 24 to 42 hours in man, presumably because the metabolically labile methyl group of tolbutamide (3) is replaced by a chlorine atom [41]. Contrary to the earlier belief that chlorpropamide is excreted unchanged in man [42], hydroxylation of the propyl side chain, especially at the 2 position, does occur in man and rat, in addition to cleavage of the propyl-nitrogen bond [41, 43]. As a result of its pharmacokinetic properties, chlorpropamide requires only once-a-day dosing; clinically it shows an antidiabetic activity at daily dose of 100 to 500 mg [37].

Chlorpropamide is unique in that it may cause 'dilutional hyponatremia' by enhancing the release of antidiuretic hormone (ADH) and by increasing renal sensitivity to ADH [44]. By contrast, glibenclamide (1) has a diuretic action and it inhibits the peripheral action of ADH in diabetes insipidus [45].

Tolbutamide (3, D-860) is one of the oldest clinically successful sulphonylureas. It has a relatively low potency [46], human doses being in the range of 500 to 3000 mg per day [37, 47], and it is extensively metabolized in man, mainly by conversion of the methyl group to a carboxylic acid group, with a plasma half-life of 4 to 8 hours [48, 49].

Tolbutamide was the sulphonylurea examined by the prospective UGDP-study, which originally tried to verify beneficial long-term effects of antidiabetic therapy. Instead of the anticipated beneficial effects, there appeared to be an increased incidence of cardiovascular deaths in the tolbutamide group compared with placebo or insulin-treated groups [50]. However, the validity of this conclusion has been questioned and the study itself has been criticized on the basis of improper design, execution and data handling. There has also been criticism of the use of a fixed dosage of tolbutamide regardless of therapeutic effect, inadequate patient randomization, insufficient baseline data and unsuitable biostatistical techniques. Furthermore, other studies suggest no detrimental effects of tolbutamide on vascular disease.

At the present time the controversy is still unresolved and it is not clear whether valid conclusions can be drawn from this study or whether conclusions concerning tolbutamide should be extrapolated to other sulphonylureas. Articles and editorials pro [51–55] and con [56–65] the UGDP study continue to appear.

Tolazamide (4, U-17835) belongs to the sulphonyl-semicarbazide family. It is active at 100 to 1000 mg per day [12], has a half-life of 7 hours in man, and is converted to active and inactive metabolites by oxidation of the aromatic methyl group, by hydroxylation of the hexahydroazepine ring in the 4-position or by conversion to *p*-toluenesulphonamide [17, 66, 67]. Tolazamide is suitable for once-a-day therapy, and it has been effective in a high percentage of patients not responding to sulphonylureas (primary and secondary failures) [68].

Gliclazide (5, S-1702, S-852) is another semicarbazide derivative which is more potent and longer acting than tolbutamide (3) [69]. Its clinical dose is 80–320 mg per day and it has a half-life of 12 hours, being metabolized by hydroxylation of the side chain [70]. This agent has been claimed to reduce platelet stickiness, to inhibit platelet aggregation and to have fibrinolytic properties and a protective effect on the microvascular system, at least in animals [71, 72]. These findings have potential implications for the therapy or prevention of diabetic vascular complications. However, another group of investigators demonstrated in animals that the inhibitory effect on platelet aggregation is shared by a wide variety of sulphonylureas and related agents and that this effect probably does not occur at drug concentrations achieved in diabetic therapy [69, 73, 74]. A recent comparison of gliclazide with glibenclamide (1) in diabetics showed that at therapeutic doses both drugs reduced platelet aggregation after low doses of ADP, but that only glibenclamide (1) reduced the platelet response to adrenaline or to collagen [75].

Acetohexamide (6) is one of the older sulphonylureas and is converted to an active metabolite, L-(−)-hydroxyhexamide (7) in man. The parent drug has a

half-life of 1.6 h and the metabolite a longer one of about 5 hours; L-(−)-4′,-transhydroxy-hydroxyhexamide (Chem. Abstr. name: 1-(4-hydroxycyclohexyl)-3-[*p*-(1-hydroxyethyl)phenyl]sulphonyl]urea) and 4′-transhydroxyacetohexamide are also metabolites [66, 76]. The clinical dose of acetohexamide is 250-1500 mg per day [47].

Glibornuride (8, Ro-6-4563, glybornuride) is one of the newer sulphonylureas which displays a relatively high potency (about 40 times tolbutamide (3) and one-fifth of glibenclamide (1)) without the magic amidoalkyl chain in the position para to the sulphonamide group [77–80]. The clinical dose of glibornuride ranges from 6.25 to 75 mg daily [37, 81]. This drug is well absorbed, its half-life is approximately 8 to 10 hours [82, 83], and it is extensively hydroxylated to inactive metabolites [84].

