SYNTHESIS AND EVALUATION OF SOME NITROGENOUS HETEROCYCLES FOR INTERACTION WITH BENZODIAZEPINE RECEPTORS

A Thesis

submitted for the

Degree of Doctor of Philosophy

in

The Australian National University

by

Jiankuo Zhang

Division of Neuroscience
The John Curtin School of Medical Resaerch
The Australian National University

Canberra
March, 1992

To my wife

Certificate of Originality

The work described in this thesis was carried out by the candidate at The Australian National University. Where the work of others was employed or quoted, appropriate references are given.

Zhang Jiankun

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr G.B. Barlin for his kind advice, encouragement and supervision, and to Prof. D.R. Curtis and Dr W.L.F. Armarego for their advice and encouragement. I also wish to thank Dr D.J. Brown sincerely for his patience and constant encouragement, and particularly for his suggestions and assistance with the preparation of this thesis.

I thank also Dr L.P. Davies for some biological test results and for assistance with Chapter I of this thesis; and Mr S.J. Ireland for determination of most of the binding data and for technical assistance. I acknowledge the kind assistance of Dr M.D. Fenn in the interpretation of the ^{1}H n.m.r. spectral data. My special thanks to Mrs E.M. McNaughton for typing this manuscript.

Finally I wish to thank the Australian National University for the award of a Post-graduate Research Scholarship and Australasian Drug Development Limited for part payment of the Overseas Student Charge.

SUMMARY

Chemical syntheses and receptor binding studies (and some structure activity comparisons) of some imidazo-[1,2-*b*]pyridazines, imidazo[1,2-*a*]pyridines, imidazo-[1,2-*a*]pyrimidines and imidazo[1,2-*a*]pyrazines are reported in this thesis. ^{1}H n.m.r. spectra are also recorded and discussed.

Several new series of 2-aryl-6-substituted-3-unsubstituted[3-dimethylaminomethyl, 3-acetamidomethyl, 3-benzamidomethyl and 3-(substituted benzamidomethyl)] imidazo[1,2-*b*]pyridazines have been prepared. The 3-unsubstituted imidazo[1,2-*b*]pyridazines were prepared from the appropriate 6-substituted pyridazin-3-amines with bromoacetyl compounds, and substituents were introduced at the 3-position by Mannich reaction or by heating with *N*-hydroxymethylamides. New 2-aryl-6-(*N*-benzyl-*N*-methylamino)-3-methoxy- and 2-aryl-3-methoxy-6-(substituted benzylamino)imidazo[1,2-*b*]pyridazines were prepared from the relevant 6-benzylaminopyridazin-3-amine 2-oxides with bromoacetyl compounds followed by diazomethane methylation of the imidazo[1,2-*b*]pyridazin-3(5*H*)-ones.

Many 6-(variously substituted)-3-methoxy-(acylaminomethyl and dimethylaminomethyl)-2-benzyl(phenethyl, biphenyl-4′-yl, 6′-methylnaphthalen-2′-yl, t-butyl, and cyclohexyl)imidazo[1,2-*b*]pyridazines have also been prepared to measure the effect of significant changes in size and conjugation of the 2-substituent. All these compounds were subsequently tested for their ability

to bind to specific binding sites in rat brain preparations. The results of the binding studies of each series of compounds are presented and discussed in each chapter. The most active compounds in displacing [^{3}H]diazepam from rat brain preparation were 3-benzamidomethyl-2-(3′,4′-methylenedioxyphenyl)-6-methylthioimidazo[1,2-*b*]pyridazine with IC_{50} 2 nM and 3-methoxy-6-(3′,4′-methylenedioxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine with IC_{50} 1 nM (*cf.* diazepam 4.2 nM). Some of these compounds which exhibit high binding affinity are being examined (by others) for pharmacological activity in rats.

The binding affinity of the imidazo[1,2-*b*]pyridazines prepared in this work varied considerably. Of the compounds with 3-benzamidomethyl(3-acetamidomethyl, or 3-dimethylaminomethyl) groups or those which were unsubstituted at the 3-position, the 3-benzamidomethyl compounds generally bound most strongly. Further substitution in the phenyl ring of the 3-benzamidomethyl group generally had a detrimental (or marginally beneficial) effect on the displacement of [^{3}H]diazepam from rat brain membrane preparations. *N*-Methylation, as in 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-phenylimidazo-[1,2-*b*]pyridazine, generaly decreased binding ability by *ca* 2-fold relative to each *N*-demethyl analogue. Amongst the compounds with variable substituents at the 2-position the order of activity was Ph > $PhCH_2$ > $PhCH_2CH_2$ > C_6H_4Ph-*p* (6′-methylnaphthalen-2′-yl, cyclo-C_6H_{11}, Bu^t).

The effects of removing N-5 from imidazo[1,2-*b*]-pyridazines and its replacement by CH, as in imidazo-[1,2-*a*]pyridines, and the effects of shifting the ring nitrogen atom at the 5-position in imidazo[1,2-*b*]-pyridazines into the 7- or 8-position in imidazo[1,2-*a*]-pyrazines and imidazo[1,2-*a*]pyrimidines, respectively, on the ability to displace [^{3}H]diazepam from rat brain membrane have been examined. Generally the imidazo[1,2-*a*]-pyridines were less active than the corresponding imidazo[1,2-*b*]pyridazines (one exception recorded), and amongst corresponding derivatives of the triazabicyclic systems the order of activity was imidazo[1,2-*b*]pyridazine >> imidazo[1,2-*a*]pyrimidine $\gtrsim$ imidazo[1,2-*a*]pyrazine.

CONTENTS

CHAPTER I

CHAPTER I INTRODUCTION TO ANXIOLYTICS AND RELATED CNS-ACTIVE COMPOUNDS

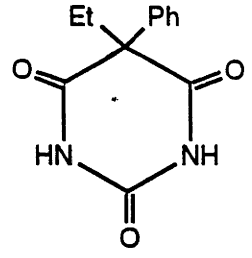
I-1 Historical background

Anxiety, which has been recognized as an inherent part of man's being, is an ubiquitous emotional state common to everyday life.[1] It is related to fear which is a response to current tangible threats; anxiety occurs in anticipation of a threat not yet present. Various studies have suggested that from about three to eight per cent of the population has clinically significant anxiety at any one time.[2] As for the pervasiveness of anxiety disorders and the extent of usage of antianxiety drugs, in the year 1984/1985 Australian medical practitioners wrote some five million prescriptions for drugs of the benzodiazepine group and cost the pharmaceutical benefit scheme some twenty-two million dollars.[3] In the USA, multicenter epidemological surveys of three communities in the period 1980-1982, showed that anxiety disorders had a life-time prevalence rate of between ten per cent and twenty-five per cent[4] and a six-month prevalence rate of between six per cent and fifteen per cent.[5] In 1985, psychotropic drugs prescribed within the United States cost approximately one billion dollars.[6]

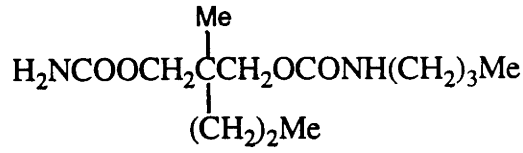
Throughout history, various classes of chemicals and plant products have been used to treat anxiety conditions. These include the barbiturates, such as phenobarbital (Fig.

I-1) amobarbital and butabarbital, propanediols [meprobamate and tybamate (Fig. I-1)] antihistamines, benzodiazepines [such as diazepam (Fig. I-1)], opiate alkaloids and belladonna derivatives.

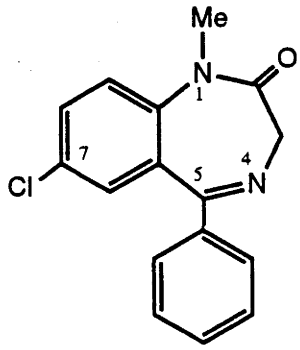
Alcohol is one of the oldest and most commonly-used antianxiety agents. Alcohol possesses anxiolytic and sedative-hypnotic properties in both laboratory animals and man, but frequently it has other socially unacceptable effects.[7] 2-Methyl-2-propyl-1,3-propanediol dicarbamate (meprobamate), synthesized by Ludwig and Piech[8] in 1951, was discovered to possess antianxiety activity three years later.[9] In 1955 the introduction of meprobamate (under the trade name 'Miltown') and 1957 (as 'Equanil') provided the



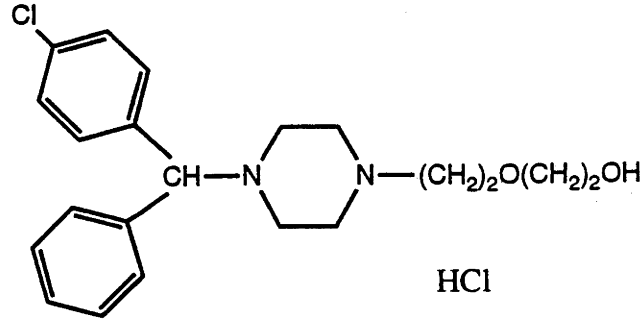
Phenobarbital



Tybamate



Diazepam

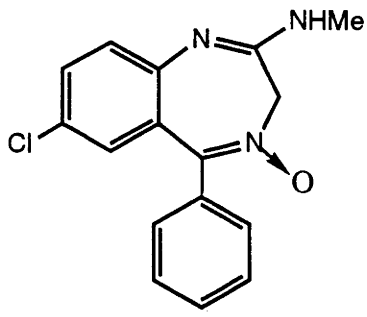


Hydroxyzine (HCl)

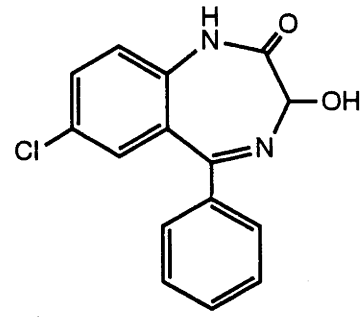
Fig. I-1

first generally useful replacement for the overly-sedating barbiturates in the treatment of anxiety.[10] Before the benzodiazepines were discovered, the substituted propanediols were considered to be important antianxiety agents. Although meprobamate has been in some disfavour of recent years, it still appears to be a reasonably effective and a reliable drug.[11] Barbiturates and various other compounds which are chemically unrelated, such as hydroxyzine hydrochloride (Fig. I-1) and chlormezanone, also have potent anxiolytic activities. In the last 85 years many hundreds of barbiturates have been prepared. These have been used as hypnotics and in lower doses as sedatives, anxiolytics and antiepileptics. Hydroxyzine hydrochloride was first synthesized by Morren *et al.* in 1954,[12] and was widely used as a sedative. The arylthiazanone derivative, chlormezanone, which was less frequently used as an antianxiety drug, was synthesized by Surrey *et al.* in 1958.[13]

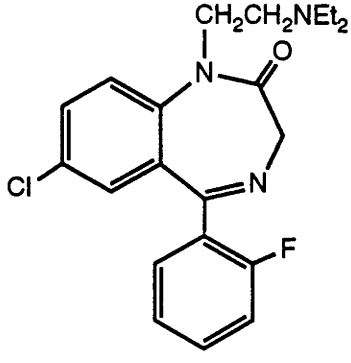
The use of the above-mentioned drugs is limited because of several side effects and of concerns about their serious toxicity at high dosage. Since the early 1960s, the use of barbiturates and other drugs as prescription anxiolytics has been displaced almost entirely by the benzodiazepines, such as chlordiazepoxide and diazepam.



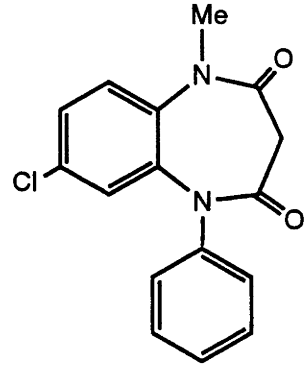
Chlordiazepoxide
(Librium, 1963)



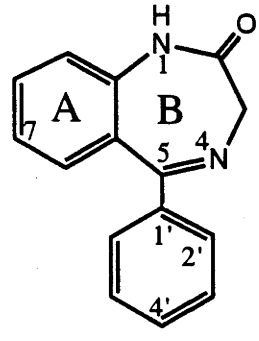
Oxazepam
(Serax,1965)



Flurazepam
(Dalmane,1970)



Clobazam
(Urbanyl,1975)



5-Phenyl-2,3-dihydro-1,4-benzodiapin-2-one

Fig. I-2

I-2 Benzodiazepines

Although the first centrally-acting 1,4-benzodiazepine compound, chlorodiazepoxide, was synthesized by Sternbach and colleagues in 1955,[14] it was not until the 1960s that animal studies carried out with chlordiazepoxide indicated that the compound had hypnotic, sedative and anticonvulsant effects similar to those of meprobamate, in mice and cats. Chlordiazepoxide (under the trade name 'Librium'; 1960, Fig. I-2) was the first 1,4-benzodiazine[15] introduced into clinical use and was soon followed by other more potent compounds such as diazepam ('Valium' 1963; Fig. I-1), oxazepam ('Serax' 1965, in USA) and flurazepam ('Dalmane' 1970; Fig. I-2).

The benzodiazepines consist of a bicyclic nucleus formed by the fusion of a benzene ring with a partially unsaturated seven-membered heterocycle containing two nitrogen atoms in the 1,4-positions. Benzodiazepines possessing biological activity usually have a phenyl group at the 5-position, and an electronegative substituent at the 7-position.[16] The benzodiazepines are extensively used in clinical practice as anxiolytics, anticonvulsants, hypnotics and muscle relaxants[17] because they are very effective and were considered to have mild side effects, were safe on overdosage and lack drug interactions with serious clinical consequences.[7] Thousands of benzodiazepine derivatives have been synthesized and screened pharmacologically; about two dozen assorted

benzodiazepine drugs are available in clinical use world-wide. The pattern of consumption for anxiolytics and hypnotics in Australia for the period 1975-1984 is shown in Fig. I-3.[7] It has been suggested that 10-20% of adults in the Western world regularly ingest antianxiety agents.[7] Chronic usage of these drugs has been reported to be widespread, with a large proportion of all 'repeat' prescriptions being for benzodiazepine drugs. Although in the short-term, benzodiazepines have proved to be beneficial in the treatment of anxiety and related disorders, their long-term use has been seriously questioned because it has been demonstrated that benzodiazepine drugs give rise to physical and psychological dependence[18] and tolerance. The benzodiazepines have achieved a world-wide use due to their wide spectrum of nervous system activity. They also set the stage for a greatly increased understanding of the pharmacology, the biochemistry and chemistry of anxiety and of central nervous system receptor structure and function.

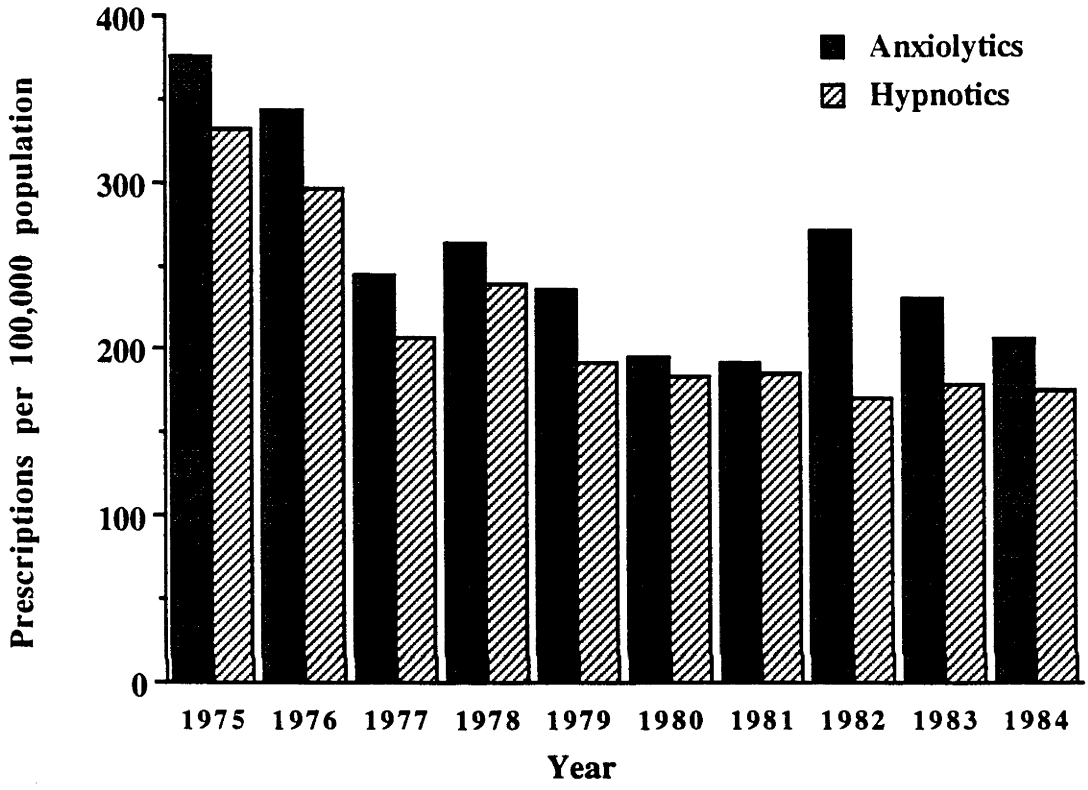


Fig. I-3 Patterns of consumption for anxiolytics and hypnotics in Australia for the 10-year period 1975-1984. The figures are based on the number of prescriptions for drugs available on the Pharmaceutical Benefits Scheme. These are not all prescriptions written for these drugs, but are representative of the pattern of use during this period. Data from Ref. 7

I-3 Structure-activity relationships for benzodiazepines

Most synthetic benzodiazepines have been shown to possess muscle relaxant and sedative properties in animals.[16] A large number of the fundamental studies of the structure-bioactivity relationships within the benzodiazepine compounds (Fig. I-2) has been summarized by Sternbach *et al.*[15,19,20,21] They suggested that, in the 1,4-benzodiazepine ring system,[14,15,19,20,21] the presence of the seven-membered imido-lactam ring and the 5-phenyl substitutent were essential; that substitution, in particular, with electronegative substituents in the 7- and 2'- positions, markedly increased biological activity; and that further substitution was advantageous only at the 1- and 3-positions.

Since the 'discovery' of specific high-affinity central nervous system (CNS) binding sites for benzodiazepines in 1977, simple *in vitro* binding tests were devised which were relatively well suited to the screening of a large series of benzodiazepines and non-benzodiazepine compounds. Appropriate studies were able to define common structural features required for high affinity binding to benzodiazepine receptors in *in vitro* assays.[22] The published binding data from a total of 29 drugs, comprising benzodiazepines and compounds from other structural classes, were used by Crippen[23] to identify common three dimensional features and determine the free energy of binding; a possible binding site model for a subset of 18

compounds, comprising 15 site points and 5 adjustable energy parameters, was deduced by applying distance geometry analysis.[24] Crippen drew the conclusion that five non-hydrogen atoms of each ligand can occupy corresponding points in the site and thus constitute a possible benzodiazepine pharmacophore.

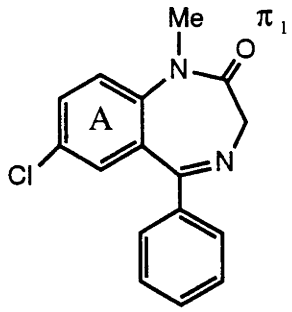
Theoretical (chemical) techniques[25,26] have been used in a study of the molecular discriminants of receptor affinity and activity of a series of benzodiazepines. Gilli and coworkers[27] developed a model based on the X-ray structures of benzodiazepines. They concluded that small conformational differences among the benzodiazepines have little effect on activity and the major determinant of activity is the electronegativity of the substituent in the 7-position because it affects the strength of potential hydrogen bonds formed at N-1.

Leow *et al.*[26] proposed that the receptor possesses three cationic sites that interact with an electron-withdrawing group at C-7, the C-2 oxo group and the imino nitrogen atom, N-4. A better model was developed by Codding and Muir;[28] it showed the positions and the types of binding groups characterized by the differences between antagonist and agonist ligands for the benzodiazepine receptor. It was notable that agonist benzodiazepines possess a consistent arrangement of the carbonyl oxygen atom, the aromatic "A" ring (Fig. I-2) and the imino nitrogen atom (N-4) and that the two electronegative atoms

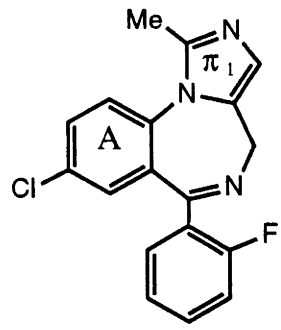
(O and N) are always on the same side of the "A" ring.[29]

Definitive studies to determine the absolute stereochemistry of the active conformation of benzodiazepines and the chiral nature of the 1,4-benzodiazepine ring system were carried out[29,30] by single-crystal X-ray and by n.m.r. analysis. These showed that the S-enantiomer of C-3 methyl substituted benzodiazepines is more active in the [^{3}H]diazepam binding, in all cases, than is the corresponding R-enantiomer.[30] Fryer and coworkers,[31,32] with the assistance of computer graphics analysis, have developed a three dimensional molecular model based on structural correlations of compounds (both benzodiazepines and non-benzodiazepines such as those shown in Fig. I-4) which possess high affinity (IC_{50} values in the low nM range) for CNS benzodiazepine binding sites. (see p.70)

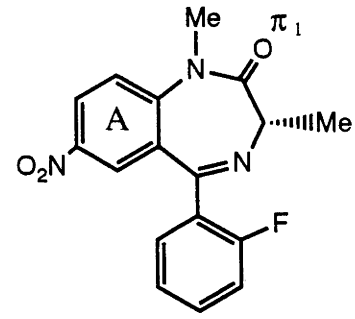
It was designed to fit all known ligands for the benzodiazepine receptor and as a predictive tool for the design of *in vitro* active drugs. The Fryer model[31] (Fig. I-5) has been helpful. In this model there are two major binding sites. The first site is an aromatic or heteroaromatic "A" ring which is capable of undergoing (π-π) stacking interactions with aromatic or heteroaromatic groups within a receptor; and the second, a proton-accepting source (π_1) bearing a spatial relationship to the "A" ring. An auxiliary binding site namely, a second electron-enriched region (π_2), also spatially related to the "A" ring,[29,31] was proposed. Other auxiliary binding



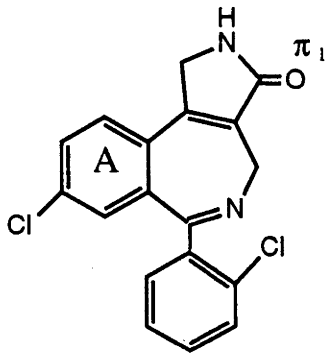
Diazepam



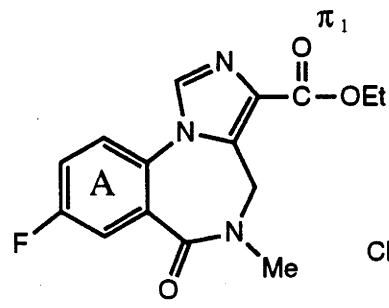
Midazolam



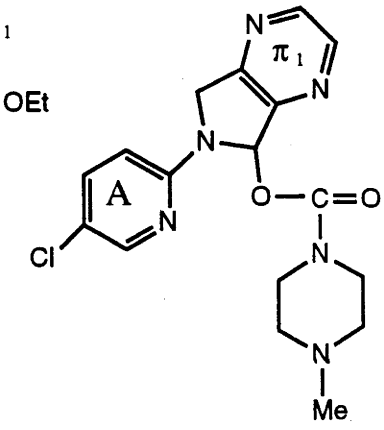
Ro 11-6896



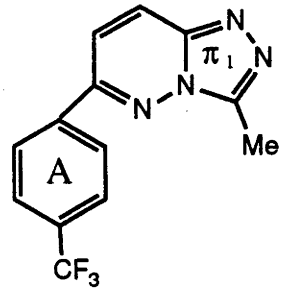
Ro 22-8515



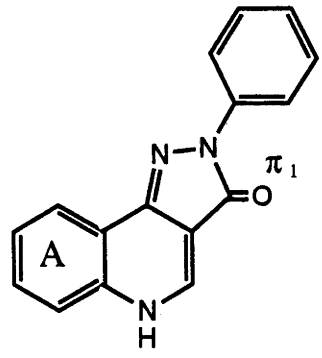
Ro 15-1788



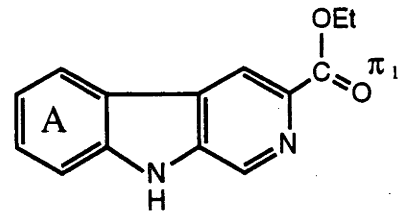
Zopiclone



CL 218,872



CGS 8216



β-CCE

Fig.I-4

sites were also envisaged.

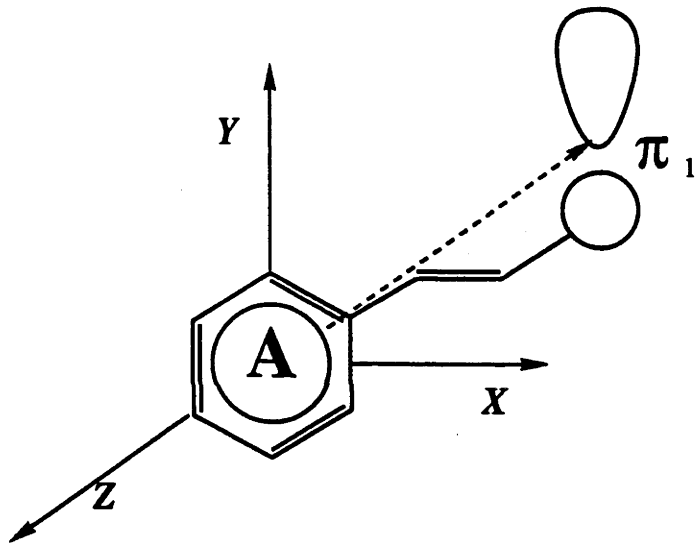
The results of calculations based on the Fryer *et al.* model are presented in (Table I-1). A relationship between the *in vitro* activity of a series of compounds and their "A" to π_1 distance was established.[31,33] Thus for compounds acting as agonists, the distance from mid "A" to π_1 is from 3Å to about 6.5Å; as antagonists the range is from about 6.5Å to 7.5Å; and as inverse agonists the distance begins at approximately 7.5Å. It was proposed that with the "A" ring placed in the xy plane of a set of cartesian coordinates with its centre at (0,0,0), the proton-attracting group for active compounds always had positive values for x,y and z coordinates (Fig. I-5).

Table I-1. Coordinates for π_1 for various ligands at the benzodiazepine receptor[A]

Compounds	X(Å)	Y(Å)	Mid "A"-π_1(Å)
Diazepam	4.50	1.76	4.91
Midazolam	3.88	2.75	4.78
R022-8515	4.83	2.36	5.44
R022-9187	3.82	2.81	4.75
R022-7244	4.71	3.85	6.11
Zopiclone	3.56	3.56	5.00
ZK93423	3.20	4.34	5.45
CGS-17867A	3.16	2.28	3.90
CL218,872	4.6	1.0	4.80

[A] Data taken from (Ref. 33)

A study of steric effects has revealed that the conformations of agonist-receptor and antagonist-receptor are different and it has been possible to differentiate the conformational requirements for both full agonists and full antagonists.[34]



(A) Aromatic or heteroaromatic ring lying in the xy plane

(π_1) Proton-accepting atom or group, mid 'A' to π_1 distance $\approx$3.0-6.5 A° (for agonists)

Mid 'A' to π_1 distance, x, y, defined as positive

Indicative of structural skeleton fixing the relationship 'A' to π_1, should be at least partially unsaturated, may be part of aromatic or heteroaromatic ring

Fig. I-5 Schematic three-dimensional model for binding of ligands at the **BZR**

I-4 Mechanisms of action of benzodiazepines

I-4.1 GABA/Benzodiazepine receptors

A receptor[35,36] is a tissue site which can selectively bind a compound of a specific chemical structure and which, following the binding, mediates a particular biological response. Most receptor sites of interest to neuroscientists are proteins which exist in the plasma membrane of neurones, with their binding sites facing toward the extracellular environment. In 1967, electrophysiologists discovered that benzodiazepines potentiate inhibitory nerve transmission at sites in which the amino acid, γ-aminobutyric acid (GABA), is the inhibitory synaptic transmitter.[35,36] Later it was shown biochemically that GABA can potentiate the binding of radioactively labelled benzodiazepines to mammalian brain membranes[37] and, conversely that benzodiazepines can potentiate GABA binding to the membranes.[38] Benzodiazepine receptors are coupled to both GABA receptors and chloride channels in a GABA-receptor/benzodiazepine-receptor/ chloride ionophore complex (Fig. I-6).[39]

It is generally accepted that this complex functions to regulate the flow of chloride ions across neuronal membranes. The nerve-cell membrane is normally impermeable to chloride ions but when the chloride channels in the membrane are opened they allow chloride ions to enter,

causing an increase in the negative potential across the membrane and making the cell more difficult to excite. The probability of chloride channel opening is markedly increased by $GABA_A$ receptor occupancy and in this GABA acts as an inhibitory neurotransmitter. Benzodiazepines alone cannot cause chloride channels to open and inhibit neurones but when GABA is released on to a neuron in the presence of the benzodiazepine, a greater chloride influx is produced through the membrane than with GABA alone. Thus benzodiazepines enhance the transfer of GABA-mediated inhibitory signals in the vertebrate CNS. The potentiating effect of benzodiazepines on GABA-mediated chloride

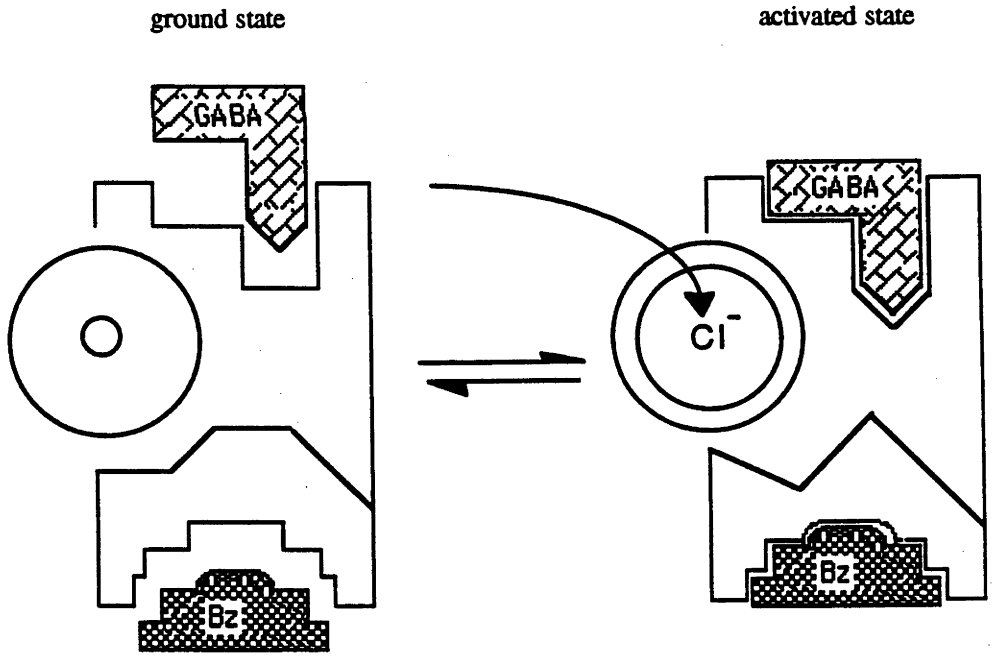


Fig.I-6 A model of the GABA-receptor/benzodiazepine-receptor/chloride ionophore complex.

conductance appears to be due to an increase in the frequency of chloride channel openings rather than on the duration of each channel opening (as is the case with barbiturates).

Benzodiazepine receptors are wide-spread and densely situated throughout the CNS, with phylogenetically-older areas (e.g. pons and medulla) containing fewer receptors than phylogenetically newer structures (e.g. cerebral cortex). Nevertheless, the regional differences in receptor density are smaller than for many other neurotransmitter receptors. This can probably be explained by functional and structural heterogeneity at these receptors in the various areas of the brain.

Although early work suggested a homogeneous population of benzodiazepine receptors in the mammalian CNS, this is now known to be incorrect. On the basis of pharmacological studies, several subclassifications of receptor sites are known to exist, and specific CNS benzodiazepine receptors have been classified into Type I and Type II sites.[40,41] Research with a group of triazolopyridazine compounds (including CL 218,872) (Fig. I-7)[40] revealed that those compounds had anxiolytic activity but possibly did not cause sedation (ataxia). This led to the proposal that the triazolopyridazine-selective receptors (Type I sites) were more closely involved in the anxiolytic actions of the benzodiazepine, whereas Type II sites were related more closely to

sedation. The subclassification of receptor sites promises to hasten the development of compounds with significantly more selective anxiolytic, sedative/hypnotic or muscle-relaxant actions. The elucidation of receptor subtypes has been aided in recent years by rapid progress in molecular biological techniques.

I-4.2 Molecular biology

To aid in understanding the possible mechanism of action of the benzodiazepines *via* GABAergic transmission, a hypothetical model of the benzodiazepine-receptor/GABA-receptor/chloride channel complex was proposed by Haefely *et al.*[42,43] They assumed that it was a tetrameric glycoprotein consisting of four identical or similar protomers (subunits). These four structurally related subunits were assembled to generate an integral ion pore which could close or open in response to small conformational changes in the subunit(s).

More recently, with the application of molecular biology cell techniques, it has been suggested that the GABA-gated chloride channel consists of a pentameric structure. It was considered initially that only two types of subunit (α and β) were needed to form channels that matched the behaviour of endogenous GABA receptors.[44] But more recent investigations showed that a third type of subunit, a γ-subunit, of the GABA-benzodiazepine receptor in the brain,[45] was required for more robust regulation of the channel by benzodiazepines.[46] Haefely and coworkers[47]

have recently proposed that the hetero-oligomeric $GABA_A$ receptor chloride ion channel complex consists of subunits (α, β, γ, δ in varying stoichiometry). These form a transmembrane anion channel gated by the primary ligand GABA and modulated by secondary (allosteric or heterotopic) ligands. The binding site for GABA is related primarily to the β-subunit, while the α-subunit includes the binding site for benzodiazepines. Each of the subunits of the GABA receptor, when expressed alone in a cultured cell (e.g. oocyte), is capable of forming a GABA-activated chloride ion channel, the opening of which is blocked by picrotoxin and potentiated by pentobarbital.[46] However, high concentrations of GABA ($\sim$ 100 μM) are needed to open these model channels. At a meeting on "Drug Action at the Molecular Level: Differences Between Agonist and Antagonists" in 1989, Schofield[48] reported that thirteen different $GABA_A$ receptor subunits had been cloned. Changing α-subunit combinations appeared to alter the affinity of receptor for ligand, while the presence of the δ_2-subunit was needed to observe the full benzodiazepine pharmacology, involving allosteric potentiation of GABA receptors and the effects of inverse agonists. There is experimental evidence supporting the idea that changing various combinations of α-subunits with a single β-subunit results in $GABA_A$ receptors with different GABA sensitivity.[49] Thus, different combinations of the various α-, β, and δ-subunits will result in a large number of pharmacologically different $GABA_A$-benzodiazepine receptor

subtypes.[40] Evidence from molecular biological investigations (as briefly outlined above) suggesting a possible multiplicity of somewhat different benzodiazepine-receptor/GABA-receptor/chloride ionophore complexes within the CNS, offers the possibility of selectively modulating specific GABA neurones in various brain regions by the synthesis of appropriate benzodiazepine-like ligands.

I-4.3 Agonists, antagonists and inverse agonists

Small amounts of ethyl β-carboline-3-carboxylate (β-CCE) have been isolated from human urine and mammalian brain extracts[39] (see Chapter I-4.4). The pharmacological effects of β-CCE are opposite to those of the clinically useful benzodiazepines. It was reported[50] that β-CCE reverses the anxiolytic effects of benzodiazepines and exhibits anxiogenic activity.[51] In contrast, the related compounds, ethyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate (ZK93423) and its 5-benzyloxy isomer (ZK91296) are anticonvulsants and have been classified as full agonist and partial agonist, respectively, at the benzodiazepine receptor. The different pharmacological activities of these benzodiazepine (and non-benzodiazepine) receptor ligands have been shown in Table I-2.

Table I-2 Action of some benzodiazepine receptor ligands

Actions			Compound
Agonists	{	Anxiolytic	Benzodiazepines Triazolopyridazines
		Anticonvulsant	CGS 9896
Antagonists	{		ZK 91296 Ro15-1788
			Ro15-1788
		Anxiogenic	CGS 8216
Inverse agonists	{	Convulsant	β-CCE β-CCM DMCM

i. Agonists

Agonists are defined as drugs which interact with their receptor to exert a biological response (i.e. they have positive intrinsic efficacy). Examples are diazepam and chlordiazepoxide which have characteristic therapeutic effects (such as anticonvulsant, sedative, muscle relaxant, anxiolytic and hypnotic activities).

ii. Antagonists

Receptor antagonists are defined as drugs with a high affinity for their receptor, but having no relevant biological effects of their own at the receptor other than

to block the effects of both agonist and inverse agonist ligands (i.e. no intrinsic efficacy).

To date, no 'pure' antagonists at benzodiazepine receptors have been reported. Ro 15-1788 (Fig. I-9) has been found to inhibit specifically [^{3}H]diazepam binding to brain synaptosomal fractions and to antagonise specifically the behavioural, electrophysiological and biochemical effects of clinically useful benzodiazepines in a variety of experimental paradigms.[22] Although many investigators have confirmed these observations, it has also been reported that Ro 15-1788 does possess some intrinsic pharmacological effects. For example, high doses of the compound have been shown to have anticonvulsant effects.[52,53] Thus although Ro 15-1788 is classified as a benzodiazepine antagonist, it may have some slight intrinsic partial agonist activity in some cases.

iii. Inverse agonists

These are drugs which combine with their receptors to exert a biological response which, when compared to agonists, have the reverse profile of activity i.e. they show a 'mirror image' biological response.

Examples are the β-carboline derivatives, such as 4-ethyl-6,7-dimethoxy-β-carboline (DMCM), which have effects opposite to those of the 'classical' benzodiazepine

agonists and possess pro-convulsant and anxiogenic properties.[52]

I-4.4 Endogenous ligands

Recent investigations have shown that several benzodiazepines occur naturally in the brain of various species, including rats and humans.[54-58] It has been suggested that benzodiazepines which occur in brain are possibly of dietary origin because they are found in numerous plants that serve as food, and also in cows milk.[56,57] It also has been suggested, with some limited evidence (e.g. from their presence in neuroblastoma or glioma cell cultures) that they may be endogenously synthesized but the biosynthetic pathways to such benzodiazepines are as yet unknown.

The isolation of *N*-demethyldiazepam, a metabolite of diazepam, has been achieved from bovine and rat brains, and purified by immuno-affinity chromatography employing a monoclonal antibody to the benzodiazepine, 3-hemisuccinyl-oxyclonazepam.[54,59] The isolated compound was identical with synthetic *N*-desmethyldiazepam in binding studies, further identified by gel filtration and reverse-phase HPLC, and finally characterized by mass spectrometry (GC-MS).[54] A compound with the same absorption spectrum and HPLC profile as oxazepam was also reported.[54,59] The same group detected benzodiazepine immunoreactivity in human brain kept in paraffin since 1940, sixteen years before benzodiazepines were first synthesized by the

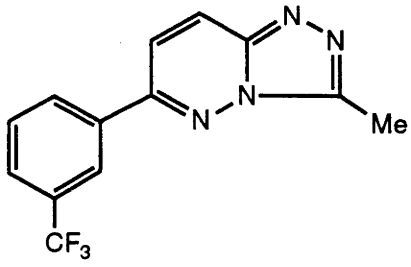
pharmaceutical industry.[54] Benzodiazepine-like immunoreactivity has been noted also in various rat tissues (liver, kidney, spleen, brain) but the CNS had the highest levels.[59] More recently, benzodiazepine-like material has been found in human milk[60] (4 to 8 mg lorazepam units/ml). Klotz also reported[61] the isolation of diazepam from cherries harvested in 1958. In an attempt to explain the origin of the naturally-occurring benzodiazepines, various plants were screened for these compounds. Diazepam and desmethyldiazepam have been identified by specific mass spectrometry (GC-MS) with selective ion monitoring (SIM)[55] in extracts from wheat. In addition to diazepam and desmethyldiazepam, several other benzodiazepine, including delorazepam, deschlorodiazepam, deloremetazepam, lormetazepam, and isodiazepam have been identified in aqueous acid extracts of wheat and of potato tubers by GC-MS.[61] Benzodiazepine-like compounds[55] have also been reported from radioreceptor binding assays (RRA) to occur in several other products (fish powder, barley, corn, millet, oats, rice, buck wheat and soya beans). The authors of this work considered it unlikely that the trace amounts of benzodiazepine found in brains would have any direct pharmacological effect.

Some other non-benzodiazepine compounds which may be possible endogenous ligands include the following. Inosine and 6-hydroxypurine (degradation products of the nucleoside, adenosine) have a relatively low affinity for benzodiazepine receptors, although there is evidence that

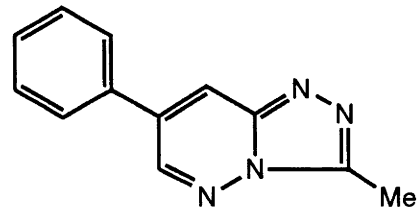
such levels may substantially increase during stress or hypoxia.[39] Ethyl β-carboline-3-carboxylate, which has been isolated from bovine brain and human urine extracts[39,62] (1.78 mg from 1800 litres of urine), binds very tightly to benzodiazepine receptors. Initially it was believed[39] to be a product formed during the extraction process but it is now considered to be an endogenous component of bovine brain.

I-5 Benzodiazepines and the development of new drugs

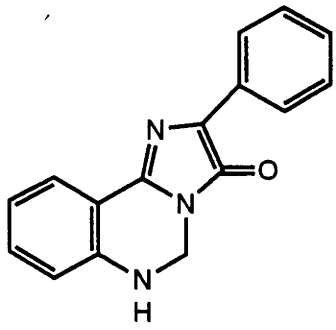
Since the discovery of chlordiazepoxide and diazepam, many thousands of benzodiazepine derivatives have been synthesized and screened for pharmacological activity with the aim of finding compounds with more selective pharmacological activities. This has been a relatively fruitful area of research. Anxioselective compounds, such as clobazam, which do not possess the undesirable side effects of some benzodiazepine derivatives, (Fig. I-2) have shown a good separation between anxiolytic and sedative dosages. A class of non-benzodiazepine compounds, which has a wide separation between anxiolytic and anticonvulsant or sedative doses, is the triazolopyridazine derivatives (e.g. CL 218,872; 3-methyl-6-(3'-trifluoromethylphenyl)-1,2,4-triazolo[4,3-b]pyridazine, Fig. I-7). Unfortunately, CL 218,872 cannot be used clinically as it has been reported to have hepatotoxic effects in animals.[42] In 1985, Bourguignon and coworkers[66] synthesized SR 95195 (3-methyl-7-phenyl-1,2,4-triazolo[4,3-*b*]pyridazine, Fig. I-7) in which the phenyl ring was attached to the 7-position (i.e. on the pyridazine ring). It has inverse agonist activity at the benzodiazine receptor site.[67] Yokoyama and coworkers[68] reported the synthesis of CGS 8216, CGS 9895 and CGS 9896 (Fig. I-7) which possessed different intrinsic activities at the benzodiazepine receptor: CGS 8216 was a potent benzodiazepine receptor antagonist,[68,69] CGS 9896 was a partial agonist (and important structural lead) and



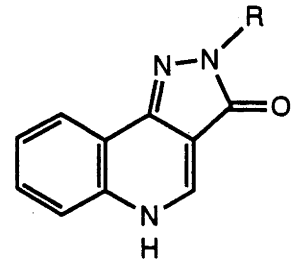
CL 218,872



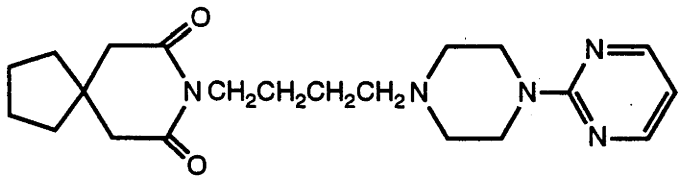
SR 95195



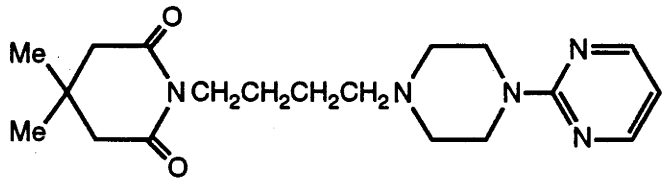
CGS 13767



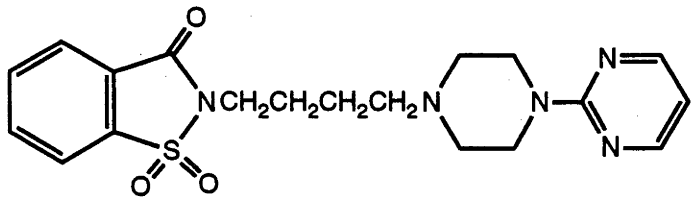
	R
CGS 8216	Ph
CGS 9895	C_6H_4Me-*p*
CGS 9896	C_6H_4Cl-*p*



Buspirone



Gepirone
(M J 13805)



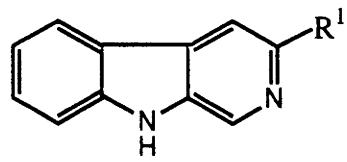
Ipsapirone
(TYXQ 7821)

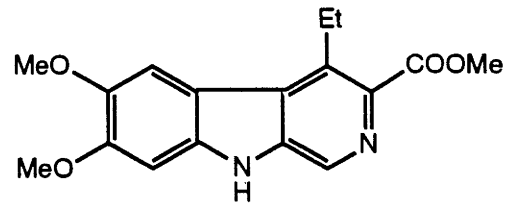
Fig. I-7

CGS 9895 was a full agonist at low, and an antagonist at high doses. Recently Francis and coworkers[70] have reported that another series of non-benzodiazepine-type compounds (e.g. CGS 13767, Fig. I-7), shows benzodiazepine binding affinity (IC_{50} value of CGS 13767 is 4 nM).

Parenthetically, a new development in the psychopharmacology of anxiety was the introduction of buspirone (Fig. I-7) as a non-benzodiazepine anxiolytic drug. This lipophilic, heterocyclic compound is one of a new class of agents known as azaspirodecanediones. Buspirone was reported initially to be effective only in female patients,[71] was less effective and took longer to work than diazepam.[72] However, more recent studies found that buspirone was effective in benzodiazepine-sensitive anxiety states,[73] but without the side-effects of sedation and dependency.[74] Chemically, buspirone is unlike the benzodiazepines and it has been found that it does not interact with either benzodiazepine or GABA binding sites. However, there is evidence to indicate that buspirone and its analogue (MJ 13805; Fig. I-7) can affect specifically 5-HT-mediated processes in the CNS.[75] A related compound, ipsapirone (Fig. I-7) also possesses anxiolytic effects in animals but does not have sedative, ataxic or anticonvulsant effects. The proposal that the anxiolytic effects of buspirone and ipsapirone are mediated by an effect on $5\text{-}HT_{1A}$ sites[76] is supported by electrophysiological and behavioural data.

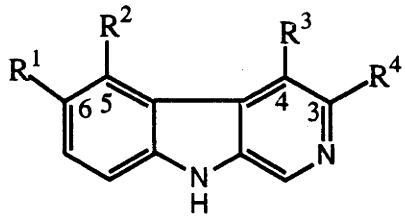
β-Carboline derivatives (Fig. I-8) also interact with





	R^1
β - CCM	COOMe
β- CCE	COOEt
FG 7142	CONHMe

DMCM



ZK	R^1	R^2	R^3	R^4
91296	H	OCH_2Ph	CH_2OMe	CO_2Et
95962	H	OPr^i	CH_2OMe	CO_2Et
93426	H	OPr^i	Me	CO_2Et
91262	H	OCH_2Ph	CH_2OMe	CO_2Et
93423	OCH_2Ph	H	CH_2OMe	CO_2Et
90886	H	OMe	Et	CO_2Et
112119	H	OCH_2Ph	CH_2OMe	CO_2CHMe_2

Fig. I-8 Some β-carboline derivatives that interact with benzodiazepine receptors

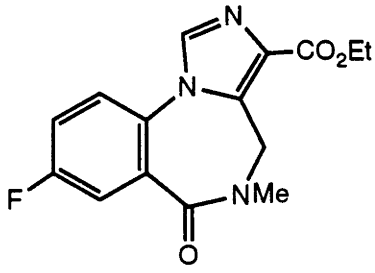
benzodiazepine receptors.[22] Ethyl β-carboline-3-carboxylate (β-CCE) was isolated from human urine and mammalian brain extracts by Braestrup *et al.*[62] in 1980. A series of unique β-carbolines has been developed and this has led to the concept of bidirectional agonism. The pharmacological effects of β-CCE are opposite to those of the clinically useful benzodiazepines. Jensen and coworkers[77] have studied the bidirectional effects of some β-carbolines on seizure activity and suggested that ZK 93423 (ethyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate and its 5-benzyloxy isomer, ZK 91296) are anticonvulsants: they have been classified as full agonist and partial agonist respectively. The inverse agonists, FG 7142 (*N'*-methyl-β-carboline-3-carboxamide), ZK 90886 (ethyl 4-ethyl-5-methoxy-β-carboline-3-carboxylate) and DMCM (methyl 4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate) have effects opposite to those of the 'agonists' and thus possess convulsant and anxiogenic properties. The antagonist, ZK 93426 (ethyl 5-isopropoxy-4-methyl-β-carboline-3-carboxylate) has no effect in these convulsant tests. The compound ZK 91296 has been reported to possess clear benzodiazepine-like agonist effects in squirrel-monkeys[78] and enhances GABA binding in rat cerebral cortex membranes.[79] The compound ZK 95962 is a partial agonist. Results of studies with the β-carbolines ZK 91296, ZK 95962 and ZK 93426 in healthy human patients have been reviewed.[80] Abecarnil (ZK 112119)[47,81,82] is effective in lower doses than diazepam in tests predictive of

antianxiety activity and in some models of epilepsy, but it is less effective in tests measuring sedation and muscle relaxation and it is weaker than ethanol and hexobarbitone.

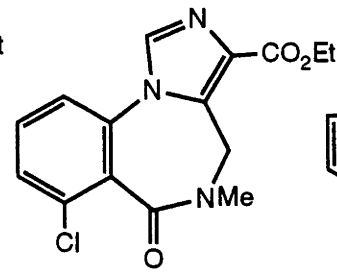
The first specific benzodiazepine antagonist, the imidazobenzodiazepine Ro 15-1788 (trade name 'Flumazenil', Fig. I-9) was reported[22] in 1981. It is largely devoid of any intrinsic activity in most behavioural and electrophysiological experiments (see Section I-4.3) but blocks all CNS effects of compounds which act through binding at benzodiazepine receptors.[83-85] Some partial agonist properties have been reported for this compound.[86] Effects such as sedation, muscle relaxation, sleep and even coma which are induced by some benzodiazepine agonist drugs can be reversed by Ro 15-1788.[87-89] The congener Ro 15-3505 (Fig. I-9) showed a higher potency in human pharmacological studies;[90] Ro 19-1880 is a full agonist and Ro 19-4603 is an inverse agonist. Bretazenil (Ro 16-6028; Fig. I-9) possesses about a ten-fold higher affinity than diazepam for the benzodiazepine receptor.[47] Its profile of activity in relation to benzodiazepine receptor full agonists has been studied.[47]

Some imidazo[1,2-*a*]pyridines and imidazo[1,2-*a*]-pyrimidines interact with benzodiazepine receptors; these have been reviewed by Gardner[91] and Haefely *et al.*[42] The pharmacology of zopiclone (Fig. I-9), an anxiolytic-hypnotic, has been examined.[92]

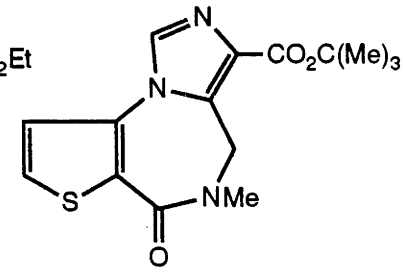
The pharmacological profile of suriclone (Fig. I-9) is that of a typical agonist,[93] but its binding



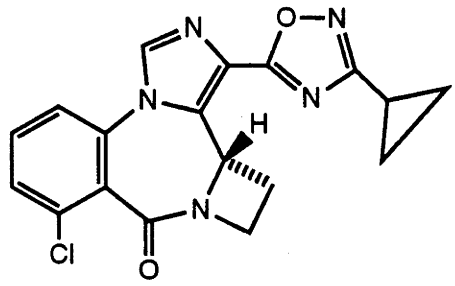
Ro 15-1788



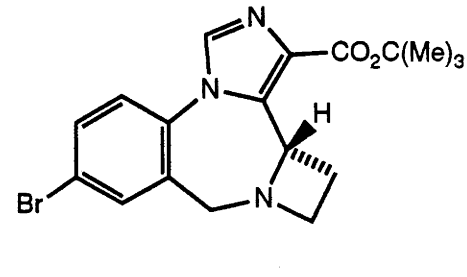
Ro 15-3505



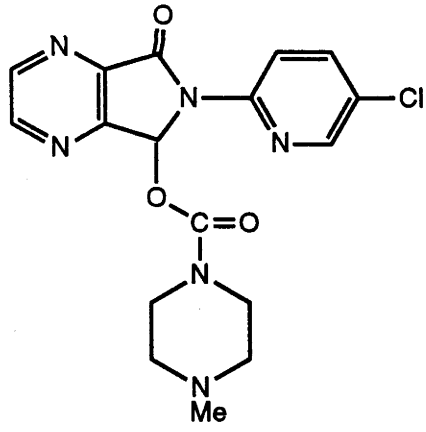
Ro 19-4603



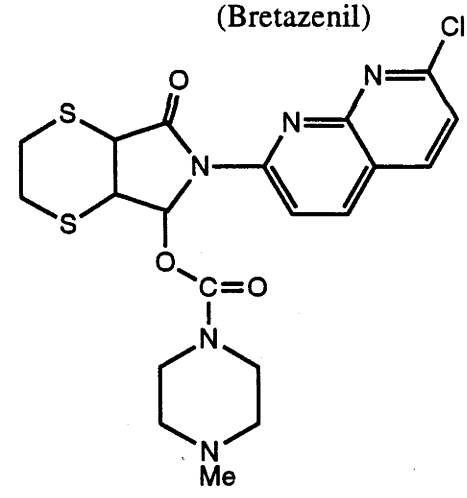
Ro 19-1880



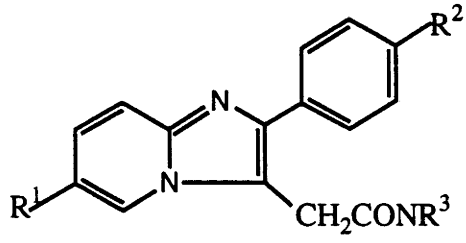
Ro 16-6028
(Bretazenil)



Zopiclone



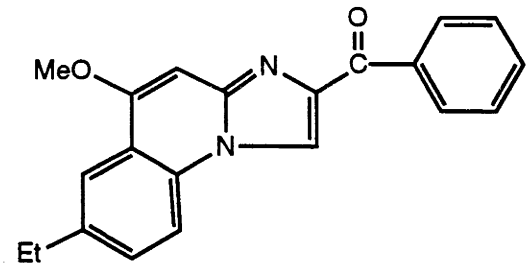
Suriclone



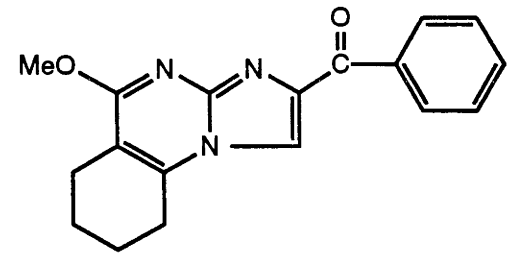
	R^1	R^2	R^3
Zolpidem	Me	Me	Me_2
Alpidem	Cl	Cl	Pr_2

Fig I-9

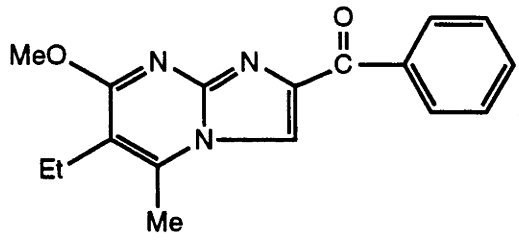
characteristics differ (independent of GABA concentration[94] and unaffected by photolabelling the receptor with the covalently bound flunitrazepam[95]) suggesting that it may bind to a novel site linked allosterically to the benzodiazepine receptor.[96] The imidazo[1,2-*a*]pyridine compounds, zolpidem and alpidem (Fig. I-9), have been claimed to possess preferential affinities for particular receptor subtypes in both animals and man. Zolpidem is a hypnoselective agent whereas alpidem has been reported to be anxiolytic in clinical studies on over 1500 patients with situational anxiety, general anxiety disorders and psychotic syndromes.[97,98] Alpidem has anxiolytic-like effects in some (but not all) animal tests, and also anticonvulsant effects without sedation and muscle relaxation.[99,100] The ketone (RU 31719; Fig. I-10) has all the pharmacological properties of a classical benzodiazepine agonist and it was orally active in a conflict screen.[101] The imidazo[1,2-*a*]pyrimidines RU 32698 (Divaplon; Fig. I-10) and RU 32514 have activity similar to that of chlordiazepoxide in various models.[47] Oxadiazole (Fig. I-10) has an IC_{50} of 630 nM for the displacement of [^{3}H]flunitrazepam from rat-brain preparations and yet displays antianxiety properties of similar potency to those of chlordiazepoxide in animal models, while demonstrating negligible myorelaxant effects.[102] Divaplon (RU 32698) shows broad anticonvulsant activity. Both RU 32698 and RU 32514, exhibit little or no activity in tests measuring sedation/motor performance impairment.



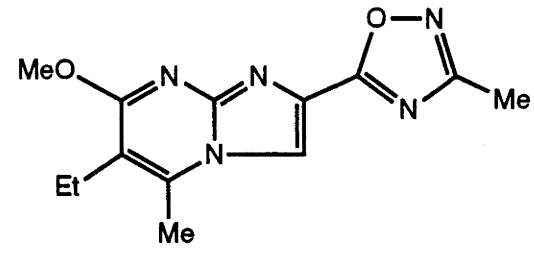
RU 31719



RU 32514



RU 32698
(Divaplon)



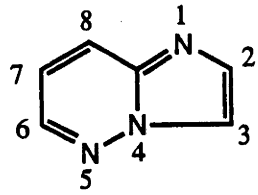
Oxadiazole

Fig. I-10 Some Imidazo[1,2-*a*]pyrimidines and an imidazo[1,2-*a*]pyridine

I-6 Syntheses and reactions of some imidazo[1,2-b]-pyridazines

I-6.1 Structure and nomenclature

Imidazo[1,2-*b*]pyridazine (**I.1**) is a heteroaromatic bicyclic system possessing 10 π-electrons; it consists of a π-excessive imidazole ring and a π-deficient pyridazine ring. The orientation of rings is as shown below with the positions numbered according to IUPAC rules.



I.1 Imidazo[1,2-*b*]pyridazine

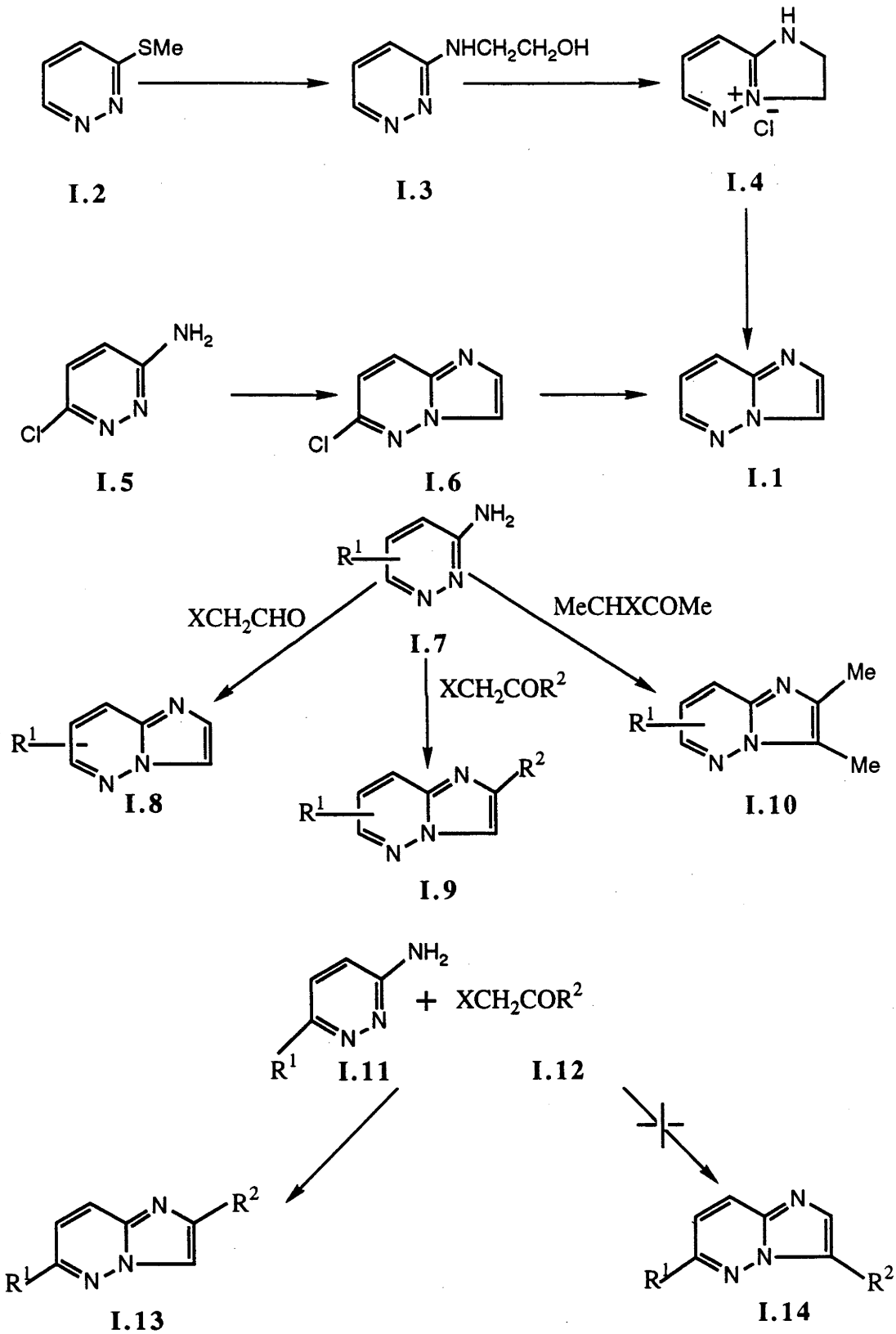
I-6.2 Syntheses

The synthesis of imidazo[1,2-*b*]pyridazines was first reported by Yoneda and coworkers[103] in 1964; and derivatives of this ring system have now attracted much attention because of their potential usefulness. Such compounds are most conveniently synthesised from suitably substituted pyridazines. The synthesis, and the physical and chemical properties have been reviewed by Tisler and

Stanovnik[104] and by Maury.[105].

The parent imidazo[1,2-*b*]pyridazine (**I.1**) has been synthesised by two methods. Armarego[106] in 1965 reported the preparation of imidazo[1,2-*b*]pyridazine (**I.1**) from 3-methylthiopyridazine (**I.2**). Compound (**I.2**) with ethanolamine gave 3-(β-hydroxyethylamino)pyridazine (**I.3**) which with thionyl chloride afforded the corresponding β-chloro compound which was cyclised to 2,3-dihydroimidazo-[1,2-*b*]pyridazinium chloride (**I.4**). This was oxidized by potassium ferricyanide to give compound (**I.1**) in 5% yield. In the second synthetic method 6-chloropyridazin-3-amine (**I.5**) was condensed with bromoacetaldehyde to give 6-chloroimidazo[1,2-*b*]pyridazine (**I.6**) which was dechlorinated, by hydrogen in the presence of palladized charcoal and triethylamine, to compound (**I.1**) in 75% yield.[107,108]

Substituted imidazo[1,2-*b*]pyridazines are usually prepared from substituted pyridazin-3-amines with an appropriate α-halogenocarbonyl compound. For example, the pyridazin-3-amine (**I.7**; R^1=H) with α-halogenoaldehydes such as bromo-,[107,109] and chloro-acetaldehyde[110,111] gave the imidazo[1,2-*b*]pyridazines (**I.8**; R^1=H); and with α-halogenoketones such as phenacyl bromide,[103,112,113] various substituted phenacyl bromides,[103,112,114] chloroacetone[111,115] and bromoacetone[113] gave 2-aryl and 2-methyl 3-unsubstituted imidazo[1,2-*b*]pyridazines (**I.9**); and 3-bromobutan-2-one[116] similarly gave the 2,3-dimethyl


SMe
NHCH2CH2OH
H
N
+N
Cl
I.2
I.3
I.4
NH2
Cl
N
I.5
I.6
I.1
NH2
R1
XCH2CHO
MeCHXCOMe
XCH2COR2
I.7
I.8
I.9
I.10
Me
R2
NH2
+ XCH2COR2
R1
I.11
I.12
I.13
I.14

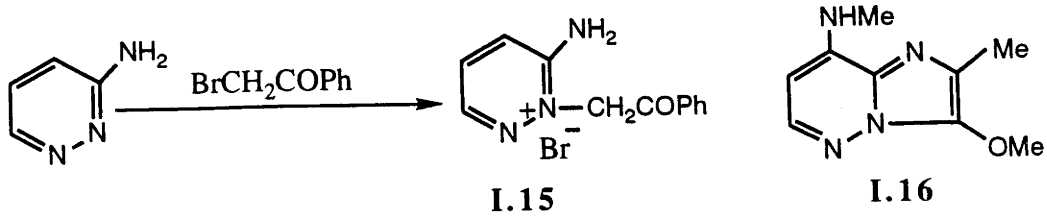
analogues (**I.10**).

In 1968, Lombardino[110] reported that the reaction of 6-methoxypyridazin-3-amine with ethyl bromopyruvate or with ethyl 2-chloroacetoacetate gave ethyl 6-methoxy-imidazo[1,2-*b*]pyridazine 2-carboxylate and ethyl 6-methoxy-2-methylimidazo[1,2-*b*]pyridazine-3-carboxylate respectively; and Ostroversnik and coworkers[117] have used 1,2-dibromoethane and ethyl chloroacetate to prepare imidazo[1,2-*b*]pyridazines. The condensation with halogeno-ketones is usually carried out in refluxing ethanol or 1,2-dialkoxyethane but reactions with halogenoaldehydes are usually carried out at room temperature. Such condensations could lead to either compound (**I.13**) or (**I.14**) (assuming the formation of C-N bonds exclusively) but, in fact, only the compound (**I.13**) was generally obtained. It has been suggested[104] that the more favoured intermediate arises from the attack by compound (**I.12**) on the ring nitrogen atom of compound (**I.11**); this is supported by the isolation of compound (**I.15**) from the condensation of pyridazin-3-amine and phenacyl bromide.[103]

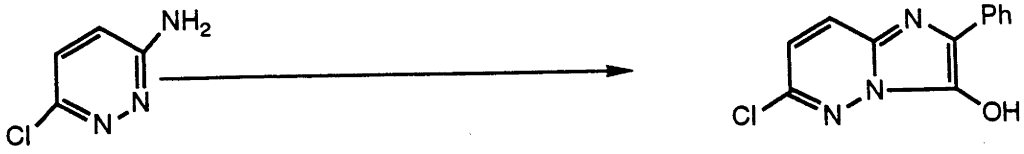
A two step mechanism has been proposed[104] involving the formation of both N-1 and N-2 quaternized intermediates of which only the N-2 quaternized compound (e.g. **I.15**) undergoes ring closure to the imidazo[1,2-*b*]pyridazine. Yields in these reactions are usually less than 60%. Simple mono- and di-aminopyridazines with iodomethane have been found to give both 1- and 2-methyl derivatives, with no evidence for quaternization at the exocyclic nitrogen

atom.[118] In 1982 Barlin and coworkers[119] reported that the condensation of 4-methylaminopyridazin-3-amine with pyruvaldehyde dimethyl acetal in methanolic hydrogen chloride gave 3-methoxy-2-methyl-8-methylaminoimidazo-[1,2-*b*]pyridazine (**I.16**) and in 1983 they described the condensation of 6-chloropyridazin-3-amine (**I.5**) with phenylglyoxal in ethanolic hydrogen chloride to give compound (**I.17**) which was methylated by diazomethane to give the 3-methoxy analogue (**I.20**; R^1=Cl, R^2=Ph). This work was extended in 1986[120] and in subsequent papers. More recently Barlin and coworkers[121,122] have described the condensation of 6-benzylamino- (and 6-phenoxy)-pyridazin 3-amine 2-oxides[121,122] (**I.18**) with phenacyl bromide to give the hydroxy compound (**I.19**) which was methylated with an excess of ethereal diazomethane to give compound (**I.20**).

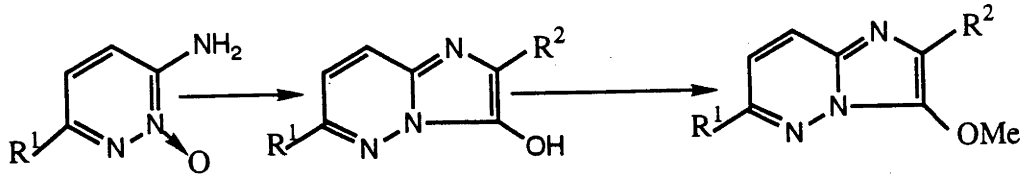
Yoshiro and coworkers[123] have reported the reactions of pyridazine (**I.21**) with tetracyanoethylene oxide to give pyridazinium dicyanomethylide (**I.22**) which condensed in the presence of sodium methoxide to give the 3-cyanoimidazo[1,2-*b*]pyridazine (**I.23**). Another synthesis has been described by Podergajs and coworkers[124] in which *N*,*N*-dimethyl-*N'*-pyridazinylformamidine (**I.24**) and analogous compounds were condensed with α-bromoacetyl ketones and gave the 3-acyl compounds (**I.25**). Stanovnik and coworkers[125] have also reported that 6-(2',2'-dimethoxyethylaminotetrazolo[1,5-*b*]pyridazine (**I.26**) in the



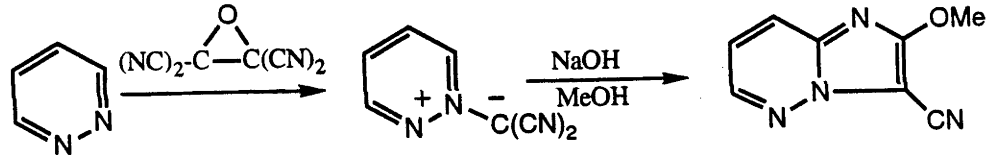
I.15 I.16



I.5 I.17

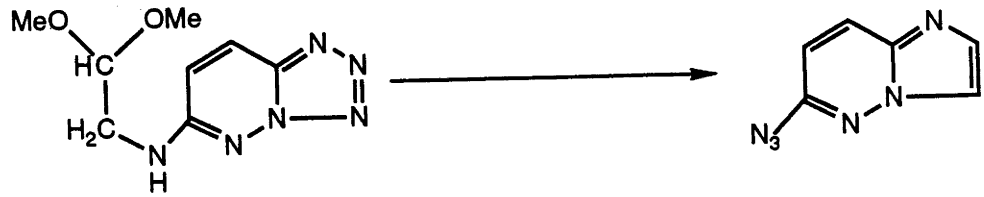


I.18 I.19 I.20



I.21 I.22 I.23

I.24 I.25



I.26 I.27

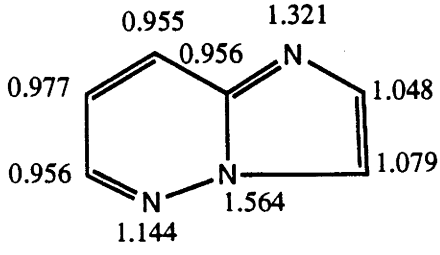
presence of polyphosphoric acid underwent simultaneous ring closure, and ring opening of the tetrazole ring, to give 6-azidoimidazo[1,2-*b*]pyridazine (**I.27**).

I-6.3 Chemical and physical properties

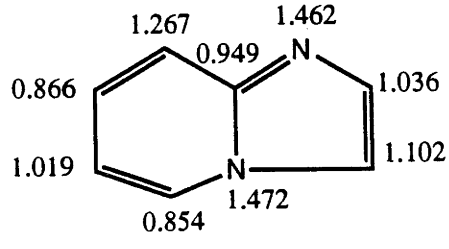
i Physical properties

HMO calculations[104] of the π-electron densities in imidazo[1,2-*b*]pyridazine and imidazo[1,2-*a*]pyridine[126] have been carried out and the results are shown in formulae (**I.28**) and (**I.29**). These indicate that in imidazo[1,2-*b*]-pyridazine and imidazo[1,2-*b*]pyridine, the 3-position is most susceptible to electrophilic substitution, whereas the 6- and 8-positions of imidazo[1,2-*b*]pyridazine (**I.28**) and the 5- and 7-position of imidazo[1,2-*a*]pyridine (**I.29**) should be the most active towards nucleophilic substitution.

Protonation studies[106] on imidazo[1,2-*b*]pyridazine (**I.28**) and imidazo[1,2-*b*]pyridine (**I.29**) showed that compounds (**I.28**) and (**I.29**) have pKa values of 4.57[106] and 6.72[127] respectively; whereas that of pridazin-3-amine and pyridin-2-amine are 5.19 and 6.82[128] respectively. It has



I.28



I.29

been claimed that imidazo[1,2-*b*]pyridazine protonates at N-1.[106] The lower basic strength of (**I.28**) relative to (**I.29**) is due to the presence of the second doubly bound ring nitrogen atom in the imidazo[1,2-*b*]pyridazine.

^{1}H n.m.r. and ^{13}C n.m.r. studies of imidazo[1,2-*b*]-pyridazine have been reported by Kobe *et al.*[108] and Pugmire *et al.*,[129,130] respectively.

ii Electrophilic reactions (excluding methylation)

The susceptibility of imidazo[1,2-*b*]pyridazine to electrophilic reactions has been studied[108,131,132] by means of chlorination with phosphorus pentachloride at elevated temperatures. The order of reactivity is 3 > 2 (in agreement with the results of HMO calculations) and 7 > 8 > 6. Mannich reactions were also effected on 6-methoxyimidazo[1,2-*b*]pyridazine and its 2-methyl derivative.[110,111] However forcing conditions were required in some instances to effect the reaction. Bromination[107,108] of imidazo[1,2-*b*]pyridazine with *N*-bromosuccinimide (NBS) or bromine in acetic and gave 3-bromoimidazo[1,2-*b*]pyridazine and nitration[131] of 2-phenyl-imidazo[1,2-*b*]pyridazine has been shown to proceed initially at the 3-position.

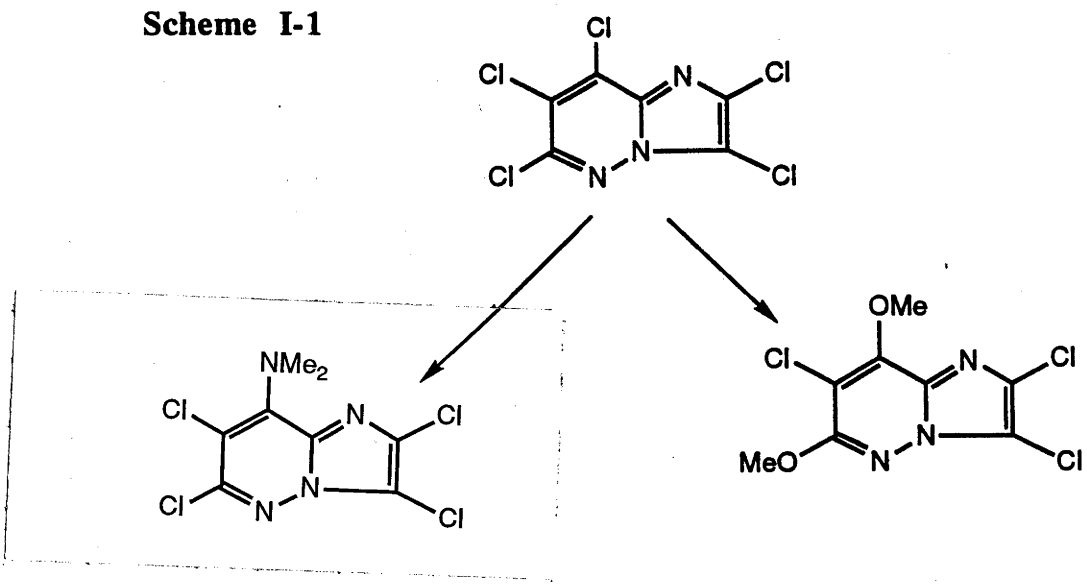
iii Nucleophilic reactions

Nucleophilic displacement of the chloro-substituent from 6-chloroimidazo[1,2-*b*]pyridazine has been investigated for the preparation of 6-variously substituted imidazo-[1,2-*b*]pyridazines. Yoneda *et al.*[103] have described

reactions of 6-chloro-2-phenylimidazo[1,2-*b*]pyridazine with hydroxide and alkoxide ions and dialkylamines to give 6-hydroxy, alkoxy and dialkylamino analogues, respectively; also the chloro substituent was removed by catalytic reduction. Stanovnik and Tisler[109,113] reported that with hydrazine hydrate the chloro substituent at the 6-position was replaced to afford the 6-hydrazino compound in 90% yield. They also reported[107] replacement of the chloro substituent by thiophenoxide ions but attempted replacements with ammonia or with potassium hydrogen sulphide were unsuccessful.

The reactions of perchloroimidazo[1,2-*b*]pyridazine (obtained from imidazo[1,2-*b*]pyridazine by the reaction with phosphorus pentachloride at 260-280°) towards dimethylamine and methoxide ions have been studied.[133] Whereas aqueous dimethylamine under reflux displaced the chloro substituent from the 8-position, methoxide ions in refluxing methanol caused replacement of the chloro substituents at both the 6- and 8-position (see Scheme I-1).

Scheme I-1

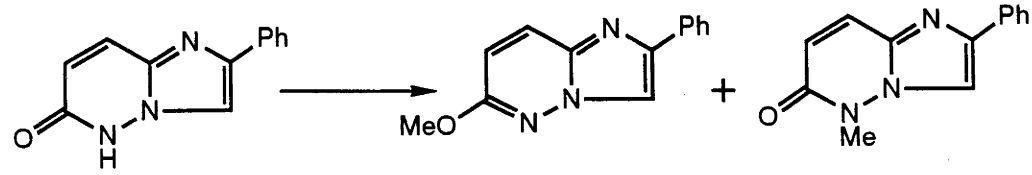


iv Methylation, methyl group migration and some cycloaddition reactions

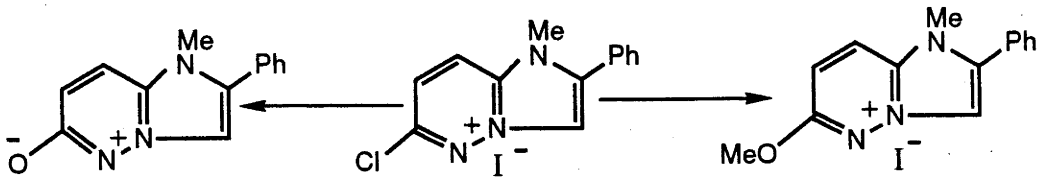
The methylation of 2-phenylimidazo[1,2-*b*]pyridazin-6(5*H*)-one (**I.30**) with methyl iodide reported in 1964 by Yoneda and coworkers[103] was reinvestigated by Furlan, Stanovnik and Tisler.[134] They found that both the 6-methoxy compound (**I.31**) and the 5-methyl compound (**I.32**) were formed and that some starting material remained. It has also been reported[120,135] that the methylation of 2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one and analogues with excess ethereal diazomethane gave the corresponding 3-methoxyimidazo[1,2-*b*]pyridazine.

Migration[134] of the methyl groups was observed when 6-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**I.31**) was heated at 240^{o} for 2 h. It gave a mixture of unchanged 6-methoxy compound (**I.31**), the *N*-methyl compound (**I.32**) and the anhydro salt (**I.33**) in the ratio of 25:61:14. Thus migration of the methyl group from oxygen occurred to the neighbouring N-5 and to the more distant N-1. When 6-chloro-1-methyl-2-phenylimidazo[1,2-*b*]pyridazin-4-ium iodide[134] (**I.34**) was treated with methoxide ions it gave, by nucleophilic substitution, the methoxy compound (**I.35**) and with hydroxide ions it gave the anhydro salt (**I.33**). When the anhydro salt[134] (**I.33**) was methylated with methyl iodide it gave a mixture of the 1,5-dimethyl compound (**I.36**) and the 6-methoxy compound (**I.35**) in the ratio of 1:5. Also when compound[134] (**I.31**) was heated with methyl iodide under pressure it gave compounds (**I.36**) and (**I.35**).

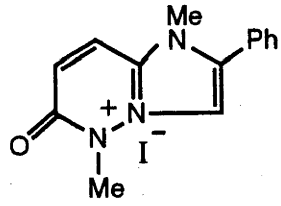
Scheme I-2



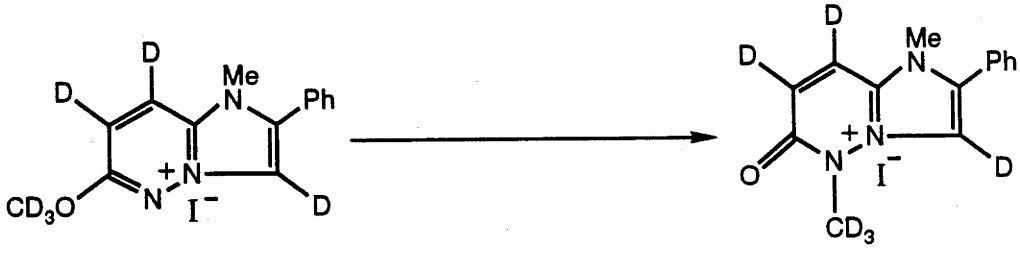
I.30 **I.31** **I.32**



I.33 **I.34** **I.35**



I.36

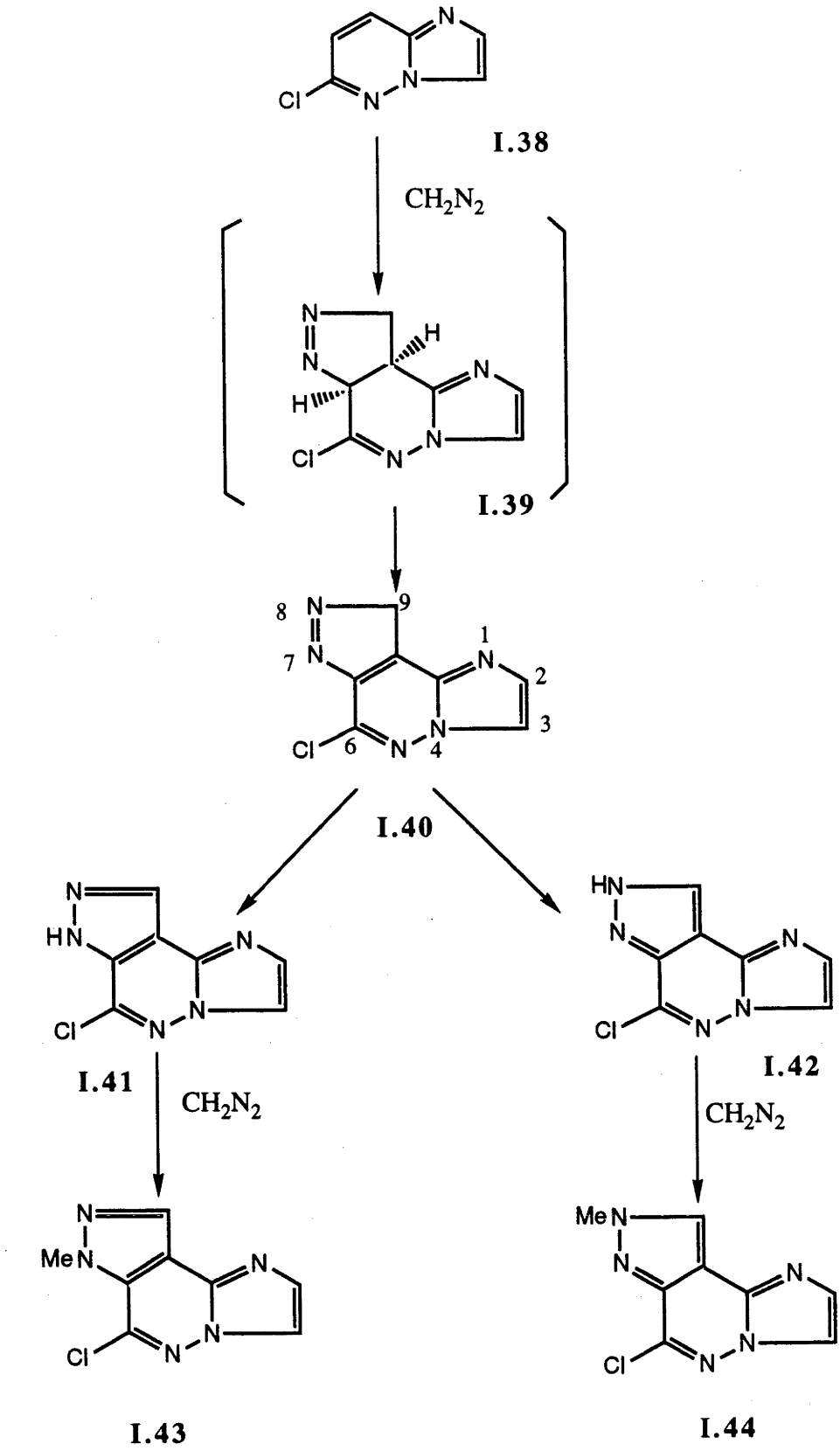


I.37

Compound (**I.36**) was also obtained[134] by thermal rearrangement of compound (**I.35**) or by quaternization of compound (**I.32**) with methyl iodide at 160°. The conversion of compound (**I.35**) to compound (**I.36**) has been studied[134] with the deutero-compound (**I.37**) and it has been shown that the CD_3 group migrated to N-5 only and that no interchange of methyl groups occurred. This methyl group migration was probably intermolecular;[134,136] and the driving force for the rearrangements was the greater stability of the amido structure.[137]

Cycloaddition reaction of imidazo[1,2-*b*]pyridazine with diazomothane,[138] 2-diazopropane,[139] and 2-diazobutane[140] have been reported; diazomethane was the most reactive. Addition occurred across the C7-C8 double bond and is highly regiospecific. The reaction of 6-chloroimidazo[1,2-*b*]pyridazine (**I.38**, Scheme I-3) in a mixture of dimethylformamide and chloroform with diazomethane is illustrated. The mechanism[138] proposed by these workers involved 1,3-dipolar cycloaddition of diazomethane across the polarized C7-C8 double bond of 6-chloroimidazo[1,2-*b*]pyridazine to give the primary cycloadduct (**I.39**) which underwent oxidative transformation to compounds (**I.40**). This was followed by 1,3 and/or 1,5-sigmatropic rearrangement to the tautomeric intermediates (**I.41** and **I.42**) and further methylation with diazomethane gave compounds (**I.43** and **I.44**).

Scheme I-3

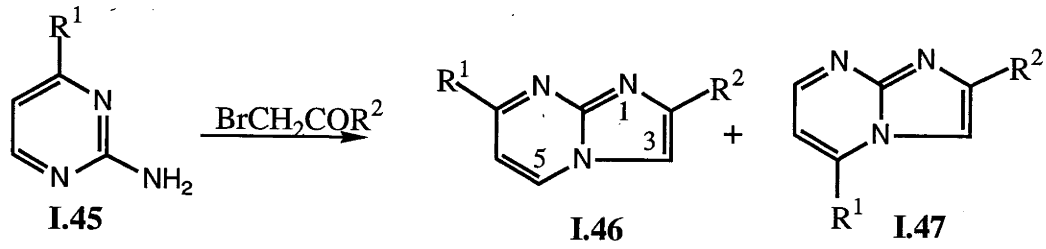


I-7 Syntheses and reactions of some imidazo[1,2-a]-pyrimidines, imidazo[1,2-a]pyrazines and imidazo[1,2-a] pyridines

Derivaties of imidazo[1,2-*a*]pyrimidines, imidazo-[1,2-*a*]pyrazines and imidazo[1,2-*a*]pyridines (10 π-electron systems) have been synthesized, usually in good yield, by condensations of the readily available pyrimidin-2-amine, pyrazin-2-amine and pyridin-2-amine with α-halogeno-carbonyl compounds.

The condensation of pyrimidin-2-amines with the α-halogenocarbonyl compounds has been described by Paudler and Kuder.[141] The structures of the products formed from these condensations were unequivocal if the pyrimidin-2-amine used was either unsubstituted or monosubstituted at position 5 or symmetrically disubstituted at positions 4 and 6. The products from a 4-substituted pyrimidin-2-amine with α-bromoacetaldehyde were either a 7-substituted (**I.46**) or a 5-substituted imidazo[1,2-*a*]pyrimidine (**I.47**).

Recently, Rival and coworkers[142] have reported the synthesis and *in vitro* antifungal activity of some imidazo[1,2-*a*]pyrimidine derivatives. The synthesis involved the condensation of a pyrimidin-2-amine with an



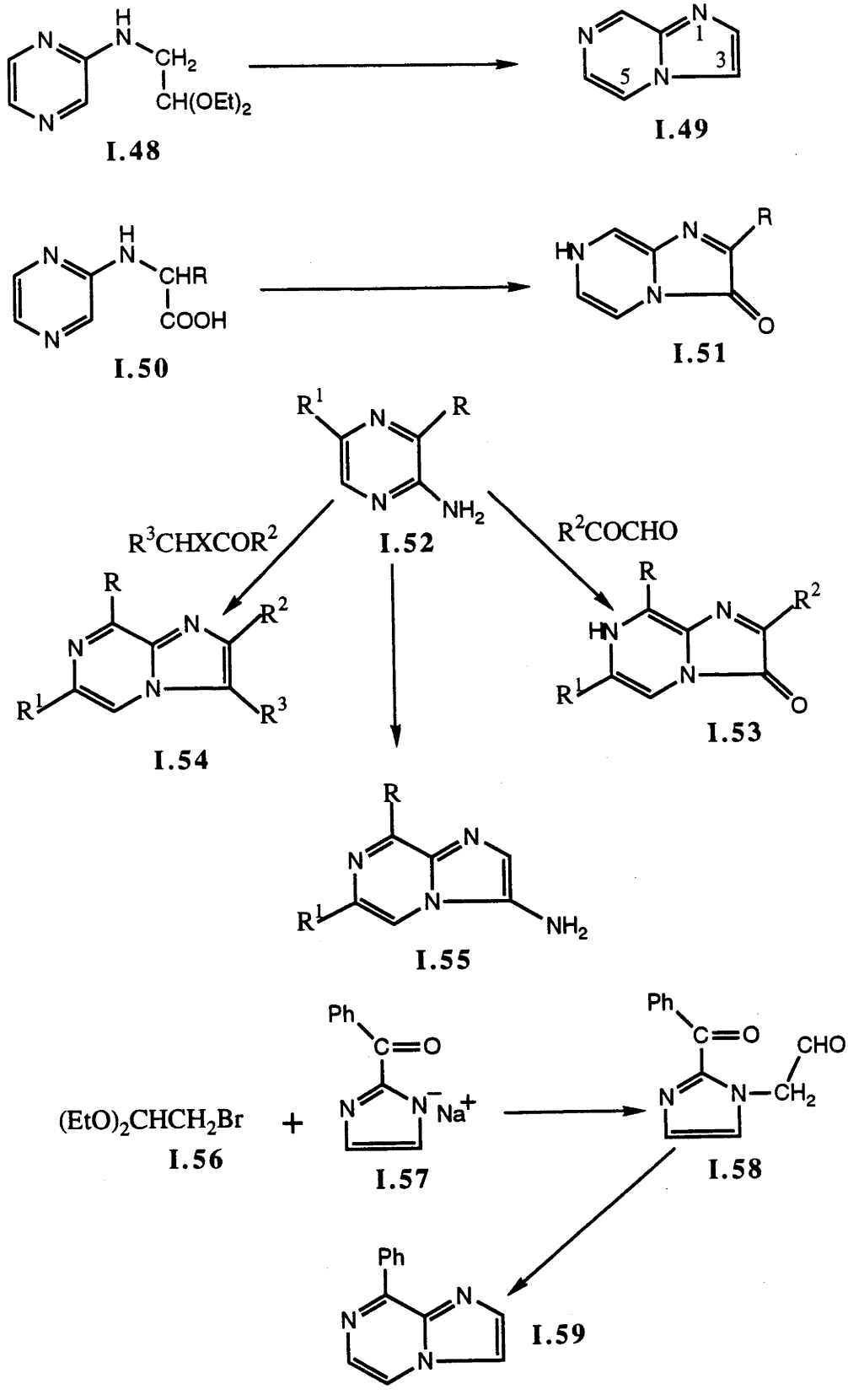
I.45 **I.46** **I.47**

appropriate α-bromoketone and then introduction (initially) of a nitro, nitroso, or bromo (by bromination with NBS in CCl_4) substituent at the 3-position.

There are two principal methods for the preparation of derivatives of imidazo[1,2-*b*]pyrazines from pyrazines. The first involves the cyclization of appropriately substituted pyrazin-2-amine. Thus cyclization of the pyrazin-2-ylaminoacetal (**I.48**, Scheme I-4) in concentrated sulphuric acid gave imidazo[1,2-*a*]pyrazine (**I.49**) in low yield,[106] and the pyrazin-2-ylaminoacetic acid (**I.50**) with sodium alkoxide afford imidazo[1,2-*a*]pyrazin-3-(2*H*)-ones (**I.51**).[143] The second involved the reaction of pyrazin-2-amine with bifunctional reagents. For example, pyrazin-2-amines (**I.52**) with α-keto aldehydes gave compounds (**I.53**),[144,145] and pyrazin-2-amines with α-halogeno-carbonyl compounds gave compounds (**I.54**).[143,146-151] Also imidazo[1,2-*a*]pyrazin-3-amine (**I.55**; $R=R^1=H$) was prepared by the reaction of pyrazin-2-amine with sodium cyanide and the bisulfite addition compound from formaldehyde.[152] Imidazo[1,2-*a*]pyrazines have also been prepared by cyclization of imidazoles. For example, 2-benzoylimidazole (**I.57**) was alkylated with compound (**I.56**) in DMF and the intermediate (**I.58**) when refluxed with acetic acid containing ammonium acetate cyclised to 8-phenyl-imidazo[1,2-*a*]pyrazine (**I.59**).[153]

The preparation of imidazo[1,2-*a*]pyridines has been achieved mostly from pyridin-2-amine by closure of the imidazole ring, but also from the imidazole by closure of

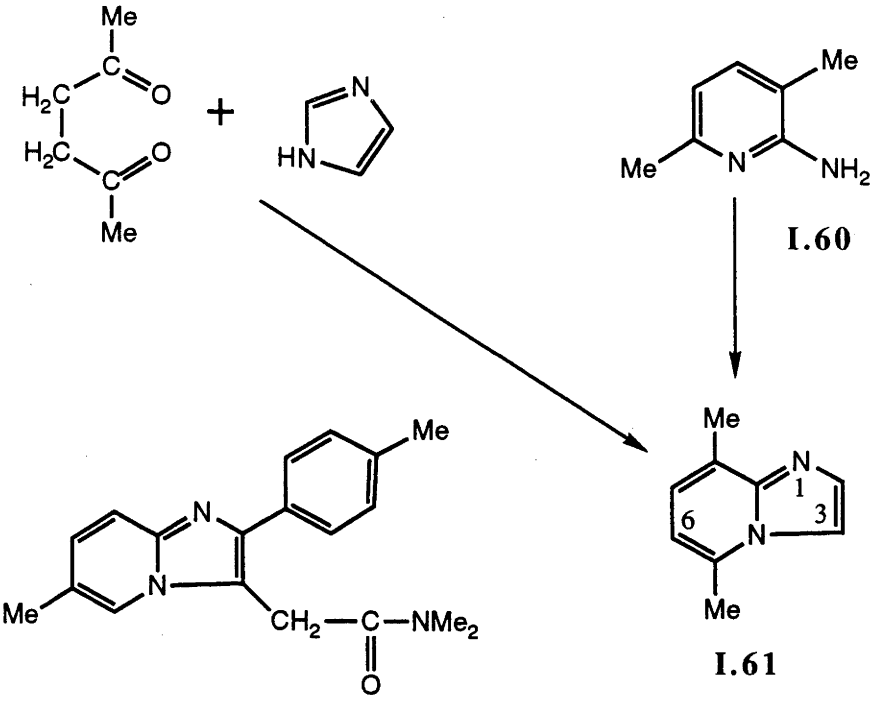
Scheme I-4


I.48
I.49
I.50
I.51
I.52
R³CHXCOR²
R²COCHO
I.53
I.54
I.55
(EtO)₂CHCH₂Br
I.56
I.57
I.58
I.59

the pyridine ring. For example, the pyridin-2-amine (**I.60**) with bromoacetaldehyde gave the imidazo[1,2-*a*]pyridine (**I.61**),[154] which was also obtained from imidazole with hexane-2,5-dione.[154]

The chemistry and structure-activity relationships of the new hypnotic agent [SL 80.0750-23N; *N*,*N*,6,-trimethyl-2-(4′-tolylimidazo[1,2-*a*]pyridine-3-acetamide (**I.62**) (hemitirtrate-Zolpidem)] has been reviewed by George and coworkers.[155] The synthesis commenced from 5-methyl-pyridin-2-amine (**I.63**), which with α-bromo-4′-methyl-acetophenone gave the substituted imidazo[1,2-*a*]pyridine (**I.64**; 93% yield), and this underwent the Mannich reaction with formaldehyde and dimethylamine in acetic acid to afford compound (**I.65**; 79% yield). Alkylation of the side-chain nitrogen of the latter with methyl iodide afforded the quaternary ammonium salt (**I.66**) which with sodium cyanide gave the corresponding nitrile (**I.67**; 81% yield). This nitrile was hydrolyzed in aqueous acid to the carboxylic acid (**I.68**; 91% yield) which was converted to the *N*,*N*-dimethylamide (**I.62**; 85% yield).

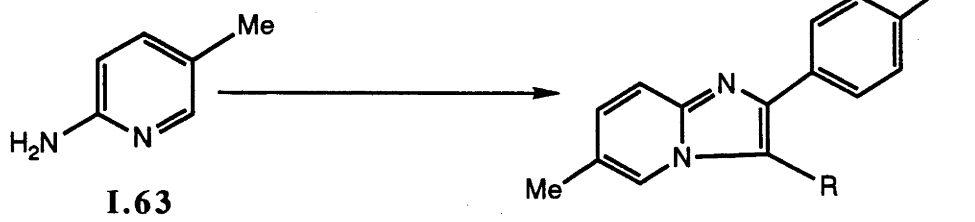
For the large scale preparation of zolpidem, a new process was developed. Thus treatment of the 3-unsubstituted imidazo[1,2-*a*]pyridine (**I.64**) with the appropriate glyoxylic amide (prepared *in situ* from the corresponding ketal) generated the α-hydroxyacetamide (**I.69**) in high yield. The latter with thionyl chloride gave compound (**I.70**) which was then reduced (under various conditions) to the acetamide (**I.62**).



I.60

I.61

I.62



I.63

I.64, R= H
I.65, R= CH_2NMe_2
I.66, R= $CH_2N^+Me_3\ I^-$
I.67, R= CH_2CN
I.68, R= CH_2COOH
I.69, R= $CH(OH)CONMe_2$
I.70, R= $CHClCONMe_2$
I.62, R= CH_2CONMe_2

I-8 Previous pharmacological and biological studies of some imidazo[1,2-b]pyridazines, imidazo[1,2-a]pyridines, imidazo[1,2-a]pyrimidines and imidazo[1,2-a]pyrazines

Pharmacological activity of some imidazo[1,2-*b*]-pyridazines was first reported by Nitta and coworkers in 1965.[156] They discovered that these heterocyclic compounds possess analgesic, sedative and antispasmodic properties and were shown to be useful as "inhibitors of central nerves". Almirante and coworkers[114] in 1966 synthesized a series of derivatives of imidazo[1,2-*b*]pyridazine (and imidazo[1,2-*a*]pyrimidine) which were found also to possess antiinflammatory, antipyretic and hypothermal activity as well as anticonvulsant activity. Three years later some imidazo[1,2-*b*]pyridazines with halogeno or alkoxy groups at C-6, alkyl group at C-2 and different Mannich side chains at C-3 were synthesized and reported to show activity against hypertension and were potent antiinflammatory agents.[111] Abignente and coworkers have reported[157] the synthesis of some imidazo[1,2-*b*]pyridazines (and imidazo[1,2-*a*]pyrimidines) with attached carboxamide or acetamide groups at the 2-position and various substituents on the six-membered ring: these were evaluated for antiinflammatory, analgesic and antipyretic activity. Bristol and Lovey[158] synthesized other imidazo[1,2-*b*]-pyridazine compounds for the treatment of ulcers.[158] Other groups have reported some 6-(pyridin-3′-yl) or 6-(3′-trifluoromethylphenyl)-2-(or 3-)alkylimidazo[1,2-*b*]-

pyridazines as anxiolytic agents[159] and some imidazopyridazinylacrylamides as anti-hypertensives, diureties and saluretics.[160] More recently, Barlin and coworkers[120-22,161-167] have made a large number of 2,3,6-trisubstituted imidazo[1,2-*b*]pyridazines for screening for more selective pharmacological activity than the benzodiazepine class of compounds in the central nervous system. They reported that certain classes of these compounds, such as 2-(4'-aminophenyl)-3-methoxy-6-(3"-methoxybenzylamino)imidazo[1,2-*b*]pyridazine[121] with IC_{50} 1.0 nM (cf [^{3}H]diazepam with IC_{50} of 4.2 nM), exhibited higher activity in the displacement of [^{3}H]diazepam from rat brain membranes than diazepam. Derivatives of imidazo[1,2-*b*]pyridazine have shown other biological actions including their use as bronchodilators,[168] inotropic,[169] antithrombotic[169] and cardiovascular agents,[170] antibiotics,[171-173] antibacterial [174-176] and antimicrobial agents,[177-179] antiprotozoal agents[180-183] herbicides and synergistic herbicides,[184-192] pesticides,[193,194] antitumour agents,[195] cardiotonic agents[196] and insecticides;[197] they have been used for control of hemorrhagic colitis in swine[198] and of foot rot, liver lesions in ruminant animals[199] and plant growth regulants.[188]

In 1965 Almirante and coworkers[114,200] also reported that some imidazo[1,2-*a*]pyridines showed analgesic, antiinflammatory, antipyretic and anticonvulsant activity. Recently other groups[201,203] have reported other

imidazo[1,2-*a*]pyridines which were useful as sedatives, anxiolytics and anticonvulsants. Other biological uses have been reported for imidazo[1,2-*a*]pyridines such as ionotropic agents,[204] synergistic herbicidal agents,[187,189-191] herbicides,[185,192] antibiotics,[172,173] and cardiotonic agents.[205]

Imidazo[1,2-*a*]pyrimidines have been reported to show analgesic,[200] antiinflammatory,[157,200] antipyretic,[200] hypothermal,[200] anticonvulsant,[200] herbicidal,[205] antithrombic,[170] cardiovascular,[170] and antifungal activity.[142]

Derivatives of imidazo[1,2-*a*]pyrazines have been examined and claims have been reported for antiinflammatory,[206,207] β-blocking,[208] uterine-relaxing[209] and antibronchospastic activity.[209] Also 3-amino-8-benzyloxy-2-methylimidazo[1,2-*a*]pyrazine[210,211] has been reported to have antiulcer activity and 2-(2′,4′-dimethoxyphenyl)imidazo[1,2-a]pyrazine and related compounds show inotropic activity.[212]

1-9 Present work

In the present work a large number of substituted imidazo[1,2-*b*]pyridazines, and some imidazo[1,2-*a*]-pyridines, -pyrimidines, and -pyrazines have been synthesised and tested for binding to rat brain plasma membrane. This research was directed to the identification of compounds (or compound types), which bound relatively strongly to benzodiazepine receptors, for further evaluation (by others) for possibly more selective pharmacological actions in the central nervous system, than have the benzodiazepine class of compound.

The research reported in this thesis includes: in Chapter II, the syntheses of 3-unsubstituted-2-aryl-6-(variously substituted)imidazo[1,2-*b*]pyridazines (from the relevant 6-substituted pyridazin-3-amine with bromoacetyl compounds) which were then converted by Mannich reaction to the 3-dimethylaminomethyl derivatives, and by reaction with *N*-hydroxymethylamides to the 3-acetamidomethyl and 3-benzamidomethyl derivatives; in Chapter III, the syntheses of 3-(substituted benzamidomethyl) derivatives by similar procedures; and in Chapter IV, details of the syntheses of 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-phenyl (and substituted phenyl)imidazo[1,2-*b*]pyridazines and some new 6-(substituted benzylamino) analogues. In Chapter V, syntheses are reported for a large number of imidazo-[1,2-*b*]pyridazines in which larger conjugating and nonconjugating aromatic substituents were incorporated at

the 2-position: these included 2-benzyl [phenethyl, (biphenyl-4′-yl) or (naphthalen-2′-yl)]-imidazo[1,2-*b*]-pyridazines.

All compounds were examined for their ability to displace [^{3}H]diazepam from rat brain plasma membrane as a measure of their ability to interact with benzodiazepine receptors of the central nervous system. The results are discussed and compared with existing data.

The role of the *N*-5 ring nitrogen atom of imidazo-[1,2-*b*]pyridazines has been examined in work described in Chapter VI. Derivatives of imidazo[1,2-*a*]pyridines, imidazo[1,2-*a*]pyrimidines, and imidazo[1,2-*a*]pyrazines have been prepared and their binding abilities compared with those of the corresponding imidazo[1,2-*b*]pyridazines.

Some compounds, prepared in the present work and which exhibit high binding affinity at benzodiazepine binding sites, have been subjected (by others) to behavioural tests in rats. These have revealed significant anxiolytic effects without sedation but these data do not form part of the present thesis.

CHAPTER II

CHAPTER II. Synthesis and binding studies of some 3-(acylaminomethyl and dimethylaminomethyl)-2-phenyl (and substituted phenyl)-6-(variously substituted)imidazo-[1,2-b]pyridazines

II-1 Introduction

In this chapter the syntheses of some 3-(acetamidomethyl, benzamidomethyl or dimethylaminomethyl)-2-phenyl(and substituted phenyl)-6-(halogeno, alkylthio, alkoxy, phenylthio, phenoxy, benzylthio, or benzyloxy) imidazo[1,2-*b*]pyridazines are described; and the results from an examination of these compounds for their ability to displace [^{3}H]diazepam from rat brain membrane preparations are described also.

This work was prompted by literature[213] reports that 3-acylaminomethyl-2-aryl-6-substituted (and unsubstituted) imidazo[1,2-*a*]pyridines showed anxiolytic, sleep-inducing, hypnotic, anticonvulsant, analgesic and antiulcer properties; that 6-aryl-2-(and 3-)dialkylaminomethyl-imidazo[1,2-*b*]pyridazines[214] were M_1-muscarinic receptor agonists, useful where cortical cholinergic deficits were manifest and particularly for Alzheimer-type dementias; and by published research work from this group with 6-(variously substituted)-2-aryl-3-methoxyimidazo[1,2-*b*]-pyridazines[120-122,161-167] (predominantely) and related compounds.

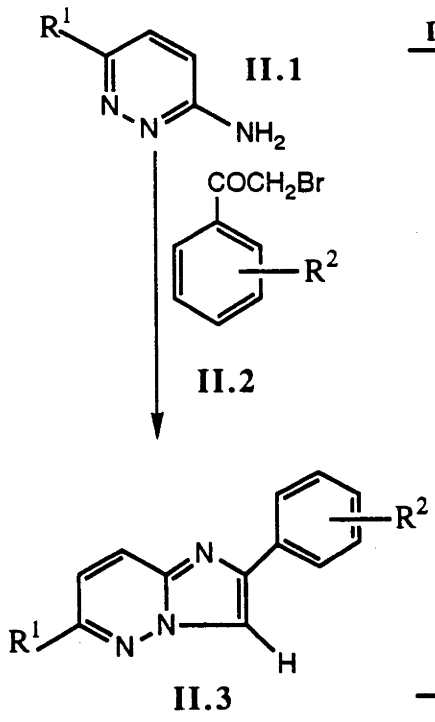
In the work described in this chapter 6-(variously substituted)-3-(acetamidomethyl, benzamidomethyl and

dimethylaminomethyl)-2-phenylimidazo[1,2-*b*]pyridazines were prepared and tested for their ability to displace [^{3}H]diazepam from rat brain plasma membrane. Attempts were then made to optimise this activity by introducing 4′-methyl and 3′,4′-methylenedioxy substituents into the 2-phenyl group.