Carbutamide (9, BZ-55) was the first sulphonylurea to be used clinically in the treatment of diabetes mellitus after Franke and Fuchs demonstrated its glucose-lowering effect in diabetics [85]. However, the toxic effects on bone marrow seen in some patients caused this drug to be withdrawn from the market in certain countries [49, 86]. Carbutamide (9) is metabolically inactivated by N-acetylation and has a half-life of 30 to 60 hours [49, 87]. The clinical dose of this drug is 500-1500 mg per day [37, 88]. Structure-activity relationships around this structure have been described [89].

Glipizide (10, glidiazinamide, K-4024) is a newer high potency sulphonylurea, differing structurally from glibenclamide (1) only in having a 5-methyl-2-pyrazinyl instead of the 5-chloro-2-methoxyphenyl group in the amide side chain [90, 91]. This drug is rapidly and completely absorbed [92] and may be superior to glibenclamide (1) in that respect [93]. It is highly effective in non-insulin dependent diabetics at doses of 2.5 to 15 mg/day and is apparently equivalent to glibenclamide (1) in this regard [94]. The kinetics of drug metabolism are complex; the plasma half-life is 3 to 4 hours and increases with time; the drug is metabolized by hydroxylation of the cyclohexyl ring [39, 92]. A comprehensive review on glipizide has recently appeared [95].

Glisepoxide (11, BS-4231) combines an isoxazole-containing amidoalkyl side chain with the semicarbazide structure of tolazamide (4) to yield a highly potent hypoglycaemic agent (300 times the potency of tolbutamide in normal subjects) [96]. In man it is well absorbed and has a relatively short half-life of 1.7 hours; 50%of the drug is excreted unchanged and the substituted benzenesulphonamide is one of the metabolites [97]. In the clinic, glisepoxide is effective at doses between 1 and 24 mg [98] with a usual daily dose of 2-12 mg [37].

Glymidine (12, glycodiazine, SH-717) differs significantly in structure from the sulphonylureas, having the urea portion replaced by an isoelectronic aminopyrimidine [99]. Immunologically, this drug lacks cross-reactivity with sulphon-

ylureas and can be used in certain diabetics who show allergic skin rashes in response to sulphonylurea therapy [100]. Like sulphonylureas, glymidine is an insulin releaser in rats [101] and in man [102]. Its half-life is 3.8 hours and it is metabolized by demethylation to the hypoglycaemic β-hydroxyethoxy derivative, which itself is excreted 70 to 90% unchanged. The half-life of this metabolite is biphasic with an initial value of 3 hours for a period of 6 hours after administration and a value of 24 hours after that. An additional metabolite is the 5-carboxymethoxysulphonamidopyrimidine [103, 104]. The clinical dose of glymidine ranges from 500 to 2000 mg [27, 105].

Gliquidone (13) is another high potency insulin-releasing sulphonylurea with an imidoalkyl side chain, which is active at 15 to 120 mg [30, 106, 107]. It is apparently well tolerated and extensively metabolized to inactive metabolites, mainly by *O*-demethylation and by hydroxylation at the 4- and 3-positons in the cyclohexyl ring. The drug has a biphasic half-life of 1.5 and 24 hours. Since only 5% of gliquidone is eliminated by the kidneys, the rest through bile into the faeces, the drug can be used in patients with renal insufficiency [108, 109].

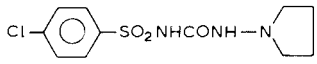
Glycopyramide (14) is a semicarbazide structurally related to chlorpropamide (2), and effective at doses between 250 and 1250 mg [110, 111].

Glybuzole (15, desaglybuzole, 1324 AN, 1395 TH, 7891 RP) is structurally unique, although it bears some resemblance to glymidine (12) with the 2-aminopyrimidine ring replaced by a thiadiazole ring. This drug is closely related to the antibacterial sulphanilylthiadiazole (16) whose hypoglycaemic activity was recognized in 1942 [112] and whose structure-activity relationships were described shortly thereafter [113]. The daily clinical dose of glybuzole is in the 1000 mg range and its rate of elimination is slow like that of chlorpropamide (2). Glybuzole lacks the antilipolytic effect produced by sulphonylureas [114–117].

Glypentide (17, UR-661) is structurally similar to glibenclamide (1), lacking the chlorine atom and having a cyclopentyl in place of the cyclohexyl ring [118, 119]. In the clinic it has been reported to be equipotent with glibenclamide (1) but with longer lasting insulin elevations [120].