II-2 Syntheses

The starting materials required for the preparation of these compounds were the relevant 6-substituted pyridazin-3-amines (**II.1**, Scheme II-1). Many of these compounds have been prepared in good yield by Barlin and Ngu[163-166] from 6-chloropyridazin-3-amine by heating with appropriate nucleophiles. The compounds (**II.1**) were then condensed with suitable bromoacetyl compounds (**II.2**) to give the key intermediates, *viz.* the 2-aryl-3-unsubstituted-6-(variously substituted)imidazo[1,2-*b*]pyridazines (**II.3a-n**). Such condensations have been used by Yoneda and coworkers[103] to prepare some 6-chloroimidazo[1,2-*b*]pyridazines. The 3-dimethylaminomethylimidazo[1,2-*b*]pyridazines (**II.4**, Scheme II-2) required for the present work were prepared from the corresponding 3-unsubstituted imidazo[1,2-*b*]pyridazines (**II.3**) by a Mannich reaction with dimethylamine and aqueous formaldehyde (or paraformaldehyde), or more conveniently by a modification thereof in which compounds (**II.3**) were heated with bis(dimethylamino)methane[215] and a catalytic amount of phosphoric acid in acetic acid at *ca* 120^{o}C. The

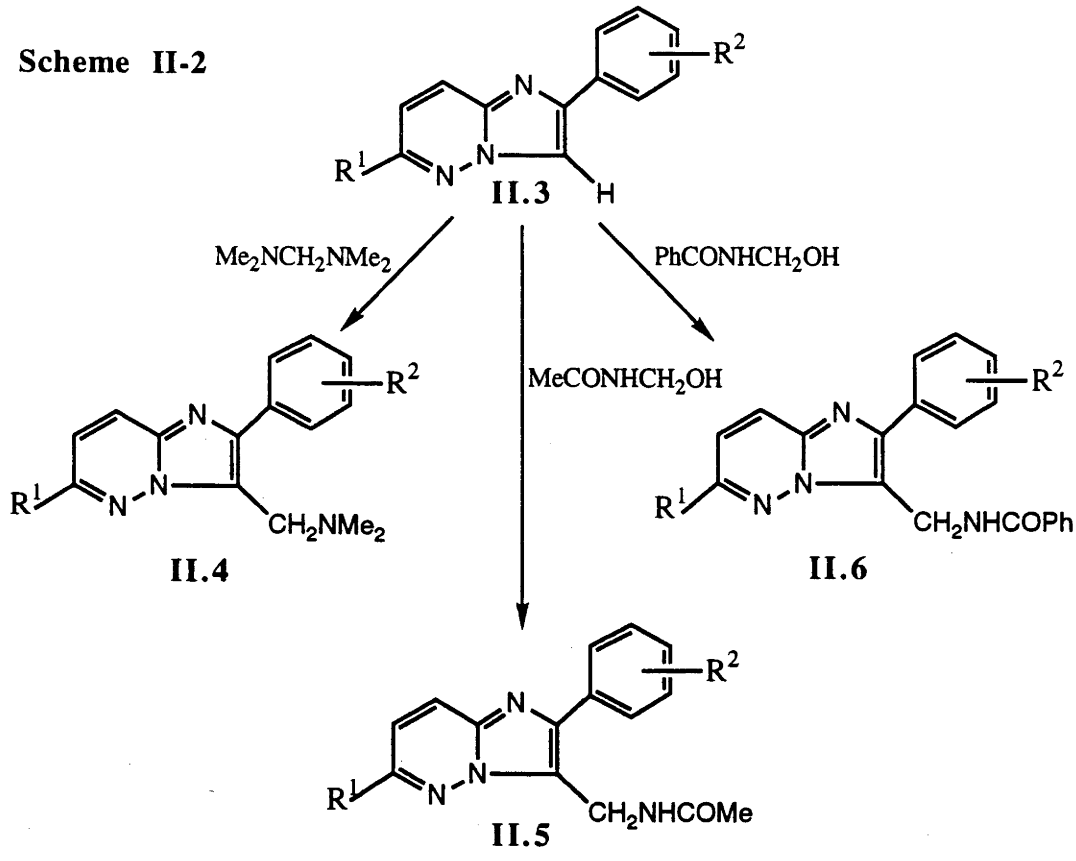
Scheme II-1



II. 3	R^1	R^2
a	F	H
b	F	Me-*p*
c	Cl	Me-*p*
d	Cl	OCH_2O-3',4'
e	SMe	H
f	SMe	Me-*p*
g	SMe	OCH_2O-3',4'
h	OMe	H
i	OMe	Me-*p*
j	SPh	H
k	SPh	Me-*p*
l	OC_6H_4OMe-*o*	H
m	$SCH_2C_6H_4OMe$-*m*	Me-*p*
n	OCH_2Ph	H
o	Cl	H
p	Cl	Cl-*p*
q	$SCH_2C_6H_4OMe$-*m*	H

latter reaction has been used by Ledincer and Hauser[215] to prepare *N,N*-dimethylaminomethylferrocene (methiodide). In this way were prepared the 3-dimethylaminomethyl-imidazo[1,2-*b*]-pyridazines (**II.4a-g**, Scheme II-2) which contained at the 6-position methylthio, methoxy, phenylthio, benzoxy and (2'-methoxyphenoxy) groups; and at the 2-position phenyl or 3',4'-methylenedioxyphenyl groups. In all cases yields of 24-74% were obtained. The reaction with bis(dimethylamino)methane gave a better yield and the products were more readily purified than by use of the standard Mannich reaction with excess dimethylamine and aqueous formaldehyde. The 3-acylaminomethylimidazo[1,2-*b*]-pyridazines containing 3-acetamidomethyl and 3-benzamidomethyl groups were prepared by a method similar to

Scheme II-2



II. 4	R^1	R^2
a.	Cl	H
b.	Cl	OCH_2O-*3',4'*
c.	SMe	H
d.	OMe	H
e.	SPh	H
f.	OC_6H_4OMe-*o*	H
g.	OCH_2Ph	H
h.	Cl	Cl-*p*
i.	SCH_2Ph	H

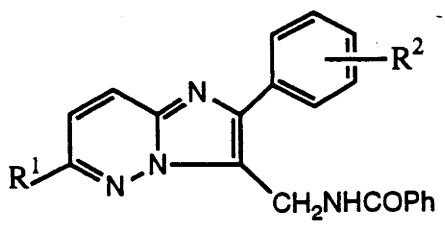
II. 5	R^1	R^2
a.	F	H
b.	Cl	H
c.	Cl	OCH_2O-*3'.4'*
d.	SMe	H
e.	OMe	H
f.	SPh	H
g.	SPh	Me-*p*
h.	OC_6H_4OMe-*o*	H
i.	OCH_2Ph	H
j.	SCH_2Ph	H

II. 6	R^1	R^2
a.	F	H
b.	Cl	Me-*p*
c.	Cl	OCH_2O-*3',4'*
d.	SMe	Me-*p*
e.	SMe	OCH_2O-*3',4'*
f.	OMe	Me-*p*
g.	SPh	H
h.	OCH_2Ph	H
i.	F	Me-*p*
j.	Cl	H
k.	SMe	H
l.	OMe	H
m.	OC_6H_4OMe-*o*	H
n.	SCH_2Ph	H

that described for the preparation of 3-acylaminomethylimidazo[1,2-*a*]pyridines.[213] Thus 3-unsubstituted-2-aryl-6-(variously substituted)imidazo-[1,2-*b*]pyridazines (**II.3**) were heated at reflux with *N*-(hydroxmethyl)acetamide or *N*-(hydroxmethyl)benzamide[216] in glacial acetic acid containing a catalytic amount of concentrated sulphuric acid to give 3-acetamidomethyl- and 3-benzamidomethyl-imidazo[1,2-*b*]pyridazines (**II.5a-i** and **II.6a-h**), respectively (Scheme II-2), which were isolated as free bases by adjusting to pH 10 with aqueous ammonia. However in certain preparations, such as that of 3-acetamidomethyl-6-(2′-methoxyphenoxy)-2-phenylimidazo-[1,2-*b*]pyridazine (**II.5h**), it was necessary to restrict the quantity of sulphuric acid in the reaction (No desired product was isolated from reactions with higher concentrations of acid). The products prepared in this work were purified by chromatography and were obtained in moderate (10-84%) yield.

The compounds (**II.3o-q**), (**II.4i**), (**II.5j**), (**II.6i-n**) and (**II.7a-i**) have been described previously: references to relevant literature are given in Table II-4.

Scheme II-3



II.7

II. 7	R^1	R^2
a.	F	H
b.	Cl	H
c.	Cl	Me-*p*
d.	SMe	H
e.	OMe	H
f.	SPh	H
g.	OC_6H_4OMe-*o*	H
h.	SCH_2Ph	H
i.	OCH_2Ph	H

II-2 ^{1}H n.m.r. spectra

All compounds prepared in this work were examined by ^{1}H n.m.r. spectroscopy at 90 MHz as described in the Experimental section. The ^{1}H n.m.r. spectrum of unsubstituted imidazo[1,2-*b*]pyridazine, in deuterochloroform, has been reported[104,108] and it shows that the protons attached to the five-membered (imidazole) ring appear as an AB quartet. The chemical shifts of protons at C-2 and C-3 were found to be at δ7.89 and 7.99, respectively, each with a coupling constant of $J_{2,3}$ 1.0 Hz. Those on the six-membered (pyridazine) ring moiety appeared as an ABX system with long-range coupling across the bridgehead atoms. The chemical shifts and coupling constants of these protons were δ9.30 (H6), 7.00 (H7), 7.95 (H8); with $J_{6,7}$ 4.5, $J_{7,8}$ 10.0, $J_{6,8}$ 2.0 and $J_{3,8}$ 0.8 Hz.

The ^{1}H n.m.r. spectrum of the 2,6-disubstituted imidazo[1,2-*b*]pyridazine, 6-chloro-2-methylimidazo[1,2-*b*]-pyridazine[108] has been reported in perdeuteroacetone (CD_3COCD_3) and the chemical shifts were observed at δ8.03, 7.30 and 8.09 for H3, H7 and H8, respectively.

Some ^{1}H n.m.r. spectral data obtained for the compounds prepared in the present work are presented for compounds (**II.3**) in Table II-1, compounds (**II.4**) in Table II-2 and compounds (**II.5**) and (**II.6**) in Table II-3. Those of the 3-unsubstituted imidazo[1,2-*b*]pyridazine (**II.3**) derivatives revealed a signal due to the proton at C-3 as a singlet in the region δ7.81-8.20. In the compounds (**II.3c**,

Table II-1 Some ^{1}H n.m.r. spectral data (δ)[A] for 3-unsubstituted-2-aryl-6-(variously substituted)imidazo-[1,2-b]pyridazines

Formula number	R^1	R^2	H3	H7	H8
II.3b	F	Me-*p*	8.11	6.85	B
3o[C]	Cl	H	8.20	7.02	7.78
3c	Cl	Me-*p*	8.18	7.03	7.89
3p[C]	Cl	Cl-*p*	8.16	7.04	7.88
3d	Cl	OCH_2O-3′,4′	8.10	7.04	7.87
3e	SMe	H	8.17	6.87	7.77
3f	SMe	Me-*p*	8.12	6.74	7.83
3g	SMe	OCH_2O-3′,4′	8.05	6.85	7.69
3h	OMe	H	8.06	6.70	7.81
3i	OMe	Me-*p*	8.01	6.67	7.80
3j	SPh	H	7.81	6.77	B
3k	SPh	Me-*p*	8.10	6.76	B
3l	OC_6H_4OMe-*o*	H	7.90	6.86	7.86
3m	$SCH_2C_6H_4OMe$-*m*	Me-*p*	8.11	6.77	B
3n	OCH_2Ph	H	8.07	6.75	B

[A]Reported as parts per million (δ) downfield from tetramethylsilane (T.M.S.) as internal standard in deuterochloroform ($CDCl_3$).
[B]Complex signal
[C]Prepared by Dr M.M.L. Ngu

II.3p and **II.3d**) with a common substituent at C-6 (e.g. chloro), insertion of substituents in the 2-phenyl group (such as methyl, chloro or methylenedioxy) had a small effect and only on the position of the signal due to H-3. The hydrogen atoms at C-3 in 2-phenyl-6-phenylthio[1,2-*b*]-pyridazine (**II.3j**) and 6-(2′-methoxyphenoxy)-2-phenyl-imidazo[1,2-*b*]pyridazine (**II.3l**) are relatively more shielded and occur at the δ7.81 and 7.90, respectively. The shielding of the hydrogen atoms at C-3 in the 6-

methylthio-2-substituted phenyl compounds (**II.3e-g**) is similar to the 6-chloro-2-substituted phenyl analogues.

Table II-2 Some ^{1}H n.m.r. spectral data (δ)[A] for 3-dimethylaminomethylimidazo[1,2-b]pyridazines

Formula number	R^1	R^2	CH_2	$(Me)_2N$	H7	H8
II.4a	Cl	H	3.97	2.32	7.05	7.89
4h[B]	Cl	Cl-*p*	3.93	2.31	7.05	7.89
4b	Cl	OCH_2O-3′,4′	3.94	2.32	7.03	7.87
4c	SMe	H	3.95	2.32	6.85	7.72
4d	OMe	H	3.92	2.32	6.67	7.79
4d	SPh	H	3.77	2.14	6.82	7.75
4f	OC_6H_4Me-*o*	H	3.64	2.09	6.92	7.90
4g	OCH_2Ph	H	3.93	2.32	6.70	7.83
4i[B]	SCH_2Ph	H	3.95	2.30	6.85	7.74

[A]Reported as parts per million (δ) downfield from tetramethylsilane (T.M.S.) as internal standard in deuterochtoroform ($CDCl_3$)

[B]Prepared by Dr M.M.L. Ngu

In the 2,3,6-trisubstituted imidazo[1,2-*b*]pyridazines reported here, the methylene protons in compounds (**II.4**) (Table II-2) and (**II.5**) (Table II-3) occurred in the ranges δ3.64-3.97 and δ4.77-5.02, respectively. The methylene and both methyl groups of the dimethylaminomethyl substituent in compound (**II.4**) appear to be more shielded by the 6-phenylthio and the 6-(2′-methoxyphenoxy) groups, and the chemical shifts of the methylene and dimethylamino groups in compound (**II.4f**) are at δ3.64 and 2.09, respectively.

Chemical shifts for the methylene protons in 6-(variously substituted)-3-benzamidomethyl-2-phenylimidazo[1,2-*b*]-pyridazines (Table II-3) were found to occur in the range δ4.63 [for the 6-(2′-methoxyphenoxy) compound (**II.6m**)] to 5.26 [for the 6-methylthio compound (**II.6k**)].

All protons on C-7 and C-8 appeared as an AB quartet with a coupling constant of $J_{7,8}$ 9.5 Hz. Chemical shifts for H7 occurred in the ranges δ6.66-7.09 (in **II.5**) and δ6.57-7.09 (in **II.6**); whereas that for H8 were at δ7.69-7.89 (in **II.3**), δ7.72-7.90 (in **II.4**), δ7.57-7.97 (in **II.5**) and δ7.88-8.06 (in **II.6**), respectively.

Table II-3 Some ^{1}H n.m.r. spectral data (δ)[A] for 3-acylaminomethylimidazo[1,2-b]pyridazines (II.5 and II.6).

Formula Number	R^1	R^2	Me	CH_2	H7	H8
II.5a	F	H	2.02	4.99	6.73	B
5b	Cl	H	2.03	5.02	7.09	7.92
5c	Cl	OCH_2O-3′,4′	2.04	4.97	7.07	7.88
5d	SMe	H	2.03	4.98	6.86	7.64
5e	OMe	H	2.02	4.97	6.70	7.76
5f	SPh	H	1.85	4.79	6.87	7.86
5g	SPh	Me-*p*	1.85	4.78	6.86	B
5h	OC_6H_4OMe-*o*	H	1.81	4.77	7.00	7.97
5i	OCH_2Ph	H	1.97	4.88	6.66	7.57
5j	SCH_2Ph	H	1.93	4.97	6.89	7.67
II.6a	F	H		5.20	6.91	B
6i[C]	F	Me-*p*		5.18	6.88	8.01
6j[C]	Cl	H		5.23	7.09	7.91
6c	Cl	OCH_2O-3′,4′		5.21	6.95	B
6b	Cl	Me-*p*		5.19	7.01	B
6k[C]	SMe	H		5.26	6.94	B
6d	SMe	Me-*p*		5.25	6.92	B
6e	SMe	OCH_2O-3′,4′		5.14	6.78	B
6l[C]	OMe	H		5.02	6.93	8.06
6f	OMe	Me-*p*		5.13	6.57	B
6g	SPh	H		5.00	6.82	B
6m[C]	OC_6H_4OMe-*o*	H		4.63	6.88	7.88
6n[C]	SCH_2Ph	H		5.18	6.77	B
6h	OCH_2Ph	H		5.19	6.74	B

[A]Reported as parts per million (δ) downfield from tetramethylsilane (T.M.S.) as internal standard in deuterochloroform ($CDCl_3$)

[B]Complex signal

[C]Prepared by Dr M.M.L. Ngu

II-4 In vitro binding studies

Three benzodiazepine radioligands, [^{3}H]flunitrazepam, [^{3}H]diazepam and [^{3}H]clonazepam, commonly are used for labelling benzodiazepine receptors.[217] [^{3}H]Diazepam and [^{3}H]flunitrazepam characteristically bind to the same species of binding sites in central nervous system receptors, they bind in a similar regional distribution and are used interchangeably for *in vitro* investigations in the mammalian central nervous system. In the work described in this thesis, [^{3}H]diazepam can also be used to determine benzodiazepine binding to peripheral-type benzodiazepine receptors in tissues such as kidney, heart and adrenal gland.

II-4.1 Biochemical characteristics of [^{3}H]diazepam binding

[^{3}H]Diazepam binds to benzodiazepine receptors from rat brain membrane with high affinity. The dissociation equilibrium constant for [^{3}H]diazepam at 0^o is *ca* 3.2 nM (dissociation equilibrium constant K_D = affinity constant = binding constant); the association and dissociation rate constants at 0^oC are 6.8×10^7 M^{-1} min^{-1} and 0.16 min^{-1}, respectively.[218] Specific [^{3}H]diazepam binding to brain membranes is saturable,[219,220] thus showing the presence of a limited number of binding sites.

Equilibrium between bound and unbound [^{3}H]diazepam is

reached within fifteen minutes in the standard binding assay ($0^{o}C$).[221]

Specific [^{3}H]diazepam binding shows a linear dependency on membrane protein (up to 3.0 mg protein using a crude extract) and a pH-optimum between pH 7.0 to pH 7.4. This binding is temperature-dependent, with the highest binding at $4^{o}C$.[221] Using membranes prepared from adult rat forebrain and [^{3}H]diazepam as ligand, an Hill coefficient near 1.0 is found indicating noncooperative interaction between benzodiazepine binding sites. Parenthetically it may be noted that further work indicated the presence of at least two pharmacologically, biochemically and functionally distinct molecular classes of binding sites[222,224] (see previous Chapter).

[^{3}H]Diazepam binding does not distinguish between these two sub-populations of benzodiazepine binding sites. However, [^{3}H]diazepam binding is known to be stereospecific.[30,219] The *in vitro* binding of the radiolabel has been observed to undergo competitive displacement by unlabelled drugs which interact isosterically with the benzodiazepine receptor site.[219,220,225] Many researchers (e.g. Braestrup and Squires[225]) have found that there is a very good correlation between [^{3}H]diazepam displacing potency IC_{50} values for a diverse range of compounds and their ED_{50} (or MED) values in several "*in vivo*" pharmacological tests that are very predictive of anxiolytic activity in man.

A similar correlation was obtained also by Möhler and Okada[219] in 1977. Receptor-binding techniques have identified a large number of non-benzodiazepine drugs which possess a similar spectrum of pharmacological activity as the benzodiazepines.

In order to assess the affinity of new compounds for benzodiazepine receptors a simple and efficient *in vitro* binding assay has been devised which involves the displacement of [^{3}H]diazepam from an appropriate brain membrane preparation. Submilligram quantities of material are required for the *in vitro* test whereas much larger quantities are required for whole animal tests. In this way one can determine rapidly, accurately and cheaply the binding of new compounds to benzodiazepine receptors, and thus facilitate structure-activity studies.

II-4.2 Results of <u>in vitro</u> testing

The 3-acylaminomethyl, 3-dimethylaminomethyl and 3-unsubstituted imidazo[1,2-*b*]pyridazines prepared in this work were tested for their ability to bind to rat brain plasma membrane. This was determined in a competitive [^{3}H]diazepam binding assay in which the ability of the compound under test to displace [^{3}H]diazepam was determined. The details of this biological test procedure are outlined in the Experimental section II-5.3. The

results are presented in Table II-4 as IC_{50} values (the concentration of drugs that result in 50% displacement of specific [^{3}H]diazepam binding in the standard assay) or the percentage displacement at a specified concentration, together with the data for some 3-methoxy analogues, prepared previously by other workers,[162,163,166,167] for comparison. The IC_{50} values are related to Ki_{app} (apparent inhibition constant) where $Ki_{app}=IC_{50}/(1+C/Kd)$, where C = concentration of [^{3}H]diazepam (0.7 ± 0.1 nM). Thus, IC_{50} values assess the affinity of the test compounds for benzodiazepine receptors. Unlabelled diazepam was used as a control.

II-4.3 Discussion of results

The results shown in Table II-4 for the *in vitro* binding by the imidazo[1,2-*b*]pyridazines reveal that the 3-unsubstituted imidazo[1,2-*b*]pyridazines (**II.3**) had lower binding potencies than the 3-(substituted aminomethyl)-imidazo[1,2-*b*]pyridazines (**II.4, II.5** or **II.6**), with the exception of 3-dimethylaminomethyl-6-chloro (and 6-benzyloxy)-2-phenyl(3′,4′-methylenedioxyphenyl and possibly 4′-chlorophenyl)imidazo[1,2-*b*]pyridazines (**II.4a**, **II.4b**, **II.4h** and **II.4g**). Whereas the 3-dimethylamino group had only a small beneficial effect in the 6-methylthio, 6-methoxy and 6-(2′-methoxyphenoxy) compounds (**II.4c**, **II.4d**

The assays were carried out by Mr S.J. Ireland and Dr L.P. Davies

Table II-4 Results for the displacement of [^{3}H]diazepam from rat brain membrane by some substituted imidazo-[1,2-b]pyridazines

Formula number	Imidazo[1,2-*b*]pyridazine	IC_{50} (nM)[A] (or % displ.[B])
II.3a	6-F-3-H-2-Ph	(21%)
3b	6-F-3-H-2-C_6H_4Me-*p*	383
5a	6-F-3-CH_2NHAc-2-Ph	679
6a	6-F-3-CH_2NHBz-2-Ph	68
6i	6-F-3-CH_2NHBz-2-C_6H_4Me-*p*	8[C]
7a	6-F-3-OMe-2-Ph	320[D]
3o	6-Cl-3-H-2-Ph	>3000[C]
3c	6-Cl-3-H-2-C_6H_4Me-*p*	(63%)
3d	6-Cl-3-H-2-C_6H_3(3′,4′-OCH_2O-)	1427
3p	6-Cl-3-H-2-C_6H_4Cl-*p*	(46%)[C]
4a	6-Cl-3-CH_2NMe_2-2-Ph	(0%)
4b	6-Cl-3-CH_2NMe_2-2-C_6H_3(3′,4′-OCH_2O-)	2043
4h	6-Cl-3-CH_2NMe_2-2-C_6H_4Cl-*p*	1370[C]
5b	6-Cl-3-CH_2NHAc-2-Ph	474[C]
5c	6-Cl-3-CH_2NHAc-2-C_6H_3(3′,4′-OCH_2O-)	152
6j	6-Cl-3-CH_2NHBz-2-Ph	140[C]
6b	6-Cl-3-CH_2NHBz-2-C_6H_4Me-*p*	18
6c	6-Cl-3-CH_2NHBz-2-C_6H_3(3′,4′-OCH_2O-)	25
7b	6-Cl-3-OMe-2-Ph	772[D]
7c	6-Cl-3-OMe-2-C_6H_4Me-*p*	148[D]
3e	6-SMe-3-H-2-Ph	(21%)
3f	6-SMe-3-H-2-C_6H_4Me-*p*	(35%)
3g	6-SMe-3-H-2-C_6H_3(3′,4′-OCH_2O-)	(40%)
4c	6-SMe-3-CH_2NMe_2-2-Ph	(30%)
5d	6-SMe-3-CH_2NHAc-2-Ph	55
6k	6-SMe-3-CH_2NHBz-2-Ph	19.5[C]
6d	6-SMe-3-CH_2NHBz-2-C_6H_4Me-*p*	7
6e	6-SMe-3-CH_2NHBz-2-C_6H_3(3′,4′-OCH_2O-)	2
7d	6-SMe-3-OMe-2-Ph	884[C]
3h	6-OMe-3-H-2-Ph	(18%)
3i	6-OMe-3-H-2-C_6H_4Me-*p*	1704
4d	6-OMe-3-CH_2NMe_2-2-Ph	(20%)
5e	6-OMe-3-CH_2NHAc-2-Ph	523
6l	6-OMe-3-CH_2NHBz-2-Ph	79[C]
6f	6-OMe-3-CH_2NHBz-2-C_6H_4Me-*p*	23
7e	6-OMe-3-OMe-2-Ph	829[C]

Table II-4 Continued

Formula number	Imidazo[1,2-*b*]pyridazine	IC_{50} (nM)[A] (or % displ.[B])
3j	6-SPh-3-H-2-Ph	(43%)
3k	6-SPh-3-H-2-C_6H_4Me-*p*	(22%)
4e	6-SPh-3-CH_2NMe_2-2-Ph	(44%)
5f	6-SPh-3-CH_2NHAc-2-Ph	24
5g	6-SPh-3-CH_2NHAc-2-C_6H_4Me-*p*	14
6g	6-SPh-3-CH_2NHBz-2-Ph	9
7f	6-SPh-3-OMe-2-Ph	117[E]
3l	6-OC_6H_4OMe-*o*-3-H-2-Ph	(45%)
4f	6-OC_6H_4OMe-*o*-3-CH_2NMe_2-2-Ph	333
5h	6-OC_6H_4OMe-*o*-3-CH_2NHAc-2-Ph	(60%)
6m	6-OC_6H_4OMe-*o*-3-CH_2NHBz-Ph	(not significant)[C]
7g	6-OC_6H_4OMe-*o*-3-OMe-2-Ph	70[F]
3g	6-$SCH_2C_6H_4$OMe-*m*-3-H-2-Ph	330[C]
3m	6-$SCH_2C_6H_4$OMe-*m*-3-H-2-C_6H_4Me-*p*	(26%)
4i	6-SCH_2Ph-3-CH_2NMe_2-2-Ph	58[C]
5j	6-SCH_2Ph-3-CH_2NHAc-2-Ph	55[C]
6n	6-SCH_2Ph-3-CH_2NHBz-2-Ph	445[C]
7k	6-SCH_2Ph-3-OMe-2-Ph	25[F]
3n	6-OCH_2Ph-3-H-2-Ph	(53%)
4g	6-OCH_2Ph-3-CH_2NMe_2-2-Ph	(31%)
5i	6-OCH_2Ph-3-CH_2NHAc-2-Ph	208
6h	6-OCH_2Ph-3-CH_2NHBz-2-2-Ph	(65%)
7i	6-OCH_2Ph-3-OMe-2-Ph	20[G]

[A] IC_{50} values (nM) are the concentrations required in the presence of 100 μM δ-aminobutyric acid to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparations
[B] At 1000 nM
[C] Prepared by Dr M.M.L. Ngu
[D] Ref. 162
[E] Ref. 167
[F] Ref. 163
[G] Ref. 166

and **II.4f**) and a negligible effect in the 6-phenylthio compound (**II.4e**), it had a significant enhancing effect in the 6-benzylthio compound (**II.4i**, IC_{50} 58 nM). All the 3-dimethylaminomethyl compounds (**II.4**) bound less strongly than their corresponding 3-methoxy analogues (**II.7**). The most active compound in this series was the 6-benzylthio-3-dimethylaminomethyl-2-phenylimidazo[1,2-*b*]pyridazine (**II.4i**, IC_{50} 58 nM) which was signicantly more active than 6-benzyloxy-3-dimethylaminomethyl-2-phenylimidazo-[1,2-*b*]pyridazine (**II.4g**, 31% displacement at 1000 nM). Likewise the 6-methylthio analogue (**II.4c**, 30% displacement at 1000 nM) was more active than the 6-methoxy compound (**II.4d**, 20% displacement at 1000 nM).

The 3-acetamidomethyl group in the 3-acetamidomethyl-imidazo[1,2-*b*]pyridazine compounds (**II.5**) had a significant beneficial effect in increasing binding compared with the 3-unsubstituted imidazo[1,2-*b*]pyridazine analogues (**II.3**). For example 3-acetamidomethyl-6-phenylthio (and 6-methylthio)-2-phenylimidazo[1,2-*b*]pyridazines (**II.5f** and **II.5d**) had IC_{50} values of 24 and 55 nM, whereas the corresponding 3-unsubstituted compounds (**II.3j** and **II.3e**) gave 43% and 21% displacement at 1000 nM, respectively. The 3-acetamidomethyl group was probably least effective in 3-acetamidomethyl-6-(2′-methoxyphenoxy)-2-phenylimidazo-[1,2-*b*]pyridazine (**II.5h**, 60% displacement at 1000 nM) compared to the 3-unsubstituted analogue (**II.3l**, 45% displacement at 1000 nM). The most active compound in this series was the 3-acetamidomethyl-6-phenylthio-2-(4′-tolyl)-

imidazo[1,2-*b*]pyridazine (**II.5g**) with IC_{50} 14 nM. The 3-acetamidomethyl-6-methylthio (and 6-benzylthio)-2-phenyl-imidazo[1,2-*b*]pyridazines (**II.5d**, IC_{50} 55 and **II.5j**, IC_{50} 55 nM) showed *ca* ten-fold and four-fold increases in binding activity as compared with their corresponding 6-methoxy and 6-benzyloxy analogues (**II.5e** and **II.5i**, IC_{50} values 523 and 208 nM), respectively.

The 3-benzamidomethyl substituent in the 6-fluoro (**II.6a**, IC_{50} 68 nM), 6-chloro (**II.6j**, IC_{50} 140 nM and **II.6c**, IC_{50} 25 nM), 6-methylthio (**II.6k**, IC_{50} 19.5 nM), 6-methoxy (**II.6l**, IC_{50} 79 nM) and 6-phenylthio (**II.6g**, IC_{50} 9 nM) compounds had a greater beneficial effect in increasing binding than the 3-acetamidomethyl group in compounds (**II.5a-f**), respectively. This effect was evidently greatest in 3-benzamidomethyl-6-fluoro-2-phenyl-imidazo[1,2-*b*]pyridazine (**II.6a**, IC_{50} 68 nM) which bound *ca* ten-fold more strongly than 3-acetamidomethyl-6-fluoro-2-phenylimidazo[1,2-*b*]pyridazine (**II.5a**, IC_{50} 679 nM). It was detrimental in the 6-(2′-methoxyphenoxy) compound (**II.6m**, displacement not significant at 1000 nM), 6-benzylthio compound (**II.6n**, IC_{50} 445 nM) and 6-benzyloxy compound (**II.6h**, 65% displacement at 1000 nM) as compared with their corresponding 3-acetamidomethyl analogues (**II.5h**, 60% displacement at 1000 nM, **II.5j**, IC_{50} 55 nM and **II.5i**, IC_{50} 208 nM), respectively. It appears that the presence of aromatic groups in all three substituents at 2-, 3- and 6-positions may be detrimental to binding; however, 3-benzamidomethyl-2-phenyl-6-phenylthioimidazo-

[1,2-*b*]pyridazine (**II.6g**, IC_{50} 9 nM) which contains a phenyl ring in all three substituents at the 2-, 3- and 6-positions, bound *ca* 3-fold more strongly than its 3-acetamidomethyl analogue (**II.5f**, IC_{50} 24 nM). The most active member of this series was the 3-benzamidomethyl-2-(3′,4′-methylenedioxyphenyl)-6-methylthioimidazo[1,2-*b*]-pyridazine (**II.6e**, IC_{50} 2 nM); and eight of these compounds (**II.6**) reported in this chapter had IC_{50} values of 25 nM or less. The 3-benzamidomethyl compounds (**II.6**), which contained two aromatic substituents as in the 6-fluoro compound (**II.6a**, IC_{50} 68 nM), the 6-chloro compounds (**II.6j**, IC_{50} 140 nM and **II.6b**, IC_{50} 18 nM), the 6-methylthio compound (**II.6k**, IC_{50} 19.5 nM) and the 6-methoxy compound (**II.6l**, IC_{50} 79 nM), were more active than the corresponding 3-methoxy compounds [(**II.7a**), IC_{50} 320; (**II.7b**), IC_{50} 772; (**II.7c**), IC_{50} 148; (**II.7d**), IC_{50} 884; and (**II.7e**), IC_{50} 829 nM, respectively] which contained only one aromatic ring at the 2-position. Consistent with the data presented above for 3-benzamidomethyl and 3-acetamidomethyl compounds, the 3-benzamidomethyl compounds which contained three aromatic groups, such as 3-benzamidomethyl-6-benzyloxy-2-phenylimidazo[1,2-*b*]-pyridazine (**II.6h**, 65% displacement at 1000 nM) was significantly less active than the 6-benzyloxy-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**II.7i**, IC_{50} 20 nM) which contained two aromatic rings. The 6-phenylthio compound (**II.6g**, IC_{50} 9 nM) proved an exception, in that it was significantly more active than its 3-methoxy analogue

(**II.7f**, IC_{50} 117 nM).[167] The results of the *in vitro* competitive binding studies reveal that the 6-substituted imidazo[1,2-*b*]pyridazines (**II.4**, **II.5** and **II.6**) which contained the 6-methylthio group were more active than their analogues which contained a 6-methoxy group. The 6-fluoro compounds were also more active than their 6-chloro analogues. The presence of an electron-donating group such as 4′-methyl or 3′,4′-methylenedioxy (or an electron-withdrawing group such as chloro) in the 2-phenyl substituent in the series of 6-fluoro (chloro, methylthio, methoxy and phenylthio)-2-phenylimidazo[1,2-*b*]pyridazines increased affinities for the receptor compared to the 2-phenyl analogues but 6-(3′-methoxybenzylthio)-2-(4′-tolyl)-imidazo[1,2-*b*]pyridazine (**II.3m**, 26% displacement at 1000 nM) was significantly less active than the 6-(3′-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (**II.3q**, IC_{50} 330 nM).

As pointed out previously, on the basis of structure-activity studies on a large number of benzodiazepines and non-benzodiazepines, Fryer[32] has suggested a model which produces the necessity, among other factors, of an aromatic or heteroaromatic ring which is spatially related to a proton-accepting group to assure effective binding of these molecules to the receptor. The aromatic ring is probably involved in π-π stacking interactions with the aromatic rings of amino acid side chains within the receptor whereas the proton-accepting group may be involved in hydrogen bonding with amino acids containing SH, NH_2, OH or imidazo NH groups within the receptors.

In the present work the "A" ring proposed in the Fryer model may be represented by the 2-aryl substituent and the π region may be located at N-1 or N-5 (or other areas). All the 6-fluoroimidazo[1,2-*b*]pyridazines listed in Table II-4 bound more strongly than the 6-chloro analogues; and 6-alkylthioimidazo[1,2-*b*]pyridazines bound more strongly than their 6-alkoxy analogues. These differences in binding ability are not dependent only on electronic effects. Whereas the Hammett function (σ_{para}) for a *p*-fluoro substituent is +0.06[226] and that for a *p*-chloro substituent is +0.23, the values for a *p*-methylthio group is 0.00 and that for a *p*-methoxy group is -0.27.[226] Obviously other factors such as bulk, shape, and lipophilicity of substituents are important in determining overall binding ability.

II-5 Experimental

II-5.1 General topics

i) Melting points (m.p.s) were taken in Pyrex capillaries with an electrothermal melting point apparatus and were uncorrected.

ii) ^{1}H n.m.r. spectra were generally recorded at 90 MHz and 30^{o} with a Jeol FX 90Q fourier-transform spectrometer, with tetramethylsilane (in $CDCl_3$, unless otherwise stated) as an internal standard (δ 0.0 ppm). Data were presented in the following order. Chemical shift (ppm) relative to tetramethylsilane; multiplicity; coupling constant (J) in Hz; assignment (if appropriate). The following abbreviations were adopted: s (singlet); d (doublet); t (triplet); dd (doublet of doublets). Exchangeable protons were identified by their disappearance upon addition of deuterium oxide.

iii) Microanalyses were conducted by the Australian National University Analytical Services Unit, Canberra. Solids for analysis were dried at 70-100^{o}C/0.1 mmHg for 12-15 h unless otherwise specified. Some compounds, analysed with water (or solvent) which could not be removed entirely without incipient decomposition.

iv) Analytical thin layer chromatography (t.l.c.) was performed on glass or aluminium sheet precoated with Merck kieselgel 60 F254 or Merck aluminium oxide 60 F254 neutral

(type E) of 0.25 mm thickness. Preparative thin layer chromatography was performed on glass plate precoated with Merck aluminium oxide 60 F254 (type E) of 1.5 mm thickness. Columns for chromatography were packed using Merck aluminium oxide 90 active neutral (0.063-0.2 mm, 70-230 mesh ASTM).

v) Ethereal diazomethane was prepared from nitrosomethylurea.[227]

vi) Reaction temperatures refer to external or bath temperatures, unless otherwise indicated.

vii) Where full experimental details are not recorded, percentage yields are given in brackets from the 6-substituted pyridazin-3-amine and the 6-substituted imidazo[1,2-*b*]pyridazine, as appropriate.

viii) The light petroleum used as a solvent had b.p. 60-80^{o} unless specified otherwise.

II-5.2 Synthetic work

The starting materials which were not available from commercial sources but which were required for the syntheses in this work were prepared according to literature procedures as indicated below.

6-Methylthio-2-phenylimidazo[1,2-b]pyridazine (II.3e)

A mixture of 6-methylthiopyridazin-3-amine[118] (1.15 g) and α-bromoacetophenone (1.63 g) in ethanol (16 ml) was refluxed for 2 h. Sodium hydrogen carbonate (0.688 g) was then added and the mixture was refluxed for 4 h. After chilling, the solid was filtered off and washed with water. The solid (1.532 g) was recrystallised from ethanol to give the *title compound*, m.p. 149-151°. (Found, for a sample dried at 60°/0.1 mmHg for 15 h: C, 64.9; H, 4.6; N, 17.4. $C_{13}H_{11}N_3S$ required C, 64.7; H 4.6; N, 17.4%). ^{1}H n.m.r. δ 2.62, s, SMe; 6.87, d, J 9Hz, H7; 7.77, d, J 9Hz, H8; 7.37-7.48 and 7.89-8.00, complex, Ph; 8.17, s, H3.

6-Chloro-2-(3',4'-methylenedioxyphenyl)imidazo-[1,2-b]pyridazine (II.3d)

A mixture of 6-chloropyridazin-3-amine (0.491 g) and α-bromo-3,4-methylenedioxyacetophenone[228] (0.937 g) in ethanol (10 ml) was refluxed for 2 h, sodium hydrogen carbonate (0.44 g) then added and the reflux continued for 2 h. After cooling, the yellow solid (0.88 g) was filtered off and the residue from the filtrate after t.l.c. (alumina; benzene) gave a further quantity (0.037 g) of product. It was recrystallised from ethanol to give the *title compound*, m.p. 232-234°. (Found: C, 57.2; H, 2.8; N, 15.1. $C_{13}H_8ClN_3O_2$ requires C, 57.0, H, 2.9; N, 15.3%). ^{1}H n.m.r.: δ 6.02, s, OCH_2O; 6.89, d, J 8.0 Hz, H5'; 7.04, d, J 9.5 Hz, H7; 7.43, bs, H2'; 7.47, dd, $J_{5',6'}$ 6 Hz, $J_{2',6'}$ 1.5 Hz, H6'; 7.87, d, J 9.5 Hz, H8; 8.10, bs, H3.

In a similar manner from 6-fluoropyridazin-3-amine,[120] 6-chloropyridazin-3-amine,[229] 6-methylthiopyridazin-3-amine,[118] 6-methoxypyridazin-3-amine,[230,231] 6-phenylthiopyridazin-3-amine,[161] 6-(2′-methoxyphenoxy)pyridazin-3-amine,[163] 6-benzylthiopyridazin-3-amine,[164] 6-(3′-methoxybenzylthio)pyridazin-3-amine[163] and 6-benzyloxypyridazin-3-amine[166] with α-bromoacetophenone, α-bromo-4′-methylacetophenone,[232] α-bromo-3′,4′-methylenedioxyacetophenone,[228] or α-bromo-4′-chloroacetophenone[232] were prepared the following compounds.

6-Fluoro-2-phenylimidazo[1,2-b]pyridazine (II.3a) (90%), m.p. 192-194° (from ethanol) (Found: c, 66.1; H, 4.2; N, 19.8%. $C_{13}H_8FN_3$. 0.25 H_20 requires C, 66.2; H, 3.9; N, 19.4%). ^{1}H n.m.r.: δ 6.87, d, J 9.5 Hz, H7; 7.30-7.60 and 7.80-8.25, complex, Ph and H3,8.

6-Fluoro-2-(4′-tolyl)imidazo[1,2-b]pyridazine (II.3b) (68%), m.p. 211-213° (from toluene) (Found: C, 68.9; H, 4.3; N, 18.4. $C_{13}H_{10}FN_3$ requires C, 68.7; H, 4.4; N, 18.5%). ^{1}H n.m.r. (CD_3SOCD_3): δ 2.33, s, Me; 7.23, d, $J_{7,8}$ 9 Hz, H7; 7.25, d, 7.90, d, $J_{2',3'}$ 8 Hz, H2′,3′,5′,6′; 8.29, dd, $J_{7,8}$ 9 Hz, J_{H8F} 10 Hz, H8; 8.74, s, H3. In $CDCl_3$: δ 2.40, s, Me; 6.85, d, J 9 Hz, H7; 7.27, d, 7.84, d, $J_{2',3'}$ 8 Hz, H2′,3′,5′,6′; 7.89-8.03, complex, H8; 8.11, s, H3.

6-Chloro-2-phenylimidazo[1,2-b]pyridazine (II,.3o) was prepared according to literature procedures.[120] It had m.p. 200-201° (lit.[103] 200°) ^{1}H n.m.r.: δ 7.02, d, J 9.5 Hz, H7; 7.78, d, J 9.5 Hz, H8; 7.26-7.57 and 7.84-8.01, complex, Ph; 8.20, s, H3.

6-Chloro-2-(4'-tolyl)imidazo[1,2-b]pyridazine (II.3c) (81%), m.p. 218-220° (from ethanol) (Found: C, 64.2; H, 4.1; N, 17.4 $C_{13}H_{10}ClN_3$ requires: C, 64.1; H, 4.1; N, 17.2%). ^{1}H n.m.r.: δ 2.41, s, Me; 7.03, d, J 9.5 Hz, H7; 7.28, d, 7.86, d, J 8 Hz, H2',3',5',6'; 7.89, d, J 9.5 Hz, H8; 8.18, s, H3.

6-Methylthio-2-(4'-tolyl)imidazo[1,2-b]pyridazine (II.3f) (61%), m.p. 125-127° (from ethanol) (Found, for a sample dried at 60°/0.1 mmHg for 15 h: C, 66.1; H, 5.2; N, 16.4. $C_{14}H_{13}N_3S$ requires C, 65.9; H, 5.1, N, 16.5%). ^{1}H n.m.r.: δ 2.39, s, Me; 2.61, s, MeS; 6.74, d, J 9.5 Hz, H7; 7.25, d, 7.71, d, J 8 Hz, H2',3',5',6'; 7.83, d, J 9.5 Hz, H8; 8.12, s, H3.

2-(3',4'-Methylenedioxyphenyl)-6-methylthioimidazo-[1,2-b]pyridazine (II.3g) (43%), m.p. 162-163° (from ethanol) (Found, for a sample dried at 95°/710 mmHg for 20 h: C, 59.0; H, 4.0; N, 14.7 $C_{14}H_{11}N_3O_2S$ requires C, 58.9, H, 3.9; N, 14.7%). ^{1}H n.m.r.: δ 2.61, s, MeS; 6.00, s, OCH_2O; 6.85, d, J 9.5 Hz, H7; 6.88, d, J 8 Hz, H5'; 7.41,

s, H2′; 7.46, dd, $J_{5',6'}$ 8 Hz, $J_{2',6'}$ 1.5 Hz, H6′; 7.69, d, J 9.5 Hz, H8; 8.05, bs, H3.

6-Methoxy-2-phenylimidazo[1,2-b]pyridazine (II.3h) (51%), m.p. 135-137° (from ethanol). (Found, for a sample dried at 60°/0.1 mmHg for 15 h: C, 69.7; H, 5.1; N, 18.9. $C_{13}H_{11}N_3O$ requires C, 69.3; H, 4.9; N, 18.7%). ^{1}H n.m.r.: δ 4.00, s, MeO; 6.70, d, J 9.5 Hz, H7; 7.38-7.46 and 7.87-7.99, complex, Ph; 7.81, d, J 9.5 Hz, H8; 8.06, s, H3.

6-Methoxy-2-(4′-tolyl)imidazo[1,2-b]pyridazine (II.3i) (66%), m.p. 139-140° (from light petroleum), (Found: C, 70.2; H, 5.6, N, 17.6. $C_{14}H_{13}N_3O$ requires C, 70.3; H, 5.5; N, 17.6%). ^{1}H n.m.r.: δ 2.39, s, Me; 4.00, s, MeO; 6.67, d, J 9.5 Hz, H7; 7.25, d, 7.81, d, J 8 Hz, H2′,3′,5′,6′; 7.80, d, J 9.5 Hz, H8; 8.01, s, H3.

2-Phenyl-6-phenylthioimidazo[1,2-b]pyridazine (II.3j) (91%), m.p. 127-129° (from ethanol) (Found, for a sample dried at 100°/710 mmHg for 20 h: C, 71.3; H, 4.3; N, 13.9. $C_{18}H_{13}N_3S$ requires C, 71.3; H, 4.3; N, 13.9%). ^{1}H n.m.r.: δ 6.77, d, J 9.5 Hz, H7; 7.38-7.99, complex, 2xPh and H8; 7.81, s, H3.

6-Phenylthio-2-(4′-tolyl)imidazo[1,2-b]pyridazine (II.3k) (85%), m.p. 162-164° (from ethanol) (Found, for a sample dried at 100°/710 mm Hg for 15 h: C, 71.7; H, 4.9; N, 13.4. $C_{19}H_{15}N_3S$ requires C, 71.9; H, 4.8; N, 13.2%). ^{1}H n.m.r.: δ

6.76, d, J 9.5 Hz, H7; 7.21-7.88, complex, 2xPh and H8; 8.10, s, H3.

6-(2′-Methoxyphenoxy)-2-phenylimidazo[1,2-b]pyridazine (II.3l) (63%), m.p. 132-134° (from toluene) (Found: C, 71.6; H, 4.5; N, 13.2. $C_{19}H_{15}N_3O_2$ requires C, 71.9; H, 4.8; N, 13.2%). ^{1}H n.m.r.: δ 3.73, s, MeO; 6.86, d, $J_{7,8}$ 9 Hz, H7; 6.88-7.91, complex, H3′,4′,5′,6′ and Ph; 7.86, d, $J_{7,8}$ 9 Hz, H8; 7.90, s, H3.

6-(3′-Methoxybenzylthio)-2-(4"-tolyl)imidazo[1,2-b]-pyridazine (II.3m) (64%), m.p. 104-105° (from light petroleum) (Found: C, 69.8; H, 5.2; N, 11.5. $C_{21}H_{19}N_3OS$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r.: δ 2.37, s, Me; 3.77, s, MeO; 4.38, s, CH_2; 6.77, d, J 9.5 Hz, H7; 6.72-7.87, complex, H2′,4′,5′,6′,2",3",5",6" and H8; 8.11, s, H3.

6-Benzyloxy-2-phenylimidazo[1,2-b]pyridazine (II.3a) (75%), m.p. 148-149° (from ethanol) (Found, for a sample dried at 100°/710 mm Hg for 12 h: C, 75.9; H, 5.0; N, 14.0. $C_{19}H_{15}N_3O$ requires C, 75.7; H, 5.0; N, 13.9%). ^{1}H n.m.r.: δ 5.38, s, CH_2; 6.75, d, $J_{9.5}$ Hz, H7; 7.37-8.00, complex, 2xPh and H8; 8.07, s, H3.

6-Chloro-3-dimethylaminomethyl-2-phenylimidazo-[1,2-b]pyridazine (II.4a)

6-Chloro-2-phenylimidazo[1,2-*b*]pyridazine (0.117 g) was added to a solution of bis(dimethylamino)methane[215]

(0.12 ml) and phosphoric acid (0.2 ml) in glacial acetic acid (2.0 ml) and the mixture stirred at 120° for 14 h. The acetic acid was evaporated under reduced pressure, the residue diluted with water, the mixture adjusted to pH 10 with aqueous ammonia and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4) and solvent evaporated and the white solid (0.109 g) was subjected to t.l.c. (alumina; chloroform/light petroleum, 2:1) and recrystallised from ethanol to give the *title compound* , m.p. 142-143° (Found: C, 62.5; H, 5.3; N, 19.3. $C_{15}H_{15}ClN_4$ requires C, 62.8; H, 5.3; N, 19.5%). ^1H n.m.r.: δ 2.32, s, 2xMe; 3.97, s, CH_2; 7.05, d, J 9.5 Hz, H7; 7.43-7.50 and 8.05-8.15, complex, Ph; 7.89, d, J 9.5 Hz, H8.

3-Dimethylaminomethyl-6-(2'-methoxyphenoxy)-2-phenyl-imidazo[1,2-b]pyridazine (II.4f)

6-(2'-Methoxyphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (0.15 g), bis(dimethylamino)methane[215] (0.2 ml), phosphoric acid (0.2 ml) and acetic acid (2.0 ml) as above gave the *title compound* (0.124 g), m.p. 140-142° [after t.l.c. (alumina; chloroform)] (Found, for a sample dried at 100°/710 mm for 5 h: C, 70.9; H, 5.6; N, 15.0. $C_{22}H_{22}N_4O_2$ requires C, 70.6; H, 5.9; N, 15.0%). ^1H n.m.r.: δ 2.09, s, Me_2N; 3.64, s, CH_2; 3.73, s, MeO; 6.92, d, J 9.5 Hz, H7; 7.00-7.46 and 7.96-8.08, complex, H3',4',5',6' and Ph; 7.90, d, J 9.5 Hz, H8.

In a similar manner the following compounds were prepared.

6-Chloro-3-dimethylaminomethyl-2-(3′,4′-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (II.4b) (28%), m.p. 154-155° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from a mixture of ethanol and benzene, and benzene and light petroleum. (Found, for a sample dried at 50°/0.1 mmHg for 15 h: C, 58.0; H, 4.5; N, 16.7. $C_{16}H_{15}ClN_4O_2$ requires C, 58.1; H, 4.6; N, 16.9%). ^{1}H n.m.r.: δ 2.32, s, Me_2N; 3.94, s, CH_2N; 6.02, s, OCH_2O; 6.92, d, J 8 Hz, H5′; 7.03, d, J 9.5 Hz, H7; 7.63, dd, $J_{5',6'}$ 8 Hz, $J_{2',6'}$ 1.5 Hz, H6′; 7.69, bs, H2; 7.87, d, J 9.5 Hz, H8.

3-Dimethylaminomethyl-6-methylthio-2-phenylimidazo[1,2-b]pyridazine (II.4c) (66%) as light yellow crystals, m.p. 131-133° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from light petroleum. (Found, for a sample dried at 60°/0.1 mmHg for 15 h: C, 64.6; H, 6.2; N, 18.6. $C_{16}H_{18}N_4S$ requires C, 64.4; H, 6.1; N, 18.8%). ^{1}H n.m.r.: δ 2.32, s, Me_2N; 2.64, s, MeS; 3.95, s, CH_2N; 6.85, d, J 9.5 Hz, H7; 7.30-7.60 and 8.02-8.14, complex, Ph; 7.72, d, J 9.5 Hz, H8.

3-Dimethylaminomethyl-6-methoxy-2-phenylimidazo-[1,2-b]pyridazine (II.4d) (58%), as yellow crystals m.p. 120-122° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from light petroleum. (Found: C, 68.1; H, 6.7; N, 20.0. $C_{16}H_{18}N_4O$ requires C, 68.1; H, 6.4; N, 19.85). ^{1}H n.m.r.: δ 2.32, s, Me_2N; 3.92,

s, CH_2N; 4.03, s, MeO; 6.67, d, J 9.5 Hz, H7; 7.30-7.50 and 8.0-8.12, complex, Ph; 7.79, d, J 9.5 Hz, H8.

3-Dimethylaminomethyl-2-phenyl-6-phenylthioimidazo-[1,2-b]pyridazine (II.4e) (38%), as yellow crystals, m.p. 126-127° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from a mixture of ethanol and light petroleum (Found, for a sample dried at 100°/710 mmHg for 20 h: C, 67.7; H, 5.5; N, 14.9. $C_{21}H_{20}N_4S$. 0.6 H_2O requires C, 67.9; H, 5.8; N, 15.1%). ^1H n.m.r.: δ 2.14, s, Me_2N; 3.77, s, CH_2N; 6.82, d, J 9.5 Hz, H7; 7.38-7.65 and 8.00-8.12, complex, 2xPh, 7.75, d, J 9.5 Hz, H8.

6-Benzyloxy-3-dimethylaminomethyl-2-phenylimidazo-[1,2-b]pyridazine (II.4g) (63%), as white crystals, m.p. 115-117° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from light petroleum. (Found, for a sample dried at 100°/710 mmHg for 3 h: C, 74.0; H, 5.9; N, 15.9. $C_{22}H_{22}N_4O$ requires C, 73.7; H, 6.2; N, 15.6%). ^1H n.m.r.: δ 2.32, s, Me_2N; 3.93, s, CH_2N; 5.43, s, CH_2O; 6.70, d, J 9.5 Hz, H7; 7.37-7.50 and 7.98-8.11, complex, 2xPh; 7.83, d, J 9.5 Hz, H8.

3-Acetamidomethyl-6-methylthio-2-phenylimidazo[1,2-b]-pridazine (II.5d)

6-Methylthio-2-phenylimidazo[1,2,-*b*]pyridazine (0.18 g) was added to a solution of *N*-(hydroxymethyl)acetamide[24], glacial acetic acid (3.0 ml) and concentrated sulphuric

acid (0.2 ml) and the mixture refluxed with stirring in an oil bath at 120° for 14 h. The acetic acid was evaporated under reduced pressure and the residue diluted with water and adjusted with ammonium hydroxide to pH 10. This mixture was extracted with chloroform, the solvent evaporated, and the product subjected to t.l.c. (alumina; chloroform, light petroleum, 3:1) to give the *title compound* (0.11 g), m.p. 247-249° (from ethanol) (Found, for a sample dried at 60°/0.1 mmHg for 15 h: C, 61.8; H, 5.3; N, 17.9. $C_{16}H_{16}N_4OS$ requires C, 61.5; H, 5.2; N, 17.9%). ^{1}H n.m.r: δ 2.03, s, MeCO; 2.61, s, MeS; 4.98, d, J 5 Hz, CH_2; 6.43, bs, NH; 6.86, d, J 9.5 Hz, H7; 7.35-7.60, complex, 7.69-7.84, complex, Ph; 7.64, d, J 9.5 Hz, H8.

In a similar manner the following compounds were prepared.

3-Acetamidomethyl-6-fluoro-2-phenylimidazo[1,2-b]-pyridazine (II.5a) (65%), m.p. 227-229° after t.l.c. (alumina; chloroform) and recrystallised from toluene and also ethanol (Found: C, 62.6; H, 4.6; N, 19.4. $C_{15}H_{12}FN_4O.0.3\ H_2O$ requires C, 62.4; H, 4.4; N, 19.4%). ^{1}H n.m.r: δ 2.02, s, MeCO; 4.99, d, J 5.5 Hz, CH_2N; 6.38, bs, NH; 6.93, d, J 9.5 Hz, H7; 7.42-7.54 and 7.87-8.15 complex, Ph and H8.

3-Acetamidomethyl-6-chloro-2-phenylimidazo[1,2-b]pyridazine (II.5b) (90%), m.p. 257-259° (from toluene) (Found: C, 60.2; H, 4.4; N, 18.5. $C_{15}H_{13}ClN_4O$ requires C, 59.9; H,

4.4; N, 18.6%). ^{1}H n.m.r: δ 2.03, s, Me; 5.02, d, J 5.5 Hz, CH_2; 7.09, d, $J_{7,8}$ 9Hz, H7; 7.39-8.02, complex, Ph; 7.92, d, $J_{7,8}$ 9 Hz, H8. Mass spectrum m/z 300(M^+) (30%), 257 (100%), 223 (44%), 103 (6%).

3-Acetamidomethyl-6-chloro-2-(3',4'-methylenedioxyphenyl)-imidazo[1,2-b]pyridazine (II.5c) (80%), as yellow crystals, m.p. 237-240° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from methanol (Found: C, 55.5; H, 3.6; N, 16.0. $C_{16}H_{13}ClN_4O_3$ requires C, 55.7; H, 3.8; N, 16.3%). ^{1}H n.m.r: δ 2.04, s, MeCO; 4.97, d, J 5.5 Hz, CH_2N; 6.01, s, OCH_2O; 6.92, d, J 8 Hz, H5'; 7.07, d, J 9.5 Hz, H7; 7.40-7.52, complex, H2',6'; 7.88, d, J 9.5 Hz, H8.

3-Acetamidomethyl-6-methoxy-2-phenylmidazo[1,2-b]-pyridazine (II.5e) (52%), as white crystals, m.p. 229-230° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from ethanol (Found: C, 64.5; H, 5.6; N, 18.6 $C_{16}H_{16}N_4O_2$ requires C, 64.8; H, 5.4; N, 18.9%). ^{1}H n.m.r: δ 2.02, s, MeCO; 4.02, s, MeO; 4.97, d, J 5 Hz, CH_2N; 6.25, bs., NH.; 6.70, d, J 9.5 Hz, H7; 7.35-7.50, complex, 7.70-7.86, complex, Ph; 7.76, d, J 9.5 Hz, H8.

3-Acetamidomethyl-2-phenyl-6-phenylthioimidazo-[1,2-b]pyridazine (II.5f) (42%), as white crystals, m.p. 213-215° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found: C, 65.8; H, 4.9;

N, 14.7. $C_{21}H_{18}N_4OS$. 0.5 H_2O requires C, 65.8; H, 5.0; N, 14.6%). ^1H n.m.r: δ 1.85, s, MeCO; 4.79, d, J 5.5 Hz, CH_2N; 6.03, bs, NH; 6.87, d, J 9.5 Hz, H7; 7.40-8.00, complex, 2xPh; 7.86, d, J 9.5 Hz, H8.

3-Acetamidomethyl-6-phenylthio-2-(4'-tolyl)imidazo-[1,2-b]pyridazine (II.5g) (80%), as white crystals, m.p. 191-193° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from toluene (Found: C, 67.8; H, 5.2; N, 14.3. $C_{22}H_{20}N_4OS$ requires C, 68.0; H, 5.2; N, 14.4%). ^1H n.m.r.: δ 1.85, s, MeCO; 2.38, s, Me; 4.78, d, J 5.5 Hz, CH_2N; 6.05, bs, NH, 6.86, d, J 9.5 Hz, H 7; 7.21-7.86, complex, 2 x Ph and H 8.

3-Acetamidomethyl-6-(2'-methoxyphenoxy)-2-phenylimidazo-[1,2-b]pyridazine (II.5h)

This compound was prepared similarly but with less sulphuric acid (0.05 ml) and the mixture was refluxed for 5 h only. The product was purified by t.l.c. (alumina; chloroform) and recrystallisation from toluene to give the *title compound* (50%), as a yellow solid, m.p. 174-175° (Found, for a sample dried at 100°/710 mmHg for 20 h: C, 68.1; H, 5.2; N, 14.2. $C_{22}H_{20}N_4O_3$ requires C, 68.0; H, 5.2; N, 14.4%). ^1H n.m.r: δ 1.81, s, MeCO; 3.82, s, MeO; 4.77, d, J 5.5 Hz, CH_2N; 6.09, bs, NH; 7.00, d, J 9.5 Hz, H7; 7.12-7.50, complex, 7.80-8.00, complex, H 3',4',5',6' and Ph; 7.97, d, J 9.5 Hz, H8.

3-Acetamidomethyl-6-benzyloxy-2-phenylimidazo-[1,2-b]pyridazine (II.5i) (22%), m.p. 195-196° after t.l.c. (alumina; chloroform) and recrystallisation from toluene (Found, for a sample dried at 100°/710 mmHg for 7h: C, 70.7; H, 5.3; N, 15.1. $C_{22}H_{20}N_4O_2$ requires C, 70.9; H, 5.4; N, 15.0%). ^{1}H n.m.r: δ 1.97, s, MeCO; 4.88, d, J 5.5 Hz, CH_2NH; 5.38, CH_2O; 6.66, d, J 9.5 Hz, H7; 7.27-7.50, complex, 7.70-7.80, complex, 2xPh; 7.57, d, J 9.5 Hz, H8.

3-Benzamidomethyl-6-fluoro-2-phenylimidazo[1,2-b]pyridazine (II.6a) A solution of *N*-(hydroxymethyl)benzamide[216] (0.114 g) in glacial acetic acid (2 ml) with concentrated sulphuric acid (0.2 ml) was heated at 50° for 15 min. 6-Fluoro-2-phenylimidazo[1,2-*b*]pyridazine (0.12 g) was added and the reaction mixture was refluxed for 20 h; evaporated under reduced pressure and the oily residue diluted with water and adjusted with aqueous ammonia to pH 10. The solution was extracted with chloroform, the extract was washed with water, dried (Na_2SO_4) and the solvent evaporated. The product was recrystallised from toluene and gave the *title compound* (0.070 g), m.p. 185-187° (Found: C, 69.2; H, 4.15; N, 15.9. $C_{20}H_{15}FN_4O$ requires C, 69.4; H, 4.4; N, 16.2%). ^{1}H n.m.r.: δ 5.20, d, J 5.5 Hz, CH_2NH; 6.91, d, J 9.5 Hz, H7, 7.40-8.19, complex, 2xPh and H8.

3-Benzamidomethyl-6-chloro-2-(4'-tolyl)imidazo-[1,2-b]pyridazine (II.6b) (84%), m.p. 251-252° after t.l.c. (alumina; chloroform) and recrystallisaton from toluene

(Found: C, 66.7; H, 4.8; N, 14.9. $C_{21}H_{17}ClN_4O$ requires C, 66.9; H, 4.5; N, 14.9%). ^1H n.m.r.: δ 2.39, s, Me; 5.19, d, J 5.5 Hz, CH_2N; 7.01, d, J 9.5 Hz, H7; 7.22-7.86, complex, H2′,3′,5′,6′, Ph and H8.

3-Benzamidomethyl-6-chloro-2-(3′,4′-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (II.6c) (10%), m.p. 212-214° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of methanol and light petroleum, (Found, for a sample dried at 105°/0.1 mmHg for 5 h: C, 62.0; H, 4.0; N, 13.5. $C_{21}H_{15}ClN_4O_3$ requires C, 62.0; H, 3.7; N, 13.8%). ^1H n.m.r.: δ 5.21, d, J 5.5 Hz, CH_2N; 6.02, s, OCH_2O; 6.95, d, J 9.5 Hz, H7; 7.00-7.97, complex, H2",5",6" and Ph and H8.

3-Benzamidomethyl-6-methylthio-2-(4′-tolyl)imidazo-[1,2-b]pyridazine (II.6d) (60%), m.p. 222-224° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene (Found, for a sample dried at 60°/0.1 mmHg for 15 h: C, 68.4; H, 5.3; N, 14.5. $C_{22}H_{20}N_4OS$ requires C, 68.0; H, 5.2; N, 14.1%). ^1H n.m.r.: δ 2.40, s, MeC; 2.61, s, MeS; 5.25, d, J 5.5 Hz, CH_2N; 6.92, d, J 9.5 Hz, H7; 7.30-7.85, complex, H2",3",5" and 6", Ph and H8.

3-Benzamidomethyl-2-(3′,4′-methylenedioxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.6e) (64%), as yellow crystals, m.p. 200-202° after t.l.c. (alumina, chloroform) and recrystallisation from toluene (Found: C, 63.2; H,

4.5; N, 13.2. $C_{22}H_{18}N_4O_3S$ requires C, 63.1; H, 4.3; N, 13.4%). ^1H n.m.r.: δ 2.56, s, MeS; 5.14, d, J 5.5 Hz, CH_2N; 5.98, s, OCH_2O; 6.78, d, J 9.5 Hz, H7; 7.21-7.51 and 7.85-7.98, complex, H2",5",6", Ph and H8.

3-Benzamidomethyl-6-methoxy-2-(4'-tolyl)imidazo-[1,2-b]pyridazine (II.6f) (64%), as white crystals m.p. 215-217° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene (Found, for a sample dried at 100°/710 mmHg for 12 h: C, 71.2; H, 5.5; N, 15.2. $C_{22}H_{20}N_4O_2$ requires C, 70.9; H, 5.4; N, 15.0%). ^1H n.m.r.: δ 2.36, s, MeC; 3.94, s, MeO; 5.13, d, J 5.5 Hz, CH_2N; 6.57, d, J 9.5 Hz, H7; 7.30-7.94, complex, H2",3",5",6", Ph and H8.

3-Benzamidomethyl-2-phenyl-6-phenylthioimidazo-[1,2-b]pyridazine (II.6g) (70%), m.p. 177-179° after t.l.c. (alumina; chloroform) and recrystallisation from toluene (Found: C, 71.7; H, 4.8; N, 12.9. $C_{26}H_{20}N_4OS$ requires C, 71.5; H, 4.6; N, 12.8%). ^1H n.m.r.: δ 5.00, d, J 5.5 Hz, CH_2N; 6.82, d, J 5.5 Hz, H7; 7.27-8.01, complex, 2xPh and H8.

3-Benzamidomethyl-6-benzyloxy-2-phenylimidazo-[1,2-b]pyridazine (II.6h) (26%), as white crystals, m.p. 193-195° after t.l.c. [alumina; chloroform then alumina, chloroform, light petroleum] and recrystallisation from toluene. (Found: C, 74.7; H, 5.3; N, 13.0. $C_{27}H_{22}N_4O_2$ requires C, 74.6; H, 5.1; N, 12.9%). ^1H n.m.r. ($CDCl_3$): δ

5.19, d, J 5.5 Hz, CH_2NH; 5.38, s, CH_2O; 6.74, d, J 9.5 Hz, H7; 7.20-7.90, complex, 3 x Ph.

II-5.3 [^{3}H]Diazepam binding assay

Young adult Spargue-Dawley rats were decapitated and their brains removed and then placed on ice. Washed synaptosmal membranes were prepared from the "p2" mitochondrial pellet according to a previously published procedure[233] and stored frozen until used. On the day of assay, membrane preparations were thawed, washed once by centrifugation and resuspended on ice-cold distilled water, then resuspended in 50 mM Tris-HCl buffer, pH 7.4, at 2^{o}C. For the receptor binding assays, aliquots of the membrane suspension (approximately 0.8 mg protein) were incubated with tritiated diazepam (86.6 Ci/mmol, 0.70 ± 0.05 nM final concentration) in a final volume of 50 mM Tris-HCl buffer containing various concentrations of the test compounds and 100 μM GABA (to stimulate the binding of the ligand to the benzodiazepine receptors in the plasma membranes). Four separate concentrations of test compounds were always used in tests on each compound. Assays were conducted on ice for an incubation period of 35 min. Nonspecific binding was determined in separated tubes by the addition of a large excess (100 μM) of unlabelled diazepam. Membranes were collected by filtration under vacuum on glass-fibre filters (Whatman GF/B, 2.5 cm) and washed with 12 ml of ice-cold buffer. Filters were placed in scintillation vials with 1 ml of water and 8 ml toluene/triton X-100

scintillation fluid; bound radioactivity was determined by conventional techniques. The imidazo[1,2-*b*]pyridazines were routinely tested at four different concentrations of 30, 100, 300 and 1000 nM; and within each experiment all assays were performed in triplicate. For each concentration of the test compounds, results were calculated as the per cent displacement of specific binding. Where specific binding was taken as the amount of radioactive diazepam bound in control tubes (no inhibitor) less the amount bound in the presence of excess unlabelled diazepam. IC_{50} values (the concentration of the drug causing 50% displacement of radioactive diazepam bound to the brain membranes, and standard assay conditions) were calculated for each test compound by using computer-assisted log-logit analysis. If the correlation coefficient of the lines of best-fit to log-logit curves was less than 0.95 for a test compound, the experiment was repeated.

Compounds were initially dissolved in dimethyl sulphoxide (DMSO) to give 4 mM stock solutions which were then serially diluted with buffer (or DMSO/buffer) and immediately added to the assay tubes. DMSO was also added to control and blank tubes so that all tubes contained the same final concentration of DMSO (0.25%).

CHAPTER III

CHAPTER III Syntheses and binding studies of some 3-(substituted benzamidomethyl)-6-fluoro (and chloro)-2-phenyl (and 4′-tolyl)imidazo[1,2-b]pyridazines

III-I Introduction

In Chapter II the synthesis of 6-(variously substituted)-3-benzamidomethyl-2-phenyl (and substituted phenyl)imidazo[1,2-*b*]pyridazines and studies of their activity in displacing [^{3}H]diazepam from its specific binding sites in rat brain were reported.

In this chapter, syntheses are described for 6-fluoro- and 6-chloro-3-(substituted benzamidomethyl)-2-phenyl (and 4′-tolyl)imidazo[1,2-*b*]pyridazines and the results of tests to determine their ability to displace [^{3}H]diazepam from rat brain membrane preparations, are given and discussed. The aim of this investigation was to determine the structure-activity relationships for this series of compounds with the hope of identifying potent and selective inhibitors of [^{3}H]diazepam binding.

Thus the effect of introducing various substituents into the 3-benzamidomethyl group in the 2-aryl-6-(fluoro and chloro)-3-benzamidomethylimidazo[1,2-*b*]pyridazines has been studied.

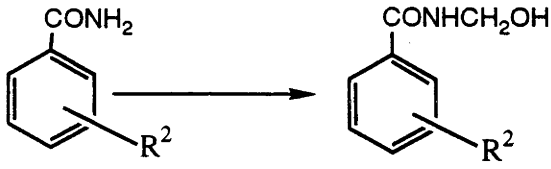
III-2 Syntheses

The starting materials required for the preparation of the title compounds (**III.3** and **III.4**, Scheme III-2) were the substituted (*N*-hydroxymethyl)benzamides (**III.1**, Scheme III-1) which were prepared from the corresponding amides by stirring with formaldehyde in aqueous potassium carbonate, and the 3-unsubstituted-6-chloro (and 6-fluoro)-2-phenyl (and 4′-tolyl)imidazo[1,2-*b*]pyridazines (**III.2**, X = Cl or F) which have been described in Chapter II.

A mixture of an *N*-(hydroxymethyl)benzamide (**III.1**) and compounds (**III.2**, Scheme III-2) in acetic acid containing sulphuric acid were heated at 120°C for *ca* 14 h to give compounds (**III.3** or **III.4**), respectively, obtained in yields which varied from 9 to 88%.

The nitro compounds (**III.4a-d**) were reduced catalytically with hydrogen over Raney nickel in ethanol to the corresponding amino compounds (**III.4e,t-v**, Scheme III-3), respectively.

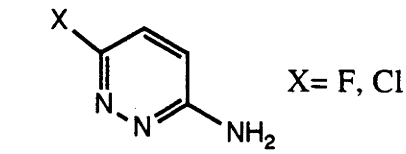
Scheme III-1



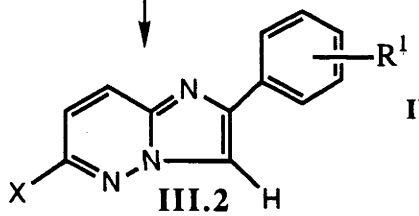
III. 1

III. 1	R^2
a.	NMe_2-*m*
b.	F-*o*
c.	F-*m*
d.	F-*p*
e.	Cl-*o*
f.	Cl-*m*
g.	Cl-*p*
h.	NO_2-*o*
i.	NO_2-*m*
j.	NO_2-*p*
k.	Me-*o*
l.	Me-*m*
m.	Me-*p*
n.	OMe-*p*

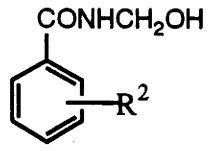
Scheme III-2

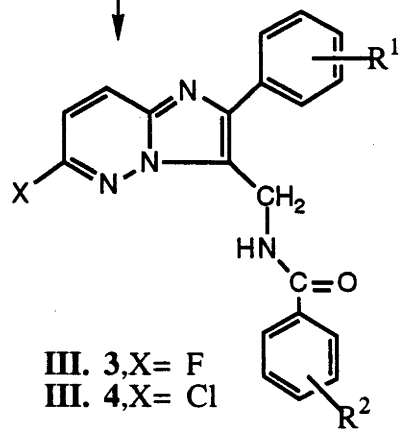


X= F, Cl



III.2





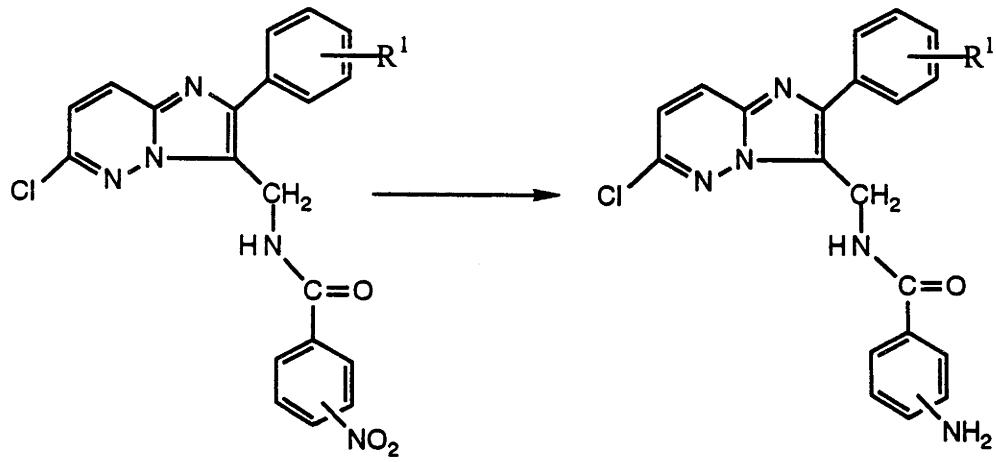
III. 3,X= F
III. 4,X= Cl

III.3	R^1	R^2
a	H	NO_2-*o*
b	H	NO_2-*m*
c	Me-*p*	NO_2-*o*
d	H	F-*o*
e	H	F-*m*
f	H	F-*p*
g	Me-*p*	F-*o*
h	Me-*p*	F-*m*
i	Me-*p*	F-*p*
j	H	Cl-*o*
k	H	Cl-*m*
l	H	Cl-*p*
m	Me-*p*	Cl-*o*
n	Me-*p*	Cl-*m*
o	Me-*p*	Cl-*p*
p	H	Me-*o*
q	H	Me-*m*
r	H	Me-*p*

III.4	R^1	R^2
a	H	NO_2-*o*
b	H	NO_2-*m*
c	H	NO_2-*p*
d	Me-*p*	NO_2-*m*
e	Me-*p*	NH_2-m
f	Me-*p*	C_5H_4N-β*
g	H	F-*o*
h	H	F-*m*
i	H	F-*p*
j	Me-*p*	F-*o*
k	Me-*p*	F-*m*
l	Me-*p*	F-*p*
m	H	Cl-*o*
n	H	Cl-*m*
o	H	Cl-*p*
p	H	Me-*m*
r	H	Me-*p*
s	H	OMe-*p*
t	H	NH_2-*o*
u	H	NH_2-*m*
v	H	NH_2-*p*
w	H	NMe_2-*m*

*Pyridin-3-yl(not substituted phenyl)

Scheme III-3



III. 4 a-d

III. 4 e,t-v.

III-3 ^{1}H n.m.r. spectra

The ^{1}H n.m.r. spectra of the 3-unsubstituted compounds (**III.2**) employed in this work have been described in Chapter II; those of compounds (**III.1**) are recorded in the Experimental section and the methylene and methyl protons are recorded in Table III-1. The signal for protons of each methylene group in compounds (**III.1**), adjacent to the NH group of the carbamoyl group, appeared as a doublet with a coupling constant of 6.5 - 7.0 Hz and a chemical shift mostly in the range δ4.93-4.97. The exceptions were the 2-fluoro compound (**III.1b**) and the nitro compounds (**III.1h-j**) in which the methylene protons appeared as a triplet with chemical shifts of δ4.99 (**III.1b**) and δ4.66-4.74 (in CD_3SOCD_3), respectively. On addition of deuterium oxide, each of these doublets and triplets collapsed to a singlet.

The spectra of the title compounds (**III.3** and **III.4**) are not recorded in the Experimental section because the signals due to the aromatic groups at the 2- and 3-positions overlap to give spectra which were not readily assigned. The chemical shifts of the methylene protons (δ4.94-5.23) are, however, recorded in Table III-2.

Table III-1 Some ^{1}H n.m.r. spectral data[A] for some N-(hydroxymethyl)benzamides

III.1	R^1	Me of R^1	CH_2[B]
a	NMe_2-*m*	2.99	4.95
b	F-*o*		4.99[C]
c	F-*m*		4.97
d	F-*p*		4.95
e	Cl-*o*		4.96
f	Cl-*m*		4.96
g	Cl-*p*		4.97
h	NO_2-o		4.66[C]
i	NO_2-*m*		4.74[C]
j	NO_2-*p*		4.74[C]
k	Me-*o*	2.46	4.93
l	Me-*m*	2.40	4.97
m	Me-*p*	2.39	4.93
n	OMe-*p*	3.86	4.95

[A]Reported as parts per million (δ) downfield from tetramethylsilane as internal standard in deuterochloroform except for compounds (**III.1h-j**) which were examined in CD_3SOCD_3

[B]Doublet unless specified otherwise

[C]Triplet

Table III-2 Some ^{1}H n.m.r. spectral data[A] for 6-fluoro (and 6-chloro)-3-(substituted benzamidomethyl)-2-phenyl-(and 4′-tolyl)-imidazo[1,2-b]pyridazines

III.3	R^1 and δ	R^2 and δ	CH_2[B]
a	H	NO_2-*o*	5.22
b	H	NO_2-*m*	5.22
c	Me-*p* 2.41	NO_2-*o*	5.17
d	H	F-*o*	5.23
e	H	F-*m*	5.17
f	H	F-*p*	5.17
g	Me-*p* 2.41	F-*o*	5.23
h	Me-*p* 2.40	F-*m*	5.16
i	Me-*p* 2.40	F-*p*	5.15
j	H	Cl-*o*	5.17
k	H	Cl-*m*	5.18
l	H	Cl-*p*	5.20
m	Me-*o* 2.41	Cl-*o*	5.20
n	Me-*m* 2.41	Cl-*m*	5.18
o	Me-*p* 2.41	Cl-*p*	5.19
p	H	Me-*o* 2.42	5.18
q	H	Me-*m* 2.36	5.17
r	H	Me-*p* 2.38	5.19

III.4			
a	H	NO_2-*o*	5.22
b	H	NO_2-*m*	5.22
c	H	NO_2-*p*	5.23
d	Me-*p* 2.41	NO_2-*m*	5.25
e	Me-*p* 2.41	NH_2-*m*	5.16
f	Me-*p* 2.40	C_5H_4N-β[C]	5.23
g	H	F-*o*	5.27
h	H	F-*m*	5.20
i	H	F-*p*	5.23
j	Me-*p* 2.41	F-*o*	5.24
k	Me-*p* 2.40	F-*m*	5.18
l	Me-*p* 2.40	F-*p*	5.18
m	H	Cl-*o*	5.25
n	H	Cl-*m*	5.22
o	H	Cl-*p*	5.23
p	H	Me-*o* 2.44	5.24
q	H	Me-*m* 2.37	5.19

Table III-2 Continued

III.4	R^1 and δ	R^2 and δ		CH_2[B]
r	H	Me-*p*	2.37	5.21
s	H	OMe-*p*	3.84	5.22
t	H	NH_2-*o*		5.21
u	H	NH_2-*m*		4.94
v	H	NH_2-*p*		5.22
w	H	NMe_2-*m*	2.99	5.24

[A]Reported as parts per million (δ) downfield from tetramethylsilane as internal standard in deuterochloroform.

[B]Doublet

[C]Pyridinyl (not substituted phenyl)

III-4 In vitro binding studies

The series of compounds prepared in this chapter were screened for their ability to bind at specific benzodiazepine receptors in the rat brain preparations using the [^{3}H]diazepam binding assay as outlined in Chapter II-5.3.

II-4.1 Results of [^{3}H]diazepam binding assays

The *in vitro* competitive binding results are shown in Table III-3 as IC_{50} values or per cent displacement at the specified concentration.

III-4.2 Discussion of results

An examination of the data in Table III-3 for the 6-fluoro compounds (**III.3**) and the 6-chloro compounds (**III.4**) revealed a wide range of activity.

The 6-fluoro compounds (**III.3**) generally bound more strongly than their 6-chloro analogues. Exceptions were the 6-fluoro-3-(nitrobenzamidomethyl) compounds (**III.3a**, IC_{50} 1743 nM and **III.3b**, IC_{50} 384 nM) and the 6-fluoro-3-(2′-toluamidomethyl compound (**III.3p**, 4% displacement at 1000 nM) as compared to the 6-chloro analogues (**III.4a**, IC_{50} 657 nM; and **III.4b**, IC_{50} 354 nM) and the 6-chloro-3-(2′-toluamidomethyl) analogue (**III.4p**, IC_{50} 623 nM).

Compounds which contained the 2-(4′-tolyl) group bound more strongly than their analogues which contained the 2-phenyl group; comparison of the IC_{50} values in

Table III-3 Results for the displacement of [^{3}H]diazepam from its specific binding sites in rat brain preparations by some 3-(substituted benzamidomethyl)-imidazo[1,2-b]pyridazines

Formula number	Imidazo[1,2-*b*] pyridazine	IC_{50}(nM)[A] (or % displ.)[B]
II.6 a	6-F-3-$CH_2NHCOPh$-2-Ph	68
II.7	6-F-3-$CH_2NHCOPh$-2-C_6H_4Me-*p*	8
III.3 a	6-F-3-$CH_2NHCOC_6H_4NO_2$-*o*-Ph	1743
b	6-F-3-$CH_2NHCOC_6H_4NO_2$-*m*-Ph	384
c	6-F-3-$CH_2NHCOC_6H_4NO_2$-*o*-2-C_6H_4Me-*p*	(25%)
d	6-F-3-$CH_2NHCOC_6H_4F$-*o*-2-Ph	114
e	6-F-3-$CH_2NHCOC_6H_4F$-*m*-2-Ph	57
f	6-F-3-$CH_2NHCOC_6H_4F$-*p*-2-Ph	77
g	6-F-3-$CH_2NHCOC_6H_4F$-*o*-2-C_6H_4Me-*p*	7
h	6-F-3-$CH_2NHCOC_6H_4F$-*m*-2-C_6H_4Me-*p*	8
i	6-F-3-$CH_2NHCOC_6H_4F$-*p*-2-C_6H_4Me-*p*	8
j	6-F-3-$CH_2NHCOC_6H_4Cl$-*o*-2-Ph	460
k	6-F-3-$CH_2NHCOC_6H_4Cl$-m-2-Ph	155
l	6-F-3-$CH_2NHCOC_6H_4Cl$-*p*-2-Ph	190
m	6-F-3-$CH_2NHCOC_6H_4Cl$-*o*-2-C_6H_4Me-*p*	60
n	6-F-3-$CH_2NHCOC_6H_4Cl$-*m*-2-C_6H_4Me-*p*	18
o	6-F-3-$CH_2NHCOC_6H_4Cl$-*p*-2-C_6H_4Me-*p*	18
p	6-F-3-$CH_2NHCOC_6H_4Me$-*o*-2-Ph	(4%)
q	6-F-3-$CH_2NHCOC_6H_4Me$-*m*-2-Ph	235
r	6-F-3-$CH_2NHCOC_6H_4Me$-*p*-2-Ph	94
II.12	6-Cl-3-$CH_2NHCOPh$-2-Ph	140
II.6 b	6-Cl-3-$CH_2NHCOPh$-2-C_6H_4Me-*p*	18
III.4 a	6-Cl-3-$CH_2NHCOC_6H_4NO_2$-*o*-Ph	657
b	6-Cl-3-$CH_2NHCOC_6H_4NO_2$-*m*-Ph	354
c	6-Cl-3-$CH_2NHCOC_6H_4NO_2$-*p*-Ph	(9%)
d	6-Cl-3-$CH_2NHCOC_6H_4NO_2$-*m*-2-C_6H_4Me-*p*	23
e	6-Cl-3-$CH_2NHCOC_6H_4NH_2$-*m*-2-C_6H_4Me-*p*	17
f	6-Cl-3-$CH_2NHCOC_5H_4N$-β-2-C_6H_4Me-*p*	34
g	6-Cl-3-$CH_2NHCOC_6H_4F$-*o*-2-Ph	145
h	6-Cl-3-$CH_2NHCOC_6H_4F$-*m*-2-Ph	66
i	6-Cl-3-$CH_2NHCOC_6H_4F$-*p*-2-Ph	97

Table III-3 Continued

Formula number	Imidazo[1,2-*b*] pyridazine	IC_{50} (nM)[A] (or % displ.)[B]
j	6-Cl-3-$CH_2NHCOC_6H_4F$-*o*-2-C_6H_4Me-*p*	22
k	6-Cl-3-$CH_2NHCOC_6H_4F$-*m*-2-C_6H_4Me-*p*	10
l	6-Cl-3-$CH_2NHCOC_6H_4F$-*p*-2-C_6H_4Me-*p*	24
m	6-Cl-3-$CH_2NHCOC_6H_4Cl$-*o*-2-Ph	(24%)
n	6-Cl-3-$CH_2NHCOC_6H_4Cl$-*m*-2-Ph	203
o	6-Cl-3-$CH_2NHCOC_6H_4Cl$-*p*-2-Ph	1961
p	6-Cl-3-$CH_2NHCOC_6H_4Me$-*o*-2-Ph	623
q	6-Cl-3-$CH_2NHCOC_6H_4Me$-*m*-2-Ph	303
r	6-Cl-3-$CH_2NHCOC_6H_4Me$-*p*-2-Ph	348
s	6-Cl-3-$CH_2NHCOC_6H_4OMe$-*p*-2-Ph	185
t	6-Cl-3-$CH_2NHCOC_6H_4NH_2$-*o*-2-Ph	145
u	6-Cl-3-$CH_2NHCOC_6H_4NH_2$-*m*-2-Ph	140
v	6-Cl-3-$CH_2NHCOC_6H_4NH_2$-*p*-2-Ph	81
w	6-Cl-3-$CH_2NHCOC_6H_4NMe_2$-*m*-2-Ph	87

[A] IC_{50} values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparations

[B] Percentage displacement at 1000 nM

Table III-3 revealed that this enhanced binding varied from 4- to 16-fold.

An examination of the 6-fluoro compounds revealed that 6-fluoro-3-(2′-fluorobenzamidomethyl)-2-(4"-tolyl)-imidazo[1,2-*b*]pyridazine (**III.3g**, IC_{50} 7 nM) bound most strongly: it was slightly stronger than the 3′- and 4′-fluoro isomers (**III.3h** and **III.3i**), respectively, (IC_{50} values 8 nM) and also slightly stronger than the unsubstituted analogue, 6-fluoro-3-benzamidomethyl-2-(4′-tolyl)imidazo[1,2-*b*]pyridazine (**II.7**, IC_{50} 8 nM). The

2′-, 3′- and 4′-fluoro substituents have only a small effect on binding ability.

The 6-fluoro-3-(2′-,3′- and 4′-chlorobenzamidomethyl) compounds (**III.3j-o**, IC_{50} values 460, 150, 190, 60, 18 and 18 nM, respectively) all bound less strongly than the corresponding 6-fluoro-3-(unsubstituted benzamidomethyl) compounds (**II.6a**, IC_{50} 68 nM; and **II.7**, IC_{50} 8 nM), respectively. A similar situation also applied to 6-fluoro-3-(substituted benzamidomethyl) compounds containing nitro and methyl substituents.

An examination of the 6-chloro compounds revealed a pattern of behaviour similar to that of the 6-fluoro analogues but with some variations. Thus 6-chloro-3-(3′-fluorobenzamidomethyl)-2-(4"-tolyl)imidazo[1,2-*b*]-pyridazine (**III.4k**, IC_{50} 10 nM) bound more strongly than the 3-(unsubstituted benzamidomethyl) analogue (**II.6b**, IC_{50} 18 nM) or its 2′- and 4′-fluoro isomers (**III.4j** and **III.4l**, IC_{50} values 22 and 24 nM, respectively). The 6-chloro-3-(3′-fluorobenzamidomethyl) compound (**III.4h**, IC_{50} 66 nM) also bound more strongly than its 2′- and 4′-fluoro isomers (**III.4g**, IC_{50} 145; and **III.4i**, IC_{50} 97 nM), respectively. The 6-chloro-3-(4′-methoxybenzamidomethyl) compound (**III.4s**, IC_{50} 185 nM) bound more strongly than its 4′-methyl analogue (**III.4r**, IC_{50} 348 nM).

Whereas the 3-(nitrobenzamidomethyl) compounds (**III.4a-c**, IC_{50} 657, 354 nM and 9% displacement at 1000 nM, respectively) possessed only weak binding ability, reduction to the corresponding amino compounds (**III.4t-v**,

IC_{50} values 145, 140 and 81 nM, respectively) increased binding properties.

N,*N*-Dimethylation of the 6-chloro-3-(3′-amino-benzamidomethyl) compound (**III.4u**, IC_{50}, 140 nM) to give the 3-(3′-*N*,*N*-dimethylaminobenzamidomethyl) analogue (**III.4w**, IC_{50} 87 nM) increased binding significantly. 6-Chloro-3-(3′-nitro- and 3′-amino-benzamidomethyl)-2-(4"-tolyl)imidazo[1,2-*b*]pyridazines (**III.4d** and **III.4e**) were outstanding with IC_{50} values of 23 and 17 nM, respectively. The 3-nicotinamidomethyl compound (**III.4f**, IC_{50} 34 nM) was less active than the corresponding 3-(3′-aminobenzamidomethyl) compound (**III.4e**, IC_{50} 17 nM).

The effect of a 2′-nitro group in 3-(substituted benzamidomethyl) compounds, e.g. (**III.3a**), (**III.3c**) and **III.4a**) was to markedly decrease activity relative to the 3′- and 4′-nitro isomers and to the corresponding unsubstituted 3-benzamidomethyl compounds. Similar reduced activity was found in compounds (**III.3j**, **III.3m**, **III.3p**, **III.4m** and **III.4p**) which each contained a 2′-chloro- or 2′-methyl group. Steric effects of relatively large groups at the 2′-positions may be responsible for the diminished binding ability observed. It has been proposed[234] that lipophilicity may be an important factor affecting the binding of benzodiazepine-like compounds. In the present work the 3′-dimethylamino compound (**IV.4w**, IC_{50} 87 nM) bound more strongly than its 3′-amino analogue (**IIIa.4u**, IC_{50} 140 nM) and the dimethylamino group would be expected to be more lipophilic than the amino group.[235]

III-5 Experimental

The general procedures and also experimental details for [^{3}H]diazepam binding assay have been recorded in Chapter II-5.1 and II-5.3.

3-Dimethylamino-N-(hydroxymethyl)benzamide (III.1a)

A mixture of 3-dimethylaminobenzamide[236] (1.0 g), potassium carbonate (0.107 g) and water (15 ml) was warmed to dissolve the solid, aqueous formaldehyde (0.6 ml; 37% solution) added and the mixture stirred at room temperature for 2 h. The solid (0.645 g) was filtered off, washed with water and recrystallised from water to give yellow crysatals of *3-dimethylamino-N-(hydroxymethyl)benzamide*, m.p. 138-140° (Found: C, 62.1; H, 7.2; N, 14.4. $C_{10}H_{14}N_2O_2$ requires C, 61.8; H, 7.3; N, 14.4%). ^{1}H.n.m.r.: δ 2.99, s, Me_2N; 4.95, d, J 6.5 Hz, CH_2N; 6.83-7.37, complex, ArH.

3-Fluoro-N-(hydroxymethyl)benzamide (III.1c)

A mixture of 3-fluorobenzamide[237] (0.5 g), potassium carbonate (0.022 g) and water (5 ml) was warmed to dissolve the solid. Aqueous formaldehyde (0.42 g) was added and the mixture stirred in an oil bath at 100° for 3h. After cooling, the solid (0.264 g) was filtered off and washed with water. It was recrystallised from benzene to give colourless crystals of the *title compound*, m.p. 103-105°. (Found, for a sample dried at 20°/0.1 mmHg for 6 h: C, 56.8; H, 4.7; N, 8.1. $C_8H_8FNO_2$

requires C, 56.8; H, 4.8; N, 8.3%). ^{1}H.n.m.r.: δ 4.97, d, J 6.5Hz, CH_2N; 7.13-7.58, complex, ArH.

2-Fluoro-N-(hydroxymethyl)benzamide (III.1b)

This compound was prepared in a similar manner from 2-fluorobenzamide[237] but the reaction mixture was heated for 6 h. The product was collected by filtration and extraction with chloroform and recrystallised from benzene to give colourless crystals of the *title compound* (61%) m.p.. 94-95°. (Found, for a sample dried at 20°/0.1 mmHg for 15 h: C, 57.0; H, 4.8; N, 8.0. $C_8H_8FNO_2$ requires C, 56.8; H, 4.8; N, 8.3%. ^{1}H.n.m.r.: δ 3.69, t, J 7.5Hz, CH_2OH; 4.99, t, J 6.5Hz, CH_2N; 7.03-8.20, complex, ArH.

2-Chloro-N-(hydroxymethyl)benzamide (III.1e)

This compound was prepared as described by Schönenberger *et al.*[238] It was recrystallised from water, subjected to t.l.c. (alumina; chloroform) and recrystallised from benzene to give the *title compound*, m.p. 117-119° (lit.[238] 106-108°) (Found: C, 52.3; H, 4.0; N, 7.5. Calc. for $C_8H_8ClNO_2$. 0.03 C_6H_6: C, 52.3; H, 4.4; N, 7.5%). ^{1}H.n.m.r.: δ 4.96, d, J 6.5Hz, CH_2N; 7.26-7.76, complex ArH.

3-Chloro-N-(hydroxymethyl)benzamide (III.1f)

This compound was prepared as described by Schönenberger *et al.*[238] It was recrystallised from aqueous ethanol, subjected to t.l.c. (alumina;

chloroform) and recrystallised from benzene to give the *title compound*, m.p. 125-127° (lit.[238] 116-118°) (Found: C, 52.1; H, 4.1; N, 7.5. Calc. for $C_8H_8ClNO_2$: C, 51.8; H, 4.3; N, 7.5%) ^{1}H.n.m.r.: δ 4.96, d, J 6.5Hz, CH_2N; 7.29-7.79, complex, ArH.

Preparation and ^{1}H.n.m.r. of other N-(hydroxymethyl)-benzamides

The following *N*-(hydroxymethyl)benzamides were prepared according to literature procedures: 2-, 3- and 4-nitro-;[239] 4-fluoro-;[238] 4-chloro-;[238] 2-methyl-;[240] 3-methyl-;[241] 4-methyl-;[238] and 4-methoxy-*N*-(hydroxymethyl)benzamides.[241]

Their ^{1}H.n.m.r. data were as follows:

2-NO_2 (**III.1h**) (CD_3SOCD_3): δ 4.66, t, J 6.5Hz, CH_2N; 5.82, t, J 7.5Hz, CH_2OH; 7.54-8.07, complex, ArH; 9.25, br, NH.

3-NO_2 (**III.1i**) (CD_3SOCD_3): δ 4.74, t, J 6.5Hz, CH_2N; 5.80, t, 7.5Hz, CH_2OH; 7.69-8.69, complex, ArH.; 9.51, br, NH.

4-NO_2 (**III.1j**) (CD_3SOCD_3): δ 4.74, t, J 6.5Hz, CH_2N; 5.81, t, J 7.5Hz, CH_2OH; 8.11, d, J 8Hz, 8.34, d, J 8Hz, ArH, 9.04, br, NH.

4-F (**III.1d**): δ 4.95, d, J 6.5Hz, CH_2N; 7.11, t, J_{HH} = J_{HF} = 8Hz, 7.81, q, J_{HH} 8 Hz, J_{HF} 5.5Hz, ArH.

4-Cl (**III.1g**): δ 4.97, d, J 6.5Hz, CH_2N; 7.42, d, 7.76, d, J 8Hz, H 2,3,5,6.

2-Me (**III.1k**): δ 2.46, s, Me; 4.93, d, J 6.5Hz, CH_2N; 6.80, br, NH; 7.19-7.46, complex, ArH.

3-Me (**III.1l**): δ 2.40, s, Me; 4.97, d, J 6.5Hz, CH_2N; 7.27-7.63, complex, ArH.

4-Me (**III.1m**) ($CDCl_3$ + D_2O): δ 2.39, s, Me; 4.93, s, CH_2N; 7.21, d, J 9Hz, 7.69, d, J 9Hz, ArH.

4-OMe (**III.1n**): δ 3.86, s, MeO; 4.95, d, J 7Hz, CH_2N; 6.93, d, J 9Hz, H 3,5; 7.77, dd, $J_{2,3}$ 9Hz, $J_{2,5}$ 1.5Hz, H 2,6.

6-Fluoro-3-(4'-fluorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.3f)

A mixture of 4-fluoro-*N*-(hydroxymethyl)benzamide (0.11 g), glacial acetic acid (3.0 ml) and concentrated sulphuric acid (10 drops) was stirred in an oil bath at 50° for 15 minutes until a solution was obtained. Then 6-fluoro-2-phenylimidazo[1,2-*b*]pyridazine (0.110 g) was added and the mixture refluxed with stirring in an oil bath at 120° for 15 h. The acetic acid was evaporated under reduced pressure, the residue diluted with water, adjusted with aqueous ammonia to pH 10, and extracted

with chloroform. The extract was dried (Na_2SO_4), the solvent evaporated and the solid obtained was subjected to t.l.c. (alumina; chloroform, light petroleum; 2:1) and recrystallised from toluene to give the *title compound* (0.068g), m.p. 225-227°. (Found, for a sample dried at 70°/710 mmHg for 24 h: C, 64.4, H, 3.9; N, 14.8. $C_{20}H_{14}F_2N_4O$. 0.5 H_2O requires C, 64.3; H, 4.0; N, 15.0%)

6-Fluoro-3-(2'-nitrobenzamidomethyl)-2-phenylimidazo-[1,2-b]pyridazine (III.3a)

A mixture of *N*-hydroxymethyl-2-nitrobenzamide (0.21 g), glacial acetic acid (4.0 ml) and concentrated sulphuric acid (0.5 ml) was stirred in an oil bath at 50° for 15 min. 6-Fluoro-2-phenylimidazo[1,2-*b*]pyridazine (0.213 g) was added and the mixture refluxed with stirring in an oil bath at 120° for 15 h. The acetic acid was evaporated under reduced pressure, the residue diluted with water and adjusted to pH 10 and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4) and evaporated, and the product recrystallised from toluene to give white crystals of the *title compound* (0.125 g), m.p. 242-245°. (Found, for a sample dried at 75°/710 mmHg for 20 h: C, 61.5; H, 3.7; N, 17.7. $C_{20}H_{14}FN_5O_3$ requires, 61.4; H, 3.6; N, 17.7%).

In a similar manner the following compounds were prepared.

6-Fluoro-3-(3′-nitrobenzamidomethyl)-2-phenylimidazo-[1,2-b]pyridazine (III.3b) (27%), m.p. 206-208° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 61.2; H, 3.8; N, 17.7. $C_{20}H_{14}FN_5O_3$ requires, C, 61.4; H, 3.6; N, 17.9%).

6-Fluoro-3-(2′-nitrobenzamidomethyl)-2-(4′′-tolyl)-imidazo[1,2-b]pyridazine (III.3c) was obtained as colourless crystals (17%), m.p. 262-264° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 62.4; H, 4.2; N, 17.5. $C_{21}H_{16}FN_5O_3$ requires C, 62.2; H, 4.0; N, 17.3%).

6-Fluoro-3-(2′-fluorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.3d) (34%), m.p. 181-183° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 65.7; H, 3.7; N, 15.2. $C_{20}H_{14}F_2N_4O$ requires C, 65.9; H, 3.9; N, 15.4%).

6-Fluoro-3-(3′-fluorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.3e) (21%), m.p. 197-199° after t.l.c. (alumina; 33% light petroleum in chloroform) and recrystallisation from toluene. (Found: C, 65.9; H, 3.9; N, 15.3. $C_{20}H_{14}F_2N_4O$ requires C, 65.9; H, 3.9; N, 15.4%).

6-Fluoro-3-(2′-fluorobenzamidomethyl)-2-(4′′-tolyl)-imidazo[1,2-b]pyridazine (III.3g) (21%), m.p. 213-214° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found, for a sample dried at 120°/710 mmHg for 16 h: C, 66.0; H, 4.3; N, 14.5. $C_{21}H_{16}F_2N_4O$. 0.1 H_2O requires C, 66.3; H, 4.3; N, 14.7%).

6-Fluoro-3-(3′-fluorobenzamidomethyl)-2-(4′′-tolyl)-imidazo[1,2-b]pyridazine (III.3h) (34%), m.p. 240-242° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from toluene. (Found, for a sample dried at 120°/710 mmHg for 15 h: C, 66.4; H, 4.3; N, 14.7. $C_{21}H_{16}F_2N_4O$ requires C, 66.7; H, 4.3; N, 14.8%).

6-Fluoro-3-(4′-fluorobenzamidomethyl)-2-(4′′-tolyl)-imidazo[1,2-b]pyridazine (III.3i) (81%), m.p. 236-237° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found, for a sample dried at 110°/0.1 mmHg for 5 h: C, 66.4; H, 4.4; N, 14.8. $C_{21}H_{16}F_2N_4O$ requires C, 66.7; H, 4.3; N, 14.8%).

3-(2′-Chlorobenzamidomethyl)-6-fluoro-2-phenyl-imidazo[1,2-b]pyridazine (III.3j) was obtained as white crystals (24%), m.p. 225-227° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 63.1; H, 3.6; N, 14.4. $C_{20}H_{14}ClFN_4O$ requires C, 63.1; H, 3.7; N, 14.7%).

3-(3'-Chlorobenzamidomethyl)-6-fluoro-2-phenyl-imidazo[1,2-b]pyridazine (III.3k) (14%), m.p. 196-197° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 63.3; H, 3.7; N, 14.5. $C_{20}H_{14}ClFN_4O$ requires C, 63.1; H, 3.7; N, 14.7%).

3-(4'-Chlorobenzamidomethyl)-6-fluoro-2-phenyl-imidazo[1,2-b]pyridazine (III.3l) was obtained as a white solid (88%), m.p. 255-257° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found, for a sample dried at 120°/710 mmHg for 16 h: C, 62.6; H, 3.6; N, 14.4. $C_{20}H_{14}ClFN_4O$. 0.1 H_2O requires C, 62.8; H, 3.7; N, 14.6%).

3-(2'-Chlorobenzamidomethyl)-6-fluoro-2-(4''-tolyl)-imidazo[1,2-b]pyridazine (III.3m) (25%), m.p. 235-236° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found, for a sample dried at 105°/0.1mmHg for 2h: C, 63.8; H, 4.1; N, 14.0. $C_{21}H_{16}ClFN_4O$ requires C, 63.9; H, 4.1; N, 14.2%).

3-(3'-Chlorobenzamidomethyl)-6-fluoro-2-(4''-tolyl)-imidazo[1,2-b]pyridazine (III.3n) as a colourless solid (26%), m.p. 239-241° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 63.6; H, 4.1; N, 14.0. $C_{21}H_{16}ClFN_4O$ requires C, 63.9; H, 4.1; N, 14.2%).

3-(4′-Chlorobenzamidomethyl)-6-fluoro-2-(4′′-tolyl)-imidazo[1,2-b]pyridazine (III.3o) (31%), m.p. 257-259° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found: C, 64.2; H, 4.3; N, 14.2. $C_{21}H_{16}ClFN_4O$ requires C, 63.9; H, 4.1; N, 14.2%).

6-Fluoro-2-phenyl-3-(2′-toluamidomethyl)imidazo[1,2-b]-pyridazine (III.3p) (44%), m.p. 226-228° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found, for a sample dried at 120°/710 mmHg for 16 h: C, 69.1; H, 4.7; N, 15.3. $C_{21}H_{17}FN_4O$. 0.2 H_2O requires C, 69.3; H, 4.8; N, 15.4%).

6-Fluoro-2-phenyl-3-(3′-toluamidomethyl)imidazo-[1,2-b]pyridazine (III.3q) (38%), m.p. 201-203° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 69.9; H, 4.7; N, 15.3. $C_{21}H_{17}FN_4O$ requires C, 70.0; H, 4.8; N, 15.5%).

6-Fluoro-2-phenyl-3-(4′-toluamidomethyl)imidazo-[1,2-b]pyridazine (III.3r) (36%), m.p. 211-213° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 69.9; H, 4.6; N, 15.4. $C_{21}H_{17}FN_4O$ requires C, 70.0; H, 4.8; N, 15.5%).

6-Chloro-3-(2′-nitrobenzamidomethyl)-2-phenylimidazo-[1,2-b]pyridazine (III.4a) (37%), m.p. 268-270° after recrystallisation from toluene. (Found: C, 59.2; H, 3.6; N, 17.0. $C_{20}H_{14}ClN_5O_3$ requires C, 58.9; H, 3.5; N, 17.2%).

6-Chloro-3-(3′-nitrobenzamidomethyl)-2-phenylimidazo-[1,2-b]pyridazine (III.4b) (28%), m.p. 220-222° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 58.7; H, 3.5; N, 16.9. $C_{20}H_{14}ClN_5O_3$ requires C, 58.9; H, 3.5; N, 17.2%).

6-Chloro-3-(4′-nitrobenzamidomethyl)-2-phenylimidazo-[1,2-b]pyridazine (III.4c) (25%), m.p. 256-258° after recrystallisation from toluene. (Found: C, 59.2; H, 3.6; N, 16.9. $C_{20}H_{14}ClN_5O_3$ requires C, 58.9; H, 3.5; N, 17.2%).

6-Chloro-3-(3′-nitrobenzamidomethyl)-2-(4′′-tolyl)-imidazo[1,2-b]pyridazine (III.4d) (51%), m.p. 238-240° (from toluene). (Found, for a sample dried at 100°/710 mmHg for 17 h: C, 59.8; H, 3.8; N, 16.4. $C_{21}H_{16}ClN_5O_3$ requires C, 59.8; H, 3.8; N, 16.6%).

6-Chloro-3-(2′-fluorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.4g) (46%), m.p. 205-207° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 63.0; H,

3.6; N, 14.5. $C_{20}H_{14}ClFN_4O$ requires C, 63.1; H, 3.7; N, 14.7%).

6-Chloro-3-(3'-fluorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.4h) (35%), m.p. 203-205° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 63.2; H, 3.6; N, 14.7. $C_{20}H_{14}ClFN_4O$ requires C, 63.1; H, 3.7; N, 14.7%).

6-Chloro-3-(4'-fluorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.4i) (37%), m.p. 226-228° after recrystallisation from toluene. (Found: C, 62.9; H, 3.7; N, 14.4. $C_{20}H_{14}ClFN_4O$ requires C, 63.1; H, 3.7; N, 14.7%).

6-Chloro-3-(2'-fluorobenzamidomethyl)-3-(4''-tolyl)-imidazo[1,2-b]pyridazine (III.4j) (51%), m.p. 222-224° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found, for a sample dried at 105°/0.1 mmHg for 6 h: C, 64.2; H, 4.2; N, 14.1. $C_{21}H_{16}ClFN_4O$ requires C, 63.9; H, 4.1; N, 14.2%).

6-Chloro-3-(3'-fluorobenzamidomethyl)-3-(4''-tolyl)-imidazo[1,2-b]pyridazine (III.4k) (28%), m.p. 231-232° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found, for a sample dried at 105°/0.1 mmHg for 6 h: C, 64.1; H, 4.2; N, 14.1. $C_{21}H_{16}ClFN_4O$ requires C, 63.9; H, 4.1; N, 14.2%).