Metahexamide (18) is one of the older agents with a greater potency than chlorpropamide (clinical daily dose 50-300 mg), with a half-life of 19-26 hours in normal man, and with an even longer half-life in diabetic patients. Thirty to 35% of the drug is excreted unchanged and 45-50% is converted to the sulphonamide. Interestingly, the amino group is not acetylated as it is in carbutamide [15].

Tolcyclamide (19, glycyclamide), is closely related to tolbutamide (3), with a cyclohexyl ring in place of the butyl group. It is much less potent than glipizide (10), being active at a daily dose of 1000 mg [121].



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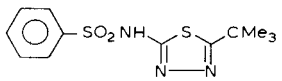

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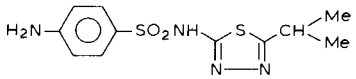

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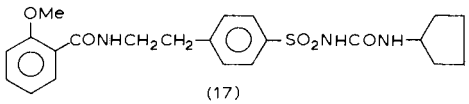

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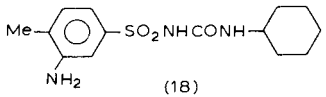

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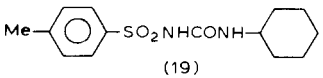

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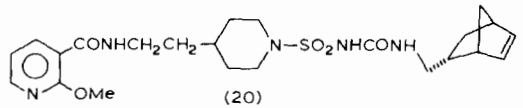
DRUGS IN CLINICAL INVESTIGATION

A wide variety of structural modifications has been carried out both on the original carbutamide (9) - tolbutamide (3) - chlorpropamide (2) - type sulphonylureas as well as on the second generation high potency sulphonylureas of the glibenclamide (1) type. Rather than presenting a comprehensive review of all these modifications (the interested reader may find such compilations in other reviews [122, 123]), this section will review newer modified sulphonylurea type agents which are not yet marketed but for which the results of clinical trials have been published. These compounds are listed in alphabetical order.

Gliamilide (20, CP-27,634) is the combination of a sulphamoylurea [124] with a high potency amidoalkyl side chain [125] and was found to be effective in non-insulin dependent diabetics at 50 and 75 mg and to have a very short plasma half-life [126].

Glicaramide (21, SQ-65993) is a glibenclamide (1) variation with a heterocyclic acyl group in place of the 2-methoxy-5-chlorobenzyl group and with similar potency [127, 128]. It may have more pronounced extra-pancreatic effects than glibenclamide (1) or tolbutamide (3) [128].



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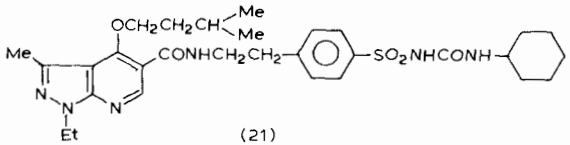

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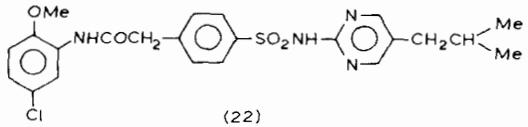

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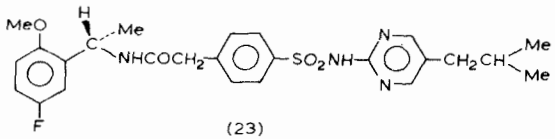

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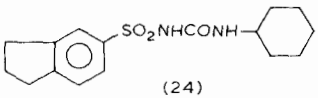

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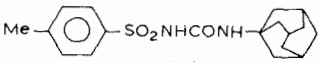

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Glidanile (22, glydanile, gliacetanile) combines a high potency-type side chain with a sulphonylaminopyrimidine structure similar to glymidine (12). Interestingly, the typical 5-chloro-2-methoxybenzamidoethyl chain is shortened by one methylene unit and the positions of the amine and carbonyl groups are reversed. This arrangement places the amide nitrogen at the same distance from the arylsulphonylurea portion as in glibenclamide (1), suggesting that the postulated secondary binding site of the high potency agents involves the nitrogen atom of the side chain. Glidanile is more potent than glymidine (12) and is rapidly metabolized and eliminated, predominantly as the tertiary isobutyl alcohol derivative which still possesses 30% of the hypoglycaemic activity of the parent drug [129, 130].

Gliflumide (23, glioptamide) is structurally related to glidanile (22), except that fluorine is substituted for the chlorine function and that an additional carbon

atom bearing a methyl group is inserted between the amino group and the aromatic ring of the side chain. This type of side chain also leads to highly potent agents in the sulphonylurea series, and it is interesting that the S-(−)-isomer is the active enantiomer in both series [131]. In man, gliflumide is more potent than glibenclamide (1) and it mimics the biological profile of glibenclamide, including its typical insulin-release pattern observed after intravenous administration [132, 133].