6-Chloro-3-(4′-fluorobenzamidomethyl)-3-(4′′-tolyl)-imidazo[1,2-b]pyridazine (III.4l) (59%), as pale yellow crystals, m.p. 232-234° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found, for a sample dried at 110°/0.1 mmHg for 5 h: C, 64.1; H, 4.4; N, 13.9. $C_{21}H_{16}ClFN_4O$ requires C, 63.9; H, 4.1; N, 14.2%).

6-Chloro-3-(2′-chlorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.4m) (40%), m.p. 247-248° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found: C, 60.7; H, 3.4; N, 13.8. $C_{20}H_{14}Cl_2N_4O$ requires C, 60.5; H, 3.5; N, 14.1%).

6-Chloro-3-(3′-chlorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.4n) (25%), m.p. 214-216° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found: C, 60.2; H, 3.6; N, 13.8. $C_{20}H_{14}Cl_2N_4O$ requires C, 60.5; H, 3.6; N, 14.1%).

6-Chloro-3-(4′-chlorobenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.4o) (27%), m.p. 257-259° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found: C, 60.8; H, 3.8; N, 14.2. $C_{20}H_{14}Cl_2N_4O$ requires C, 60.5; H, 3.6; N, 14.1%).

6-Chloro-2-phenyl-3-(2'-toluamidomethyl)imidazo-[1,2-b]pyridazine (III.4p) (36%), m.p. 249-251° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found, for a sample dried at 60°/0.1 mmHg for 40 h: C, 66.8; H, 4.7; N, 14.7. $C_{21}H_{17}ClN_4O$ requires C, 66.9; H, 4.5; N, 14.9%).

6-Chloro-2-phenyl-3-(3'-toluamidomethyl)imidazo-[1,2-b]pyridazine (III.4q) (32%), m.p. 204-206° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 67.3; H, 4.4; N, 14.8. $C_{21}H_{17}ClN_4O$ requires C, 66.9; H, 4.5; N, 14.9%).

6-Chloro-2-phenyl-3-(4'-toluamidomethyl)imidazo-[1,2-b]pyridazine (III.4r) (53%), m.p. 237-239° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found: C, 67.2; H, 4.5; N, 14.8. $C_{21}H_{17}ClN_4O$ requires C, 66.9; H, 4.5; N, 14.9%).

6-Chloro-3-(4'-methoxybenzamidomethyl)-2-phenyl-imidazo[1,2-b]pyridazine (III.4s) (9%), m.p. 227-228° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene. (Found, for a sample dried at 110°/710 mmHg for 2 h: C, 64.3; H, 4.4; N, 14.0. $C_{21}H_{17}ClN_4O_2$ requires C, 64.2; H, 4.4; N, 14.3%).

3-(2'-Aminobenzamidomethyl)-6-chloro-2-phenylimidazo-[1,2-b]pyridazine (III.4t)

6-Chloro-3-(2'-nitrobenzamidomethyl)-2-phenylimidazo[1,2-*b*]pyridazine (0.056 g) in ethanol (20 ml) with Raney nickel was shaken with hydrogen at room temperature and pressure until uptake ceased. The catalyst was filtered on kieselguhr and the solvent evaporated. The product was recrystallised from toluene to give the *title compound* (0.040 g) (77%), m.p. 210-212°. (Found, for a sample dried at 100°/710 mmHg for 14 h: C, 64.3; H, 4.4; N, 18.2. $C_{20}H_{16}ClN_5O$ requires C, 63.6; H, 4.3; N, 18.5%).

3-(3'-Aminobenzamidomethyl)-6-chloro-2-phenylimidazo-[1,2-b]pyridazine (III.4u)

6-Chloro-3-(3'-nitrobenzamidomethyl)-2-phenylimidazo[1,2-*b*]pyridazine (0.073 g) in ethanol (20 ml) with Raney nickel was shaken with hydrogen at room temperature and pressure until uptake ceased. The catalyst was filtered off on celite and the solvent evaporated. The product was recrystallised from toluene to give yellow crystals of the *title compound* (0.035 g, 52%), m.p. 211-213°. (Found: C, 64.0; H, 4.3; N, 18.4. $C_{20}H_{16}ClN_5O$ requires C, 63.6; H, 4.3; N, 18.5%).

3-(4'-Aminobenzamidomethyl)-6-chloro-2-phenylimidazo-[1,2-b]-pyridazine (III.4v) (18%), m.p. 279-281° after recrystallisation from ethanol. (Found, for a sample

dried at 100°/710 mmHg for 10 h: C, 63.4; H, 4.4; N, 18.4. $C_{20}H_{16}ClN_5O$ requires C, 63.6; H, 4.3; N, 18.5%).

6-Chloro-3-(3′-dimethylaminobenzamidomethyl)-2-phenylimidazo[1,2-b]pyridazine (III.4m) (21%), m.p. 185-187° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of benzene and cyclohexane. (Found, for a sample dried at 110°/0.1 mmHg for 4 h: C, 64.8; H, 5.0; N, 17.1. $C_{22}H_{20}ClN_5O$ requires C, 65.1; H, 5.0; N, 17.3%).

3-(3′-Aminobenzamidomethyl)-6-chloro-2-(4′′-tolyl)imidazo[1,2-b]pyridazine (III.4e) (70%), m.p. 206-208° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found, for a sample dried at 105°/710 mmHg for 24 h: C, 66.6; H, 5.1; N, 16.3. $C_{21}H_{18}ClN_5O$. 0.4 C_7H_8 requires C, 66.7; H, 5.0; N, 16.3%).

6-Chloro-3-(nicotinamidomethyl)-2-(4′-tolyl)imidazo[1,2-b]pyridazine (III.4f) (7%) was prepared in a similar manner from 6-chloro-2-(4′-tolyl)imidazo[1,2-*b*]pyridazine and *N*-(hydroxymethyl)nicotinamide.[242] It was purified by t.l.c. (alumina; chloroform) and recrystallised from toluene to give light yellow crystals, m.p. 212-214° (Found: C, 63.6; H, 4.4; N, 18.5. $C_{20}H_{16}ClN_5O$ requires C, 63.6; H, 4.3; N, 18.5%).

CHAPTER IV

CHAPTER IV Syntheses and binding studies of some 6-(N-benzyl-N-methylamino) and 6-(substituted benzylamino)-3-methoxy (and unsubstituted)-2-arylimidazo[1,2-b]-pyridazines

IV-1 Introduction

Some 6-benzylamino [and 6-(methoxybenzylamino)]-3-methoxy-2-phenyl (substituted phenyl and pyridinyl)imidazo-[1,2-*b*]pyridazines have been prepared[121] previously from 6-benzylamino [or 6-(methoxybenzylamino)]pyridazin-3-amine 2-oxides. Studies on structure-activity relationships of some 2-aryl-3-methoxy-6-(various substituted)imidazo [1,2-*b*]-pyridazines indicated that the 6-(methoxybenzyl-amino) compounds were in general more effective in the displacement of [^{3}H]diazepam than imidazo[1,2-*b*]-pyridazines with other substituents at the 6-position.

In the work described in this chapter the study has been extended to 2-aryl-3-methoxy-6-(3′-methoxy-, 3′,4′-dimethoxy-, 3′,4′-methylenedioxy- and 2′- and 4′-chlorobenzylamino)imidazo[1,2-*b*]pyridazines to examine the electronic (and other) effects of substituents in both the benzylamino group and the 2-aryl group; and also to the 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-phenyl (substituted phenyl or pyridinyl)imidazo[1,2-*b*]pyridazines to examine the steric and electronic effects of *N*-methylation and of substitution in the 2-aryl group. The results from this

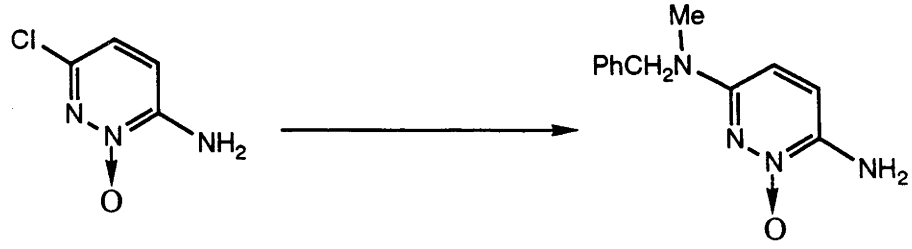
study have been compared with binding activities exhibited by derivatives of 3-methoxy-6-(methoxybenzylamino)imidazo[1,2-*b*]pyridazines reported previously.[121]

In addition, the syntheses and binding affinity of 6-(3′,4′-methylenedioxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine and of some 3-alkoxy-6-(3′-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]-pyridazines have been investigated.

IV-2 Syntheses

The intermediate required for the synthesis of the title compounds in this chapter was 6-chloropyridazin-3-amine 2-oxide which was more reactive towards nucleophilic substitution than the 6-chloropyridazin-3-amine.[121] In 1989, Barlin and coworkers[121] reported the preparation of 6-benzylamino(and methoxybenzylamino)-3-methoxy-2-arylimidazo[1,2-*b*]pyridazines from 6-chloropyridazin-3-amine 2-oxide (**IV.1**). Replacement of the 6-chloro substituent in compound (**IV.1**) by the relevant benzylamine gave the intermediate 6-benzylaminopyridazin-3-amine 2-oxide which when heated with α-bromoacetyl compounds such as α-bromoacetophenone in ethanol gave the corresponding 2-phenylimidazo[1,2-*b*]pyridazin-3-ols (such as **IV.3**). These compounds were treated directly with diazomethane and gave the corresponding 3-methoxy compounds.

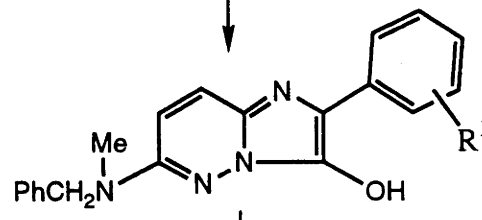
Scheme IV-1



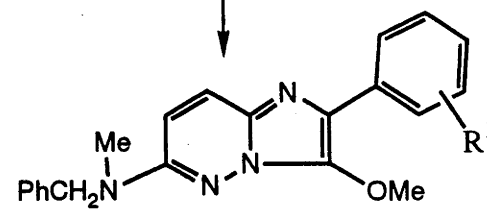
IV.1

IV.2

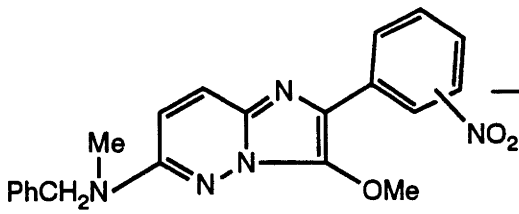
IV.4	R^1
a	H
b	NO_2-*m*
c	NO_2-*p*
d	F-*o*
e	F-*m*
f	F-*p*
g	Cl-*o*
h	Cl-*p*
i	CF_3-*m*
j	CF_3-*p*
k	Me-*o*
l	Me-*m*
m	Me-p
n	OMe-*p*
o	OCH_2O-*3',4'*
p	NH_2-*m*
q	NH_2-*p*
r	C_5H_4N-β*
s	C_5H_4N-γ*



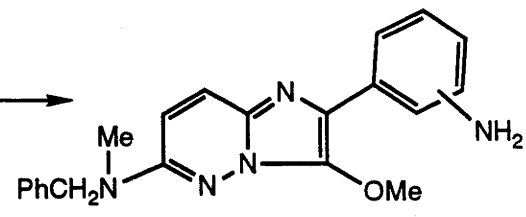
IV.3



IV.4



IV.4 b,c



IV.4 p,q

* Pyridinyl (not substituted phenyl)

IV-2.1 Syntheses of some 6-(N-benzyl-N-methylamino)-3-methoxy-2-arylimidazo[1,2-b]pyridazines

6-Chloropyridazin-3-amine 2-oxide was found to react with excess *N*-benzyl-*N*-methylamine in a sealed reaction vessel at 170°C for 16 h to give 6-(*N*-benzyl-*N*-methylamino)pyridazin-3-amine 2-oxide (**IV.2**) in 50% yield. The latter, when heated with α-bromoacetyl compounds such as α-bromoacetophenone in ethanol, gave the 6-(*N*-benzyl-*N*-methylamino)-2-phenylimidazo[1,2-*b*]pyridazin-3-ols (**IV.3**). Compounds (**IV.3**) were methylated directly with diazomethane and gave corresponding methoxy derivatives (**IV.4**) in 3-58% yield.

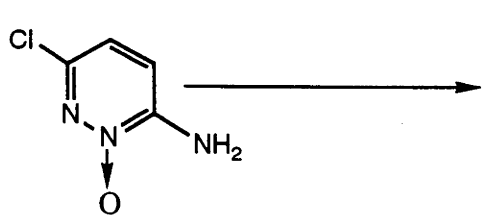
The 2-(pyridinyl) compound [**IV.4r** and **s**] were prepared in low yield by an analogous method.

The nitro compounds (**IV.4b** and **c**) were reduced by iron powder in aqueous methanolic hydrochloric acid to the corresponding amino compounds (**IV.4p** and **q**).

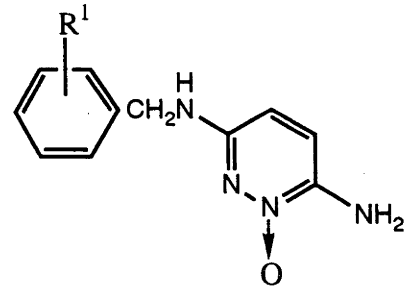
IV-2.2 Syntheses of some 6-(substituted benzylamino)-3-methoxy-2-(substituted phenyl and pyridinyl)imidazo-[1,2-b]pyridazines

The general method for the synthesis of the compounds (**IV.5**) was described by Barlin and coworkers.[121] In an analogous manner, 6-chloropyridazin-3-amine 2-oxide was heated at 142-145°C for 12-40 h with 2-chlorobenzylamine, 4-chlorobenzylamine and 3,4-methylenedioxybenzylamine, respectively, to give compounds (**IV.5**). These compounds (**IV.5**), with α-bromoacetyl compounds (as above), underwent cyclisation to give the hydroxy compounds (**IV.6**) which were

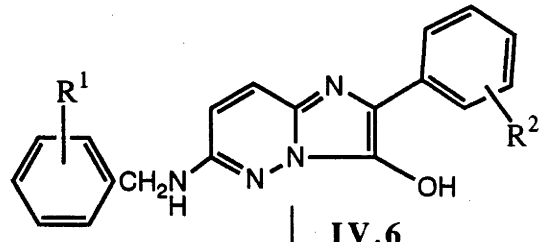
Scheme IV-2



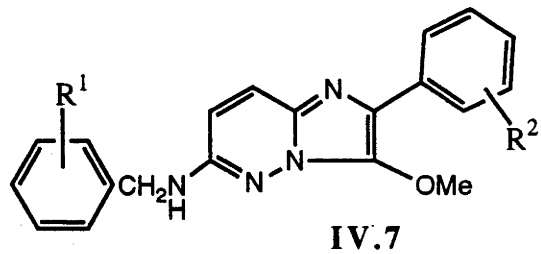
IV.1



IV.5



IV.6



IV.7

IV.7	R^1	R^2
a	OMe-*m*	OMe-*p*
b	OMe-*m*	Cl-*o*
c	OMe-*m*	Cl-*m*
d	OMe-*m*	Cl-*p*
e	OMe-*m*	OCH_2O-*3'',4''*
f	$(OMe)_2$-*m,p*	Me-*p*
g	$(OMe)_2$-*m,p*	OCH_2O-*3'',4''*
h	OCH_2O-*3',4'*	OCH_2O-*3'',4''*
i	Cl-*o*	Me-*p*
j	Cl-*o*	Cl-*p*
k	Cl-*o*	NO_2-*m*
l	Cl-*o*	NO_2-*p*
m	Cl-*o*	C_5H_4N–β*
n	Cl-*p*	H
o	Cl-*p*	Me-*p*
p	Cl-*p*	OCH_2O-*3'',4''*

*Pyridin-3-yl (not substituted phenyl)

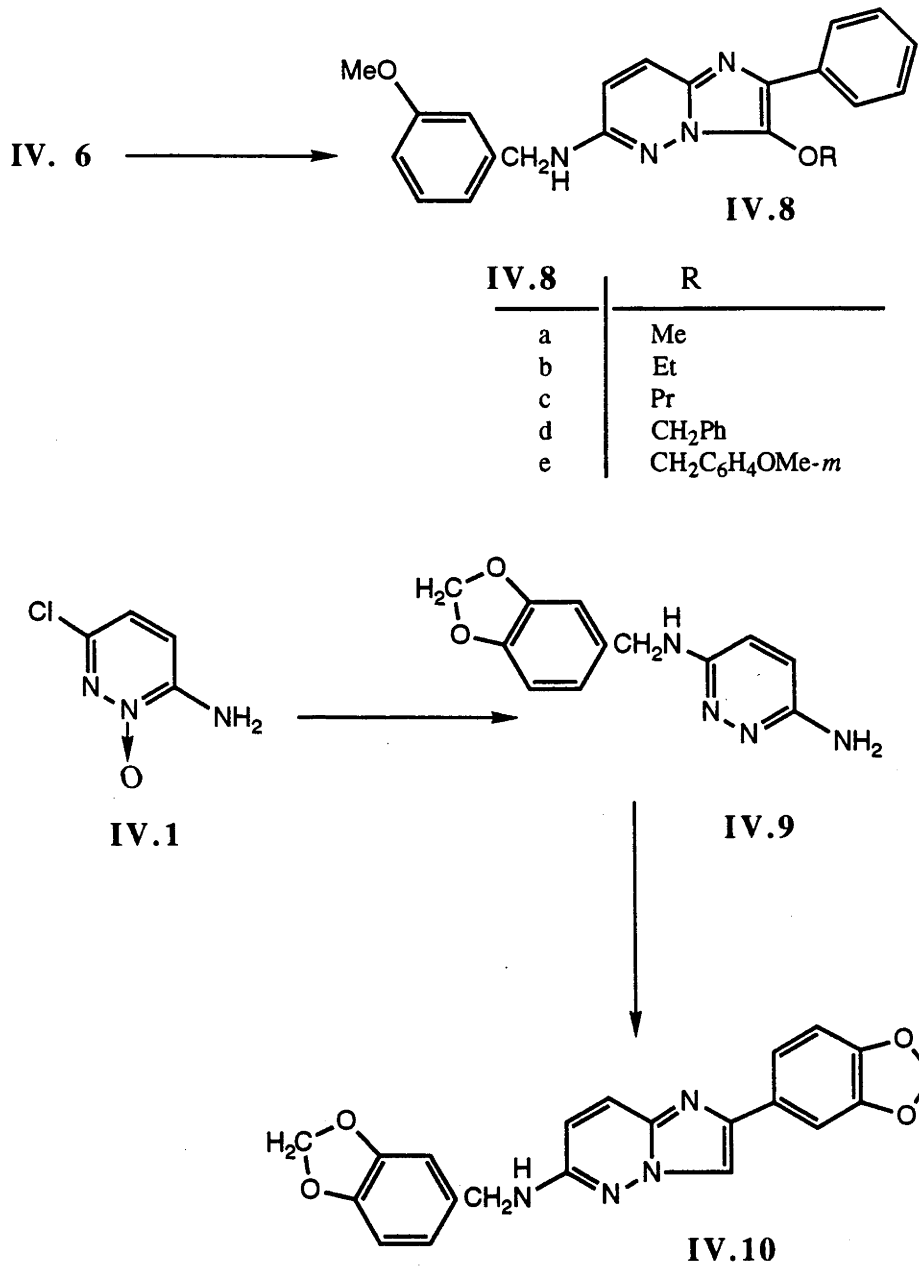
subsequently methylated with diazomothane to give the methoxy compounds (**IV.7a-p**).

6-(3′-Methoxybenzylamino)-3-(3"-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (**IV.8e**) was also prepared from 6-(3′-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]-pyridazin-3-ol by heating with 3-methoxybenzyl chloride in dimethylformamide at 100°.

IV-2.3 Synthesis of 6-(3′,4′-methylenedioxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-b]pyridazine

6-(Benzylamino)pyridazin-3-amine 2-oxides have generally been prepared from 6-chloropyridazin-3-amine 2-oxides and the appropriate benzylamine by heating at 160° for 16-20 h.[121] However, when 6-chloropyridazin-3-amine 2-oxide was heated with excess 3,4-methylenedioxybenzylamine at 160° for 14 h it gave, as an oil, 6-(3′,4′-methylenedioxybenzylamino)pyridazin-3-amine (**IV.9**) by replacement of the 6-chloro substituent and removal of the *N*-oxide group, perhaps by intermolecular *N*-oxidation. This product with α-bromo-3,4-methylenedioxyacetophenone gave 6-(3′,4′-methylenedioxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine (**IV.10**) as the hydrobromide.

Scheme IV-3


IV. 6
MeO
CH2N
H
N
N
N
OR
IV.8
IV.8 | R
a | Me
b | Et
c | Pr
d | CH2Ph
e | CH2C6H4OMe-m
Cl
N
N
O
NH2
IV.1
H2C
O
O
CH2N
H
N
N
NH2
IV.9
H2C
O
O
CH2N
H
N
N
O
CH2
O
IV.10

IV-3 ^{1}H n.m.r. spectra

The ^{1}H n.m.r. spectral data of some 2-aryl-6-(*N*-benzyl-*N*-methylamino)-3-methoxyimidazo[1,2-*b*]pyridazines (**IV.4**) and 2-aryl-3-methoxy-6-(substituted benzylamino)-imidazo[1,2-*b*]pyridazines (**IV.7**) are reported in Tables IV-1 and IV-2, respectively, and may be compared with those for some 2-aryl-3-methoxy-6-(methoxybenzylamino)imidazo-[1,2-*b*]pyridazines reported[121] previously. The signal due to the 3-methoxy group in compounds (**IV.4** and **IV.7**) appeared in the range δ4.00-4.15 except for the 2-(2′-tolyl) compound (**IV.4k**, δ3.92) and the 2-(2′-chlorophenyl) compound (**IV.7b**, δ3.98). Generally downfield shifts were observed in this signal with increased electron-withdrawing power of the substituent in the 2-aryl group (with due allowance for positional effects). This is probably due to extended conjugation to the C_2-C_3 double bond.

The chemical shifts for the protons of the *N*-methyl group of compounds (**IV.4**) occurred in the range δ3.13-3.22, and the signals for protons of the benzylic methylene group in compounds (**IV.4**) are sharp singlets in the range δ4.69-4.78. However, those of the benzylic methylene group in compounds (**IV.7**) generally appeared as a doublet with a chemical shift in the range δ4.50-4.58, except for the 6-(2′-chlorobenzylamino) compounds (**IV.7i-m**) in which the signal was in the range δ4.70-4.72 possibly due to steric effects. The protons of the methylene group in compounds (**IV.4**) were more deshielded than those in compounds (**IV.7**), and the doublet given by compounds (**IV.7**) collapsed to a

Table IV-1 Some ^{1}H n.m.r. spectral data[A] for 2-aryl-6-(N-benzyl-N-methylamino)-3-methoxy imidazo[1,2-b]pyridazines

IV.4	NMe	3-OMe	CH_2	H7	H8
a	3.18	4.07	4.74	6.66	7.60
b	3.22	4.15	4.78	6.75	7.65
c	3.22	4.14	4.78	6.78	7.67
d	3.14	4.06	4.71	6.65	7.60
e	3.16	4.08	4.72	6.66	7.57
f	3.16	4.06	4.73	6.65	7.57
g	3.18	4.01	4.75	6.68	7.61
h	3.19	4.07	4.76	6.69	7.60
i	3.20	4.10	4.76	6.71	7.61
j	3.22	4.11	4.78	6.78	7.73
k	3.18	3.92	4.75	6.67	7.60
l	3.18	4.07	4.74	6.66	7.61
m	3.13	4.04	4.69	6.60	7.55
n	3.17	4.06	4.74	6.64	7.58
o	3.16	4.05	4.73	6.64	7.56
p	3.15	4.05	4.72	6.63	7.58
q[B]	3.17	4.05	4.75	6.65	7.62
r[C]	3.21	4.11	4.77	6.72	7.62
s	3.20	4.14	4.76	6.75	7.60

[A]Reported as parts per million (δ downfield from tetramethylsilane as internal standard in deuterochloroform

[B]Pyridin-2-yl group (not substituted phenyl)

[C]Pyridin-3-yl group (not substituted phenyl)

singlet on addition of deuterium oxide thus confirming coupling between the benzylic methylene and the amino proton in compounds (**IV.7**). In general, the amino proton in compounds (**IV.7**) appeared as a broad triplet, with a chemical shift of δ4.65-5.24.

The protons on C-7 and C-8 of the compounds (**IV.4** and **IV.7**) appeared as an AB quartet with a coupling constant of $J_{7,8}$ 9.5 Hz. The chemical shifts of these protons in compounds (**IV.4**) are in the range δ6.60-6.78 and 7.55-7.67 and in compounds (**IV.7**) the signals for H7 and H8 were in

the range δ6.20-6.47 and 7.49-7.64, respectively. The signal due to H-7 in compounds (**IV.4**) was downfield of that in compounds (**IV.7**). The downfield shift of H-7 in compounds (**IV.4**) is probably due to decreased conjugation of the lone pair on nitrogen across the pyridazine ring due to the steric effects in the *N*-methyl compounds.

The aromatic protons of the phenyl substituents on C-2 and C-6 of the imidazo[1,2-*b*]pyridazine ring usually appeared complex. However, the assignments for these protons in para-substituted phenyl groups at the 2-position in the compounds (**IV.4**) were readily carried out.

Table IV-2 Some ^{1}H n.m.r. spectral data[A] for 2-aryl-3-methoxy-6-(substituted benzylamino)imidazo[1,2-b]-pyridazines

IV.7	3-OMe	CH_2[B]	NH[C]	H7	H8
a	4.04	4.58	4.73	6.36	7.55
b	3.98	4.58	4.88	6.20	7.58
c	4.08	4.58	5.03	6.47	7.64
d	4.04	4.56	4.91	6.39	7.52
e	4.04	4.57	4.86	6.43	D
f	4.08	4.55	-	6.37	7.58
g	4.08	4.55	-	6.36	7.56
h	4.07	4.50	4.65	6.36	7.54
i	4.05	4.70	4.83	6.40	7.60
j	4.05	4.70	4.92	6.42	7.57
k	4.10	4.70	5.24	6.47	7.53
l	4.13	4.72	5.01	6.47	7.57
m[E]	4.08	4.72	5.11	6.44	7.56
n	4.01	4.53	5.09	6.36	7.49
o	4.00	4.56	4.91	6.36	7.52
p	4.00	4.57	4.80	6.36	7.53

[A]Reported as parts per million (δ) downfield from tetramethylsilane as internal standard in deuterochloroform
[B]Doublet
[C]Broad triplet
[D]H8 complex
[E]Pyridin-3-yl

IV-4 *In vitro* binding studies

The binding studies on the compounds prepared in this chapter were carried out using the [^{3}H]diazepam binding assay as outlined in Chapter II-5.3.

IV-4.1 Results of [^{3}H]diazepam assay

The results for the *in vitro* displacement studies by the compounds (**IV.4** and **IV.7**) are shown in Table IV-3 as IC_{50} values (or per cent displacement) at the concentration specified in the presence of 100 μM γ-aminobutyric acid (GABA).

IV-4.2 Discussion of results

The results are recorded (in Table IV-3) from investigations of the displacement of [^{3}H]diazepam from rat brain membranes by the 6-(*N*-benzyl-*N*-methylamino)-imidazo[1,2-*b*]pyridazines (**IV.4a-s**), the 6-(substituted benzylamino)imidazo[1,2-*b*]pyridazines (**IV.7a-p**) and 6-(3′,4′-methylenedioxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine (**IV.10**) prepared in this chapter.

Data for some 6-(benzylamino and methoxybenzylamino)-3-alkoxy-2-phenylimidazo[1,2-*b*]pyridazines reported[121] previously are also given in Table IV-3 for comparison.

Table IV-3 Results for the displacement of [^{3}H]diazepam from its specific binding sites in rat brain preparation by some 6-(N-benzyl-N-methylamino) and 6-(substituted benzylamino)imidazo[1,2-b]pyridazines and some reference compounds

Formula number	Substituted imidazo[1,2-*b*]pyridazine	IC_{50} (nM)[A] (or % displ.)[B]
	6-$NHCH_2$Ph-3-OMe-2-Ph	9[C]
IV.4a	6-$NMeCH_2$Ph-3-OMe-2-Ph	18.9
b	6-$NMeCH_2$Ph-3-OMe-2-$C_6H_4NO_2$-*m*	166
c	6-$NMeCH_2$Ph-3-OMe-2-$C_6H_4NO_2$-*p*	>>300
d	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4F-*o*	9.8
e	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4F-*m*	20.7
f	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4F-*p*	33
g	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4Cl-*o*	(74%)
h	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4Cl-*p*	329
i	6-$NMeCH_2$Ph-3-OMe-2-$C_6H_4CF_3$-*m*	683
j	6-$NMeCH_2$Ph-3-OMe-2-$C_6H_4CF_3$-*p*	(30%)
k	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4Me-*o*	(57%)
l	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4Me-*m*	111
m	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4Me-*p*	266
n	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4OMe-*p*	152
o	6-$NMeCH_2$Ph-3-OMe-2-C_6H_3(3′,4′-OCH_2O-)	46
p	6-$NMeCH_2$Ph-3-OMe-2-$C_6H_4NH_2$-*m*	11.3
q	6-$NMeCH_2$Ph-3-OMe-2-$C_6H_4NH_2$-*p*	187
r	6-$NMeCH_2$Ph-3-OMe-2-C_5H_4N-β	69
s	6-$NMeCH_2$Ph-3-OMe-2-C_6H_4N-γ	(15.8%)
IV.7a	6-$NHCH_2C_6H_4OMe$-*m*-3-OMe-2-C_6H_4OMe-*p*	8
b	6-$NHCH_2C_6H_4OMe$-*m*-3-OMe-2-C_6H_4Cl-*o*	37
c	6-$NHCH_2C_6H_4OMe$-*m*-3-OMe-2-C_6H_4Cl-*m*	17
d	6-$NHCH_2C_6H_4OMe$-*m*-3-OMe-2-C_6H_4Cl-*p*	9
e	6-$NHCH_2C_6H_4OMe$-*m*-3-OMe-2-C_6H_3(3″,4″-OCH_2O-)	1.5
f	6-$NHCH_2C_6H_3(OMe)_2$-3′,4′-3-OMe-2-C_6H_4Me-*p*	5
g	6-$NHCH_2C_6H_3(OMe)_2$-3′,4′-3-OMe-2-C_6H_3-(3″,4″-OCH_2O-)	2
h	6-$NHCH_2C_6H_3$(3′,4′-OCH_2O-)-3-OMe-2-C_6H_3-(3″,4″-OCH_2O-)	1

Table IV-3 Continued

Formula number	Substituted imidazo[1,2-*b*]pyridazine	IC_{50} (nM)[A] (or % displ.)[B]
i	6-$NHCH_2C_6H_4Cl$-*o*-3-OMe-2-C_6H_4Me-*p*	120
j	6-$NHCH_2C_6H_4Cl$-*o*-3-OMe-2-C_6H_4Cl-*p*	114
k	6-$NHCH_2C_6H_4Cl$-*o*-3-OMe-2-$C_6H_4NO_2$-*m*	112
l	6-$NHCH_2C_6H_4Cl$-*o*-3-OMe-2-$C_6H_4NO_2$-*p*	(25%)[D]
m	6-$NHCH_2C_6H_4Cl$-*o*-3-OMe-2-C_6H_4N-β	20
n	6-$NHCH_2C_6H_4Cl$-*p*-3-OMe-2-Ph	
o	6-$NHCH_2C_6H_4Cl$-*p*-3-OMe-2-C_6H_4Me-*p*	38
p	6-$NHCH_2C_6H_4Cl$-*p*-3-OMe-2-C_6H_3(3",4"-OCH_2O-)	10
IV.10	6-$NHCH_2C_6H_3$(3',4'-OCH_2O-)-3-H-2-C_6H_3-(3",4"-OCH_2O-)	8
IV.8a	6-$NHCH_2C_6H_4OMe$-*m*-3-OMe-2-Ph	2.9[C]
b	6-$NHCH_2C_6H_4OMe$-*m*-3-OEt-2-Ph	5[E]
c	6-$NHCH_2C_6H_4OMe$-*m*-3-OPr-2-Ph	120[E]
d	6-$NHCH_2C_6H_4OMe$-*m*-3-OCH_2Ph-2-Ph	(8%)[E]
e	6-$NHCH_2C_6H_4OMe$-*m*-3-$OCH_2C_6H_4OMe$-*m*-2-Ph	(0%)

[A] IC_{50} values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparations
[B] At 1000 nM unless specified otherwise
[C] Ref. 121
[D] At 30 nM
[E] Kindly prepared by Mr S.J. Ireland

i Discussion of binding results for the 2-aryl-6-(N-benzyl-N-methylamino)-3-methoxyimidazo[1,2-b]pyridazines

An examination of the results for the 6-(*N*-benzyl-*N*-methylamino)imidazo[1,2-*b*]pyridazines reveals that 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-phenylimidazo[1,2-*b*]-pyridazine (**IV.4a**; IC_{50} 18.9 nM) was *ca* half as active as its *N*-demethyl analogue, 6-benzylamino-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine (IC_{50} 9 nM).[121] The lower potency for the 6-(*N*-benzyl-*N*-methylamino) derivative may be due to

the steric hindrance by the *N*-methyl substituent which limits interaction of the compound (**IV.4a**) with the benzodiazepine receptor relative to the corresponding 6-benzylamino analogue; or it may indicate that the N-H group of the 6-benzylamino compounds interacts with the receptor, for example by forming hydrogen bonds. In both of these circumstances the 6-benzylamino compounds would be expected to bind more strongly than their *N*-methyl derivatives. However, it should be noted that 3-methoxy-6-phenethylamino-2-phenylimidazo[1,2-*b*]pyridazine[121] (IC_{50} 800 nM) binds much less strongly than its *N*-methyl derivative.

The effects of substitution in the 2-phenyl substituent of 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**IV.4a**; IC_{50} 18.9 nM) with electron-donating or electron-withdrawing groups have been examined. Strong electron-withdrawing groups such as *para*-nitro (σ_{para} = 0.78)[226] and *para*-trifluoromethyl decreased binding activity. For example, a comparison of 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-(4′-nitrophenyl)imidazo-[1,2-*b*]pyridazine (**IV.4c**; IC_{50} >>300 nM) with the parent compound (**IV.4a**; IC_{50} 18.9 nM) reveals that the *para*-nitro compound is significantly less active. This effect is greater than in 6-(3′-methoxybenzylamino)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**IV.8a**; IC_{50} 2.9 nM) and its *para*-nitro derivative,[121] 6-(3′-methoxybenzylamino)-3-methoxy-2-(4"-nitrophenyl)imidazo[1,2-*b*]pyridazine (IC_{50} 13 nM), but less than in 6-(2′-methoxybenzylamino)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (IC_{50} 1.8 nM)[121] and its *para*-nitro derivative (20.6% displacement at 10 nM).[121]

The receptor site appears to tolerate the small electron-withdrawing fluoro substituent at 2′-position in the 2-phenyl ring. For example, the effect of a fluoro substituent in the 6-(*N*-benzyl-*N*-methylamino)-2-(2′-,3′- and 4′-fluorophenyl)-3-methoxyimidazo[1,2-*b*]pyridazines (**IV.4d-f**; IC_{50} values 9.8, 20.7 and 33 nM, respectively) was to increase activity in the 2-(2′-fluorophenyl) compound relative to the parent 2-phenyl compound (**IV.4a**; IC_{50} 18.9 nM) but to decrease activity slightly in the 2-(3′- and 4′-fluorophenyl) isomers. In a comparison of the 6-(3′-methoxybenzylamino)-3-methoxy-2-(2"-,3"- and 4"-fluorophenyl)imidazo[1,2-b]pyridazines[121] with their parent compounds, the 2"- and 4"-fluoro-substituents enhanced activity by *ca* two-fold but the 3"-fluoro-substituent decreased activity by *ca* five-fold.[121] The 2-(4′-fluorophenyl) compound (**IV.4f**; IC_{50} 33 nM) bound more strongly than the 2-(4′-chlorophenyl) analogue (**IV.4h**, IC_{50} 329 nM).

The 2′- and 4′-chloro substituent in compounds (**IV.4g**, 74% displacement at 1000 nM; and **IV.4h**, IC_{50} 329 nM) decreased binding significantly relative to the 2-phenyl compound (**IV.4a**; IC_{50} 18.9 nM). A similar situation prevailed with 2-(4′-chlorophenyl)-3-methoxy-6-(2′-methoxybenzylamino)imidazo[1,2-*b*]pyridazine (IC_{50} 16 nM) relative to its 2-phenyl analogue (IC_{50} 1.8 nM),[121] and likewise the 3′- and 4′-trifluoromethyl compounds (**IV.4i**; IC_{50} 683 nM and **IV.4j**; 30% displacement at 1000 nM respectively) exerted considerable deactivating effects

which were greater than in the 6-(3′-methoxybenzylamino) compounds (IC_{50} values 55 and 24 nM).[121]

Electron-donating groups present in the 2-phenyl substituent of 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-(2′- 3′- and 4′-tolyl), 2-(4′-methoxyphenyl) and 2-(3′,4′-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazines (**IV.4k-o**) decreased binding ability relative to the 2-(unsubstituted phenyl) compound (**IV.4a**), but the 3′,4′-methylenedioxy group in compound (**IV.4o**; IC_{50} 46 nM) was less deactivating than a 4′-methyl or 4′-methoxy group as in compound (**IV.4m**; IC_{50} 266 nM or **IV.4n**; IC_{50} 152 nM).

2-(3′-Aminophenyl)-6-(*N*-benzyl-*N*-methylamino)-3-methoxyimidazo[1,2-*b*]pyridazine (**IV.4p**; IC_{50} 11.3 nM) was *ca* twice as active as the parent 2-phenyl analogue (**IV.4a**; IC_{50} 18.9 nM) but in the 2-(4′-aminophenyl) isomer (**IV.4q**; IC_{50} 187 nM), the binding ability was decreased ten-fold. However, the 6-(3′-methoxybenzylamino)-3-methoxy-2-(3"- and 4"-aminophenyl)imidazo[1,2-*b*]pyridazines (IC_{50} values 1.8 and 1.0 nM respectively) were both more active than the 2-(unsubstituted phenyl) analogue (IC_{50} 2.9 nM).[121]

The 2-(3′- and 4′-aminophenyl) compounds (**IV.4p**; IC_{50} 11.3 nM and **IV.4q**; IC_{50} 187 nM) were significantly more active than the corresponding nitro compounds (**IV.4b**; IC_{50} 166 nM and **IV.4c**; IC_{50} >>300 nM).

Replacement of the 2-phenyl group of 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**IV.4a**) with a pyridin-3-yl or pyridin-4-yl group resulted in a reduction in binding potency [as with the 2-(3′- and

4′-nitro or 3′- and 4′-trifluoromethyl) analogues] and these compounds (**IV.4r**; IC_{50} 69 nM and **IV.4s**; 15.8% displacement at 1000 nM) were less active than the 2-(3′- and 4′-aminophenyl) analogues (**IV.4p**; IC_{50} 11.3 nM and **IV.4q**; IC_{50} 187 nM), respectively. In the 6-(2′- and 3′-methoxybenzylamino)-3-methoxy-2-[pyridin-3"-(and 4"-)yl] imidazo[1,2-*b*]pyridazines[121] the 2-(pyridin-3"-yl) compounds (IC_{50} values 3 and 2.1 nM, respectively) had approximately the same activity as the 2-phenyl analogues (IC_{50} values 1.8 and 2.9 nM, respectively) but the 3-methoxy-6-(3′-methoxybenzylamino)-2-(pyridin-4"-yl) compound (GBLD 258; IC_{50} 5.8 nM) was less active.[121]

In the series of 6-(*N*-benzyl-*N*-methylamino) compounds reported here it was found that the effect of *para*-substituents in the 2-phenyl group was a lower binding affinity for benzodazepine receptors than that for the corresponding *meta*-substituted isomers.

ii Discussion of binding results for the 3-(alkoxy and unsubstituted)-2-aryl-6-(substituted benzylamino)-imidazo[1,2-b]pyridazines

The effect of substitution by methoxy groups in the 6-benzylamino substituent of 2-aryl-6-benzylamino-3-methoxy-imidazo[1,2-*b*]pyridazines has been investigated previously[121] and we have now examined the effect of some substituents in the 2-aryl group. The activity of 3-methoxy-6-(3′-methoxybenzylamino)-2-(4"-methoxyphenyl)-imidazo[1,2-*b*]pyridazine (**IV.7a**; IC_{50} 8 nM) was *ca* half that of 3-methoxy-6-(3′-methoxybenzylamino)-2-phenyl-

imidazo[1,2-*b*]pyridazine (**IV.8a**; IC_{50} 2.9 nM).[121] A similar situation existed with the 6-benzylthio[164] (and 6-benzyloxy)[166]-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazines and their 2-(4′-methoxyphenyl) analogues, but in the 6-(3′-methoxybenzylthio) analogues[165] the 2-phenyl and 2-(4′-methoxyphenyl) compounds (GBLD 190 and GBLD 205) both had IC_{50} 11 nM. A comparison of 2-(4′-methoxyphenyl) with 2-(4′-tolyl) groups revealed that compound (**IV.7a**; IC_{50} 8 nM) was *ca* 2.5 fold less active than 3-methoxy-6-(3′-methoxybenzylamino)-2-(4"-tolyl)imidazo[1,2-*b*]pyridazine (GBLD 308; IC_{50} 3.2 nM)[121] whereas in the 6-(*N*-benzyl-*N*-methylamino) compounds described above, compound (**IV.4n**; IC_{50} 152 nM) was twice as active as its 2-(4′-tolyl) analogue (**IV.4m**; IC_{50} 266).

An examination of the activity of the 2-(chlorophenyl) compounds (**IV.7b,c,d**; IC_{50} values 37, 17 and 9 nM, respectively), revealed the 2-(4′-chlorophenyl) isomer to be the more active [as with the 6-(benzylthio)-2-(2′- and 4′-chlorophenyl)-3-methoxyimidazo[1,2-*b*]pyridazines[164] (GBLD 148 and 147; IC_{50} values 198 and 48 nM, respectively)].

The 6-(3′-methoxybenzylamino) compound (**IV.7d**; IC_{50} 9 nM) was also more active than 2-(4′-chlorophenyl)-3-methoxy-6-(2′-methoxybenzylamino)imidazo[1,2-*b*]pyridazine (GBLD 309; IC_{50} 16 nM).[121]

The results for the chloro compounds differ from those for the fluoro analogues, 2-(2′-,3′- and 4′-fluorophenyl)-3-methoxy-6-(3"-methoxybenzylamino)imidazo[1,2-*b*]-pyridazines (GBLD 283, 216 and 274; IC_{50} values 1.5, 15 and

1.5 nM respectively), in which the 2′- and 4′-fluoro isomers were equally active. In all cases the 2-(chlorophenyl) compounds were significantly less active than the 2-(fluorophenyl) analogues. Amongst the 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-(2′-,3′- and 4′-fluorophenyl)imidazo[1,2-*b*]pyridazines (**IV.4d-f**; IC_{50} values 9.8, 20.7 and 33 nM, respectively), the 2-(2′-fluorophenyl) isomer was the most active. The 3′,4′-methylenedioxy group in the aromatic substituent at the 2-position has been observed previously[122] to engender significant activation. Similarly 3-methoxy-6-(3′-methoxybenzylamino)-2-(3",4"-methylenedioxyphenyl)-imidazo[1,2-*b*]pyridazine (**IV.7e**; IC_{50} 1.5 nM) was found to be *ca* twice as active as its 2-phenyl or 2-(4′-tolyl) analogues (GBLD 231 and 308; IC_{50} values 2.9 and 3.2 nM)[121] and it was *ca* five-fold more active than its 2-(4′-methoxyphenyl) analogue (**IV.7a**). A similar situation exists in the corresponding 6-(2′-methoxyphenyl) compounds[122] but its effect was slightly greater in the foregoing example. However, in the 6-(*N*-benzyl-*N*-methylamino) compounds (**IV.4a, m-o**; IC_{50} values 18.9, 266, 152 and 46 nM, respectively), the effect of inserting a 4′-methyl, 4′-methoxy, or 3′,4′-methylenedioxy group in the 2-phenyl substituent was deactivating.

In the 6-(3′,4′-dimethoxybenzylamino)-3-methoxy-2-(substituted phenyl)imidazo[1,2-*b*]pyridazines, a 2-(4"-tolyl)substituent as in compound (**IV.7f**; IC_{50} 5 nM) was deactivating relative to its 2-phenyl analogue (GBLD 253; IC_{50} 3.4 nM);[121] a similar situation applied between 3-

methoxy-6-(3′-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]-pyridazine and its 2-(4"-tolyl) analogue.[121] The activity of 6-(3′,4′-dimethoxybenzylamino)-3-methoxy-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine (**IV.7g**; IC_{50} 2 nM) was found to be greater than that of the 2-(4′-tolyl) analogue (**IV.7f**; IC_{50} 5 nM).

Comparison of the 6-(3′,4′-dimethoxybenzylamino) substituent in compound (**IV.7g**; IC_{50} 2 nM) with a 6-(3′,4′-methylenedioxybenzylamino) group in compound (**IV.7h**; IC_{50} 1 nM) revealed that the methylenedioxy compound bound more strongly.

Whereas the effect of an electron-donating methoxy group in 6-(2′- or 3′-methoxybenzylamino) compounds increased binding ability,[121] an electron-withdrawing chloro substituent in the 6-(2′- or 4′-chlorobenzylamino) analogues (**IV.7**) was to decrease binding ability. 6-(4′-Chlorobenzylamino)-3-methoxy-2-(4"-tolyl)imidazo-[1,2-*b*]pyridazine (**IV.7o**; IC_{50} 38 nM) was more active than the 6-(2′-chlorobenzylamino) isomer (**IV.7i**; IC_{50} 120 nM) by *ca* three-fold. In the 6-(4′-chlorobenzylamino) compounds (**IV.7o** and **p**; IC_{50} values 38 and 10 nM respectively), 6-(4′-chlorobenzylamino)-3-methoxy-2-(3",4"-methyl-enedioxyphenyl)imidazo[1,2-*b*]pyridazine (**IV.7p**) bound more strongly by *ca* four-fold. There was little difference in the activity of the 2-(4′-tolyl), 2-(4′-chlorophenyl) and 2-(3′-nitrophenyl) compounds (**IV.7i-k**; IC_{50} values 120, 114 and 112 nM, respectively), but the 2-(pyridin-3′-yl) compound (**IV.7m**; IC_{50} 20 nM) was significantly more active in binding.

The effect of replacing the 3-methoxy group of 3-methoxy-6-(3′-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]-pyridazine[121] (**IV.8a**; IC_{50} 2.9 nM) with other alkoxy substituents was also examined. The results of *in vitro* displacement by compounds (**IV.8a-e**; IC_{50} values 2.9, 5, 120 nM and 8% and 0% displacements at 1000 nM, respectively) showed that stronger binding occurred with the smaller 3-methoxy group.

Comparison of 6-(3′,4′-methylenedioxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine (**IV.10**; IC_{50} 8 nM) with its 3-methoxy analogue (**IV.7h**; IC_{50} 1 nM) revealed that the 3-unsubstituted compound (**IV.10**) was less active by eight-fold but compound (**IV.10**) was significantly more active than the 3-unsubstituted compounds described in Chapter II.

IV-5 Experimental

The general procedure, and experimental details for the [^{3}H]diazepam binding assay, are recorded in Chapter III-5.3, except that analytical samples were dried at 60-90°0/0.1 mmHg for 5-20 h unless otherwise specified.

6-(N-Benzyl-N-methylamino)pyridazin-3-amine 2-oxide (IV.2)

6-Chloropyridazin-3-amine 2-oxide[243] (0.4 g) and *N*-benzyl-*N*-methylamine (4.0 ml) were heated in a screw-top Teflon-lined stainless steel reaction vessel at 170° for 16 h. After cooling the reaction mixture was diluted with ether and the ether solution decanted from the thick oil which was washed with more ether. The oil was dissolved in chloroform and chromatographed over a column of alumina. It was eluted firstly with chloroform and then with a mixture of methanol in chloroform (1:9). The yellow band which was eluted in the latter gave a solid which was recrystallised from acetone to give yellow crystals of the *title compound* (0.316 g), m.p. 171-172° (Found: C, 62.3; H, 6.3; N, 24.2. $C_{12}H_{14}N_4O$ requires C, 62.6; H, 6.1; N, 24.3%). ^{1}H n.m.r.: δ 3.06, s, MeN; 4.64, s, CH_2N; 6.52, d, 7.10, d, J 9 Hz, H 4,5; 7.23, complex, Ph.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-phenylimidazo-[1,2-b]pyridazine (IV.4a)

A mixture of 6-(*N*-benzyl-*N*-methylamino)pyridazin-3-amine 2-oxide (0.189 g), phenacyl bromide (0.205 g) and

ethanol (9.0 ml) was refluxed with stirring for 4 h. The solvent was evaporated and the solid residue stirred with an ethereal solution of diazomethane (from 2.5 g nitrosomethylurea) at 0° and then at room temperature overnight. The mixture was evaporated to dryness and the residue subjected to t.l.c. (alumina; chloroform) and the product (0.015 g) recrystallised from a mixture of ethanol and light petroleum to give the *title compound*, m.p. 146-147° (Found: C, 72.8; H, 6.1; N, 16.1. $C_{21}H_{20}N_4O$ requires C, 73.2; H, 5.9; N, 16.3%). ^{1}H n.m.r.: δ 3.18, s, MeN; 4.08, s, MeO; 4.74, s, CH_2N; 6.72, d, J 9.5 Hz, H 7; 7.30-8.14, complex, 2 x Ph; 7.65, d, J 9 Hz, H 8.

In a similar manner the following compounds were prepared from 6-(*N*-benzyl-*N*-methylamino)pyridazin-3-amine 2-oxide[243] and α-bromo-2-fluoroacetophenone,[165] α-bromo-3(and 4)-fluoroacetophenone,[121,244] α-bromo-2(and 4)-chloroacetophenone,[244] α-bromo-3(and 4)-trifluoromethylacetophenone,[165] α-bromo-2(3 and 4)-methylacetophenone (prepared from the corresponding methylacetophenones by bromination in ether[232,245]) α-bromo-4-methoxyacetophenone,[244] α-bromo-3,4-methylenedioxyacetophenone,[228] 3(and 4)-bromoacetylpyridine (hydrobromides),[165] and α-bromo-3(and 4)-nitroacetophenone.[121]

6-(N-Benzyl-N-methylamino)-2-(2'-fluorophenyl)-3-methoxyimidazo[1,2-b]pyridazine (IV.4d) (53%),as a thick oil after t.l.c. (alumina; chloroform, light petroleum, 3:1) (Found: C, 68.5; H, 5.4; N, 15.7. $C_{21}H_{19}FN_4O$ 0.2 H_2O requires C,

68.7; H, 5.3; N, 15.3%). ^{1}H n.m.r.: δ 3.14, s, MeN; 4.06, s, MeO; 4.71, s, CH_2N; 6.65, d, J 9 Hz, H 7; 7.25-7.83, complex, ArH; 7.60, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-2-(3'-fluorophenyl)-3-methoxy-imidazo[1,2-b]pyridazine (IV.4e) (46%),as an oil after t.l.c. (alumina; chloroform, light petroleum, 3:1) (Found: C, 69.1; H, 5.5; N, 15.7. $C_{21}H_{19}FN_4O$ requires C, 69.6; H, 5.3; N, 15.5%). ^{1}H n.m.r.: δ 3.16, s, MeN; 4.08, s, MeO; 4.72, s, CH_2N; 6.66, d, J 9 Hz, H 7; 6.96-7.87, complex, H 2',4',5',6' and Ph; 7.57, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-2-(4'-fluorophenyl)-3-methoxy-imidazo[1,2-b]pyridazine (IV.4f) (10%), m.p. 108-109° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 69.9; H, 5.5; N, 15.6. $C_{21}H_{19}FN_4O$ requires C, 69.6; H, 5.3; N, 15.5%). ^{1}H n.m.r.: δ 3.17, s, MeN; 4.07, s, MeO; 4.73, s, CH_2N; 6.66, d, J 9 Hz, H 7; 7.00-8.14, complex, H 2',3',5',6' and Ph; 7.57, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-2-(2'-chlorophenyl)-3-methoxy-imidazo[1,2-b]pyridazine (IV.4g) (49%), after t.l.c. (alumina; chloroform, light petroleum, 1:2) (Found: C, 66.9; H, 5.3; N, 14.8. $C_{21}H_{19}ClN_4O$ requires C, 66.6; H, 5.1; N, 14.8%). ^{1}H n.m.r.: δ 3.18, s, MeN; 4.01, s, MeO; 4.75, s, CH_2N; 6.68, d, J 9 Hz, H 7; 7.30-7.60, complex, H 3',4',5',6' and Ph; 7.61, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-2-(4'-chlorophenyl)-3-methoxyimidazo[1,2-b]pyridazine (IV.4h) (31%), m.p. 115-117° after t.l.c. (alumina; chloroform, light petroleum, 1:3) and recrystallisation from light petroleum (Found: C, 66.9; H, 5.0; N, 14.6. $C_{21}H_{19}ClN_4O$ requires C, 66.6; H, 5.1; N, 14.8%). ^{1}H n.m.r.: δ 3.16, s, MeN; 4.31, s, MeO; 4.73, s, CH_2N; 6.66, d, J 9 Hz, H 7; 7.29, s, Ph; 7.37, d, $J_{2',3'}$ 9 Hz, $H_{2',6'}$; 7.57, d, J 9 Hz, H 8; 8.03, d, $J_{2',3'}$ 9 Hz, H3',5'.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(3'-trifluoromethyl)imidazo[1,2-b]pyridazine (IV.4i) (58%) as an oil after t.l.c. (alumina; chloroform) (Found: C, 64.2; H, 4.6; N, 14.0. $C_{22}H_{19}F_3N_4O$ requires C, 64.1; H, 4.6; N, 14.0%). ^{1}H n.m.r.: δ 3.20, s, MeN; 4.10, s, MeO; 4.76, s, CH_2N; 6.71, d, J 9 Hz, H 7; 7.31-8.38, complex, H 2',4',5',6' and Ph; 7.62, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(4'-trifluoromethylphenyl)imidazo[1,2-b]pyridazine (IV.4j) (18%), m.p. 105-107° after t.l.c. (alumina; chloroform, benzene, 2:1) (Found, for a sample dried at 80°/0.1 mmHg for 40 h: C, 64.4; H, 4.7; N, 13.5. $C_{22}H_{19}F_3N_4O$ requires C, 64.1; H, 4.6; N, 13.6%). ^{1}H n.m.r.: δ 3.22, s, MeN; 4.11, s, MeO; 4.78, s, CH_2N; 6.78, d, J 9 Hz, H 7; 7.32, s, 7.74, d, J 9 Hz, H 8; 7.68, d, Ph; 8.23, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(2′-tolyl)imidazo-[1,2-b]pyridazine (IV.4k) (38%) after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 74.0; H, 6.5; N, 15.8. $C_{22}H_{22}N_4O$ requires C, 73.7; H, 6.2; N, 15.6%). ^{1}H n.m.r.: δ 2.45, s, Me; 3.19, s, MeN; 3.92, s, MeO; 4.76, s, CH_2N; 6.67, d, J 9 Hz, H 7; 7.30-7.87, complex, H 3′,4′,5′,6′ and Ph; 7.60, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(3′-tolyl)imidazo-[1,2-b]pyridazine (IV.4l) (39%) after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 73.5; H, 5.9; N, 15.6. $C_{22}H_{22}N_4O$ requires C, 73.7; H, 6.2; N, 15.6%). ^{1}H n.m.r.: δ 2.42, s, MeC; 3.18, s, MeN; 4.07, s, MeO; 4.74, s, CH_2N; 6.66, d, J 9 Hz, H 7; 7.06-7.95, complex, H 2′,4′,5′,6′ and Ph; 7.61, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(4′-tolyl)imidazo-[1,2-b]pyridazine (IV.4m) (44%) as an oil after t.l.c. (alumina; chloroform, light petroleum, 3:1 and alumina; chloroform, benzene, 1:1) (Found, for a sample dried at 80°/0.1 mmHg for 48 h: C, 73.7; H, 6.4. $C_{22}H_{22}N_4O$ requires C, 73.7; H, 6.2%). ^{1}H n.m.r.: δ 2.38, s, MeC; 3.17, s, MeN; 4.06, s, MeO; 4.74, s, CH_2N; 6.65, d, J 9 Hz, H 7; 7.29, s, Ph; 7.24, d, 7.99, d, $J_{2',3'}$ 8 Hz, H 2′,3′,5′,6′; 7.61, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(4′-methoxy-phenyl)imidazo[1,2-b]pyridazine (IV.4p) (24%) as a thick oil after t.l.c. (alumina; chloroform, light petroleum,

2:1) (Found: C, 69.7; H, 5.9, N, 14.7. $C_{22}H_{22}N_4O_2$. 0.2 H_2O requires C, 69.9; H, 6.0; N, 14.8%). ^1H n.m.r.: δ 3.17, s, MeN; 3.84, s, 4′-OMe; 4.06, s, 3-OMe; 4.74, s, CH_2N; 6.64, d, J 9 Hz, H 7; 6.98, d, 8.03, d, $J_{2',3'}$ 7 Hz, H 2′,3′,5′,6′; 7.30, s, Ph; 7.57, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(3′,4′-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (IV.4q) (59%) after t.l.c. (alumina; chloroform, light petroleum, 1:2) (Found: C, 67.7; H, 5.3, N, 14.4. $C_{22}H_{20}N_4O_3$ requires C, 68.0; H, 5.2; N, 14.4%). ^1H n.m.r.: δ 3.16, s, MeN; 4.05, s, MeO; 4.73, s, CH_2N; 5.96,s, OCH_2O; 6.63, d, J 9 Hz, H 7; 7.55, d, J 9 Hz, H 8; 6.83-7.66, complex, H 2′,5′,6′ and Ph.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(pyridin-4′-yl)-imidazo[1,2-b]pyridazine (IV.4s)

A solution of 6-(*N*-benzyl-*N*-methylamino)pyridazine-3-amine 2-oxide (0.2 g) in ethanol (5.0 ml) was added to a mixture of 4-bromoacetylpyridine hydrobromide[165] and sodium hydrogen carbonate (0.08 g) in ethanol (5.0 ml) and the mixture refluxed with stirring in an oil bath for 7.5 h. The ethanol was evaporated under reduced pressure and the oily residue stirred with excess ethereal diazomethane in ice and then at room temperature overnight. After evaporation of the solvent the product was subjected to t.l.c. (alumina; chloroform, carbon tetrachloride, 3:10; developed three times) to give the *title compound* (0.008 g) m.p. 135-137° (Found, for a sample dried at 70°/0.1 mmHg for 48 h: C, 66.6; H, 6.0, N, 19.5. $C_{20}H_{19}N_5O$. 1.0 H_2O

requires C, 66.1; H, 5.8; N, 19.3%). ^{1}H n.m.r.: δ 3.20, s, MeN; 4.14, s, MeO; 4.76, s, CH_2N; 6.75, d, J 9 Hz, H 7; 7.29-8.76, complex, H 2′,3′,5′,6′ and Ph; 7.60, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-pyridin-3′-yl-imidazo[1,2-b]pyridazine (IV.4r) was prepared similarly; after t.l.c. (alumina; chloroform) it was obtained as a thick oil (10%) (Found, for a sample dried at 100°/0.1 mmHg for 12 h: C, 68.1; H, 5.8, N, 19.4. $C_{20}H_{19}N_5O$. 0.7 $CHCl_3$ requires C, 68.1; H, 5.4; N, 19.8%). ^{1}H n.m.r.: δ 3.21, s, MeN; 4.11, s, MeO; 4.77, s, CH_2N; 6.72, d, J 9 Hz, H 7; 7.61, d, J 9 Hz, H 8; 6.31-9.33, complex, H 2′,4′,5′,6′ and Ph.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(3′-nitrophenyl)-imidazo[1,2-b]pyridazine (IV.4b)

A solution of α-bromo-3-nitroacetophenone[121] (0.42 g) in ethanol (5.0 ml) was added to a hot solution of 6-(*N*-benzyl-*N*-methylamino)pyridazin-3-amine 2-oxide (0.350 g) in ethanol (10 ml) and the mixture refluxed with stirring in an oil bath for 0.5 h. Sodium hydrogen carbonate (0.064 g) was then added and the reflux continued for 4 h. It was chilled, diluted with water (10 ml), adjusted to pH 7, and the red-brown precipitate (0.442 g) filtered off and washed with water and ether and dried.

This product (0.202 g) was suspended in a little methanol and stirred with excess ethereal diazomethane in ice and then at room temperature overnight. The solvent

was evaporated and the product subjected to t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallised from methanol to give yellow crystals of the *title compound* (0.137 g), m.p. 128-130° (Found: C, 64.8; H, 5.0, N, 18.0. $C_{21}H_{19}N_5O_3$ requires C, 64.8; H, 4.9; N, 18.0%). ^{1}H n.m.r.: δ 3.22, s, MeN; 4.15, s, MeO; 4.78, s, CH_2N; 6.75, d, J 9 Hz, H 7; 7.32-8.95, complex, Ph and H 2′,3′,5′,6′; 7.65, d, J 9 Hz, H 8.

6-(N-Benzyl-N-methylamino)-3-methoxy-2-(4′-nitrophenyl)-imidazo[1,2-b]pyridazine (IV.4c)

To a warm solution of 6-(*N*-benzyl-*N*-methylamino)-pyridazin-3-amine 2-oxide (0.349 g) in ethanol (10 ml) was added a solution of 4-nitrophenacyl bromide (0.42 g) in ethanol (5.0 ml) and the mixture refluxed in an oil bath at 90° for 5.5 h. It was then chilled, diluted with water (10 ml) and the red-brown precipitate (0.206 g) filtered off, washed with water followed by ether, and dried.

This product in a little methanol was stirred with excess ethereal diazomethane cooled in ice overnight. The solvent was evaporated and the product recrystallised from methanol to give yellow crystals of the *title compound* (0.062 g), m.p. 184-185° (Found: C, 64.4; H, 4.9, N, 17.8. $C_{21}H_{19}N_5O_3$ requires C, 64.8; H, 4.9; N, 18.0%). ^{1}H n.m.r.: δ 3.22, s, MeN; 4.14, s, MeO; 4.78, s, CH_2N; 6.78, d, J 9 Hz, H 7; 7.32, s, Ph; 7.67, d, J 9 Hz, H 8; 8.27, s, H 2′,3′,5′,6′.

2-(3′-Aminophenyl)-6-(N-benzyl-N-methylamino)-3-methoxy-imidazo[1,2-b]pyridazine (IV.4n)

A solution of 6-(*N*-benzyl-*N*-methylamino)-3-methoxy-2-(3′-nitrophenyl)imidazo[1,2-*b*]pyridazine (0.361 g) in methanol (65 ml) was added over 5 minutes to a rapidly stirred mixture of reduced iron powder (0.56 g), methanol (35 ml), water (7.0 ml) and concentrated hydrochloric acid (2.8 ml) at 85-90° and the stirring continued at this temperature for 3 h. The mixture was filtered through celite, which was then washed with hot methanol. The filtrate was evaporated and the residue diluted with water, adjusted to pH 7 and extracted with chloroform. The extract was washed with water and evaporated and the product subjected to t.l.c. (alumina; chloroform, light petroleum, 1:1) to give the *title compound* (0.203 g) (Found: C, 70.0; H, 6.2, N, 19.3. $C_{21}H_{21}N_5O$ requires C, 70.2; H, 5.9; N, 19.5%). ^{1}H n.m.r.: δ 3.16, s, MeN; 3.44, s, NH_2; 4.06, s, MeO; 4.72, s, CH_2N; 6.64, d, J 9 Hz, H 7; 7.29-7.49, complex, H 2′,4′,5′,6′ and Ph; 7.56, d, J 9 Hz, H 8.

2-(4′-Aminophenyl)-6-(N-benzyl-N-methylamino)-3-methoxy-imidazo[1,2-b]pyridazine (IV.4o) (30%) as a thick oil after t.l.c. (alumina; chloroform) (Found: C, 63.2; H, 5.3, N, 17.1. $C_{21}H_{21}N_5O$. 0.4 $CHCl_3$ requires C, 63.1; H, 5.3; N, 17.2%). ^{1}H n.m.r.: δ 3.17, s, MeN; 3.50, br, NH_2; 4.05, s, MeO; 4.75, s, CH_2N; 6.65, d, J 9 Hz, H 7; 6.75, d, 7.90, d, J 7 Hz, H 2′,3′,5′,6′; 7.30, s, Ph; 7.62, d, J 9 Hz, H 8.

6-(3′,4′-Methylenedioxybenzylamino)pyridazin-3-amine 2-oxide (IV.5)

A mixture of 6-chloropyridazin-3-amine 2-oxide (0.5 g) and 3,4-methylenedioxybenzylamine (2.018 g) was heated in a Teflon lined screw-top reaction vessel at 145° for 12 h. The mixture was dissolved in ethanol and added to a column of alumina and developed with chloroform to remove excess methylenedioxybenzylamine. It was then eluted with ethanol and the product (0.277 g) recrystallised from ethanol to give the *title compound*, m.p. 160-162° (Found: C, 55.6; H, 4.6; N, 21.7. $C_{12}H_{12}N_4O_3$ requires C, 55.4; H, 4.6; N, 21.5%). ^{1}H n.m.r. (CD_3SOCD_3). 4.21, d, J 5.5 Hz, CH_2; 5.86, br, NH; 5.97, s, OCH_2O; 6.50, d, J 9.5 Hz, H7; 7.07, d, J 9.5 Hz, H8; 6.82-6.89, complex, H2′,5′,6′.

3-Methoxy-6-(3′-methoxybenzylamino)-2-(4"-methoxyphenyl)-imidazo[1,2-b]pyridazine (IV.7a)

A mixture of 6-(3′-methoxybenzylamino)pyridazin-3-amine 2-oxide[121] (0.1 g) and 4-methoxyphenacyl bromide (0.11 g) in ethanol (2.0 ml) was refluxed with stirring in an oil bath for 4 h. The solvent was evaporated and the residue stirred with ethereal diazomethane in ice and at room temperature overnight. The crude product was subjected to t.l.c. (alumina; chloroform, light petroleum, 2:1) and gave the *title compound* as an oil (0.039 g) (Found: C, 67.5; H, 5.7; N, 14.2. $C_{22}H_{22}N_4O_3$ requires C, 67.7; H, 5.7; N, 14.4). ^{1}H n.m.r.: δ 3.81, s, 3.85, s, 3′-OMe 4"-OMe;

4.04, s, 3-OMe; 4.58, d, CH_2; 6.36, d, J 9.5 Hz, H7; 6.80-8.06, complex, H2′,4′,5′,6′,2",3",5",6" and 8.

2-(2′-Chlorophenyl)-3-methoxy-6-(3"-methoxybenzylamino)-imidazo[1,2-b]pyridazine (IV.7b) (40%) as an oil after t.l.c. (alumina; chloroform, light petroleum, 1:1) and the product extracted with chloroform (Found: C, 62.7; H, 5.2; $C_{21}H_{19}ClN_4O_2$. 0.1 $CHCl_3$ requires C, 62.3; H, 4.7. ^1H n.m.r.: δ 3.81, s, 3"-OMe; 3.98, s, 3-OMe; 4.58, d, J 5.5 Hz, CH_2; 4.88, br, NH; 6.42, d, J 9.5 Hz, H7; 6.80-7.63, complex, H3′,4′,5′,6′,2",4",5",6" and 8.

3-(3′-Chlorophenyl)-3-methoxy-6-(3"-methoxybenzylamino)-imidazo[1,2-b]pyridazine (IV.7c) (28%), m.p. 191-193° after t.l.c. (alumina, chloroform) and recrystallisation from ethanol (Found: C, 64.0; H, 5.0; N, 13.9. $C_{21}H_{19}ClN_4O_2$ requires C, 63.9; H 4.9; N, 14.2%). ^1H n.m.r.: δ 3.81, s, 3"-OMe; 4.08, s, MeO; 4.58, d, J 5.5 Hz, CH_2; 6.47, d, J 9.5 Hz, H7; 6.79-8.08, complex, H2′,4′,5′,6′,2",4",5",6" and 8.

2-(4′-Chlorophenyl)-3-methoxy-6-(6"-methoxybenzylamino)-imidazo[1,2-b]pyridazine (IV.7d) (58%), m.p. 162-163° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of toluene and cyclohexane (Found: C, 63.6; H, 5.1; N, 13.9. $C_{21}H_{19}ClN_4O_2$ requires C, 63.9; H, 4.9; N, 14.2%). ^1H n.m.r.: δ 3.79, s, 3′-OMe; 4.04, s, 3-OMe; 5.55, d, J 5.5 Hz, CH_2; 4.91, t, NH; 6.39, d, J 9.5 Hz, H7; 6.78-8.06, complex, H2′,3′,5′,6′,2",4",5",6" and 8.

3-Methoxy-6-(3′-methoxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (IV.7e) (25%) as an oil after t.l.c. (alumina; chloroform, developed twice) (Found, for a sample dried at 25° and 0.6 mmHg for 10 h: C, 62.1; H, 5.3; N, 12.8. $C_{22}H_{20}H_4O_4$. 1.0 H_2O requires C, 62.5; H, 5.2; N, 13.3%). ^{1}H n.m.r.: δ 3.80, s, 3′-OMe; 4.03, s, 3-OMe; 4.56, d, J 5.5 Hz, CH_2; 4.81, t, NH; 5.98, s, OCH_2O; 6.37, d, J 9.5 Hz, H7, 6.78-7.66, complex, H2′,4′,5′,6′,2",5",6" and 8.

3-Methoxy-6-(3′,4′-dimethoxybenzylamino)-2-(4"-tolyl)-imidazo[1,2-b]pyridazine (IV.7f) (49%), as yellow crystals, m.p. 150-152° after t.l.c. (alumina, chloroform) and recrystallisation from a mixture of acetone and cyclohexane (Found: C, 68.5; H, 6.3; N, 14.0. $C_{23}H_{24}N_4O_3$ requires C, 68.3; H, 6.0; N, 13.9%). ^{1}H n.m.r.: δ 2.38, s, MeC; 3.88, s, 3.89, s, 3′,4′-$(OMe)_2$; 4.08, s, 3-OMe; 4.55, bs, CH_2N; 6.37, d, J 9.5 Hz, H7; 6.78-8.03, complex, H2′,5′,6′,2",3",5",6" and 8.

3-Methoxy-6-(3′,4′-dimethoxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (IV.7g)

A mixture of 6-(3′,4′-dimethoxybenzylamino)pyridazin-3-amine 2-oxide[121] (0.1 g) and 3′,4′-methylenedioxyphenacyl bromide[228] (0.1 g) in ethanol (2.0 ml) was refluxed with stirring in an oil bath for 5 h. The solvent was evaporated, the residue suspended in methanol (2.0 ml) and stirred with excess ethereal diazomethane in ice and at

room temperature overnight. The solvent was then evaporated and the resulting oil subjected to t.l.c. (alumina; chloroform). The product (0.084 g) obtained was recrystallised from ethanol to give the *title compound* (0.047 g) as yellow crystals, m.p. 182-184° (Found: C, 63.9; H, 5.2; N, 13.0. $C_{23}H_{22}N_4O_5$ requires C, 63.6; H, 5.1; N, 12.9%). ^{1}H n.m.r.: δ 3.88, s, 3.89, s, 3′,4′-$(OMe)_2$; 4.08, s, 3-OMe; 4.55, bs, CH_2; 5.98, s, OCH_2O; 6.37, d, J 9.5 Hz, H7; 6.78-7.67, complex, H2′,5′,6′,2",5",6" and 8.

3-Methoxy-6-(3′,4′-methylenedioxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (IV.7h) (6%) as an oil after t.l.c. (alumina; chloroform, twice) (Found, for a sample dried at 20° and 0.1 mmHg for 16 h: C, 59.4; H, 3.7; N, 12.4. $C_{22}H_{18}N_4O_5$. 0.25 $CHCl_3$ requires C, 59.6; H, 4.1; N, 12.5%). ^{1}H n.m.r.: δ 4.07, s, MeO; 4.50, d, J 5.5 Hz, CH_2N; 4.68, bs, NH; 5.95, s, 5.98, s, 2xOCH_2O; 6.35, d, J 9.5 Hz, H7; 6.72-7.67, complex, H2′,5′,6′,2",5",6" and 8.

6-(2′-And 4′-chlorobenzylamino)pyridazin-3-amine 2-oxides were prepared by Mr S.J. Ireland.

6-(2′-Chlorobenzylamino)-3-methoxy-2-(4"-tolyl)imidazo-[1,2-b]pyridazine (IV.7i) (21%) as an oil after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 66.4; H, 5.2; N, 14.6. $C_{21}H_{19}ClN_4O$ requires C, 66.6; H, 5.1; N, 14.8%). It crystallised from cyclohexane as yellow crystals, m.p. 97-98° with some solvent included. ^{1}H n.m.r.: δ 2.38, s, MeC; 4.04, s, MeO; 4.71, d, J 5.5 Hz,

CH_2N; 6.38, d, J 9.5 Hz, H7; 7.17-8.01, complex, H3′,4′,5′,6′,2",3",5",6" and 8.

6-(2′-Chlorobenzylamino)-2-(4"-chlorophenyl)-3-methoxy-imidazo[1,2-b]pyridazine (IV.7j) (30%) as an oil from t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 60.3; H, 3.9; N, 14.3. $C_{20}H_{16}Cl_2N_4O$ requires C, 60.2; H, 4.0; N, 14.0%). It crystallised from cyclohexane as yellow crystals, m.p. 87-88° but even after drying at 65° and 0.1 mmHg for 20 h it analysed for 0.15 molecules of included cyclohexane. ^1H n.m.r.: δ 4.05, s, Me; 4.70, d, J 5.5 Hz, CH_2N; 4.92, bs, NH; 6.40, d, J 9.5 Hz, H7; 7.17-8.06, complex, H3′,4′,5′,6′,2",3",5",6" and 8.

6-(2′-Chlorobenzylamino)-3-methoxy-2-(3"-nitrophenyl)-imidazo[1,2-b]pyridazine (IV.7k) (20%) as an oil from t.l.c. (alumina; chloroform), which on standing gave yellow crystals, m.p. 184-186° (Found: C, 58.6; H, 4.2; N, 16.9. $C_{20}H_{16}ClN_5O_3$ requires C, 58.6; H,, 3.9; N, 17.1%). ^1H n.m.r.: δ 4.10, s, MeO; 4.69, d, J 5.5 Hz, CH_2; 5.24, t, NH; 6.46, d, J 9.5 Hz, H7; 7.15-8.91, complex, H3′,4′,5′,6′,2",4",5",6" and 8.

6-(2′-Chlorobenzylamino)-3-methoxy-2-(4"-nitrophenyl)-imidazo[1,2-b]pyridazine (IV.7l) (15%), m.p. 226-228° after t.l.c. (alumina; chloroform) and recrystallisation from ethanol (Found: C, 58.9; H, 3.9; N, 16.8. $C_{22}H_{16}Cl_5O_3$ requires C, 58.6; H, 3.9; N, 17.1%). ^1H n.m.r.: δ 4.11, s,

MeO; 4.72, d, J 5.5 Hz, CH_2; 5.01, t, NH; 6.47, d, J 9.5 Hz, H7; 7.19-8.25, complex, H3′,4′,5′,6′,2",3",5",6" and 8.

6-(2′-Chlorobenzylamino)-3-methoxy-2-(pyridin-3"-yl)-imidazo[1,2-b]pyridazine (IV.7m) (9%) as a crystalline solid, m.p. 148-150° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of acetone and light petroleum (Found: C, 62.1; H, 4.5; N, 19.1. $C_{19}H_{16}Cl_5O$ requires C, 62.4; H, 4.4; N, 19.1%). ^{1}H n.m.r.: δ 4.08, s, MeO; 4.71, d, J 5.5 Hz, CH_2N; 5.10, bs, NH; 6.45, d, J 9.5 Hz, H7; 7.18-9.28, complex, H3′,4′,5′,6′,8 and pyridinyl.

6-(4′-Chlorobenzylamino)-3-methoxy-2-phenylimidazo-[1,2-b]pyridazine (IV.7n) (43%), m.p. 158-160° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from ethanol (Found: C, 66.0; H, 4.8; N, 15.2. $C_{20}H_{17}ClN_4O$ requires C, 65.8; H, 4.7; N, 15.4). ^{1}H n.m.r.: δ 4.00, s, Me; 4.53, d, J 5.5 Hz, CH_2; 5.09, t, NH; 6.35, d, J 9.5 Hz, H7; 7.29-8.12, complex, H2′,3′,5′,6′,8 and Ph.

6-(4′-Chlorobenzylamino)-3-methoxy-2-(4′-tolyl)imidazo-[1,2-b]pyridazine (IV.7o) (25%) as a yellow solid m.p. 143-145° after t.l.c. (alumina, chloroform) and recrystallisation from a mixture of acetone and cyclohexane (Found: C, 66.7; H, 5.1; N, 14.6. $C_{21}H_{19}ClN_4O$ requires C, 66.6; H, 5.1; N, 14.8%). ^{1}H n.m.r.: δ 2.38, s, MeC; 4.00, s, MeO; 4.55, d, J 5.5 Hz, CH_2; 4.91, t, NH; 6.35, d, J 9.5 Hz, H7; 7.19-8.01, complex, H2′,3′,5′,6′,2",3",5",6" and 8.

6-(4'-Chlorobenzylamino)-3-methoxy-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (IV.7p) (20%), as yellow crystals m.p. 124-126° after t.l.c. (alumina, chloroform) and recrystallisation from a mixture of ethanol and cyclohexane (Found: C, 61.7; H, 4.5; N, 13.4. $C_{21}H_{17}ClN_4O_3$ requires C, 61.7; H, 4.2; N, 13.7%). ^1H n.m.r.: δ 4.00, s, MeO; 4.57, d, J 5.5 Hz, CH_2; 4.80, t, NH; 5.98, s, OCH_2O; 6.37, d, J 9.5 Hz, H7; 6.83-7.66, complex, H2',3',5',6',2",5",6" and 8.

6-(3',4'-Methylenedioxybenzylamino)-2-(3",4"-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (IV.10)

A mixture of 6-chloropyridazin-3-amine 2-oxide (1.0 g) and 3,4-methylenedioxybenzylamine (5.0 g) was heated at 160° for 16 h. The reaction mixture was dissolved in ethanol and then added to a column of alumina (in chloroform) and then eluted with chloroform and then ethanol. The ethanol eluate gave an oil (0.110 g) (Found: C, 58.7; H, 5.0. $C_{12}H_{12}N_4O_2$ requires C, 59.0; H, 5.0) ^1H n.m.r. (CD_3SOCD_3). δ 4.33, d, 5.5 Hz, CH_2; 5.35, br, NH_2; 5.96, s, OCH_2O; 6.35, br, NH; 6.67-6.90 complex, ArH.

A mixture of this product (0.106 g) and 3,4-methylenedioxyphenacyl bromide[1] (0.100 g) in ethanol (2.0 ml) was refluxed in an oil bath with stirring from 5 h. After cooling, the precipitate was filtered off and washed with ethanol to give a solid (0.1 g), m.p. 237-239° (Found: C, 53.2; H, 3.5; N, 11.6. $C_{21}H_{16}N_4O_4$.HBr requires C, 53.7; H, 3.7; N, 11.9%).

This solid was suspended in water and neutralised, the solvent evaporated and the residue subjected to t.l.c. (alumina; chloroform). The product (0.062 g) was recrystalled from ethanol to give the *title compound* (0.043 g), m.p. 224-226° (Found: C, 65.3; H, 4.1; N, 14.2. $C_{21}H_{16}N_4O_4$ requires C, 64.9; H, 4.2; N, 14.4%). ^{1}H n.m.r.: δ 4.47, br, CH_2; 5.96, s, 5.99, s, 2xOCH_2O; 6.42, d, J 9.5 Hz, H7; 6.81-7.70, complex, H2′,5′,6′,2",5",6" and H8; 7.86, s, H3.

6-(3′-Methoxybenzylamino)-3-(3"-methoxybenzyloxy)-2-phenylimidazo[1,2-b]pyridazine (IV.8e)

A mixture of 6-(3′-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.664 g) and phenacyl bromide (0.537 g) in ethanol (6.0 ml) was refluxed with stirring for 7 h. The solvent was then evaporated, the residue diluted with acetone (3 ml) and the solid (0.627 g) filtered off and washed with acetone.

Part of this solid (0.1 g) with 3-methoxybenzyl chloride (0.71 g) and potassium carbonate (0.1 g) in dimethylformamide (2.0 ml) was stirred in an oil at 100° for 4 h. It was then diluted with water (20 ml) and extracted with ether and the product subjected to t.l.c. (alumina; chloroform) (three times) to give as an oil the *title compound* (0.021 g) (Found: C, 70.7; H, 5.9; N, 11.4. $C_{28}H_{26}N_4O_3$ 0.5 H_2O requires C, 70.7; H, 5.9; N, 11.8%. ^{1}H n.m.r.: δ 3.42, s, CH_2O; 3.70, s, 3"-OMe; 3.76, s, 3′-OMe; 4.39, d, J 5 Hz, CH_2; 4.49, br, NH; 6.32, d, J 9.5 Hz, H7; 6.65-7.84, complex, H2′,4′,5′,6′,2",4",5",6" and H8.

CHAPTER V

CHAPTER V Syntheses and binding studies of some 2-benzyl(phenethyl, biphenyl-4′-yl, 6′-methylnaphthalen-2′-yl, t-butyl and cyclohexyl)-3-methoxy(acylaminomethyl and dimethylaminomethyl)-6-(variously substituted)imidazo-[1,2-b]pyridazines

V-1 Introduction

In this chapter a large number of 6-(variously substituted)-3-methoxy (unsubstituted, dimethylaminomethyl, acetamidomethyl and benzamidomethyl)-2-benzyl (phenethyl, biphenyl-4′-yl, 6′-methylnaphthalen-2′-yl, t-butyl and cyclohexyl)imidazo[1,2-*b*]pyridazines have been prepared and their ability to displace [^{3}H]diazepam from its specific binding sites in rat brain membrane has been examined. In imidazo[1,2-*b*]pyridazine derivatives, it has been shown that a 2-aryl substituent is a requirement for significant binding.[167] This 2-aryl group may correspond to the "A" ring in the Fryer model (see Chapter I-3).

The aim of the present investigation was to examine structure-binding activity relationships in 2,3,6-trisubstituted imidazo[1,2-*b*]pyridazines in which the potential "A" to π_1 distance was varied by inserting benzyl, phenethyl, biphenyl, 6′-methylnaphthalen-2′-yl (and cyclohexyl and t-butyl) groups at the 2-position, and to compare their activity with analogous compounds which contained 2-phenyl (substituted phenyl and pyridinyl) groups.

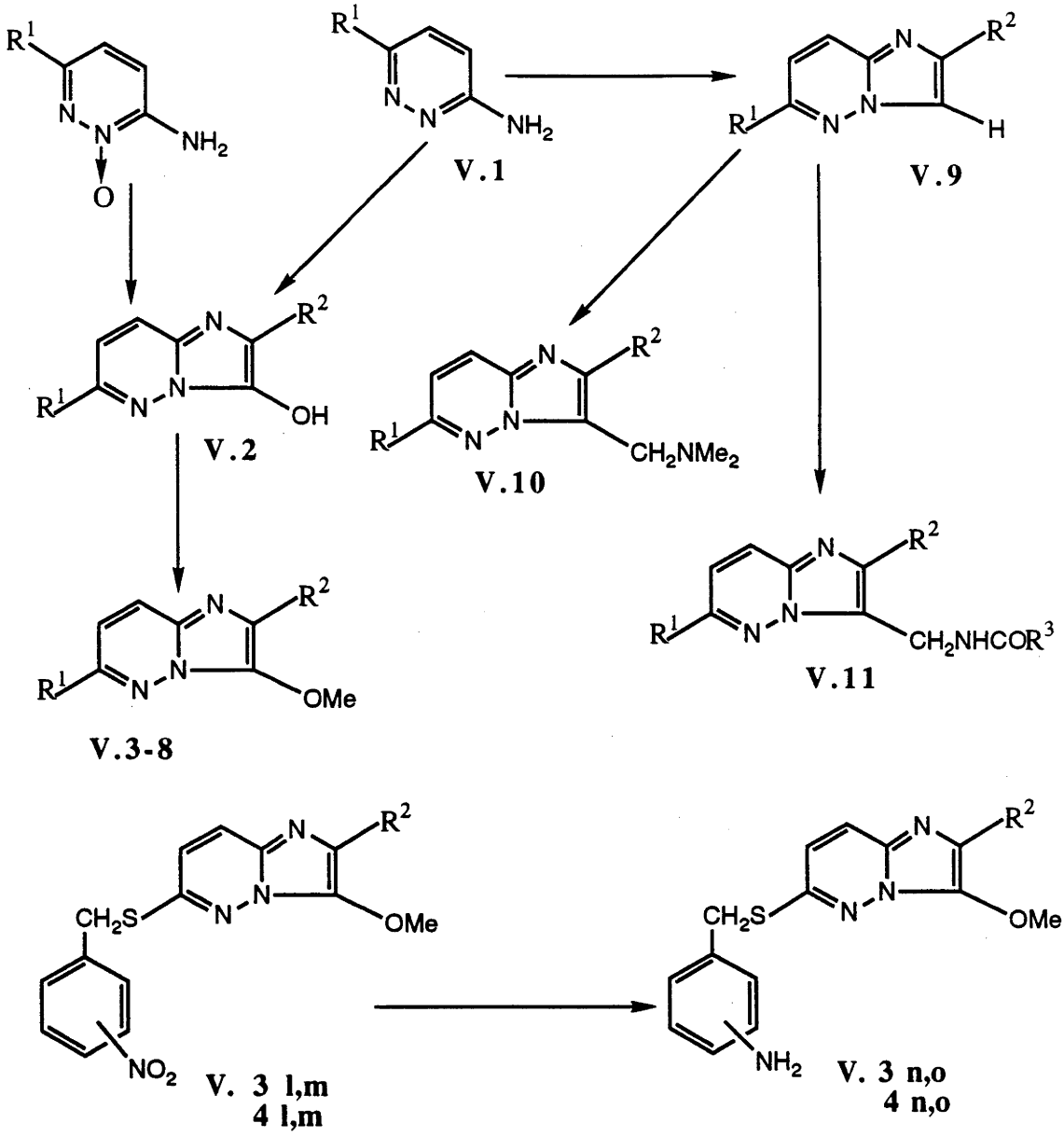
V-2 Syntheses

The general methods for syntheses of 6-(variously substituted)-3-methoxy-2-benzyl(phenethyl, biphenyl-4′-yl, substituted biphenyl-4′-yl, 6′-methylnaphthalen-2′-yl, cyclohexyl and t-butyl)imidazo[1,2-*b*]pyridazines (**V.3-8**, Scheme V-1) and 6-(variously substituted)-3-unsubstituted (and dimethylaminomethyl, acetamidomethyl, and benzamidomethyl)-2-benzyl(phenethyl, biphenyl-4′-yl, cyclohexyl and t-butyl)imidazo[1,2-*b*]pyridazines (**V.9-11**) are as outlined in Scheme V-1.

The compounds (**V.3-8**) were prepared generally from the relevant 6-substituted pyridazin-3-amine[161,163-165,229,230] with the appropriate glyoxals followed by methylation with diazomethane or from the relevant 6-substituted pyridazin-3-amine 2-oxides, such as 6-(2′-methoxyphenoxy)pyridazin-3-amine 2-oxide, with the appropriate bromoacetyl compound followed by methylation.

Benzylglyoxal[246] was prepared from mandelic acid, phenethylglyoxal[247] from hydrocinnamic acid, biphenyl-4-ylglyoxal[248] from 4-acetylbiphenyl, 4′-nitrobiphenyl-4-ylglyoxal from 4-nitrobiphenyl through 4-acetyl-4′-nitrobiphenyl,[249] t-butylglyoxal[250] from pinacolone, cyclohexylglyoxal[251] from cyclohexyl chloride through cyclohexyl methyl ketone,[252] 1-bromo-3-phenylpropanone[253] from phenylacetyl chloride, 1-bromo-4-phenylbutan-2-one[254] from 3-phenylpropionic acid, α-bromo-4-phenylacetophenone[255] from 4-phenylacetophenone, 1-bromo-

Scheme V-1



V	R^2
3a-u	CH_2Ph
4a-r	CH_2CH_2Ph
5a-i	C_6H_4Ph-*p*,or $C_6H_4(C_6H_4NO_2$-*p'*)-*p*
6a,b	$(C_{10}H_6Me$-*6'*$)$–β
7a-e	Bu^t
8a-d	C_6H_{11}-*cyclo*
9a-s	CH_2Ph, CH_2CH_2Ph, C_6H_4Ph-*p*, Bu^t or C_6H_{11}-*cyclo*
10a-n	CH_2Ph, CH_2CH_2Ph, C_6H_4Ph-*p*, Bu^t or C_6H_{11}-*cyclo*
11a-j	CH_2Ph, CH_2CH_2Ph, C_6H_4Ph-*p*, Bu^t or C_6H_{11}-*cyclo*

3,3-dimethylbutan-2-one[256] from pinacolone, and α-bromomethyl cyclohexyl ketone[257] by bromination of cyclohexyl methyl ketone.

The compounds (**V.9**) were prepared from the relevant pyridazin-3-amine and bromoacetyl compounds; and when compounds (**V.9**) were heated with bis(dimethylamino)-methane and phosphoric acid in acetic acid at 120°, the 3-dimethylaminomethyl compounds (**V.10**) were obtained.

The 3-acetamidomethyl and 3-benzamidomethyl compounds (**V.11**) were prepared from compounds (**V.9**) by heating with *N*-(hydroxymethyl)acetamide or *N*-(hydroxymethyl)benzamide in acetic acid containing a catalytic amount of concentrated sulphuric acid.

2-Benzyl (and phenethyl)-3-methoxy-6-(nitrobenzylthio)imidazo[1,2-*b*]pyridazines (**V.31,m** and **V.41, m**) were reduced with iron in aqueous hydrochloric acid to the corresponding 6-(aminobenzylthio) compounds (**V.3n,o** and **V.4n,o**; in Scheme V-1) respectively.

V-3 ^{1}H n.m.r. spectra

The ^{1}H n.m.r. spectra of the compounds reported in this chapter are recorded in the Experimental section. In deuterochloroform, the signal due to the protons of the 3-methoxy group in the 2-benzyl compounds (**V.3a-r**) was at δ3.80-3.96, and that in the 2-phenethyl compounds (**V.4a-o**) at δ3.75-3.87; these were upfield of the signals for some corresponding 2-phenyl and 2-(biphenyl-4′-yl) compounds at δ4.01-4.17 and 4.02-4.19, respectively. The signals due to the methylene of the 2-benzyl group and the ethylene of the 2-phenethyl group in the above compounds were at δ4.09-4.11 and 3.06-3.07 respectively.

The protons H7 and H8 in compounds (**V.3a-r** and **V.4a-o**) appeared as an AB quartet within the small chemical shift ranges of δ6.68-6.76 for H7 and δ7.53-7.61 for H8, with coupling constants of 9.0-9.5 Hz.

The ^{1}H n.m.r. spectra of compounds (**V.7-11**) revealed the signal due to the 2-t-butyl group in the range δ1.41-1.55; that for the 2-cyclohexyl group at δ1.26-2.90; the 3-H of 3-unsubstituted imidazo[1,2-*b*]pyridazines (**V.9**) was at δ7.55-7.72; and for substituents at 3-position the dimethyl and the methylene of the 3-dimethylaminomethyl group at δ2.07-3.35 and 3.55-4.01, respectively; the methylene of the 3-acetamidomethyl group as a doublet at δ4.39-4.99, acetyl at δ1.75-1.99; and the methylene of the 3-benzamidomethyl group as a doublet at δ4.60-5.26.

V-4 *In vitro* binding studies

The compounds prepared in this work were examined in the [^{3}H]diazepam binding assay outlines in Chapter II-5.3.

V-4.1 Results of [^{3}H]diazepam binding assay

The results of the above *in vitro* displacement studies from rat brain membrane by the compounds described in this chapter are shown in Table V-1 for the 3-methyoxyimidazo[1,2-*b*]pyridazines and in Table V-2 for 3-benzamidomethyl (acetamidomethyl, dimethylaminomethyl and unsubstituted)imidazo[1,2-*b*]-pyridazines provided the activity of these compounds generally exceded 5% displacement of [^{3}H]diazepam at 1000 nM. Some literature data for some 2-phenyl analogues are also included at the beginning of Table V-1 for comparison.

V-4.2 Discussion of binding results

An examination of the data in Table V-1 for the series of the 2-benzylimidazo[1,2-*b*]pyridazines, revealed that 2-benzyl-3-methoxy-6-(3'-methylbenzylthio)imidazo-[1,2-*b*]pyridazine (**V.3f**, IC_{50} 93 nM) was the most active compound.

Table V-1 Results for the displacement of [^{3}H]diazepam from rat brain membranes by some 2-benzyl(phenethyl, biphenyl-4′-yl, 6′-methylnaphthalen-2′-yl, t-butyl and cyclohexyl)-3-methoxy-6-(variously substituted)imidazo-[1,2-b]pyridazines (V.3-8)

Formula number		Imidazo[1,2-*b*]pyridazine	IC_{50} (nM)[A] (or % displ.)[B]
		6-Benzylthio-3-methoxy-2-phenyl-compounds	
		6-SCH_2Ph-3-OMe-2-Ph	25[C]
		6-$SCH_2C_6H_4$OMe-*o*-3-OMe-2-Ph	9[D]
		6-$SCH_2C_6H_4$OMe-*m*-3-OMe-2-Ph	11[D]
		6-$SCH_2C_6H_4$OMe-*p*-3-OMe-2-Ph	55[D]
		6-Variously substituted-3-methoxy-2-phenyl compounds	
		6-Cl-3-OMe-2-Ph	772[E]
		6-OC_6H_4OMe-*p*-3-OMe-2-Ph	998[D]
		6-SPh-3-OMe-2-Ph	117[F]
V.3		**6-Benzylthio-3-methoxy-2-benzyl compounds**	
	a	6-SCH_2Ph-3-OMe-2-CH_2Ph	197[G]
	b	6-$SCH_2C_6H_4$OMe-*o*-3-OMe-2-CH_2Ph	208
	c	6-$SCH_2C_6H_4$OMe-m-3-OMe-2-CH_2Ph	114
	d	6-$SCH_2C_6H_4$OMe-*p*-3-OMe-2-CH_2Ph	499
	e	6-$SCH_2C_6H_4$Me-*o*-3-OMe-2-CH_2Ph	425
	f	6-$SCH_2C_6H_4$Me-*m*-3-OMe-2-CH_2Ph	93
	g	6-$SCH_2C_6H_4$Cl-*o*-3-OMe-2-CH_2Ph	352
	h	6-$SCH_2C_6H_4$Cl-*m*-3-OMe-2-CH_2Ph	277
	i	6-$SCH_2C_6H_4$Cl-*p*-3-OMe-2-CH_2Ph	417
	j	6-$SCH_2C_6H_3Cl_2$-*o*,*p*-3-OMe-2-CH_2Ph	(41%)
	k	6-$SCH_2C_6H_4NO_2$-*o*-3-OMe-2-CH_2Ph	126
	l	6-$SCH_2C_6H_4NO_2$-*m*-3-OMe-2-CH_2Ph	193
	m	6-$SCH_2C_6H_4NO_2$-*p*-3-OMe-2-CH_2Ph	151
	n	6-$SCH_2C_6H_4NH_2$-*m*-3-OMe-2-CH_2Ph	136
	o	6-$SCH_2C_6H_4NH_2$-*p*-3-OMe-2-CH_2Ph	181

Table V-1 Continued

Formula number		Imidazo[1,2-*b*]pyridazine	IC_{50}(nM)[A] (or % displ.)[B]
		6-Picolylthio-3-methoxy-2-benzyl compounds	
	p	6-$SCH_2C_5H_4N$-α-3-OMe-2-CH_2Ph	363
	q	6-$SCH_2C_5H_4N$-β-3-OMe-2-CH_2Ph	372
	r	6-$SCH_2C_5H_4N$-γ-3-OMe-2-CH_2Ph	390
		6-Variously substituted 3-methoxy-2-benzyl compounds	
	s	6-OC_6H_4OMe-*p*-3-OMe-2-CH_2Ph	(51%)
	t	6-OC_6H_4Cl-*p*-3-OMe-2-CH_2Ph	(27%)
	u	6-SPh-3-OMe-2-CH_2Ph	203
		6-Benzylthio-3-methoxy-2-phenethyl compounds	
V.4	**a**	6-$SCH_2C_6H_4OMe$-*o*-3-OMe-2-CH_2CH_2Ph	(45%)
	b	6-$SCH_2C_6H_4OMe$-*m*-3-OMe-2-CH_2CH_2Ph	>>1000
	c	6-$SCH_2C_6H_4OMe$-*p*-3-OMe-2-CH_2CH_2Ph	(5.9%)
	d	6-$SCH_2C_6H_4Me$-*o*-3-OMe-2-CH_2CH_2Ph	(24%)
	e	6-$SCH_2C_6H_4Me$-*m*-3-OMe-2-CH_2CH_2Ph	(3.8%)
	f	6-$SCH_2C_6H_4Me$-*p*-3-OMe-2-CH_2CH_2Ph	(2.2%)
	g	6-$SCH_2C_6H_4Cl$-*o*-3-OMe-2-CH_2CH_2Ph	(6.3%)
	h	6-$SCH_2C_6H_4Cl$-*m*-3-OMe-2-CH_2CH_2Ph	(29%)
	i	6-$SCH_2C_6H_4Cl$-*p*-3-OMe-2-CH_2CH_2Ph	(4.3%)
	j	6-$SCH_2C_6H_3Cl_2$-*o*,p-3-OMe-2-CH_2CH_2Ph	>>1000
	k	6-$SCH_2C_6H_4NO_2$-*o*-3-OMe-2-CH_2CH_2Ph	(32%)
	l	6-$SCH_2C_6H_4NO_2$-*m*-3-OMe-2-CH_2CH_2Ph	(31%)
	m	6-$SCH_2C_6H_4NO_2$-*p*-3-OMe-2-CH_2CH_2Ph	(13%)
	n	6-$SCH_2C_6H_4NH_2$-*m*-3-OMe-2-CH_2CH_2Ph	(43%)
	o	6-$SCH_2C_6H_4NH_2$-*p*-3-OMe-2-CH_2CH_2Ph	(31%)
		6-Variously substituted-3-methoxy-3-phenethyl compounds	
	p	6-OC_6H_4OMe-*p*-3-OMe-2-CH_2CH_2Ph	(9.2%)
	q	6-OC_6H_4Cl-*p*-3-OMe-2-CH_2CH_2Ph	(2.2%)
	r	6-SPh-3-OMe-2-CH_2CH_2Ph	>>1000

Table V-1 Continued

Formula number		Imidazo[1,2-*b*]pyridazine	IC_{50}(nM)[A] (or % displ.)[B]
		6-Variously substituted 3-methoxy-2-(4′-biphenyl), t-butyl or cyclohexyl compounds	
V.5	**b**	6-$SCH_2C_6H_4OMe$-*o*-3-OMe-2-C_6H_4Ph-*p*	(4%)
	c	6-$SCH_2C_6H_4OMe$-*m*-3-OMe-2-C_6H_4Ph-*p*	(4%)
	e	6-Cl-3-OMe-2-C_6H_4Ph-*p*	(8.9%)
V.7	**a**	6-SCH_2Ph-3-OMe-2-Bu^t	(5%)
	b	6-Cl-3-OMe-2-Bu^t	(12%)
V.8	**c**	6-OC_6H_4OMe-*o*-3-OMe-2-C_6H_{11}	(8%)

[A] IC_{50} values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparations
[B] At 1000 nM
[C] Ref. 164
[D] Ref. 165
[E] Ref. 162
[F] Ref. 167
[G] Kindly prepared by Mr S.J. Ireland

A comparison of the 2-benzyl compounds (**V.3**) with their 2-phenyl analogues revealed that 2-benzyl-3-methoxy-6-(4′-methoxyphenoxy)imidazo[1,2-*b*]pyridazine (**V.3s**, 51% displacement at 1000 nM) was approximately as active as 3-methoxy-6-(4′-methoxyphenoxy)-2-phenyl-imidazo[1,2-*b*]-pyridazine[165] (IC_{50} 988 nM; see Table V-1) and 2-benzyl-3-methoxy-6-phenylthioimidazo[1,2-*b*]-pyridazine (**V.3u**, IC_{50} 203 nM) was *ca* half as active as 3-methoxy-2-phenyl-6-phenylthioimidazo[1,2-*b*]pyridazine (IC_{50} 117 nM; see Table V-1). However the 2-benzyl compounds generally were significantly less active. This

lower activity varied from *ca* 10 to 20-fold for the 2-benzyl-3-methoxy-6-(2′,3′ and 4′-methoxybenzylthio)imidazo[1,2-*b*]pyridazines (in comparison to their 2-phenylimidazo[1,2-*b*]pyridazine analogues). It was *ca* 8-fold for 2-benzyl-6-benzylthio-3-methoxyimidazo[1,2-*b*]-pyridazine (**V.3a**; IC_{50} 197 nM) in comparison to its 2-phenyl analogue (IC_{50} 25 nM; see Table V-1).[164]

Amongst 3-unsubstituted 2-benzyl-6-(2′,3′- and 4′-methoxybenzylthio)imidazo[1,2-*b*]pyridazines (**V.9b-d**; 34%, 27% and 12% displacement at 1000 nM; in Table V-2) the 6-(2′-methoxybenzylthio) compound (**V.9b**) was the most active; and 2-benzyl-6-(3′-methoxybenzylthio)imidazo-[1,2-*b*]pyridazine (**V.9c**; 27% displacement at 1000 nM) was markedly less active than its 2-phenyl analogue (**II.3g**; IC_{50} 330 nM); see Chapter II). 2-Benzyl-6-phenylthio-imidazo[1,2-*b*]pyridazine (**V.9h**; 24% displacement at 1000 nM) was half as active as 2-phenyl-6-phenylthioimidazo-[1,2-*b*]pyridazine (**II.3j**; 43% displacement at 1000 nM, see Chapter II).

Similar lower activities were also noted for the 3-dimethylaminomethyl (and 3-benzamidomethyl)-2-benzyl-imidazo[1,2-*b*]pyridazines as compared to their 2-phenyl analogues. For example, 2-benzyl-3-dimethylaminomethyl-6-(2′-methoxyphenoxy)imidazo[1,2-*b*]pyridazine (**V.10d**; 6.7% displacement at 1000 nM) was much less active than 3-dimethylaminomethyl-6-(2′-methoxyphenoxy)-2-phenyl-imidazo[1,2-*b*]pyridazine (**II.4f**; IC_{50} 333 nM, see Chapter II).

Table V-2. Results for the displacement of [^{3}H]diazepam from rat brain membranes by some 3-benzamidomethyl (acetamidomethyl, dimethylaminomethyl and unsubstituted)imidazo[1,2-b]pyridazines (V.9-11).

Formula number	Imidazo[1,2-*b*]pyridazine	IC_{50}(nM)^A (or % displ.)^B
2-Benzyl compounds		
V.9b	6-$SCH_2C_6H_4OMe$-*o*-3-H-2-CH_2Ph	(34%)
10a	6-$SCH_2C_6H_4OMe$-*o*-3-CH_2NMe_2-2-CH_2Ph	(19%)
9c	6-$SCH_2C_6H_4OMe$-*m*-3-H-2-CH_2Ph	(27%)
10b	6-$SCH_2C_6H_4OMe$-*m*-3-CH_2NMe_2-2-CH_2Ph	(31.5%)
9d	6-$SCH_2C_6H_4OMe$-*p*-3-H-2-CH_2Ph	(12%)
10c	6-$SCH_2C_6H_4OMe$-*p*-3-CH_2NMe_2-2-CH_2Ph	(3.8%)
9e	6-Cl-3-H-2-CH_2Ph	(1%)
11a	6-Cl-3-CH_2NHBz-2-CH_2Ph	>>1000
10d	6-OC_6H_4OMe-*o*-3-CH_2NMe_2-2-CH_2Ph	(6.7%)
9f	6-OC_6H_4OMe-*p*-3-H-2-CH_2Ph	(15%)
10e	6-OC_6H_4OMe-*p*-3-CH_2NMe_2-2-CH_2Ph	(17%)
9g	6-OC_6H_4Cl-*p*-3-H-2-CH_2Ph	(0%)
10f	6-OC_6H_4Cl-*p*-3-CH_2NMe_2-2-CH_2Ph	(19%)
9h	6-SPh-3-H-2-CH_2Ph	(24%)
10g	6-SPh-3-CH_2NMe_2-2-CH_2Ph	(33%)
2-Phenethyl compounds		
9j	6-SCH_2Ph-3-H-2-CH_2CH_2Ph	(2%)
10h	6-SCH_2Ph-3-CH_2NMe_2-2-CH_2CH_2Ph	(3.1%)
11b	6-SCH_2Ph-3-CH_2NHAc-2-CH_2CH_2Ph	(16%)
11c	6-SCH_2Ph-3-CH_2NHBz-2-CH_2CH_2Ph	(12%)
9k	6-$SCH_2C_6H_4OMe$-*p*-3-H-2-CH_2CH_2Ph	(8%)
10i	6-$SCH_2C_6H_4OMe$-*p*-3-CH_2NMe_2-2-CH_2CH_2Ph	>>1000
9l	6-Cl-3-H-2-CH_2CH_2Ph	(3%)
10j	6-Cl-3-CH_2NMe_2-2-CH_2CH_2Ph	>>1000
11d	6-Cl-2-CH_2NHAc-2-CH_2CH_2Ph	(8.7%)
11e	6-Cl-3-CH_2NHBz-2-CH_2CH_2Ph	(22%)

Table V-2 Continued

Formula number	Imidazo[1,2-*b*]pyridazine	IC_{50}(nM)[A] (or % displ.)[B]
9m	6-SPh-3-H-2-CH_2CH_2Ph	(7%)
11f	6-SPh-3-CH_2NHAc-2-CH_2CH_2Ph	(11%)

[A] IC_{50} Values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparations

[B] At 1000 nM

The 2-(phenethyl)imidazo[1,2-*b*]pyridazines were also found to be significantly less active than the corresponding 2-benzylimidazo[1,2-*b*]pyridazines. This lower activity of the 2-(phenethyl) compound was *ca* five-fold in 3-methoxy-6-(4′-methoxyphenoxy)-2-(phenethyl)-imidazo[1,2-*b*]pyridazine (**V.4p**; 9.2% displacement at 1000 nM) and 3-methoxy-6-(2′-methoxybenzylthio)-2-(phenethyl)-imidazo[1,2-*b*]pyridazine (**V.4a**; 45% displacement at 1000 nM) relative to their 2-benzyl analogues (**V.3s**, 51% displacement at 1000 nM and **V.3b**; IC_{50} 208 nM); but probably the biggest difference was observed between 3-methoxy-6-(3′-methylbenzylthio)-2-(phenethyl)imidazo-[1,2-*b*]pyridazine (**V.4e**; 3.8% displacement at 1000 nM) and its 2-benzyl analogue (**V.3f**; IC_{50} 93 nM). Qualitatively, the pattern of activities amongst the 2-benzyl (and 2-phenethyl)-3-methoxy-6-(substituted benzylthio)imidazo[1,2-*b*]pyridazines was generally similar to that observed for their 2-phenyl analogues.[164]

All 2-(biphenyl-4′-yl), 2-(substituted biphenyl-4′-yl), 2-(6′-methylnaphthalen-2′-yl), 2-t-butyl and 2-cyclohexylimidazo[1,2-*b*]pyridazines showed low binding ability. The data for 3-methoxy-6-(2′-methoxybenzylthio)-2-phenethylimidazo[1,2-*b*]pyridazine (**V.4a**; 45% displacement at 1000 nM) and for 3-methoxy-6-(2′-methoxybenzylthio)-2-(biphenyl-4"-yl)-imidazo[1,2-*b*]pyridazine (**V.5b**; 4% displacement at 1000 nM) reveals that the 2-phenethyl compound bound much more strongly than the 2-(biphenyl-4"-yl) analogue.

Whereas 3-benzamidomethyl-6-chloro-2-phenethyl-imidazo[1,2-*b*]pyridazine (**V.11e**; 22% displacement at 1000 nM) bound more strongly than its 3-unsubstituted analogue (**V.9l**; 3% displacement at 1000 nM), both 3-benzamidomethyl-2-benzyl-6-chloroimidazo[1,2-*b*]pyridazine (**V.11a**; IC_{50} >>1000 nM) and its 3-unsubstituted analogue (**V.9e**; 1% displacement at 1000 nM) did not show significant activity at 1000 nM. Also the 3-dimethyl-aminomethyl group did not engender significant activation in these 2-benzyl- and 2-phenethyl-imidazo[1,2-*b*]-pyridazines.