Glihexamide (24, SQ-15860) is an older sulphonylurea with a modified aryl ring. It is in the potency range of tolbutamide (3) [134] and does not seem to offer any particular advantage.

The adamantyl congener (25) of tolbutamide (3) was found to be a rapid-onset hypoglycaemic agent in the potency range of chlorpropamide (2), with an average effective daily dose of 400 mg [135].

STRUCTURE-ACTIVITY RELATIONSHIPS OF SULPHONYLUREAS AND RELATED AGENTS

It is evident from the preceding section, as well as from other, more general reviews, that despite considerable manipulation of the sulphonylurea type structure, hypoglycaemic activity can be maintained, although there are large differences in potency between the various derivatives. The basic active structure is depicted by (26), with A being aryl, typically substituted in the 4-position with methyl, chlorine, amino or, in the high potency series, with aryl-$CONH(CH_2)_n$ or aryl-$(CH_2)_nNHCO(CH_2)_m$. Alternatively, A can be a 1-piperidine ring, again optionally substituted with high potency-type side chains in the 4-position. B is typically CONHR where R is alkyl (especially Pr, Bu), cycloalkyl (especially cyclohexyl, cyclopentyl), or alkylamino or dialkylamino in the semicarbazide series. Alternatively, B can be a heterocyclic ring such as 2-pyrimidine or 2-(1,3,4-thiadiazole). Radical variations from these modifications seem to lead to decreased activity. While there are certainly large differences in potency and pharmacokinetic properties between the various structures, it is not clear whether the claims that certain agents are qualitatively superior to others are valid in clinical practice.

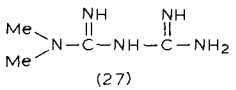
(26)

MECHANISM OF ACTION OF SULPHONYLUREAS

The mechanism of action of sulphonylureas is still not totally clear. While they do release insulin, especially after the first dose, insulin levels in patients on chronic therapy with a variety of sulphonylureas, e.g. glibenclamide (1) [136–138], chlorpropamide (2) [139–141], tolazamide (4) [142], or acetohexamide (6) [143], may return to pretreatment levels while blood glucose levels remain lowered. This effect may be due to the suppression of glucagon levels [144] or due to extrapancreatic effects of sulphonylureas, such as inhibition of gluconeogenesis [145] or potentiation of the effect of insulin in the periphery [146, 147]. On the other hand, recent studies with chlorpropamide (2) [148], glipizide (10) [149, 149a] and gliclazide (5) [150] found that postprandial insulin levels can remain elevated even after chronic therapy of diabetic patients. Excellent reviews on the various aspects of sulphonylurea function have appeared [15, 151–154]. A newer theory, based on studies with chlorpropamide (2) [155] and with glibenclamide (1) [156], suggests that sulphonylureas may increase the number of insulin receptors to a normal level. On balance, it would appear that a combination of pancreatic insulin release and of peripheral insulin potentiation, possibly at the insulin receptor level, are responsible for the antidiabetic action of sulphonylureas.

BIGUANIDES

The only non-sulphonylurea drugs which have proven useful in antidiabetic therapy are the biguanides, which can be used either alone or in combination with sulphonylureas (see *Figure 6.1*). Only three agents are marketed at the present time (in order of commercial importance, see *Table 6.2*): metformin (27), chenformin (28), and buformin (29).



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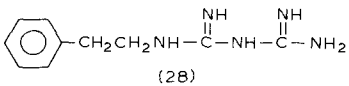

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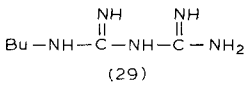


(29)

Two factors have recently emerged which adversely affect the use of biguanides. Phenformin (28), was one of the drugs examined in the UGDP study, and again, as in the case of tolbutamide (3), enhanced cadiovascular mortality was observed instead of the anticipated beneficial effects [14, 157]. These findings were judged to be severe enough to warrant an early termination of the phenformin study. Again, the conclusions of the UGDP study group have aroused controversy, and its conclusions have been questioned [158]. It is certainly interesting that a group from England found a reduced incidence of myocardial infarction in a 5 year study of phenformin [61] and that another group found a slight but insignificant beneficial effect of phenformin on survival in patients with coronary heart disease [159]. It is, therefore, not clear at this time whether the use of phenformin is associated with increased cardiovascular mortality only in diabetics or whether the findings with phenformin can be generalized to other biguanides.