In the 3-acetamidomethyl, 3-benzamidomethyl and 3-unsubstituted-6-chloro-2-phenethylimidazo[1,2-*b*]-pyridazines (**V.11d,e** and **9l**; 8.7%, 22% and 3% displacement at 1000 nM respectively) the order of activity was 3-benzamidomethyl > 3-acetamidomethyl > 3-hydrogen, consistent with that found in the 2-phenyl analogues (**II.6j**, IC_{50} 140 nM, **II.5b**, IC_{50} 474 nM and **II.3o**, IC_{50} >3000 nM respectively, see Chapter II); but

in the 6-benzylthio-2-phenethylimidazo[1,2-*b*]pyridazines, 3-benzamidomethyl-6-benzylthio-2-phenethylimidazo-[1,2-*b*]pyridazine (**V.11c**; 12% displacement at 1000 nM) was slightly weaker than the corresponding 3-acetamidomethyl compound (**V.11b**; 15% displacement at 1000 nM).

In summary, it appears that the activity of interaction with benzodiazepine receptors by derivatives of 2-phenyl(or substituted phenyl)imidazo[1,2-*b*]-pyridazine is effectively destroyed in the 2-t-butyl and 2-cyclohexyl analogues; and replacement of the 2-phenyl group by larger and non-conjugating group such as 2-benzyl, 2-phenethyl or by the larger conjugating 2-(biphenyl-4′-yl) or (6′-methylnaphthalen-2′-yl) groups markedly decreases binding ability which is in the order phenyl > benzyl > phenethyl > biphenyl-4′-yl.

Despite the low activity of many of these compounds in tests in the central nervous system, some of these compounds proved quite active (IC_{50} values <10 nM) in binding to peripheral (mitochondrial) tissue (personal communication from Dr L.P. Davies).

V-5 Experimental

The general procedure and experimental details for the [^{3}H]diazepam binding assay are recorded in Chapter II-5.3 except that samples of compounds prepared in this chapter for analysis were dried at 55-90°/0.1 mmHg for 5-20 h unless otherwise specified.

Benzylglyoxal

This compound[246] was prepared from mandelic acid through acetylmandelic acid,[258] acetylmandelyl chloride (^{1}H n.m.r.: δ 2.21, s, MeCO; 6.08, s, CH; 7.47, s, Ph) and 1-acetoxy-3-diazo-1-phenylacetone.[246] (^{1}H n.m.r.: δ 2.18, s, MeCO; 5.46, s, CHN_2; 6.00, s, CH; 7.39, s, Ph). The product was recrystallised from light petroleum and had m.p. 116-119° (lit.[246] 119-121°) ^{1}H n.m.r.: δ 6.18, s, 6.63, s, CH_2; 7.36-7.91, complex, Ph, 9.26, s, CHO.

Phenethylglyoxal

This compound was prepared from hydrocinnamic acid (3-phenylpropionic acid) through hydrocinnamyl chloride,[259] diazomethyl phenethyl ketone[247] and the α-keto-triphenyl phosphazine.[247]

The product had b.p. 79-80°/1 mmHg (lit. 80°/1 mmHg) ^{1}H n.m.r.: δ 2.95-3.05, complex, CH_2CH_2; 7.21-7.33, complex, Ph; 9.75, s, CHO.

Biphenyl-4-ylglyoxal

This compound was prepared from 4-acetylbiphenyl by oxidation with selenium dioxide in dioxane. The product

was recrystallised from water and had m.p. 119-121° (lit.[248] 114-117°) ^{1}H n.m.r.: δ 7.45-8.34, complex, PhC_6H_4; 9.70, s, CHO.

4'-Nitrobiphenyl-4-ylglyoxal

This compound was prepared from 4-nitrobiphenyl through 4-acetyl-4'-nitrobiphenyl[249] which was oxidised with selenium dioxide in aqueous dioxane. The product crystallised from methanol as yellow crystals (38%), m.p. 174-176° (Found, for a sample dried at 50°/710 mmHg for 5 h: C, 63.9; H, 4.1; N, 5.5. $C_{14}H_9NO_4$. 0.5 MeOH requires C, 64.2; H, 4.1; N, 5.2%). ^{1}H n.m.r.: δ 3.58, s, MeO, 7.73-8.40, complex, $C_6H_4C_6H_4$; 9.69, s, CHO.

t-Butylglyoxal

This compound was prepared by oxidation[250] of pinacolone with selenium dioxide in methanol. The product was distilled (b.p. 113-118°/710 mmHg, lit.[250] 114-115°) mixed with water and the hemihydrate recrystallised from benzene. It had m.p. 78-80° with prior softening (lit.[250] 91-92° with softening at 85°; lit.[260] 85°).

Cyclohexylglyoxal

This compound[251] was prepared from cyclohexyl chloride through cyclohexyl methyl ketone[252] by oxidation with selenium dioxide in aqueous dioxane. The product was distilled and had b.p. 78-90°/40 mmHg (lit.[251] 71-72°/17 mmHg) ^{1}H n.m.r.: δ 1.30-2.69, complex, C_6H_{11}; 9.23, s, CHO.

1-Bromo-3-phenylpropanone

This compound was prepared from phenylacetyl chloride as described by Lohrisch *et al.*[253]. It had b.p. 100-104°/0.9 mmHg (lit. 65°/0.04 mmHg).

1-Bromo-4-phenylbutan-2-one

This compound was prepared from 3-phenylpropionic acid as described by Bestmann *et. al.*[254]. It was distilled (b.p. 102-120°/0.1 mmHg) and had m.p. 32-33° (lit.[254] 47-48°). ^{1}H n.m.r.: δ 2.96, s, CH_2CH_2; 3.83, s, CH_2Br; 7.24, s, Ph.

α-Bromo-4-phenylacetophenone

This compound[255] was prepared from 4-phenyl-acetophenone by bromination in acetic acid. The product was filtered off and had ^{1}H n.m.r.: δ 4.48, s, CH_2; 7.41-8.12, complex, PhC_6H_4.

1-Bromo-3,3-dimethylbutan-2-one

This compound was prepared by bromination of pinacolone in anhydrous ether as described by Boyer and Straw.[256] It had b.p. 46-50°/1.5 mmHg (lit.[256] 59°/4 mmHg) ^{1}H n.m.r.: δ 1.20, s, Bu^t; 4.16, s, CH_2.

α-Bromomethyl cyclohexyl ketone

This compound was prepared by bromination[257] of cyclohexylmethyl ketone in methanol as described for 1-

methyl-3-methyl-2-butanone.[257] ^{1}H n.m.r.: δ 1.28-2.71, complex, C_6H_{11}; 3.96, s, CH_2.

2-Benzyl-3-methoxy-6-(2′-methoxybenzylthio)imidazo-[1,2-b]pyridazine (V.3b)

Benzylglyoxal (0.060 g) was added to a solution of 6-(2′-methoxybenzylthio)pyridazin-3-amine (0.1 g) and concentrated hydrochloric acid (0.06 ml) in ethanol (2.0 ml) and the mixture was refluxed with stirring in an oil bath overnight.

After cooling in ice a solution of ethereal diazomethane was added and the mixture stirred at 20° overnight. The solvents were evaporated and the product subjected to t.l.c. (alumina; chloroform-light petroleum, 1:1) and recrystallised from light petroleum to give the *title compound* (0.020 g), m.p. 131-133° (Found, for a sample dried at 60°/0.1 mmHg for 2 h: C, 67.4; H, 5.7; N, 10.7. $C_{22}H_{21}N_3O_2S$ requires C, 67.5; H, 5.4; N, 10.7%). ^{1}H n.m.r.: δ 3.87, s, 3.96, s, 2′-OMe, 3-OMe; 4.11, s, CH_2; 4.48, s, CH_2S; 6.70, d, J 9 Hz, H7; 6.81-7.54, complex, Ph and H3′,4′,5′,6′; 7.53, d, J 9 Hz, H8.

3-Methoxy-6-(3′-nitrobenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4l)

A mixture of 6-(3′-nitrobenzylthio)pyridazin-3-amine (0.2 g), phenylethylglyoxal (0.15 g), hydrochloric acid (0.12 ml) and ethanol (4.0 ml) was refluxed with stirring overnight, and then cooled in ice. Excess ethereal diazomethane solution was then added and stirring

continued overnight. The solvents were removed and the product subjected to t.l.c. (alumina; chloroform) to give the *title compound* (0.112 g) (Found: C, 62.9; H, 5.0; N, 13.6. $C_{22}H_{20}N_4O_3S$ requires C, 62.8; H, 4.8; N, 13.3%). ^1H n.m.r.: δ 3.06, s, CH_2CH_2; 3.79, s, MeO; 4.49, s, CH_2S; 6.73, d, J 9 Hz, H7; 7.22-8.37, complex, Ph and H2′,4′,5′,6′; 7.59, d, J 9 Hz, H8.

6-(3′-Aminobenzylthio)-3-methoxy-2-phenethylimidazo-[1,2-b]pyridazine (V.4n)

A solution of 3-methoxy-6-(3′-nitrobenzylthio)-2-(phenethyl)imidazo[1,2-*b*]pyridazine (0.11 g) in methanol (10 ml) was added over 5 minutes to a rapidly stirred mixture of iron powder (0.4 g), methanol (12 ml), water (4.0 ml) and concentrated hydrochloric acid (0.8 ml) at 80-85° and the mixture maintained at that temperature for 3 h. The mixture was filtered hot and the residue washed with hot methanol. The combined filtrates were evaporated to dryness, the residue diluted with water and adjusted to pH 7 and extracted with chloroform. The product was subjected to t.l.c. (alumina; chloroform) and gave the *title compound* (0.075 g) as an oil. (Found: C, 68.0; H, 6.0; N, 14.4. $C_{22}H_{22}N_4OS$ requires C, 67.7; H, 5.7; N, 14.4%). ^1H n.m.r.: δ 3.06, s, CH_2CH_2; 3.80, s, MeO; 4.34, s, CH_2S; 6.51-7.22, complex, Ph and H2′,4′,5′,6′; 6.71, d, J 9 Hz, H7; 7.56, d, J 9 Hz, H8.

2-(Biphenyl-4'-yl)-6-chloro-3-methoxyimidazo-[1,2-b]pyridazine (V.5e)

Biphenyl-4-ylglyoxal (0.18 g) was added to a mixture of 6-chloropyridazin-3-amine (0.1 g), ethanol (2.0 ml) and concentrated hydrochloric acid (0.2 ml) and the mixture refluxed with stirring in an oil bath at 95° for 5 h. The volatile material was then removed under reduced pressure, the residue diluted with methanol (2.0 ml), chilled in ice, and stirred with excess ethereal diazomethane overnight. The solvents were evaporated and the residue subjected to t.l.c. (alumina; chloroform) and the product recrystallised from light petroleum to give the *title compound* (0.074 g), m.p. 166-167° (Found: C, 68.0; H, 4.2; N, 12.5. $C_{19}H_{14}ClN_3O$ requires C, 68.0; H, 4.2; N, 12.5%). ^{1}H n.m.r.: δ 4.19, s, MeO; 7.00, d, J 9 Hz, H7; 7.26-8.27, complex, C_6H_4Ph and H8.

2-t-Butyl-3-methoxy-6-(2'-methoxyphenoxy)imidazo[1,2-b]-pyridazine (V.7d)

1-Bromo-3,3-dimethylbutan-2-one (0.14 g) was added to a warm solution of 6-(2'-methoxyphenoxy)pyridazin-3-amine 2-oxide (0.1 g) in ethanol (2.0 ml) and the mixture refluxed with stirring in an oil bath for 7 h. The reaction mixture was evaporated under reduced pressure, the residue dissolved in methanol (2 ml), cooled in ice, and stirred with excess ethereal diazomethane in ice and then at 20° overnight. The volatile material was removed under reduced pressure and the residue subjected to t.l.c. (alumina; chloroform) to give the *title compound*

(0.060 g), m.p. 106-107° (Found: C, 66.1; H, 6.7; N, 13.0. $C_{18}H_{21}N_3O_3$ requires C, 66.0; H, 6.5; N, 12.8%). ^{1}H n.m.r.: δ 1.41, s, Bu^t; 3.77, s, 3.79, s, 2′-OMe, 3-OMe; 6.75, d, J 9 Hz, H7; 6.99-7.26, complex, H3′,4′,5′,6′; 7.77, d, J 9 Hz, H8.

2-Benzyl-6-benzylthioimidazo[1,2-b]pyridazine (V.9a)

6-Benzylthiopyridazin-3-amine (0.217 g), 1-bromo-3-phenylpropanone (0.33 g) and ethanol (5.0 ml) were refluxed with stirring in an oil bath at 95° overnight. The solvent was evaporated and the residue subjected to t.l.c. (alumina; light petroleum, chloroform, 1:1) to give the *title compound* (0.124 g) (Found: C, 72.3; H, 5.3; N, 12.7. $C_{20}H_{17}N_3S$ requires C, 72.5; H, 5.2; N, 12.7%). ^{1}H n.m.r.: δ 4.14, s, CH_2; 4.37, s, CH_2S; 6.77, d, J 9 Hz, H7; 7.31, complex, 2xPh; 7.55, s, H3; 7.64, d, J 9 Hz, H8.

6-(4′-Methoxybenzylthio)-2-phenethylimidazo[1,2-b]-pyridazine (V.9k)

A mixture of 6-(4′-methoxybenzylthio)pyridazin-3-amine (1.0 g), 1-bromo-4-phenylbutan-2-one (0.92 g) and ethanol (15 ml) was refluxed with stirring for 2 h. Sodium hydrogen carbonate (0.18 g) was then added and the reflux continued overnight. The solvent was evaporated and the residue diluted with water and extracted with chloroform. The extract was dried (Na_2SO_4), the solvent evaporated and the product subjected to t.l.c. (alumina; chloroform, light petroleum, 7:3) and recrystallised from

light petroleum to give the *title compound* (0.058 g), m.p. 93-94° (Found: C, 70.2; H, 5.9; N, 11.3. $C_{22}H_{21}N_3OS$ requires C, 70.4; H, 5.6; N, 11.2%). ^1H n.m.r.: δ 3.12, s, CH_2CH_2; 3.79, s, MeO; 4.36, s, CH_2S; 6.77-7.76, complex, ArH.

2-Benzyl-6-(4'-chlorophenoxy)-3-dimethylaminomethyl-imidazo[1,2-b]pyridazine (V.10f)

2-Benzyl-6-(4'-chlorophenoxy)imidazo[1,2-*b*]-pyridazine (0.046 g) was added to a solution of bis(dimethylamino)methane (0.1 g),[215] phosphoric acid (0.2 ml) and acetic acid (2 ml) and the mixture was refluxed with stirring in an oil bath at 120° overnight. It was cooled, diluted with water, adjusted with aqueous sodium hydroxide to pH 12, and extracted with chloroform. After drying (Na_2SO_4) the solvent was evaporated and the oil was subjected to t.l.c. (alumina; chloroform, light petroleum, 7:3) to give the *title compound* (0.042 g) (Found: C, 67.0; H, 5.7; N, 14.2. $C_{22}H_{21}ClN_4O$ requires C, 67.3; H, 5.4; N, 14.3%). ^1H n.m.r.: δ 2.17, s, Me_2N; 3.60, s, CH_2N; 4.18, s, CH_2; 6.80, d, J 9 Hz, H7; 7.13-7.43, complex, Ph and H2',3',5',6'; 7.85, d, J 9 Hz, H8.

3-Acetamidomethyl-2-phenethyl-6-phenylthioimidazo[1,2-b]-pyridazine (V.11f)

2-Phenethyl-6-phenylthioimidazo[1,2-*b*]pyridazine (0.6 g) was added to a solution of *N*-(hydroxymethyl)-acetamide[216] (0.3 g), acetic acid (4.0 ml) and concentrated sulphuric acid (0.3 ml) and the mixture

refluxed with stirring in an oil bath at 120° overnight. The acetic acid was evaporated under reduced pressure and the residue diluted with water, adjusted to pH 11 with aqueous ammonia, and the mixture extracted with chloroform. The solvent was evaporated and the residue was subjected to t.l.c. (alumina, chloroform). The product was recrystallised from a mixture of acetone and light petroleum and gave the *title compound* (0.025 g), m.p. 134-136°. (Found: C, 68.4; H, 5.6; N, 14.2. $C_{23}H_{22}N_4OS$ requires C, 68.6; H, 5.5; N, 13.9%). ^1H n.m.r.: δ 1.78, s, MeCO; 3.08, s, CH_2CH_2; 4.39, d, J 6 Hz, CH_2N; 6.81, d, J 9 Hz, H7; 7.20-7.60, complex, 2xPh, 7.69, d, J 9 Hz, H8.

3-Benzamidomethyl-2-benzyl-6-chloroimidazo[1,2-b]-pyridazine (V.11a)

2-Benzyl-6-chloroimidazo[1,2-*b*]pyridazine (0.16 g) was added to a solution of *N*-(hydroxymethyl)benzamide[216] (0.1 g) in acetic acid (3.0 ml) containing sulphuric acid (0.2 ml) and the mixture was refluxed in an oil bath at 120° overnight. The acetic acid was evaporated under reduced pressure and the residue diluted with water, adjusted with aqueous sodium hydroxide to pH 11 and extracted with chloroform. The solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform) and recrystallised from acetone/light petroleum to give the *title compound* (0.032 g), m.p. 177-178°. (Found: C, 66.7; H, 4.7; N, 14.8. $C_{21}H_{17}ClN_4O$ requires C, 66.9; H, 4.5; N, 14.9%). ^1H n.m.r.: δ 4.33, s, CH_2; 4.98, d, J 6 Hz, CH_2N;

6.98, d, J 9 Hz, H7; 7.18-7.72, complex, 2xPh; 7.78, d, J 9 Hz, H8.

The following compounds were prepared in a similar manner.

2-Benzyl-3-methoxy-6-(4'-methoxyphenoxy)imidazo[1,2-b]-pyridazine (V.3s) (23%), m.p. 89-90° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum. (Found: C, 70.2; H, 5.5; N, 11.6. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r.: δ 3.79, s, 3.81, s, 4'-OMe, 3-OMe; 4.07, s, CH_2; 6.68, d, J 9 Hz, H7; 6.89, d, 7.12, d, J 9 H7, H2',3',5',6'; 7.26, bs, Ph; 7.71, d, J 9 Hz, H8.

2-Benzyl-6-(4'-chlorophenoxy)-3-methoxy[1,2-b]pyridazine (V.3t) (36%), as colourless crystals m.p. 102-103° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from light petroleum (Found: C, 65.8; H, 4.3; N, 11.2. $C_{20}H_{16}ClN_3O_2$ requires C, 65.7; H, 4.4; N, 11.5%). ^{1}H n.m.r.: δ 3.78, s, MeO; 4.07, s, CH_2; 6.72, d, J 9 Hz, H7; 7.16-7.42, complex, Ph and H2',3',5',6'; 7.75, d, J 9 Hz, H8.

2-Benzyl-3-methoxy-6-phenylthioimidazo[1,2-b]pyridazine (V.3u) (34%) after t.l.c. (alumina; chloroform, light petroleum, 1:1 then alumina; ether/light petroleum (b.p. 40-60°), 1:1, developed twice). (Found: C, 69.1; H, 5.2; N, 12.2. $C_{20}H_{17}N_3OS$ requires C, 69.1; H, 4.9; N, 12.1%).

^{1}H n.m.r.: δ 3.83, s, MeO; 4.09, s, CH_2; 6.65, d, J 9 Hz, H7; 7.19-7.66, complex, 2xPh; 7.57, d, J 9 Hz, H8.

2-Benzyl-3-methoxy-6-(2'-methylbenzylthio)imidazo[1,2-b]-pyridazine (V.3e) (51%) after t.l.c. (alumina; chloroform) (Found: C, 70.1; H, 5.9; N, 11.0. $C_{22}H_{21}N_3OS$ requires C, 70.4; H, 5.6; N, 11.2%). ^{1}H n.m.r.: δ 2.43, s, Me; 3.93, s, MeO; 4.11, s, CH_2; 4.45, s, CH_2S; 6.69, d, J 9 Hz, H7; 7.17-7.28, complex, Ph and H3',4',5',6; 7.54, d, J 9 Hz, H8.

2-Benzyl-3-methoxy-6-(3'-methylbenzylthio)imidazo[1,2-b]-pyridazine (V.3f) (36%) after t.l.c. (alumina; chloroform and alumina; chloroform, light petroleum, 1:1) (Found: C, 70.0; H, 5.9; N, 11.1. $C_{22}H_{21}N_3OS$ requires C, 70.4; H, 5.6; N, 11.2%). ^{1}H n.m.r.: δ 2.33, s, Me; 3.92, s, MeO; 4.11, s, CH_2; 4.41, s, CH_2S; 6.73, d, J 9 Hz, H7; 7.11-7.35, complex, H 2',4',5',6' and Ph; 7.58, d, J 9 Hz, H8.

2-Benzyl-3-methoxy-6-(3'-methoxybenzylthio)imidazo-[1,2-b]pyridazine (V.3c) (28%) after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 67.4; H, 5.7; N, 10.7. $C_{22}H_{21}N_3O_2S$ requires C, 67.5; H, 5.4; N, 10.7%). ^{1}H n.m.r.: δ 3.76, s, 3.90, s, 3'-OMe, 3-OMe; 4.10, s, CH_2; 4.40, s, CH_2S; 6.69, d, J 9 Hz, H7; 6.84-7.28, complex, Ph and H2',4',5',6'; 7.54, d, J 9 Hz, H8.

2-Benzyl-3-methoxy-6-(4'-methoxybenzylthio)imidazo-[1,2-b]pyridazine (V.3d) (40%) after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 67.4; H, 5.7; N, 10.5. $C_{22}H_{21}N_3O_2S$ requires C, 67.5; H, 5.4; N, 10.7%). ^{1}H n.m.r.: δ 3.77, s, 3.94, s, 4'-OMe, 3-OMe; 4.10, s, CH_2; 4.39, s, CH_2S; 6.69, d, J 9 Hz, H7; 6.73, d, 7.38, d, J 8.5 Hz, H2',3',5',6'; 7.35, complex, Ph 7.54, d, J 9 Hz, H8.

2-Benzyl-6-(2'-chlorobenzylthio)-3-methoxyimidazo[1,2-b]-pyridazime (V.3g) (22%) after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found, for a sample dried at 50°/0.1 mmHg for 24 h: C, 63.3; H, 4.8; N, 10.4. $C_{21}H_{18}ClN_3OS$ requires C, 63.7; H, 4.6; N, 10.6%). ^{1}H n.m.r.: δ 3.92, s, MeO; 4.11, s, CH_2; 4.55, s, CH_2S; 6.71, d, J 9 Hz, H7; 7.13-7.67, complex, Ph and H3',4',5',6'; 7.55, d, J 9 Hz, H8.

2-Benzyl-6-(3'-chlorobenzylthio)-3-methoxyimidazo[1,2-b]-pyridazine (V.3h) (29%) as a colourless solid, m.p. 124-126° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum. (Found: 63.8; H, 4.6; N, 10.8. $C_{21}H_{18}ClN_3OS$ requires C, 63.7; H, 4.6; N, 10.6%). ^{1}H n.m.r.: δ 3.88, s, MeO; 4.11, s, CH_2; 4.40, s, CH_2S; 6.74, d, J 9 Hz, H7; 7.15-7.35, complex, Ph and H2',4',5',6'; 7.61, d, J 9 Hz, H8.

2-Benzyl-6-(4'-chlorobenzylthio)-3-methoxyimidazo[1,2-b]-pyridazine (V.3i) (27%) after t.l.c. (alumina, chloroform). (Found: 63.3; H, 4.8; N, 10.2. $C_{21}H_{18}ClN_3OS$ requires C, 63.7; H, 4.6; N, 10.6%). ^1H n.m.r.: δ 3.90, s, MeO; 4.10, s, CH_2; 4.39, s, CH_2S; 6.70, d, J 9 Hz, H7; 7.21-7.37, complex, Ph and H2',3',5',6'; 7.56, d, J 9 Hz, H8.

2-Benzyl-6-(2',4'-dichlorobenzylthio)-3-methoxyimidazo-[1,2-b]pyridazine (V.3j) (10%), m.p. 95-97° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum (Found: C, 58.3; H, 4.0; N, 9.7. $C_{21}H_{17}Cl_2N_3OS$ requires C, 58.6; H, 4.0; N, 9.8%). ^1H n.m.r.: δ 3.92, s, MeO; 4.11, s, CH_2; 4.51, s, CH_2S; 6.71, d, J 9 Hz, H7; 7.21-7.54, complex, Ph and H3',5',6'; 7.56, d, J 9 Hz, H8.

2-Benzyl-3-methoxy-6-(2'-nitrobenzylthio)imidazo-[1,2-b]pyridazine (V.3k) (22%), m.p. 118-120° after t.l.c. (alumina, chloroform) and recrystallisation from light petroleum. (Found: C, 62.4;, H, 4.7; N, 13.8. $C_{21}H_{18}N_4O_3S$ requires C, 62.1; H, 4.5; N, 13.8%). ^1H n.m.r.: δ 3.91, s, MeO; 4.10, s, CH_2; 4.79, s, CH_2S; 6.68, d, J 9 Hz, H7; 7.20-8.10, complex, Ph and H3',4',5',6' and 8.

2-Benzyl-3-methoxy-6-(3'-nitrobenzylthio)imidazo[1,2-b]-pyridazine (V.3l) (44%), m.p. 94-96° after t.l.c. (alumina; chloroform) and recrystallisation from light

petroleum. (Found: C, 62.4, H, 4.5; N, 14.0. $C_{21}H_{18}N_4O_3S$ requires C, 62.0; H, 4.5; N, 13.8%). ^1H n.m.r.: δ 3.90, s, MeO; 4.10, s, CH_2; 4.49, s, CH_2S; 6.72, d, J 9 Hz, H7; 7.28-8.38, complex, Ph and H2′,4′,5′,6′,8.

2-Benzyl-3-methoxy-6-(4′-nitrobenzylthio)imidazo[1,2-b]-pyridazine (V.3m) (12%), m.p. 99-101° after t.l.c. (alumina, chloroform) and recrystallisation from light petroleum. (Found: C, 61.9; H, 4.5; N, 13.7. $C_{21}H_{18}N_4O_3S$ requires C, 62.1; H, 4.5; N, 13.8%). ^1H n.m.r.: δ 3.87, s, MeO; 4.11, s, CH_2; 4.50, s, CH_2S; 6.74, d, J 9 Hz, H7; 7.26-8.23, complex, Ph and H2′,3′,5′,6 and 8.

6-(3′-Aminobenzylthio)-2-benzyl-3-methoxyimidazo[1,2-b]-pyridazine (V.3n) (77%), m.p. 102-104° after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum (Found: C, 67.4; H, 5.7; N, 14.8. $C_{21}H_{20}N_4OS$ requires C, 67.0; H, 5.4; N, 14.9%). ^1H n.m.r.: δ 3.34, br, NH_2; 3.92, s, MeO; 4.09, s, CH_2; 4.34, s, CH_2S; 6.51-7.30, complex, Ph and H3′,4′,5′,6′ and 7; 7.54, d, J 9 Hz, H8.

6-(4′-Aminobenzylthio)-2-benzyl-3-methoxyimidazo[1,2-b]-pyridazine (V.3o) (95%), m.p. 101-103° after t.l.c. (alumina, chloroform) and recrystallisation from light petroleum (Found, for a sample dried at 55°/0.1mmHg for 2 h: C, 67.2; H, 5.7; N, 15.0. $C_{21}H_{20}N_4OS$ requires C, 67.0; H, 5.4; N, 14.9%). ^1H n.m.r.: δ 3.95, s, MeO;

4.10, s, CH_2; 4.35, s, CH_2S; 6.56-7.27, complex, Ph and H2′,3′,5′,6′ and 7; 7.53, d, J 9 Hz, H 8.

2-Benzyl-3-methoxy-6-(pyridin-2′-ylmethylthio)imidazo-[1,2-b]pyridazine (V.3p) (18%), after t.l.c. (alumina, chloroform) (Found: C, 66.0; H, 5.3; N, 15.8. $C_{20}H_{18}N_4OS$ requires C, 66.3; H, 5.0; N, 15.5%). ^1H n.m.r.: δ 3.88, s, MeO; 4.09, s, CH_2; 4.58, s, CH_2S; 6.76, d, J 9 Hz, H 7; 7.25-8.59, complex, Ph and H3′,4′,5′,6′,8; 7.55, d, J 9 Hz, H8.

2-Benzyl-3-methoxy-6-(pyridin-3′-ylmethylthio)imidazo-[1,2-b]pyridazine (V.3q) (10%), after t.l.c. (alumina, chloroform) (Found: C, 65.9; H, 5.3; N, 15.2. $C_{20}H_{18}N_4OS$ requires C, 66.3; H, 5.0; N, 15.5%). ^1H n.m.r.: δ 3.90, s, MeO; 4.10, s, CH_2; 4.42, s, CH_2S; 6.71, d, J 9 Hz, H7; 7.26-7.86, complex, Ph and H2′,4′,5′,6′; 7.57, d, J 9 Hz, H8.

2-Benzyl-3-methoxy-6-(pyridin-4′-ylmethylthio)imidazo-[1,2-b]pyridazine (V.3r) (18%), after t.l.c. (alumina, chloroform) (Found: C, 66.5; H, 5.3; N, 15.7. $C_{20}H_{18}N_4OS$ requires C, 66.3; H, 5.0; N, 15.5%). ^1H n.m.r.: δ 3.80, s, MeO; 4.09, s, CH_2; 4.39, s, CH_2S; 6.73, d, J 9 Hz, H7; 7.27-8.54, complex, Ph and H2′,3′,5′,6′; 7.58, d, J 9 Hz, H8.

3-Methoxy-6-(4′-methoxyphenoxy)-2-phenethylimidazo-[1,2-b]pyridazine (V.4p) (39%), after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum. (Found: C, 70.4; H, 5.9; N, 11.4. $C_{22}H_{21}N_3O_3$ requires C, 70.4; H, 5.6; N, 11.2%). ^{1}H n.m.r.: δ 3.05, s, CH_2CH_2; 3.67, s, 4′-OMe; 3.83, s, 3-OMe; 6.72, d, J 9 Hz, H7; 6.91, d, 7.09, d, J 9 Hz, H2′,3′,5′,6′; 7.23, bs, Ph; 7.75, d, J 9 Hz, H8.

6-(4′-Chlorophenoxy)-3-methoxy-2-phenethylimidazo[1,2-b]-pyridazine (V.4q) (47%), after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum. (Found: C, 66.4; H, 4.8; N, 11.2. $C_{21}H_{18}ClN_3O_2$ requires C, 66.4; H, 4.8; N, 11.1%). ^{1}H n.m.r.: δ 3.06, s, CH_2CH_2; 3.66, s, MeO; 6.74, d, J 9 Hz, H7; 7.10-7.44, complex, Ph and H2′,3′,5′,6′; 7.80, d, J 9 Hz, H8.

3-Methoxy-6-(2′-methylbenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4d) (46%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 70.5; H, 6.3; N, 10.4. $C_{23}H_{23}N_3OS$ requires C, 70.9; H, 6.0; N, 10.8%). ^{1}H n.m.r.: δ 2.44, s, MeC; 3.07, s, CH_2CH_2; 3.82, s, MeO; 4.45, s, CH_2S; 6.72, d, J 9 Hz, H7; 7.19-7.43, complex, Ph and H3′,4′,5′,6′; 7.57, d, J 9 Hz, H8.

3-Methoxy-6-(3′-methylbenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4e) (37%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 70.9; H,

6.1; N, 10.7. $C_{23}H_{23}N_3OS$ requires C, 70.9; H, 6.0; N, 10.8%). ^1H n.m.r.: δ 2.33, s, MeC; 3.07, s, CH_2CH_2; 3.81, s, MeO; 4.41, s, CH_2S; 6.73, d, J 9 Hz, H7; 7.11-7.28, complex, Ph and H2′,4′,5′,6′; 7.57, d, J 9 Hz, H8.

3-Methoxy-6-(4′-methylbenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4f) (27%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 70.8; H, 6.2; N, 10.7. $C_{23}H_{23}N_3OS$ requires C, 70.9; H, 6.2; N, 10.7%). ^1H n.m.r.: δ 2.31, s, MeC; 3.07, s, CH_2CH_2; 3.81, s, MeO; 4.40, s, CH_2S; 6.72, d, J 9 Hz, H7; 7.07-7.40, complex, Ph and H2′,3′,5′,6′; 7.56, d, J 9 Hz, H8.

3-Methoxy-6-(2′-methoxybenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4a) (23%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 67.7; H, 5.8; N, 10.7. $C_{23}H_{23}N_3O_2S$ requires C, 68.1; H, 5.7; N, 10.4%). ^1H n.m.r.: δ 3.07, s, CH_2CH_2; 3.84, s, 3.87, s, 2′-OMe, 3-OMe; 4.47, s, CH_2S; 6.72, d, J 9 Hz, H7; 6.80-7.45, complex, Ph and H3′,4′,5′,6′; 7.56, d, J 9 Hz, H8.

6-(2′-Chlorobenzylthio)-3-methoxy-2-phenethylimidazo-[1,2-b]pyridazine (V.4g) (35%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 64.1; H, 5.1; N, 10.0. $C_{22}H_{18}ClN_3OS$ requires C, 64.2; H, 4.5; N, 10.2%). ^1H n.m.r.: δ 3.07, s, CH_2CH_2; 3.80, s, MeO; 4.55, s, CH_2S; 6.72, d, J 9 Hz, H7; 7.13-7.66, complex, Ph and H3′,4′,5′,6′; 7.57, d, J 9 Hz, H8.

6-(3′-Chlorobenzylthio)-3-methoxy-2-phenethylimidazo-[1,2-b]pyridazine (V.4h) (37%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 65.1; H, 4.8; N, 10.6. $C_{22}H_{18}ClN_3OS$ requires C, 64.8; H, 4.4; N, 10.3%). ^{1}H n.m.r.: δ 3.07, s, CH_2CH_2; 3.78, s, MeO; 4.39, s, CH_2S; 6.72, d, J 9 Hz, H7; 7.22-7.49, complex, Ph and H2′,4′,5′,6′; 7.58, d, J 9 Hz, H8.

3-Methoxy-6-(3′-methoxybenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4b) (29%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 67.6; H, 5.9; N, 10.1. $C_{23}H_{23}N_3O_2S$ requires C, 68.1; H, 5.7; N, 10.4%). ^{1}H n.m.r.: δ 3.07, complex, CH_2CH_2; 3.80, s, 3′-OMe, 3-OMe; 4.42, s, CH_2S; 6.73, d, J 9 Hz, H8; 6.86-7.23, complex, Ph and H2′,4′,5′,6′; 7.58, d, J 9 Hz, H8.

3-Methoxy-6-(4′-methoxybenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4c) (45%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 67.6; H, 5.9; N, 10.0. $C_{23}H_{23}N_3O_2S$ requires C, 68.1; H, 5.7; N, 10.4%). ^{1}H n.m.r.: δ 3.07, s, CH_2CH_2; 3.78, s, 3.82, s, 4′-OMe, 3-OMe; 4.39, s, CH_2S; 6.72, d, J 9 Hz, H7; 6.79-7.42, complex, Ph and H2′,3′,5′,6′; 7.56, d, J 9 Hz, H8.

6-(4′-Chlorobenzylthio)-3-methoxy-2-phenethylimidazo-[1,2-b]pyridazine (V.4i) (30%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 64.2; H, 4.8; N, 10.3. $C_{22}H_{18}ClN_3OS$ requires C, 64.8; H, 4.5; N, 10.3%). ^{1}H n.m.r.: δ 3.07, s, CH_2CH_2; 3.79, s, MeO;

4.39, s, CH_2S; 6.72, d, J 9 Hz, H7; 7.22-7.37, complex, Ph and H2′,3′,5′,6′; 7.58, d, J 9 Hz, H8.

6-(2′,4′-Dichlorobenzylthio)-3-methoxy-2-phenethyl-imidazo[1,2-b]pyridazine (V.4j) (27%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 59.6; H, 4.2; N, 9.6. $C_{22}H_{19}Cl_2N_3OS$ requires C, 59.5; H, 4.3; N, 9.5%). ^{1}H n.m.r.: δ 3.07, s, CH_2CH_2; 3.80, s, MeO; 4.50, s, CH_2S; 6.72, d, J 9 Hz, H7; 7.15-7.43, complex, Ph and H3′,5′,6′; 7.59, d, J 9 Hz, H8.

3-Methoxy-6-(2′-nitrobenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4k) (33%), after t.l.c. (alumina; chloroform). (Found: C, 62.8; H, 4.8; N, 13.3. $C_{33}H_{20}N_4O_3S$ requires C, 62.8; H, 4.8; N, 13.3%). ^{1}H n.m.r.: δ 3.06, s, CH_2CH_2; 3.79, s, MeO; 4.79, s, CH_2S; 6.70, d, J 9 Hz, H7; 7.22-8.11, complex, Ph and H3′,4′,5′,6′; 7.57, d, J 9 Hz, H8.

3-Methoxy-6-(4′-nitrobenzylthio)-2-phenethylimidazo-[1,2-b]pyridazine (V.4m) (50%), after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 62.9; H, 5.1; N, 13.1. $C_{22}H_{20}N_4O_3S$ requires C, 62.8; H, 4.8; N, 13.3%). ^{1}H n.m.r.: δ 3.06, s, CH_2CH_2; 3.75, s, MeO; 4.48, s, CH_2S; 6.72, d, J 9 Hz, H7; 7.22, s, Ph; 7.59, d, J 9 Hz, H8; 7.65, d, 8.15, d, J 8.5 Hz, H2′,3′,5′,6′.

6-(4′-Aminobenzylthio)-3-methoxy-2-phenethylimidazo-[1,2-b]pyridazine (V.4o) (41%), m.p. 117-119° after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum. (Found, for a sample dried at 50°/0.1 mm Hg for 3 h: C, 67.3; H, 5.7; N, 14.1. $C_{22}H_{22}N_4OS$ requires C, 67.7; H, 5.7; N, 14.3%). ^{1}H n.m.r.: δ 3.07, s, CH_2CH_2; 3.83, s, MeO; 4.34, s, CH_2S; 7.57, br, NH_2; 6.71, d, J 9 Hz, H7; 7.20-7.28, complex, Ph and H2′,3′,5′,6′; 7.55, d, J 9 Hz, H8.

3-Methoxy-2-phenethyl-6-phenylthioimidazo[1,2-b]-pyridazine (V.4r) (46%), after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 69.3; H, 5.6; N, 11.1. $C_{21}H_{19}N_3OS$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r.: δ 3.06, s, CH_2CH_2; 3.70, s, Me; 6.67, d, $J_{7,8}$ 9 Hz, H7; 7.21-7.60, complex, ArH; 7.60, d, $J_{7,8}$ 9 Hz, H8.

3-Methoxy-2-(4"-nitrobiphenyl-4′-yl)-6-phenoxyimidazo-[1,2-b]pyridazine (V.5i) (25%), m.p. 225-227° after t.l.c. (alumina; chloroform, light petroleum, 2:3) and recrystallisation from methanol. (Found: C, 68.8; H, 4.3; N, 12.7. $C_{25}H_{18}N_4O_4$ requires C, 68.5; H, 4.1; N, 12.8%). ^{1}H n.m.r.: δ 4.01, s, MeO; 6.90, d, J 9 Hz, H7; 7.25-8.36, complex, Ph, $C_6H_4C_6H_4$ and H8.

2-(Biphenyl-4′-yl)-3-methoxy-6-phenylthioimidazo[1,2-b]-pyridazine (V.5f) (33%), m.p. 127-128° after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found, for

a sample dried at 20°/0.1 mmHg for 20 h: C, 73.1; H, 4.8; N, 10.3. $C_{25}H_{19}N_3OS$ requires C, 73.2; H, 4.7; N, 10.3%). ^1H n.m.r.: δ 4.02, s, MeO; 6.72, d, J 9 Hz, H7; 7.41-8.23, complex, Ph, C_6H_4Ph and H8.

6-Benzyloxy-2-(biphenyl-4'-yl)-3-methoxyimidazo[1,2-b]-pyridazine (V.5g) (17%), m.p. 131-133° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum (Found: C, 76.7; H, 5.2; N, 10.6. $C_{26}H_{21}N_3O_2$ requires C, 76.6; H, 5.2; N, 10.3%). ^1H n.m.r.: δ 4.14, s, MeO; 5.45, s, CH_2O; 6.68, d, J 9 Hz, H7; 7.38-8.23, complex, Ph, C_6H_4Ph and H8.

6-Benzylthio-2-(biphenyl-4'-yl)-3-methoxyimidazo[1,2-b]-pyridazine (V.5a) (19%), m.p. 178-179° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum (Found: C, 73.9; H, 5.2; N, 10.1. $C_{26}H_{21}N_3OS$ requires C, 73.7; H, 5.0; N, 9.9%). ^1H n.m.r.: δ 4.13, s, MeO; 4.50, s, CH_2S; 6.79, d, J 9 Hz, H7; 7.25-8.25, complex, Ph, C_6H_4Ph and H8.

2-(Biphenyl-4'-yl)-3-methoxy-6-(2''-methoxybenzylthio)-imidazo[1,2-b]pyridazine (V.5b) (30%) after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 71.7; H, 5.4; N, 9.3. $C_{27}H_{23}N_3O_2S$ requires C, 71.5; H, 5.1; N, 9.3%). ^1H n.m.r.: δ 3.89, s, 2'-OMe; 4.19, s, 3-OMe; 4.33, s, CH_2S; 6.78, d, J 9 Hz, H7; 6.86-8.26, complex, C_6H_4Ph, H3',4',5',6 and 8.

2-(Biphenyl-4'-yl)-3-methoxy-6-(3''-methoxybenzylthio)-imidazo-[1,2-b]pyridazine (V.5c) (27%) after t.l.c. (alumina; chloroform, light petroleum, 1:1) (Found: C, 71.2; H, 5.2; N, 9.0. $C_{27}H_{23}N_3O_2S$ requires C, 71.5; H, 5.1; N, 9.3%). ^{1}H n.m.r.: δ 3.80, s, 3'-OMe; 4.13, s, 3-OMe; 4.47, s, CH_2S; 6.78, d, J 9 Hz, H7; 7.00-8.25, complex, C_6H_4Ph, H2',4',5',6' and 8.

2-(Biphenyl-4'-yl)-3-methoxy-6-(4''-methoxybenzylthio)-imidazo-[1,2-b]pyridazine (V.5d) (18%) after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum. (Found: C, 71.5; H, 5.2; N, 9.3. $C_{27}H_{23}N_4OS$ requires C, 71.5; H, 5.1; N, 9.3%). ^{1}H n.m.r.: δ 3.80, s, 4'-OMe; 4.17, s, 3-OMe; 4.46, s, CH_2S; 6.78, d, J 9 Hz, H7; 6.82-8.26, complex, C_6H_4Ph, H2',3',5',6 and 8.

6-Benzylthio-3-methoxy-2-(4"-nitrobiphenyl-4'-yl)imidazo-[1,2-b]pyridazine (V.5h) (35%), m.p. 209-211° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from ethanol. (Found: C, 66.3; H, 4.4; N, 12.0. $C_{26}H_{20}N_4O_3S$ requires C, 66.6; H, 4.3; N, 12.0%). ^{1}H n.m.r.: δ 4.19, s, MeO; 4.54, s, CH_2S; 7.10, d, J 9 Hz, H7; 7.28-8.37, complex, Ph, $C_6H_4C_6H_4$ and H8.

2-t-Butyl-6-fluoro-3-methoxyimidazo[1,2-b]pyridazine (V.7b) (33%), m.p. 83-85° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and sublimation. (Found: C, 59.0; H, 6.2; N, 18.6. $C_{11}H_{14}FN_3O$ requires

C, 59.2; H, 6.3; N, 18.8%). ^{1}H n.m.r.: δ 1.44, s, Bu^t; 4.06, s, MeO; 6.71, d, J 9 Hz, H7; 7.86, dd, $J_{7,8}$ 9 Hz, J_{HF} 7 Hz, H8.

2-t-Butyl-6-chloro-3-methoxyimidazo[1,2-b]pyridazine (V.7c) (30%), m.p. 82-84° after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from light petroleum. (Found, for a sample dried at 25°/0.1 mm Hg for 3 h: C, 55.2; H, 6.0; N, 17.2. $C_{11}H_{14}ClN_3O$ requires C, 55.1; H, 5.9; N, 17.5%). ^{1}H n.m.r.: δ 1.45, s, Bu^t; 4.07, s, MeO; 6.90, d, J 9 Hz, H7; 7.76, d, J 9 Hz, H8.

6-Benzyloxy-2-t-butyl-3-methoxyimidazo[1,2-b]pyridazine (V.7e) (30%) after t.l.c. (alumina; chloroform, light petroleum, 1:1). (Found: C, 69.3; H, 7.0; N, 13.3. $C_{18}H_{21}N_3O_2$ requires C, 69.4; H, 6.8; N, 13.5%). ^{1}H n.m.r.: δ 1.43, s, Bu^t; 4.01, s, MeO; 5.41, s, CH_2O; 6.61, d, J 9 Hz, H7; 7.30-7.45, complex, Ph; 7.71, d, J 9 Hz, H8.

6-Benzylthio-2-t-butyl-3-methoxyimidazo[1,2-b]pyridazine (V.7a) (39%) after t.l.c. (alumina; chloroform). (Found, for a sample dried at 50°/0.1 mm Hg for 15 h: C, 66.2; H, 6.7; N, 12.8. $C_{18}H_{21}N_3OS$ requires C, 66.0; H, 6.5; N, 12.8%). ^{1}H n.m.r.: δ 1.44, s, Bu^t; 3.99, s, MeO; 4.46, s, CH_2S; 6.73, d, J 9 Hz, H7; 7.25-7.55, complex, Ph; 7.62, d, J 9 Hz, H8.

2-Cyclohexyl-6-fluoro-3-methoxyimidazo[1,2-b]pyridazine (V.8a) (10%) after t.l.c. (alumina; chloroform, light petroleum, 1:1, then alumina; benzene). (Found, for a sample dried at 50°/0.1 mmHg for 2 h: C, 62.4; H, 6.4; N, 16.6. $C_{13}H_{16}FN_3O$ requires C, 62.6; H, 6.5; N, 16.9%). ^1H n.m.r.: δ 1.26-2.87, complex, C_6H_{11}; 4.07, s, MeO; 6.75, d, J 9 Hz, H7; 7.89, dd, $J_{7,8}$ 9 Hz, J_{HF} 7 Hz, H8.

6-Chloro-2-cyclohexyl-3-methoxyimidazo[1,2-b]pyridazine (V.8b) (40%) after t.l.c. (alumina; chloroform, light petroleum, 1:1, then alumina; benzene). (Found, for a sample dried at 50°/0.1 mmHg for 2 h: C, 58.7; H, 6.3; N, 15.8. $C_{13}H_{16}ClN_3O$ requires C, 58.7; H, 6.3; N, 15.8%). ^1H n.m.r.: δ 1.26-2.88, complex, C_6H_{11}; 4.09, s, MeO; 6.91, d, J 9 Hz, H7; 7.74, d, J 9 Hz, H8.

2-Cyclohexyl-3-methoxy-6-(2'-methoxyphenoxy)imidazo-[1,2-b]pyridazine (V.8c) (41%) after t.l.c. (alumina; chloroform). (Found: C, 67.4; H, 6.7; N, 12.2%. $C_{21}H_{25}N_3O_2S$ requires C, 68.0; H, 6.6; N, 11.9%). ^1H n.m.r.: δ 1.20-2.80, complex, C_6H_{11}; 3.77, s, 3.81, s, 2'-OMe, 3-OMe; 6.73, d, J 9 Hz, H7; 6.98-7.26, complex, H3',4',5',6'; 7.73, d, J 9 Hz, H8.

6-Benzyloxy-2-cyclohexyl-3-methoxyimidazo[1,2-b]-pyridazine (V.8d) (22%) after t.l.c. (alumina; chloroform). (Found: C, 70.9; H, 7.1; N, 12.3. $C_{20}H_{23}N_3O_2$ requires C, 71.2; H, 6.9; N, 12.5%). ^1H n.m.r.: δ 1.26-1.90, complex, C_6H_{11}; 4.01, s, MeO;

5.40, s, CH_2O; 6.60, d, J 9 Hz, H7; 7.43, m, Ph; 7.66, d, J 9 Hz, H8.

2-Benzyl-6-chloroimidazo[1,2-b]pyridazine (V.9e) (58%), m.p. 128-129° (from ethanol). (Found: C, 64.3; H, 4.1; N, 17.2. $C_{13}H_{10}ClN_3$ requires C, 64.1; H, 4.1; N, 17.2%). ^1H n.m.r.: δ 4.20, s, CH_2; 7.09, d, J 9 Hz, H7; 7.31, s, Ph; 7.62, s, H3; 7.95, d, J 9 Hz, H8.

2-Benzyl-6-(4'-chlorophenoxy)imidazo[1,2-b]pyridazine (V.9g) (46%) as colourless crystals, m.p. 84-86° [after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum]. (Found, for a sample dried at 20°/0.1 mm Hg for 15h: C, 68.3; H, 4.2; N, 12.6. $C_{19}H_{14}ClN_3O$ requires C, 68.0; H, 4.2; N, 12.5%). ^1H n.m.r.: δ 4.11, s, CH_2; 6.81, d, J 9 Hz, H7; 7.05-7.45, complex, Ph and H3, 2',3',5',6'; 7.84, d, J 9 Hz, H8.

2-Benzyl-6-(4'-methoxyphenoxy)imidazo[1,2-b]pyridazine (V.9f) (32%), m.p. 68-70° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum. (Found: C, 72.3; H, 5.3; N, 12.7. $C_{20}H_{17}N_3O_2$ requires C, 72.5; H, 5.2; N, 12.7%). ^1H n.m.r.: δ 3.80, s, MeO; 4.10, s, CH_2; 6.78, d, J 9 Hz, H7; 6.89, d, 7.09, d, J 9 Hz, H2',3',5',6'; 7.26, bs, Ph; 7.33, s, H3; 7.79, d, J 9 Hz, H8.

2-Benzyl-6-phenylthioimidazo[1,2-b]pyridazine (V.9h) (76%) after t.l.c. (alumina; chloroform, light petroleum, 3:2). (Found: C, 71.8; H, 4.8; N, 13.4. $C_{19}H_{15}N_3S$ requires C, 71.9; H, 4.8; N, 13.2%). ^{1}H n.m.r.: δ 4.13, s, CH_2; 6.71, d, J 9 Hz, H7; 7.28-7.70, complex, 2 x Ph and H3,8.

2-Benzyl-6-benzyloxyimidazo[1,2-b]pyridazine (V.9i) (73%), m.p. 96-97° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum. (Found: C, 76.6; H, 5.8; N, 13.4%. $C_{20}H_{17}N_3O$ requires C, 76.2; H, 5.4; N, 13.3%). ^{1}H n.m.r.: δ 4.13, s, CH_2; 5.30, s, CH_2O, 6.67, d, J 9 Hz, H7; 7.31-7.42, complex, 2 x Ph and H3; 7.72, d, J 9 Hz, H8.

2-Benzyl-6-(2'-methoxybenzylthio)imidazo[1,2-b]pyridazine (V.9b) (43%), after t.l.c. (alumina; chloroform, light petroleum, 1:1). (Found: C, 70.1; H, 5.6; N, 11.7. $C_{21}H_{19}N_3OS$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r.: δ 3.84, s, MeO; 4.14, s, CH_2; 4.40, s, CH_2S; 6.75, d, J 9 Hz, H7; 6.87-7.42, complex, Ph and H3',4',5',6'; 7.55, s, H3; 7.61, d, J 9 Hz, H8.

2-Benzyl-6-(3'-methoxybenzylthio)imidazo[1,2-b]pyridazine (V.9c) (36%), after t.l.c. (alumina; chloroform, light petroleum, 1:1). (Found: C, 69.6; H, 5.5; N, 11.6. $C_{21}H_{19}N_3OS$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r.: δ 3.78, s, MeO; 4.15, s, CH_2; 4.35, s, CH_2S;

6.79, d, J 9 Hz, H7; 6.93-7.31, complex, Ph and H2′,4′,5′,6′; 7.55, s, H3; 7.67, d, J 9 Hz, H8.

2-Benzyl-6-(4′-methoxybenzylthio)imidazo[1,2-b]pyridazine (V.9d) (51%), as yellow crystals, m.p. 82-84° after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum). (Found, for a sample dried at 50°/0.1 mm Hg for 6 h: C, 69.7; H, 5.4; N, 11.6. $C_{21}H_{19}N_3OS$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r.: δ 3.78, s, MeO; 4.15, s, CH_2; 4.33, s, CH_2S; 6.76, d, J 9 Hz, H7; 6.78-7.37, complex, Ph and H2′,3′,5′,6′; 7.55, s, H3; 7.63, d, J 9 Hz, H8.

6-Chloro-2-phenethylimidazo[1,2-b]pyridazine (V.9l) (86%), m.p. 99-101° after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found, for a sample dried at 20°/0.1 mmHg for 3 h: C, 65.8; H, 4.8; N, 15.9. $C_{14}H_{12}ClN_3$ requires C, 65.2; H, 4.7; N, 16.3%). ^{1}H n.m.r.: δ 3.14, s, CH_2CH_2; 7.06, d, J 9 Hz, H7; 7.25, s, Ph; 7.63, s, H3; 7.92, d, J 9 Hz, H8.

2-Phenethyl-6-phenylthioimidazo[1,2-b]pyridazine (V.9m) (95%) after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: C, 72.3; H, 5.4; N, 12.9. $C_{20}H_{17}N_3S$ requires C, 72.5; H, 5.2; N, 12.7%). ^{1}H n.m.r.: δ 3.09, s, CH_2CH_2; 6.70, d, J 9 Hz, H7; 7.23-7.71, complex, 2xPh and H3,8.

6-Benzyloxy-2-phenethylimidazo[1,2-b]pyridazine (V.9n) (85%), m.p. 109-110° after t.l.c. (alumina; chloroform, light petroleum, 7:3) and recrystallisation from light petroleum. (Found: C, 76.5; H, 6.2; N, 12.9. $C_{21}H_{19}N_3O$ requires C, 76.6; H, 5.8; N, 12.8%). ^{1}H n.m.r.: δ 3.12, s, CH_2CH_2; 5.34, s, CH_2O; 6.76, d, J 9 Hz, H7; 7.26-7.90, complex, 2xPh and H3; 7.85, d, J 9.0 Hz, H8.

6-Benzylthio-2-phenethylimidazo[1,2-b]pyridazine (V.9j) (95%), after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: C, 72.8; H, 5.5; N, 12.0. $C_{21}H_{19}N_3S$ requires C, 73.0; H, 5.5; N, 12.2%). ^{1}H n.m.r.: δ 3.10, s, CH_2CH_2; 4.39, s, CH_2S; 6.78, d, J 9 Hz, H7; 7.26-7.38, complex, 2xPh; 7.59, s, H3; 7.65, d, J 9 Hz, H8.