A second and perhaps more important factor in the decline of biguanide use is the fact that the incidence of fatal lactic acidosis following biguanide use was underestimated. Although the incidence of lactic acidosis is relatively low and appears to occur primarily in patients with insufficient renal (interference with drug excretion and lactate metabolism), cardiac, or hepatic (interference with lactate metabolism and with phenformin (28) metabolism) function, the outcome is frequently fatal [160–163]. It has been recommended that biguanides not be used in the treatment of diabetics and this has caused certain drug regulatory agencies, such as the FDA in the USA, to withdraw biguanides from the market [164, 165].

BIGUANIDES IN CLINICAL USE

Metformin (27) is emerging as the biguanide of choice based on claims that it has a lower incidence of lactic acidosis than phenformin (28) [166, 167, 167a]. However, metformin also causes lactic acidosis in patients with renal insufficiency [168, 169]. Its clinical dose is 500-3000 mg [170]. It has been reported to have a plasma half-life of 2.8 hours and to be excreted unchanged in man [171]. More recent data obtained with ^{14}C-labelled drug show a plasma half-life of 1.7 hours [172].

Phenformin (28) was the first biguanide which was used extensively in the clinic, and structure-activity relationships in this series have been investigated [173–177]. More recently, its use has declined as a consequence of the negative findings in the UGDP study and because of the greater awareness of potentially fatal episodes of lactic acidosis with this drug, which has led to its withdrawal from the market in several countries. The clinical dose of phenformin is up to

4 times 25 mg daily or 50 to 100 mg in the timed-release form [170]. Its half-life was originally found to be 3.2 hours [178], but a longer half-life of 11 hours has been determined more recently with a gas-liquid chromatography assay [179]; it is metabolized by *p*-hydroxylation in the phenyl ring [178, 180].

Buformin (29) is a more recent entry into the field of biguanides. Several cases of lactic acidosis have also been reported after ingestion of this drug [181, 182], mostly in patients with renal insufficiency. Its daily clinical dose is in the range of 50 to 300 mg [170] and its plasma half-life is 3.8–5.5 hours; the drug is excreted unchanged [180, 183, 184].

MECHANISM OF ACTION OF BIGUANIDES

In animals, the hypoglycaemic activity of biguanides varies according to the species: guinea-pig and monkey show a marked effect, rats are moderately sensitive, and dogs are insensitive [185]. Biguanides have no effect in normal man on blood glucose levels, but they are hypoglycaemic in fasting man and in diabetics [186, 187]. A multitude of biological actions has been associated with biguanides: they have been claimed to suppress gluconeogenesis [188, 189], possibly by interfering with oxidative processes [190], to suppress postprandial plasma glucagon levels [190a], to inhibit the uptake of glucose from the intestinal tract [191–193] and to enhance glucose utilization in the periphery [194–196]. Workers from Schering have shown that, in susceptible animals, gluconeogenesis suppression is a high dose phenomenon, and that at doses approximating clinical levels potentiation of the effect of insulin on peripheral glucose uptake and oxidation is the likely mechanism of action [197]. Experiments with ^{14}C labelled glucose in man have confirmed that glucose oxidation is enhanced in normal and diabetic subjects after treatment with phenformin (28) [198, 198a], although another group found the opposite or inconsistent effects on glucose oxidation in normal or obese subjects [199, 200]. It was recently shown that the acute effect of relatively high doses of phenformin in diabetes is a reduction of hepatic glucose output, postulated to be due mainly to decreased glycogenolysis [200a]. However, the relative significance of lowered hepatic glucose production or of enhanced peripheral glucose utilization to the long-term clinical effect of phenformin in diabetics is still not clear from these studies. The inhibitory effect of biguanides on glucose uptake from the intestine certainly contributes to a lowering of the postprandial blood-glucose peak although there is probably only a delay, but not a net decrease, of glucose uptake [201]. On balance, it is likely that both the intestinal glucose uptake delay and the enhanced peripheral disposal of glucose contribute to the antidiabetic effect of biguanides. In addition, biguanides cause weight loss, possibly by an anorexigenic effect, which can be

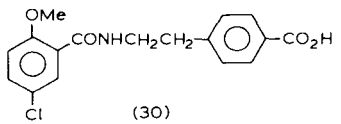
exploited in obese diabetics [202–204]. A good review of the effects of biguanides in man has appeared [205].

SELECTED STRUCTURALLY NOVEL HYPOGLYCAEMIC AGENTS

Inclusion of compounds in this section from the vast number of hypoglycaemic agents reported in the literature is somewhat arbitrary and is based on an assessment of their structural interest and clinical potential.

CARBOXYLIC ACIDS

An intriguing newer structure is meglitinide (30, HB-699) which is related to glibenclamide (1), with a carboxyl group replacing the acidic sulphonylurea function. HB-699 and its higher homologue (31, HB-093) are insulin releasers, although their potency is much lower than that of glibenclamide (1) and more in the range of tolbutamide (3) [206, 207]. It should be interesting to see whether or not (30) will prove efficacious in man. Similarly, a benzoic acid (32) substituted with the side chain of gliflumide (23) has hypoglycaemic properties, and as in the case of the sulphonylaminopyrimidine, the S-enantiomer is more potent than the R-enantiomer. This suggests that the high potency side chain of both sulphonylureas and benzoic acids interact at the same receptor [208].



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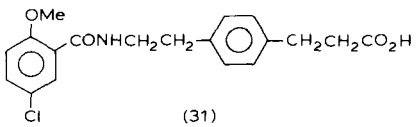

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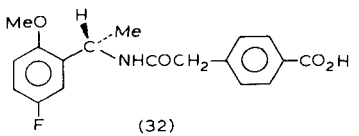

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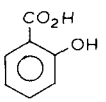

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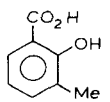
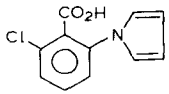


(34)

Salicylates (33, 34) have been used long ago to improve glucose control in diabetics, possibly via their insulin-releasing or antilipolytic effects. However, high doses are required and the clinical benefits appear to be marginal [209]. The structure activity relationships of salicylic acids and related compounds have been explored in rats [210–212].

The benzoic acid derivative (35) has been reported to stimulate glucose utilization, but it apparently had side effects and caused tachyphylaxis in animals [213, 214].

2-Mercaptopicolinic acid (36) and several derivatives are apparently gluconeogenesis inhibitors which lower glucose levels in animals [215]. However, in man, (36) lacked hypoglycaemic potency and caused dermatitis in 2 out of 10 patients [123].



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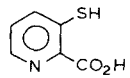

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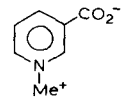

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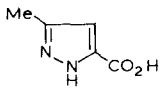

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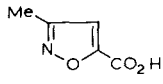

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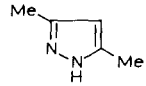
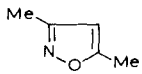


(40)

Trigonelline (37), a quarternary nicotinic acid derivative, showed transient hypoglycaemic effects at 500 mg in 5 out of 10 patients [216].

A series of heterocyclic carboxylic acids (38), (39) and their metabolic precursors (40), (41) generated some excitement a few years ago because these antilipolytic compounds lowered blood glucose in diabetics. However, tolerance developed rapidly to this effect and these agents proved unsuitable for long term diabetic therapy [217–221].



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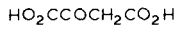

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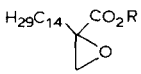

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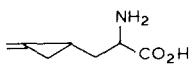

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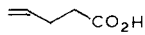


(47)

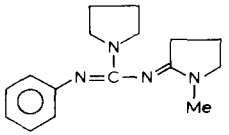
Among aliphatic carboxylic acids, dichloroacetic acid (42), in the form of its di-isopropylammonium or sodium salt, has been shown to lower blood glucose [222], and to remedy the lactate elevations found after phenformin treatment [223, 224]. However, the recent finding that (42) is mutagenic [225] will probably preclude clinical application of this agent. Its half-life was much shorter in man than in rat or dog [225a]. Oxalacetate (43) lowered blood glucose and urinary glucose output in diabetics when given as the sodium salt at doses of 100–1000 mg daily. It was especially effective in older and obese patients with a short duration of the disease; its mechanism of action is unknown [226]. A long chain oxirane carboxylic acid (44, McN-3802) and its methyl ester (45, McN-3716) are reported to be inhibitors of carnitine acyl transferase and to be hypoglycaemic in fasted, diabetic, or fat-fed animals, by enhancing peripheral glucose oxidation [227, 228]. Mechanistically, these agents are claimed to differ from hypoglycin A (46) and 4-pentenoic acid (47), two hypoglycaemic inhibitors of fatty acid oxidation which did not find clinical use because they caused liver toxicity (fatty infiltration of the liver) [229].

AMIDINES AND GUANIDINES

Among non-acidic hypoglycaemic agents, there are several intriguing guanidines and amidines. Pirogliride (48, McN-3495), although its structure is somewhat remininscent of biguanides, is reported to have a different activity profile and mechanism of action. Although it does not normally cause sulphonylurea-like increases in peripheral insulin levels, it can release insulin at high doses. In contrast to biguanides, it improves tolerance to intravenous as well as oral glucose loads, it does not interfere with intestinal glucose uptake, and it does not increase lactate levels. It is interesting that the hypoglycaemic potency of (48) is about ten times higher in monkeys than in rats or mice [230, 231]. Clinical studies are in progress [232], and it should be interesting to determine if this agent will prove to be of lasting value in antidiabetic therapy.

Recently, several papers have appeared on a series of cyclic guanidines, exemplified by (49), the structure-activity relationships of which have been summarized [233]. Compound (49) is apparently an insulin releaser, but it is not yet clear whether any of the compounds from this series have advanced to clinical trials.

In a clinical study MK-270 (50, PIDH) was compared with phenformin (28) and shown to be active only in diabetics who were not well controlled on sulphonylureas [234]. In normal subjects 400 mg of (50) showed activity and side effects similar to phenformin (28) at 200 mg [235]. No further reports have appeared on this drug.



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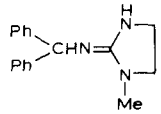

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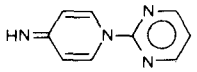

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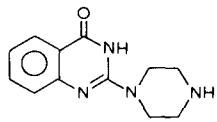
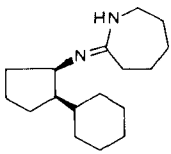


(51)

Centpiperalone (51) is a piperazine derivative related to cyclic guanidines and in the potency range of tolbutamide (3); it is probably an insulin releaser [236]. It lowers blood glucose in rat, rabbit and dog, but not in monkey or guinea-pig [237]. No clinical studies have been published as yet, although it is reportedly in Phase I [238].

From a series of hypoglycaemic and insulin releasing lactamimides, RMI 11894 (52), was selected for clinical trials [239]. No clinical reports have issued on this compound but cardiovascular toxicity has been observed in dogs [240]. Some related lactamimides, RMI-11493 (53), and RMI-10026 (54) are claimed to be hypoglycaemic and in preclinical stages of development [241].

A series of α-alkoxyamidines, exemplified by (55), displayed hypoglycaemic and natriuretic activity in animals, but the poor separation of activity form toxicity exhibited by these compounds prevented clinical studies [242].



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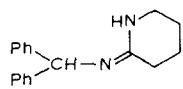

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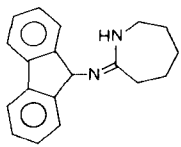

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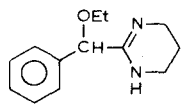

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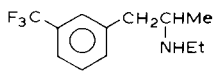
MISCELLANEOUS AGENTS

Several drugs which are primarily marketed for non-diabetic indications have shown hypoglycaemic activity in man. The anorectic agent fenfluramine (56) has been shown to improve glucose tolerance and to lower fasting blood sugar in diabetics in numerous studies. These effects are not necessarily associated with weight loss and have been postulated to involve increased peripheral glucose utilization in the presence of insulin [243]. A fenfluramine analogue, 780 SE (57), has also been shown to improve glucose tolerance in insulin-independent diabetics, an effect again unrelated to weight loss. Potentiation of insulin-mediated glucose utilization in the periphery has also been postulated as the mechanism of action [244]. The hypolipaemic agent halofenate (58) at a dose of 500-1500 mg daily reduces the requirements for sulfonylureas in diabetics, and this effect seems to be mediated by interference with the metobolism of the sulphonylurea and not by a direct hypoglycaemic effect [245–247]. The hypolipaemic drug clofibrate (59), on the other hand, which also transiently potentiates the effects of sulphonylureas at a dose of 1000 mg bid [246], has been shown to lower fasting and postprandial glucose in diabetics when given alone in a 7 day study, supposedly by increasing insulin sensitivity [248]; however, it had no effect on fasting blood glucose in longer term (48 weeks) studies [246, 247].



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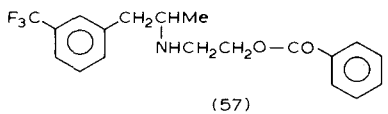

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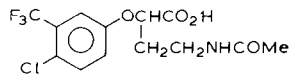

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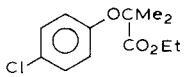
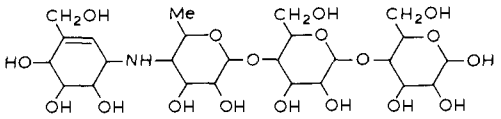


(59)

Experiments in normal subjects suggested that the glucosidase and amylase inhibitor acarbose (BAY g 5421, 60), used at about 75 mg, should be of value in decreasing postprandial blood glucose peaks [249, 250]. A study in diabetics showed that addition of 6 times 50 mg of (60) daily to the usual sulphonylurea or insulin regimen led to a further decrease in blood glucose values [251]. In a double blind cross-over study in obese non-insulin dependent diabetics, acarbose at three times 100 mg daily dose had a beneficial effect on postprandial blood glucose levels and on urinary glucose excretion [251a]. Somatostatin (61), a tetradecapeptide which was originally isolated from the hypothalamus [252],

but which occurs also in the D-cell of the pancreas [253], suppresses the secretion of growth hormone, glucagon and insulin [254]. Infusions of (61) reduced insulin requirements and glucose fluctuations in insulin-dependent diabetics [255, 256], but caused only a transient hypoglycaemia followed by hyperglycaemia in insulin-independent diabetics [257]. Infusions in normal subjects also resulted in hyperglycaemia [258]. It remains to be seen whether analogues of (61), such as D-Trp^{8}, D-Cys^{14}-somatostatin, which more specifically suppress glucagon, will be of value in the management of diabetes [259, 260].



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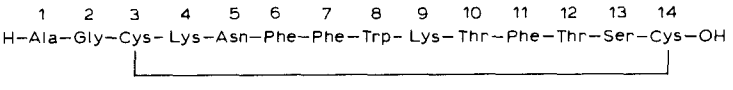
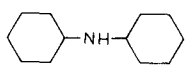


(61)

Guar gum, a gel-forming, unabsorbable carbohydrate, flattened the postprandial blood glucose peak [261] and decreased the urinary glucose output in diabetics when given at a daily dose of 25 g [262], presumably by interfering with food absorption from the intestinal tract. However, in a three month study in obese diabetic outpatients, guar gum produced gastrointestinal discomfort with no beneficial effect on blood glucose or body weight, suggesting that it is unlikely to have a useful role in clinical practice [263].

Glucose tolerance factor, a chromium-containing complex of nicotinic acid and amino acids (glycine, cysteine, glutamic acid) occurring in brewer's yeast, has been postulated to act as a cofactor of insulin at the insulin receptor [264]. Conceivably, it could be of value in diabetics who are postulated to have a deficiency of this factor, although this remains to be proven in well controlled clinical studies.

Surprisingly, very simple aliphatic amines such as dicyclohexylamine (62) have recently been shown to be hypoglycaemic in normal and diabetic animals, presumably by enhancing peripheral glucose utilization in the presence of insulin [265, 266].



(62)

In summary, while many structurally distinct agents have shown intriguing hypoglycaemic activity, time will tell whether any of these compounds will prove to be of value in the therapy of diabetes mellitus. Hopefully, some of these agents will succeed. However, it is remarkable and discouraging that so many non-sulphonylurea and non-biguanide compounds have ultimately failed to demonstrate clinical utility because of lack of efficacy or because of untolerable side effects.

CONCLUSION

The severest consequence of diabetes mellitus is the development of chronic complications, believed to be the result of excessive blood glucose excursions. Thus, the attempt to control blood glucose remains the major objective of antidiabetic therapy. Current treatment with oral hypoglycaemic agents only partially fulfills this goal and the introduction of the high potency sulphonylureas has probably led to nothing more than incremental improvements in diabetic therapy. Hopefully some of the newer research entities discussed in the last section will provide alternative and improved oral diabetic therapy. In addition, newer methods of monitoring blood glucose control, such as measurements of glycosylated haemoglobins [267, 268], may lead to better utilization of existing hypoglycaemic agents. On another front, the miniaturization of glucose monitoring and insulin delivery sytems may make such devices portable and practical and may lead to more ideal approaches towards normalization of blood glucose, especially in severely diabetic patients, but implementation of this approach still seems to be many years away [269]. Similarly, islet transplants will have to surmount rejection problems before that method can become practical [269], although progress is being made in this respect [269a].

It is conceivable that newer insights into the etiology of diabetes mellitus, such as the role of viral infections, communicability and hereditary factors, will provide alternative and/or preventative therapies. In addition, direct therapy of chronic complications may be possible, *e.g.*, by preventing excessive glycosylation of proteins [270], by preventing basement membrane thickening [271, 272], or, an approach perhaps closer to fruition, by preventing the formation of sorbitol with aldose reductase inhibitors [4]. Lastly, a better understanding of the mechanism of action of existing agents and an increasing knowledge of the biology and metabolic control mechanisms of blood glucose may ultimately lead to better hypoglycaemic agents.

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