2-(Biphenyl-4′-yl)-6-chloroimidazo[1,2-b]pyridazine (V.9p) (88%), m.p. 275-276° (from ethanol-chloroform). (Found: C, 70.2; H, 4.2; N, 13.5. $C_{18}H_{12}ClN_3$ requires C, 70.7, H, 4.0; N, 13.7%). ^{1}H n.m.r.: δ 7.01-8.26, complex, ArH.

2-(Biphenyl-4′-yl)-6-(2"-methoxyphenoxy)imidazo[1,2-b]-pyridazine (V.9q) (88%), m.p. 209-211° after t.l.c. (alumina; chloroform) and recrystallisation from methanol. (Found: 76.1; H, 4.9; N, 11.0. $C_{25}H_{19}N_3O_2$ requires C, 76.2; H, 4.9; N, 10.7%). ^{1}H n.m.r.: δ 3.80, s, MeO; 6.93, d, J 9 Hz, H7; 6.99-8.0, complex, C_6H_4 Ph and H3,8,3′,4′,5′,6′.

2-(Biphenyl-4'-yl)-6-(2"-methoxybenzylthio)imidazo-[1,2-b]pyridazine (V.9o) (93%) as yellow crystals. m.p. 174-176° (from methanol). (Found: C, 72.7; H, 5.0; N, 9.6. $C_{26}H_{21}N_3O_5$. 0.5 MeOH requires C, 72.4; H, 5.3; N, 9.6%). ^{1}H n.m.r.: δ 3.89, s, MeO; 4.48, s, CH_2S; 6.83, d, J 9 Hz, H7; 6.80-8.23, complex, C_6H_4Ph, H3",4",5",6",3 and 8.

2-t-Butyl-6-chloroimidazo[1,2-b]pyridazine (V.9r) (84%), m.p. 132-133° (from cyclohexane). (Found, for a sample dried at 20°/0.1 mmHg for 24 h: C, 57.5; H, 6.0; N, 20.2. $C_{10}H_{12}ClN_3$ requires C, 57.3; H, 5.8; N, 20.0%). ^{1}H n.m.r.: δ 1.41, s, Bu^t; 6.99, d, J 9 Hz, H7; 7.72, s, H3; 7.85, d, J 9 Hz, H8.

6-Chloro-2-cyclohexylimidazo[1,2-b]pyridazine (V.9s) (78%), m.p. 106-107° (from light petroleum) (Found, for a sample dried at 50°/0.1 mmHg for 5 h: C, 61.4; H, 6.1; N, 17.9. $C_{12}H_{14}ClN_3$ requires C, 61.1; H, 6.0; N, 17.8%). ^{1}H n.m.r.: δ 1.37-2.77, complex, C_6H_{11}; 7.00, d, J 9 Hz, H7; 7.69, s, H3; 7.84, d, J 9 Hz, H8.

2-Benzyl-3-dimethylaminomethyl-6-(2'-methoxyphenoxy)-imidazo[1,2-b]pyridazine (V.10d) (52%), after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: C, 71.5; H, 6.1; N, 14.2. $C_{23}H_{24}N_4O_2$ requires C, 71.1; H, 6.2; N, 14.4%). ^{1}H n.m.r.: δ 2.07, s, Me_2N; 3.56, s, CH_2N; 3.73, s, MeO; 4.17, s, CH_2Ph; 6.83, d, J 9 Hz, H7;

6.90-7.27, complex, Ph and H3′,4′,5′ and 6′; 7.81, d, J 9 Hz, H8.

2-Benzyl-3-dimethylaminomethyl-6-(4′-methoxyphenoxy)-imidazo[1,2-b]pyridazine (V.10e) (57%) after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: 71.1; H, 6.5; N, 14.6. $C_{23}H_{24}N_4O_2$ requires C, 71.1; H, 6.2; N, 14.4%). ^{1}H n.m.r.: δ 2.18, s, Me_2N; 3.63, s, CH_2N; 3.83, s, MeO; 4.18, s, CH_2Ph; 6.77, d, J 9 Hz, H7; 6.91, d, 7.16, d, J 8.5 Hz, H2′,3′,5′,6′; 7.26, s, Ph; 7.81, d, J 9 Hz, H8.

2-Benzyl-3-dimethylaminomethyl-6-phenylthioimidazo-[1,2-b]pyridazine (V.10g) (75%) after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: 70.3; H, 6.2; N, 15.0. $C_{22}H_{22}N_4S$ requires C, 70.6; H, 5.9; N, 15.0%). ^{1}H n.m.r.: δ 2.17, s, Me_2N; 3.66, s, CH_2N; 4.19, s, CH_2Ph; 6.75, d, J 9 Hz, H7; 7.25-7.56, complex, 2xPh; 7.66, d, J 9 Hz, H8.

2-Benzyl-3-dimethylaminomethyl-6-(2′-methoxybenzylthio)-imidazo[1,2-b]pyridazine (V.10a) (45%), after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: C, 68.6; H, 6.6; N, 13.5. $C_{24}H_{26}N_4OS$ requires C, 68.9; H, 6.3; N, 13.4%). ^{1}H n.m.r.: δ 2.31, s, Me_2N; 3.84, s, CH_2N; 3.81, s, MeO; 4.21, s, CH_2Ph; 4.49, s, CH_2S; 6.77, d, J 9 Hz, H7; 6.87-7.56, complex, Ph and H3′,4′,5′,6′; 7.62, d, J 9 Hz, H8.

2-Benzyl-3-dimethylaminomethyl-6-(3′-methoxybenzylthio)-imidazo[1,2-b]pyridazine (V.10b) (52%), after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: C, 68.7; H, 6.6; N, 13.1. $C_{24}H_{26}N_4OS$ requires C, 68.9; H, 6.3; N, 13.4%). ^{1}H n.m.r.: δ 2.29, s, Me_2N; 3.78, s, MeO and CH_2N; 4.21, s, CH_2Ph; 4.43, s, CH_2S; 6.79, d, J 9 Hz, H7; 6.99-7.33, complex, Ph and H2′,4′,5′ and 6′; 7.65, d, J 9 Hz, H8.

2-Benzyl-3-dimethylaminomethyl-6-(4′-methoxybenzylthio)-imidazo[1,2-b]pyridazine (V.10c) (45%) after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: 68.4; H, 6.7; N, 13.1. $C_{24}H_{26}N_4OS$ requires C, 68.9; H, 6.3; N, 13.4%). ^{1}H n.m.r.: δ 2.31, s, Me_2N; 3.78, s, MeO; 3.81, s, CH_2N; 4.21, s, CH_2Ph; 4.41, s, CH_2S; 6.77, d, J 9 Hz, H7; 6.84, d, 7.39, d, J 8.5 Hz, H2′,3′,5′,6′; 7.27, s, Ph; 7.64, d, J 9 Hz, H8.

6-Chloro-3-dimethylaminomethyl-2-phenethylimidazo-[1,2-b]pyridazine (V.10j) (54%) after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: C, 64.9; H, 6.2; N, 17.8. $C_{17}H_{19}ClN_4$ requires C, 64.9; H, 6.1; N, 17.8%). ^{1}H n.m.r.: δ 2.26, s, Me_2N; 3.13, s, CH_2CH_2; 3.66, s, CH_2N; 7.00, d, J 9 Hz, H7; 7.23, s, Ph; 7.82, d, J 9 Hz, H8.

6-Benzylthio-3-dimethylaminomethyl-2-phenethylimidazo-[1,2-b]pyridazine (V.10h) (28%) after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: 72.0; H, 6.7;

N, 13.9. $C_{24}H_{26}N_4S$ requires C, 71.6; H, 6.5; N, 13.9%). ^1H n.m.r.: δ 2.26, s, Me_2N; 3.11, s, CH_2CH_2; 3.68, s, CH_2N; 4.46, s, CH_2S; 6.80, d, J 9 Hz, H7; 7.25-7.45, complex, 2xPh; 7.67, d, J 9 Hz, H8.

3-(Dimethylaminomethyl)-6-(4'-methoxybenzylthio)-2-phenethylimidazo[1,2-b]pyridazine (V.10i) (38%) after t.l.c. (alumina; chloroform, light petroleum, 7:3). (Found: C, 69.0; H, 6.8; N, 12.7. $C_{25}H_{28}N_4OS$ requires C, 69.4; H, 6.5; N, 13.0%). ^1H n.m.r.: δ 2.27, s, Me_2N; 3.11, s, CH_2CH_2; 3.68, s, CH_2N; 3.79, s, MeO; 4.41, s, CH_2S; 6.78, d, J 9 Hz, H7; 6.84, d, 7.39, d, J 8.5 Hz, H2',3',5',6'; 7.24, s, Ph; 7.66, d, J 9 Hz, H8.

2-(Biphenyl-4'-yl)-6-chloro-3-dimethylaminomethylimidazo-[1,2-b]pyridazine (V.10k) (59%), m.p. 177-179° after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum. (Found: 69.5; H, 5.3; N, 15.2%. $C_{21}H_{19}ClN_4$ requires C, 69.5; H, 5.3; N, 15.4%). ^1H n.m.r.: δ 2.35, s, Me_2N; 4.01, s, CH_2N; 7.05, d, J 9 Hz, H7; 7.25-8.24, complex, C_6H_4Ph; 7.91, d, J 9 Hz, H8.

2-(Biphenyl-4'-yl)-3-dimethylaminomethyl-6-(2"-methoxy-phenoxy)imidazo[1,2-b]pyridazine (V.10l) (29%), m.p. 149-151° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of acetone and light petroleum. (Found: C, 74.2; H, 6.1; N, 12.1. $C_{28}H_{26}N_4O_2$ requires C, 74.6; H, 5.8; N, 12.4%). ^1H n.m.r.: δ 2.12,

s, Me_2N; 3.68, s, CH_2N; 3.75, s, MeO; 6.93, d, J 9 Hz, H7; 7.00-8.71, complex, C_6H_4Ph, H3′,4′,5′,6′ and 8.

2-t-Butyl-6-chloro-3-dimethylaminomethylimidazo-[1,2-b]pyridazine (V.10m) (37%) after t.l.c. (alumina; chloroform, light petroleum, 3:1). (Found: 58.4; H, 7.3; N, 21.1. $C_{13}H_{19}ClN_4$ requires C, 58.5; H, 7.2; H, 21.0%). ^1H n.m.r.: δ 1.51, s, Bu^t; 2.26, s, Me_2N; 3.92, s, CH_2N; 6.95, d, J 9 Hz, H7; 7.82, d, J 9 Hz, H8.

6-Chloro-2-cyclohexyl-3-dimethylaminomethylimidazo-[1,2-b]pyridazine (V.10n) (64%) after column chromatography. (Found: C, 61.3; H, 7.5; N, 18.9. $C_{15}H_{21}ClN_4$ requires C, 61.5; H, 7.2; N, 19.1%). ^1H n.m.r.: δ 1.39-2.90, complex, C_6H_{11}; 2.33, s, Me_2N; 3.82, s, CH_2N; 6.98, d, J 9 Hz, H7; 7.81, d, J 9 Hz, H8.

3-Acetamidomethyl-6-chloro-2-phenethylimidazo[1,2-b]-pyridazine (V.11d) (39%), m.p. 144-146° after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum. (Found: C, 61.9; H, 5.4; N, 16.7. $C_{17}H_{17}ClN_4O$ requires C, 62.1; H, 5.1; N, 17.0%). ^1H n.m.r.: δ 1.89, s, MeCO; 3.12, complex, CH_2CH_2; 4.53, d, J 5.5 Hz, CH_2N; 7.02, d, J 9 Hz, H7; 7.20, s, Ph; 7.83, d, J 9 Hz, H8.

3-Acetamidomethyl-6-benzylthio-2-phenethylimidazo-[1,2-b]pyridazine (V.11b) (90%), m.p. 146-148° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of acetone and light petroleum. (Found: 6.91; H,

5.7; N, 13.4. $C_{24}H_{24}N_4OS$ requires C, 69.2; H, 5.8; N, 13.5%). ^1H n.m.r.: δ 1.75, s, MeCO; 3.08, s, CH_2CH_2; 4.40, s, CH_2S; 4.50, d, J 5.5 Hz; CH_2N; 6.84, d, J 9 Hz, H7; 7.18-7.42, complex, 2xPh; 7.66, d, J 9 Hz, H8.

3-Acetamidomethyl-2-t-butyl-6-chloroimidazo[1,2-b]-pyridazine (V.11h) (8%), m.p. 159-161° after t.l.c. (alumina; chloroform) and recrystallisation from cyclohexane. (Found: C, 54.7; H, 6.0; N, 19.2. $C_{13}H_{17}ClN_4O$ requires C, 54.6; H, 6.2; N, 19.6%). ^1H n.m.r.: δ 1.50, s, Bu^t; 1.99, s, MeCO; 4.99, d, J 5.5 Hz, CH_2N; 7.03, d, J 9 Hz, H7; 7.89, d, J 9 Hz, H8.

3-Acetamidomethyl-6-chloro-2-cyclohexylimidazo[1,2-b]-pyridazine (V.11i) (31%), m.p. 189-190° after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from a mixture of methanol and cyclohexane. (Found: C, 58.9; H, 6.5; N, 18.1. $C_{15}H_{19}ClN_4O$ requires C, 58.7; H, 6.2; N, 18.3%). ^1H n.m.r.: δ 1.26-3.03, complex, C_6H_{11}; 1.98, s, MeCO; 4.79, d, J 5.5 Hz, CH_2N; 7.02, d, J 9 Hz, H7; 7.86, d, J 9 Hz, H8.

3-Benzamidomethyl-6-chloro-2-phenethylimidazo[1,2-b]-pyridazine (V.11e) (23%), as a white solid, m.p. 159-160° after t.l.c. (alumina; ether, then chloroform) (Found, for a sample dried at 110°/0.1 mmHg for 6 h: C, 67.9; H, 4.9; N, 14.6. $C_{22}H_{19}ClN_4O$ requires C, 67.6; H, 4.9; N, 14.3%). ^1H n.m.r.: δ 3.19, complex, CH_2CH_2; 4.75, d, J

5.5 Hz, CH_2; 6.65, bs, NH; 7.05, d, J 9 Hz; 7.20-7.76, complex, 2 x Ph; 7.89, d, J 9 Hz, H8.

3-Benzamidomethyl-6-benzylthio-2-phenethylimidazo[1,2-b]-pyridazine (V.11c) (14%), as a white solid m.p. 151-152° after t.l.c. (alumina; ether, then chloroform and alumina; chloroform) and recrystallisation from cyclohexane. (Found, for a sample dried at 110°/710 mmHg for 6 h: C, 72.5; H, 5.8; N, 11.6. $C_{29}H_{26}N_4OS$ requires C, 72.8; H, 5.5; N, 11.7%). ^{1}H n.m.r.: δ 3.14, complex, CH_2CH_2; 4.36, s, CH_2S; 4.75, d, J_{CH_2NH} 5.5 Hz, CH_2; 6.86, d, J 9 Hz, H7; 7.16-7.56, complex, 2 x Ph; 7.69, d, J 9 Hz, H8.

3-Benzamidomethyl-6-chloro-2-(biphenyl-4'-yl)imidazo-[1,2-b]pyridazine (V.11g) (26%), m.p. 232-234° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of acetone and light petroleum. (Found: C, 71.1; H, 4.4; N, 12.6. $C_{26}H_{19}ClN_4O$ requires C, 71.15; H, 4.4; N, 12.8%). ^{1}H n.m.r.: δ 5.26, d, J 5.5 Hz, CH_2N; 7.12, d, J 9 Hz, H7; 7.40-8.18, complex, Ph, C_6H_4Ph and H8.

3-Benzamidomethyl-6-chloro-2-cyclohexylimidazo[1,2-b]-pyridazine (V.11j) (67%), m.p. 178-179° after t.l.c. (alumina; chloroform) and recrystallisation from toluene. (Found: 65.4; H, 5.9; N, 15.1. $C_{20}H_{21}ClN_4O$ requires C, 65.1; H, 5.7; N, 15.2%). ^{1}H n.m.r.: δ 1.43-3.07, complex, C_6H_{11}; 5.02, d, J 5.5 Hz, CH_2N; 7.06, d, J 9 Hz, H7; 7.39-7.81, complex, Ph; 7.93, d, J 9 Hz, H8.

6'-Methylnaphthalen-2'-ylglyoxal

6'-Methyl-2'-acetonaphthone was oxidised with selenium dioxide in dioxane by a procedure similar to that described for the preparation of phenylglyoxal.[261] The product was obtained as an oil which, after heating with water and cooling, gave crystals, m.p. 113-115°. Part of this product was sublimed at 120°/0.1 mmHg and gave a yellow solid, m.p. 84-86°. (Found: C, 73.4; H, 5.5. $C_{13}H_{10}O_2$. 0.8 H_2O requires C, 73.6; H, 5.6%). ^1H n.m.r.: δ 2.56, s, Me; 7.37-8.84, complex, ArH; 9.75, s, CHO.

6-Chloro-3-methoxy-2-(6'-methylnaphthalen-2'-yl)imidazo-[1,2-b]pyridazine (V.6a)

A mixture of 6-chloropyridazin-3-amine (0.1 g), 6'-methylnaphthalen-2'-ylglyoxal (0.153 g), ethanol (2.0 ml) and concentrated hydrochloric acid (0.15 ml) was refluxed for 15 h. The solvent was evaporated under reduced pressure and stirred with excess ethereal diazomethane in ice and then at room temperature overnight. The ether was evaporated and the resulting oil was subjected to t.l.c. (alumina; chloroform) and the product recrystallised from light petroleum to give yellow crystals of the *title compound*, (0.070 g 28%), m.p. 117-118°. (Found: C, 67.1; H, 4.2; N, 13.1. $C_{18}H_{14}ClN_3O$ requires C, 66.8; H, 4.4; N, 13.0%.) ^1H n.m.r.: δ 2.50, s, Me; 4.16, s, MeO; 6.90, d, J 9 Hz, H7; 7.25-8.72, complex, H8 and naphthalenyl.

3-Methoxy-2-(6'-methylnaphthalen-2'-yl)-6-methyl-thioimidazo[1,2-b]pyridazine (V.6b) (34%), m.p. 177-178° after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum. (Found: C, 68.3; H, 5.0; N, 12.5. $C_{19}H_{17}N_3OS$ requires C, 68.0; H, 5.1; N, 12.5%). ^1H n.m.r.: δ 2.52, s, MeC; 2.68, s, MeS; 4.21, s, MeO; 6.81, d, J 9.5 Hz, H7; 7.26-8.54, complex, H8, and naphthalenyl.

CHAPTER VI

CHAPTER VI Syntheses and binding studies of some substituted imidazo[1,2-b]pyridazines and related imidazo[1,2-a]pyridines, imidazo[1,2-a]pyrimidines and imidazo[1,2-a]pyazines

VI-1 Introduction

The search for antianxiety agents without non-specific central nervous system (CNS) depressant side effects has stimulated the synthesis of new chemical structures, such as imidazo[1,2-*a*]pyridine and imidazo[1,2-*a*]pyrimidine compounds, which interact with the benzodiazepine receptors. They have been reported to show pharmacological profiles different from those of benzodiazepines.[101,102,262] Almirante and coworkers[114,200] reported the syntheses and some pharmacological properties of derivatives of imidazo[1,2-*a*]pyridine, imidazo-[1,2-*a*]pyrimidines and imidazo[1,2-*b*]pyridazine. These compounds were examined for analgesic, antiinflammatory, antipyretic and anticonvulsant activity. For example, 2-(4'-methoxyphenyl)imidazo[1,2-*a*]pyridine[200] could abolish tonic extensor seizures produced by supramaximal electroshock and antagonize convulsions elicited by pentamethylenetetrazole. Series of compounds with the imidazo[1,2-*a*]pyridine structure have recently been developed[201,202,213,263-267] and shown to have anticonvulsant, hypnotic, sedative and anxiolytic activity. Within this class, zolpidem (Fig. I-9, in Chapter I) has been used as an hypnotic agent, and alpidem (Fig. I-9; in

Chapter I) as an anxiolytic compound. Zolpidem inhibited [^{3}H]diazepam binding to membranes from different brain areas (IC_{50} values in the low nanomolar range) and it was four-fold more potent at displacing [^{3}H]diazepam in cerebellar than in hippocampal neurones.[262] It was suggested that this imidazo[1,2-*a*]pyridine derivative may stimulate preferentially the Type I benzodiazepine receptor. Amongst a large number of different imidazo-[1,2-*a*]pyridine compounds prepared and tested, a series of 2-phenyl(and substituted phenyl)-imidazo[1,2-*a*]pyridin-3-ylacetamides were investigated by George and coworkers.[155] The influences of further substitution in the pyridine ring, in the 2-phenyl group and in the acetamide side chain were examined.[155] The sedative-hypnotic properties were investigated by the effect of these compounds on the relative potency of drug-induced electrocorticographic (ECoG) sleep. These workers found that in 3-dialkylaminomethylimidazo[1,2-*a*]pyridines, the 6-methyl derivatives were more potent than the corresponding 6-chloro compounds; and a shift of the methyl group from the 6-position to the 7- and 8-positions gave compounds which were devoid of activity. The effect of substitution in the 2-phenyl group and its effect on hypnotic activity was examined in detail: *para* substituted phenyl compounds had strong sedative effects. The influence of the nature of the *para* substituent in the 2-phenyl group was very important. Electron-withdrawing groups decreased or abolished the activity, whereas electron-donating groups increased sedative activity in the following order: methyl

> methoxy ≃ chloro > acetamido.[268] A bulky group, such as t-butyl, and an hydrophilic function, such as an hydroxymethyl group decreased or abolished sedative effects. Profiling of Alpidem (Fig. I-9; in Chapter I) has revealed that it is a potent ligand at the Bz_1 (W1) and the peripheral benzodiazepine (Bz_p, W3) receptor subtypes,[268] whereas it has low affinity for Bz_2 (W2) receptors. Alpidem bound to the Bz_1 and Bz_p receptor subtypes from human brain with Kd of 1.67 nM and 0.3 nM, respectively.[268] Its high affinity and high selectivity for the benzodiazepine receptor Type I and the peripheral benzodiazepine receptor (Bz_p) may explain its selective anxiolytic activity.[268]

Almirante and coworkers[114] in 1966 reported the syntheses and testing of some imidazo[1,2-*a*]pyrimidines with analgesic, antiinflammatory, antipyretic and anticonvulsant activity. They found that several compounds of this series of imidazo[1,2-*a*]pyrimidines offered some protection against supramaximal electroshock and/or pentamethylenetetrazole induced seizures.[114] A large number of 5,6,7-trisubstituted imidazo[1,2-*a*]pyrimidin-2-yl ketones[101,269] as potential nonsedative anxiolytics and 2-oxadiazolyl- and 2-thiazolyl-imidazo[1,2-*a*]pyrimidines[102] as agonists and inverse agonists at benzodiazepine receptors have been reported by Tully and coworkers.[102] They selected the most active of the 2-oxadiazolyl-imidazo[1,2-*a*]pyrimidines, as revealed in the conflict test, for further pharmacological study.[102]

In this chapter, some imidazo[1,2-*a*]pyridines containing various substituents at C-3 and C-6 [such as 2-aryl-3-benzamidomethyl(acetamidomethyl, methoxy and unsubstituted)-6-chloro(alkoxy, alkylthio and phenylthio)-imidazo[1,2-*a*]pyridines, and some of the corresponding imidazo[1,2-*b*]pyridazines, imidazo[1,2-*a*]-pyrimidines and imidazo[1,2-*a*]pyrazines] have been prepared and examined for their ability to bind at benzodiazepine receptors. The aim of the work described in this chapter was to examine the effect on biological activity of changes in the heterocyclic nucleus, such as the replacement of N-5 by CH in substituted imidazo[1,2-*b*]pyridazines to give imidazo[1,2-*a*]pyridines, and to assess the effect of shifting the ring nitrogen at the 5-position in imidazo[1,2-*b*]pyridazines to the 7- and 8-position, as in imidazo[1,2-*a*]pyrazines and imidazo[1,2-*a*]pyrimidines. The biological results are discussed in connection with these changes.

VI-2 Syntheses

The various heterobicyclic nuclei required in this work were prepared from the respective α-aminomono-heterocycle by fusion with a two carbon unit, followed by further elaboration to give the appropriate derivative.

In this way imidazo[1,2-*a*]pyridines were prepared from pyridin-2-amines, imidazo[1,2-*a*]pyrimidines from pyrimidin-2-amines, and imidazo[1,2-*a*]pyrazines from pyrazin-2-amines.

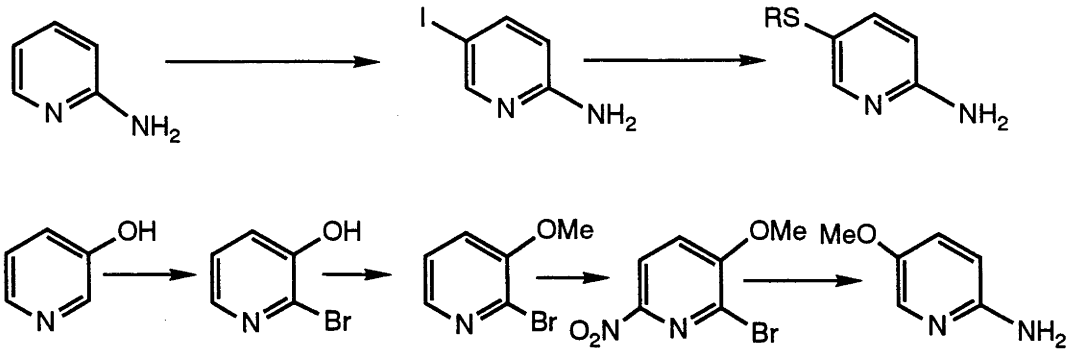
VI-2.1 Syntheses of some 3-acylaminomethyl (and methoxy)-6-chloro(methoxy, methylthio, propylthio and phenylthio)-2-phenyl(and substituted phenyl)imidazo-[1,2-a]pyridines

The starting materials required for the preparation of the compounds (**VI.3** and **VI.5**) were appropriate 5-substituted pyridin-2-amines. 5-Chloropyridin-2-amine is available commercially. Pyridin-2-amine was iodinated to give 5-iodopyridine-2-amine[270] (Scheme VI-1) and subsequently the iodo-substituent was replaced readily by methanethiolate, propanethiolate and benzenethiolate ions.[271] 5-Methoxypyridin-2-amine[272] (Scheme VI-1) was prepared from pyridin-3-ol through 2-bromopyridin-3-ol, 2-bromo-3-methoxypyridine[273] and 2-bromo-3-methoxy-6-nitropyridine[272] which, with palladium on charcoal and hydrazine hydrate, gave the desired compound.

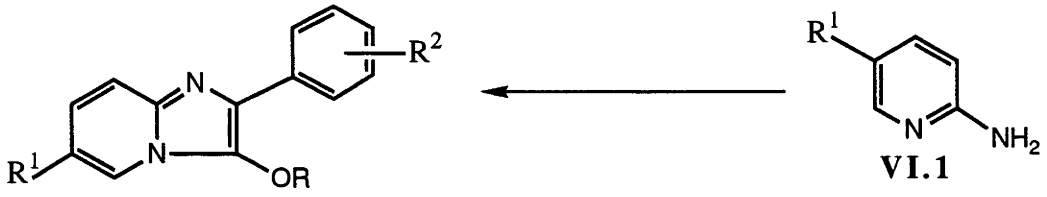
Compounds (**VI.4** and **VI.5**; Scheme VI-2) were prepared readily by procedures similar to those reported for analogous compounds in the literature.[213,274]

The 5-substituted pyridin-2-amines (**VI.1**) condensed with 4-tolylglyoxal in ethanol containing hydrochloric acid to give the 6-substituted-2-(4′-tolyl)imidazo[1,2-*a*]-pyridin-3-ols (**VI.2**).[275] These crude imidazo[1,2-*a*]-pyridin-3-ols were methylated with diazomethane, in a manner similar to that described for the preparation of 6-alkylthio- and 6-arylthio-3-methoxy-2-phenylimidazo[1,2-*b*]-pyridazines,[161] and gave the 3-methoxyimidazo[1,2-*b*]-pyridines (**VI.3**).

Scheme VI-1



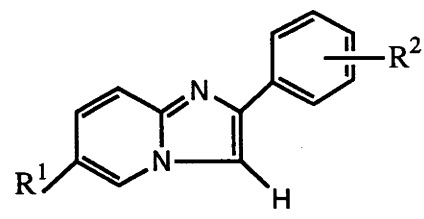
Scheme. VI-2



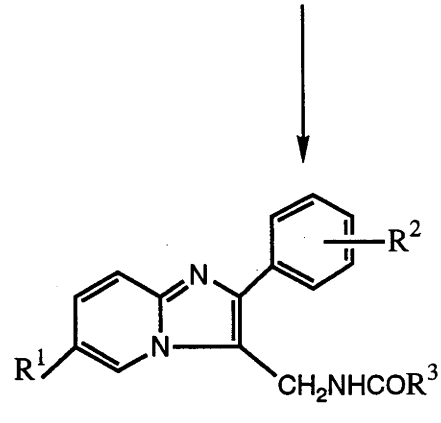
VI.1

VI.2 R=H , **VI.3** R=Me

VI.	R^1	R^2	R^3
3a	Cl	Me-*p*	
5a	Cl	Me-*p*	Ph
4a	Cl	Me-*p*	
3b	OMe	Me-*p*	
5b	OMe	Me-*p*	Ph
4b	OMe	Me-*p*	
3c	OMe	OCH_2O-*3',4'*	
5c	OMe	OCH_2O-*3',4'*	
3d	SMe	Me-*p*	
5d	SMe	Me-*p*	Ph
3e	SPr	Me-*p*	
5e	SPr	Me-*p*	Ph
3f	SPh	H	
5f	SPh	H	Me
5g	SPh	H	Ph
3g	SPh	Me-*p*	
5h	SPh	Me-*p*	Me
5i	SPh	Me-*p*	Ph
4c	SPh	Me-*p*	



VI.4

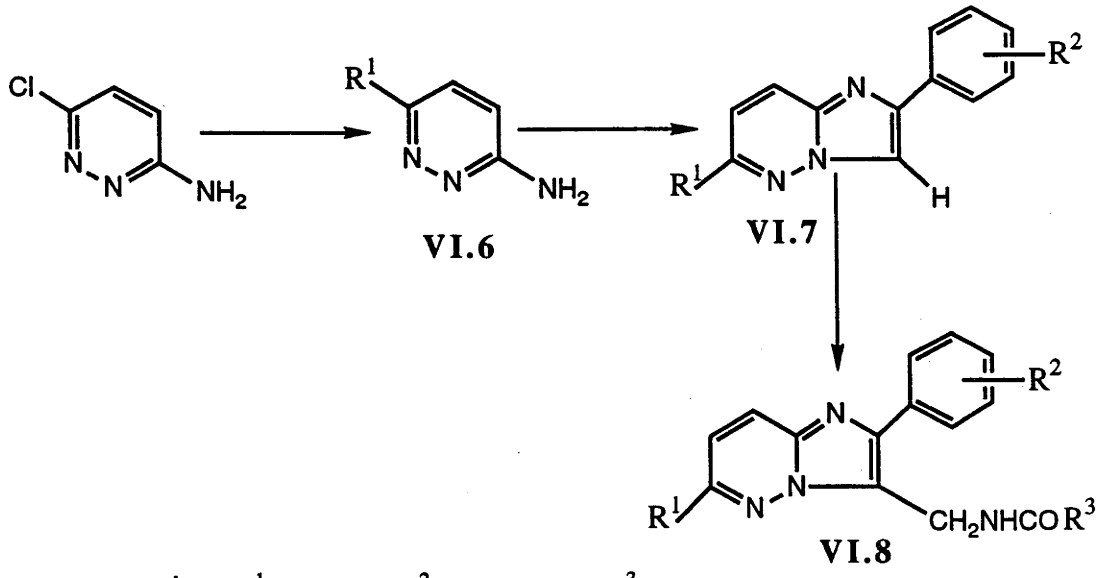


VI.5

VI-2.2 Syntheses of some 3-benzamidomethyl(naphthalen-2'-ylamidomethyl and unsubstituted)-2-(3',4'-methylene-dioxyphenyl and 4'-tolyl)-6-propylthio (and methylthio)-imidazo[1,2-b]pyridazines

The starting materials (**VI.6**), 6-methylthio- and 6-propylthio-pyridazin-3-amines, required for the preparations in this section were prepared by literature procedures.[161] The key intermediates, 3-unsubstituted imidazo[1,2-*b*]pyridazines (**VI.7**), were prepared from the 6-substituted pyridazin-3-amine by heating with the appropriate bromoacetyl compounds.[103] The compounds (**VI.8**) were prepared by heating the compounds (**VI.7**) with *N*-

Scheme VI-3



VI.	R^1	R^2	R^3
7a	SPr	Me-p	H
7b	SPr	OCH_2O-3',4'	H
8a	SPr	Me-p	$CH_2NHCOPh$
8b	SPr	OCH_2O-3',4'	$CH_2NHCOPh$
8c	SMe	OCH_2O-3',4'	$CH_2NHCOC_{10}H_7$-β

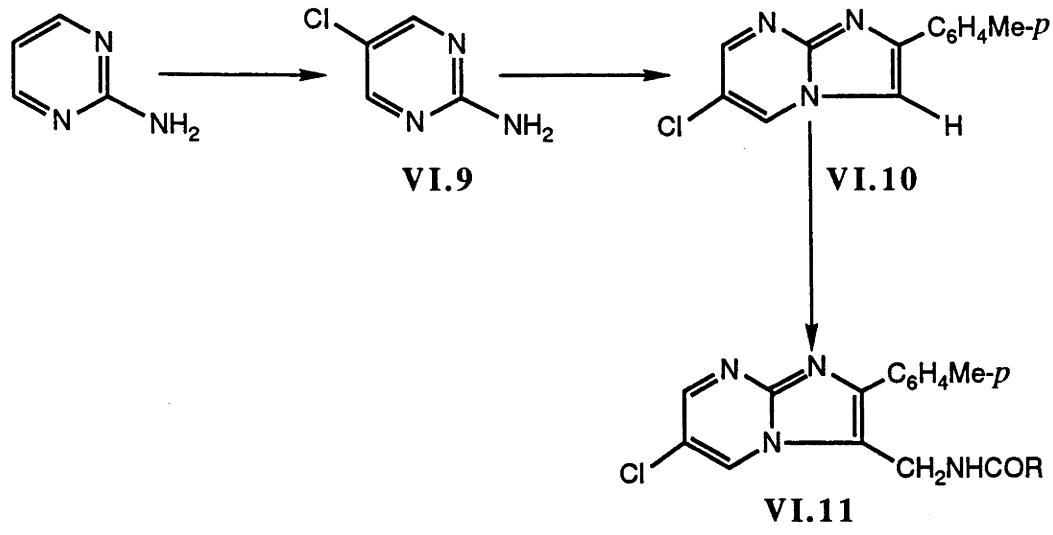
(hydroxymethyl)amides (by procedures similar to those described in Chapter II).

VI-2.3 Syntheses of some 3-acetamidomethyl(benzamidomethyl, methoxy and unsubstituted)-2-(4'-tolyl)-imidazo[1,2-a]pyrimidines and imidazo[1,2-a]pyrazines

The starting material required for the preparation of 3-acylaminomethyl-2-aryl-6-chloroimidazo[1,2-*a*]pyrimidines was 5-chloropyrimidin-2-amine (**VI.9**) It was prepared by the literature procedure from pyrimidin-2-amine by chlorination with *N*-chlorosuccinimide.[276] 5-Chloropyrimidin-2-amine condensed with α-bromo-4'-methylacetophenone[232] in ethanol and gave 6-chloro-2-(4'-tolyl)imidazo[1,2-a]pyrimidine (**VI.10**). When this was heated with *N*-(hydroxymethyl)acetamide or *N*-(hydroxymethyl)benzamide in glacial acetic acid containing a catalytic amount of concentrated sulphuric acid, it gave 3-acetamidomethyl (or 3-benzamidomethyl)-6-chloro-2-(4'-tolyl)imidazo[1,2-*a*]pyrimidines (**VI.11a** and **b**; Scheme VI-4).

The starting material required for the preparation of 6-chloro-3-methoxy (and benzamidomethyl)-2-(4'-tolyl)-imidazo[1,2-*a*]pyrazines was 5-chloropyrazin-2-amine. Its preparation was first attempted by direct chlorination of pyrazinamine with *N*-chlorosuccinimide in refluxing chloroform (a procedure used successfully to prepare 5-chloropyrimidin-2-amine above). It gave a low yield of a

Scheme VI-4



	R
VI. 11a	Me
11b	Ph

mixture of 5-chloropyrazin-2-amine, 3-chloropyrazin-2-amine and a dichloropyrazin-2-amine.

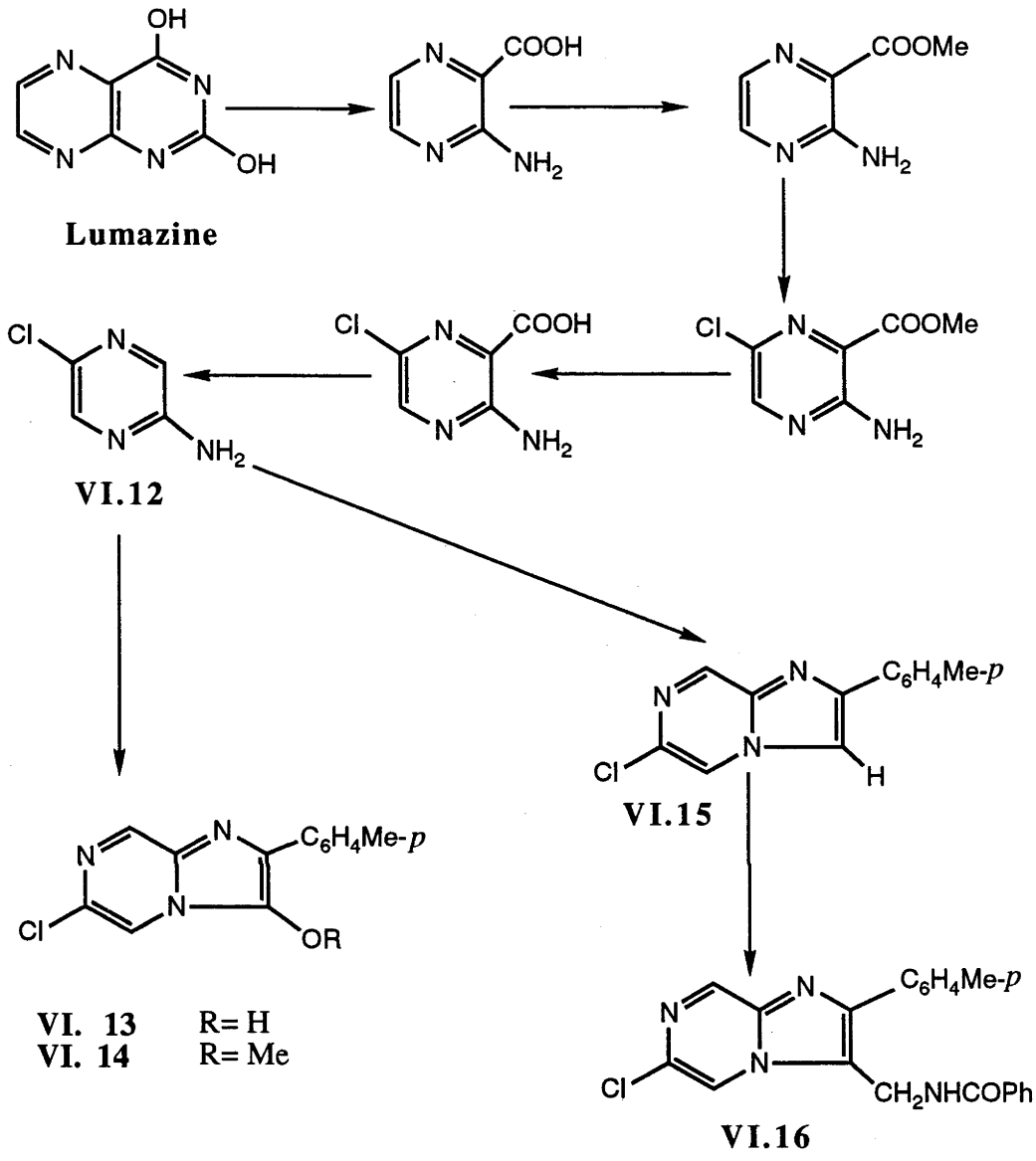
The 5-chloropyrazin-2-amine was therefore prepared by literature procedures from lumazine.[277-280] Lumazine (2,4-dihydroxypteridine) (Scheme VI-5) was decomposed by aqueous sodium hydroxide at elevated temperatures to 3-aminopyrazine-2-carboxylic acid in which the carboxylic acid group was used as a temporary blocking group during electrophilic substitution. This compound was esterified with methanol containing sulphuric acid and the resulting methyl 3-aminopyrazine-2-carboxylate was chlorinated in aqueous acetic acid to give methyl 2-amino-5-chloropyrazine-3-carboxylate.[278,279] This was then

hydrolysed and decarboxylated to give 5-chloropyrazin-2-amine.[280]

5-Chloropyrazin-2-amine (**VI.12**) condensed with 4-tolylglyoxal in ethanol with hydrochloric acid to give 6-chloro-2-(4′-tolyl)imidazo[1,2-*a*]pyrazin-3-ol (**VI.13**), which was methylated with diazomethane to give the 3-methoxy compound (**VI.14**).

5-Chloropyrazin-2-amine and α-bromo-4′-methylacetophenone readily gave 6-chloro-2-(4′-tolyl)imidazo[1,2-*a*]pyrazine (**VI.15**) which with *N*-(hydroxymethyl)benzamide gave 3-benzamidomethyl-6-chloro-2-(4′-tolyl)imidazo[1,2-*a*]pyrazine (**VI.16**).

Scheme VI-5


OH
N
N
N
N
OH
Lumazine
COOH
NH2
COOMe
Cl
VI.12
C6H4Me-p
OR
VI. 13 R= H
VI. 14 R= Me
H
VI.15
CH2NHCOPh
VI.16

VI-3 ^{1}H n.m.r. Spectra

The ^{1}H n.m.r. spectra of the 2-aryl-6-substituted imidazo[1,2-*a*]pyridines (**VI.4**) were determined and some data are listed in Table VI-1. An examination of the data for compounds (**VI.4**) revealed that the signal due to the protons of H-3 appeared in the range δ 7.70-7.84 for the 6-chloro(methoxy, methylthio, propylthio and phenylthio)-2-phenyl(4′-tolyl and 3′,4′-methylenedioxyphenyl)-imidazo[1,2-*a*]pyridines (**VI.4a-g**) and the protons H5, H7 and H8 of the pyridine ring appeared in the range δ 7.64-8.29 for H5, δ 6.95-7.91 for H7 and δ 7.52-8.00 for H8, with coupling constants of $J_{5,7}$ 2.0-2.5 Hz and $J_{7,8}$ 9.0-9.5 Hz.

The chemical shifts for H5, H7 and H8 in 6-phenylthio-2-(4′-tolyl)imidazo[1,2-a]pyridine (**VI.4g**; δ 8.27, 7.80 and 7.89 respectively) are significantly downfield of those in 6-methylthio-2-(4′-tolyl)imidazo[1,2-*a*]pyridine (**VI.4d**; δ 8.10, 7.18 and 7.59 respectively) due to the stronger electron-withdrawing properties of the phenylthio group (σ_{para} 0.07)[226] relative to the methylthio group (σ_{para} 0.00)[226]. The electron-donating properties of the methoxy group are evident in the ^{1}H n.m.r. of 6-methoxy-2-(4′-tolyl)imidazo[1,2-*a*]pyridine (**VI.4b**) in which the signal due to H5, H7 and H8 are at δ 7.64, 6.95 and 7.52, respectively, all upfield of those in the 6-methylthio analogue (**VI.4d**).

The methylene protons in the 3-acylaminomethyl-2-aryl-

Table VI-1 Some ^{1}H n.m.r. spectral data[A] for 2-aryl-3-methoxy (and unsubstituted)-6-variously substituted imidazo[1,2-a]pyridines

Compound	H3	H5	H7	H8
VI.4a	7.78	8.14	7.01	7.56
4b	7.77	7.64	6.95	7.52
4c	7.70	7.64	6.98	7.54
4d	7.79	8.10	7.18	7.59
4e	7.79	8.19	7.18	7.66
4f	7.84	8.29	7.91	8.00
4g	7.80	8.27	7.80	7.89

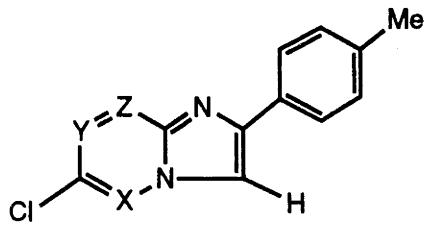
[A]Reported as parts per million (δ) downfield from T.M.S. as internal standard in deuterochloroform

6-(variously substituted)imidazo[1,2-*a*]pyridines (**VI.5f** and **VI.5h**) and (**VI.5a-c, g** and **i**) occurred as doublets (described in the Experimental section) in the ranges δ 4.85-4.86 and δ 5.04-5.12, respectively.

An examination of the data in Table VI-2 for the 3-unsubstituted imidazo[1,2-*b*]pyridazine (**II.3c**; Chapter II), imidazo[1,2-*a*]pyrazine (**VI.15**) and imidazo[1,2-*a*]pyrimidine (**VI.10**) revealed that the signal due to H-3 was further downfield in compound (**II.3c**) and was more upfield in those compounds in which the influence of a second ring nitrogen atom was more remote, as in compounds (**VI.15**) and (**VI.10**).

The chemical shifts of the methylene group in the corresponding 3-benzamidomethyl derivatives (**VI.6b, VI.16, VI.11b** and **VI.5a**), reported in the Experimetal section, reveal a similar pattern.

Table VI-2 Some ^1H n.m.r. spectral data[A] for 6-chloro-2-(4'-tolyl)imidazo[1,2-b]pyridazine, imidazo[1,2-a]pyrazine, imidazo[1,2-a]pyrimidine and imidazo[1,2-a]pyridine



Compounds	H3
X=N, Y=Z=CH **(II.3c)**	8.18
Y=N, X=Z=CH **(VI.15)**	7.91
Z=N, X=Y=CH **(VI.10)**	7.76
X=Y=Z=CH **(VI.4a)**	7.78

[A]Reported as parts per million (δ) downfield from T.M.S. as internal standard in deuterochloroform

VI-4 *In vitro* binding results

The compounds prepared in this chapter were tested in the [^{3}H]diazepam binding assay as described in Chapter II-5.3.

VI-4.1 Results of [^{3}H]diazepam binding assay

The results of these binding studies were given in Table VI-3 as IC_{50} values (or % displacement at the concentration specified). The results for the imidazo-[1,2-*a*]pyridine compounds (**VI.3,4** and **5**) are presented first, followed by those for the imidazo[1,2-*b*]pyridazines, imidazo[1,2-*a*]pyrimidines and imidazo[1,2-*a*]pyrazines.

VI-4.2 Discussion of results

The results are shown in Table VI-3 for the *in vitro* binding by the imidazo[1,2-*a*]pyridines (**VI.3, 4** and **5**), imidazo[1,2-*b*]pyridazines (**VI.7** and **8**), imidazo[1,2-*a*]-pyrimidines (**VI.10** and **11**) and imidazo[1,2-*a*]pyrazines (**VI.14-16**).

These revealed that among the series of 6-substituted imidazo[1,2-*a*]pyridines (**VI.3,4** and **5**), 3-benzamidomethyl-6-chloro-2-(4′-tolyl)imidazo[1,2-*a*]pyridine (**VI.5a**; IC_{50} 11 nM) had the strongest binding potency; and that the 2-aryl-6-chloro(methoxy, methylthio, propylthio and phenylthio)-3-unsubstituted imidazo[1,2-*a*]pyridines were all less active than the 3-acetamidomethyl, 3-benzamidomethyl and 3-methoxy analogues.

Table VI-3 Results for the displacement of [^{3}H]diazepam from rat brain by some substituted imidazo[1,2-a]pyridines (VI.3-5), imidazo[1,2-b]pyridazines (VI.7-8), imidazo-[1,2-a]pyrimidines (VI.10-11) and imidazo[1,2-a]pyrazines (VI.14-16).

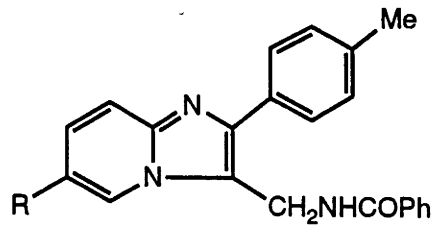
Formula number	Compounds	IC_{50} (nM)[A] (or % displ. at 1000 nM)
	Substituted imidazo[1,2-a]pyridines	
VI.4a	6-Cl-3-H-2-C_6H_4Me-*p*	(49%)
5a	6-Cl-3-CH_2NHBz-2-C_6H_4Me-*p*	11
3a	6-Cl-3-OMe-2-C_6H_4Me-*p*	146[B]
4b	6-OMe-3-H-2-C_6H_4Me-*p*	(9%)
5b	6-OMe-3-CH_2NHBz-2-C_6H_4Me-*p*	25
3b	6-OMe-3-OMe-2-C_6H_4Me-*p*	329
4c	6-OMe-3-H-2-C_6H_3(3′,4′-OCH_2O-)	980
5c	6-OMe-3-CH_2NHBz-2-C_6H_3(3′,4′-OCH_2O-)	16
4d	6-SMe-3-H-2-C_6H_4Me-*p*	(22%)
5d	6-SMe-3-CH_2NHBz-2-C_6H_4Me-*p*	27
4e	6-SPr-3-H-2-C_6H_4Me-p	(23%)
5e	6-SPr-3-CH_2NHBz-2-C_6H_4Me-*p*	155
4f	6-SPh-3-H-2-Ph	(2%)
5f	6-SPh-3-CH_2NHAc-2-Ph	455
5g	6-SPh-3-CH_2NHBz-2-Ph	(37%)
4g	6-SPh-3-H-2-C_6H_4Me-*p*	(0%)
5h	6-SPh-3-CH_2NHAc-2-C_6H_4Me-*p*	558
5i	6-SPh-3-CH_2NHBz-2-C_6H_4Me-*p*	(24%)
3c	6-SPh-3-OMe-2-C_6H_4Me-*p*	(11%)
	Substituted imidazo[1,2-b]pyridazines	
7a	6-SPr-3-H-2-C_6H_4Me-*p*	(53%)
8a	6-SPr-3-CH_2NHBz-2-C_6H_4Me-*p*	24
7b	6-SPr-3-H-2-C_6H_3(3′,4′-OCH_2O-)	254
8b	6-SPr-3-CH_2NHBz-2-C_6H_3(3′,4′-OCH_2O-)	8
8c	6-SMe-3-($CH_2NHCOC_{10}H_7$-β)[C]-2-C_6H_3-(3″,4″-OCH_2O-)	6

Table VI.3 Continued

Formula number	Compounds	IC_{50} (nM)[A] (or % displ.at 1000 nM)
	Substituted imidazo[1,2-a]pyrimidines	
10	6-Cl-3-H-2-C_6H_4Me-*p*	(0%)
11a	6-Cl-3-CH_2NHAc-2-C_6H_4Me-*p*	742
11b	6-Cl-3-CH_2NHBz-2-C_6H_4Me-*p*	504
11c	6-Cl-3-OMe-2-C_6H_4Me-*p*	(18%)[B,D]
	Substituted imidazo[1,2-a]pyrazines	
15	6-Cl-3-H-2-C_6H_4Me-*p*	(3%)
16	6-Cl-3-CH_2NHBz-2-C_6H_4Me-*p*	(6%)
14	6-Cl-3-OMe-2-C_6H_4Me-*p*	(10%)

[A] IC_{50} values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparation
[B] Prepared by Dr M.M.L. Ngu
[C] *β*-Naphthamidomethyl
[D] At 3000 nM

The 3-benzamidomethyl-6-chloro(methoxy, methylthio, propylthio and phenylthio)-2-(4′-tolyl)imidazo[1,2-*a*]-pyridines provided an interesting binding profile; the protency of the displacement of [^{3}H]diazepam from rat brain membrane by these compounds was in the order chloro (**VI.5a**; IC_{50} 11 nM) > methoxy (**VI.5b**; IC_{50} 25 nM) > methylthio (**VI.5d**; IC_{50} 27 nM) > propylthio (**VI.54**; IC_{50} 155 nM) > phenylthio (**VI.5i**; 24% displacement at 1000 nM) (Fig. VI-1). Clearly binding ability is not directly related to the electronic effects, and in these series increasing group size in the alkylthio or arylthio compounds was detrimental.



Compound Number	R	IC_{50}(nM) (or % displacement)
VI.5a	Cl	11
VI.5b	OMe	25
VI.5d	SMe	27
VI.5e	SPr	155
VI.5i	SPh	(24% at 1000nM)

Fig.VI-1

A comparison of the 2-(3′,4′-methylenedioxyphenyl) group with the 2-(4′-tolyl)substituent in 3-benzamidomethyl-6-methoxy-2-[(3′,4′-methylenedioxyphenyl) and 2-(4′-tolyl)]imidazo[1,2-*a*]pyridines (**VI.5c**, IC_{50} 16 nM; and **VI.5b**, IC_{50} 25 nM, respectively) revealed that the 2-(3′,4′-methylenedioxyphenyl) substituent was more effective than the 2-(4′-tolyl) group. However in the 3-(acetamidomethyl and benzamidomethyl)-2-phenyl (and 4′-tolyl)-6-phenylthioimidazo[1,2-*a*]pyridines (**VI.5f-i**) the 2-phenyl compounds (**VI.5f,g**; IC_{50} 455 nM and 37% displacement at 1000 nM respectively) were slightly more effective than those containing the 2-(4′-tolyl) substituent (**VI.5h,i**; IC_{50} 558 nM and 24% displacement at 1000 nM respectively).

A comparison of the imidazo[1,2-*a*]pyridines with the corresponding imidazo[1,2-*b*]pyridazines revealed that 3-benzamidomethyl-6-methoxy-2-(4′-tolyl)imidazo[1,2-*a*]-pyridine (**VI.5b**; IC_{50} 25 nM) had approximately the same activity as 3-benzamidomethyl-6-methoxy-2-(4′-tolyl)-

imidazo[1,2-*b*]pyridazine (**II.6f**; IC_{50} 23 nM in Chapter II), whereas 3-benzamidomethyl-6-chloro-2-(4′-tolyl)imidazo-[1,2-*b*]pyridine (**VI.5a**; IC_{50} 11 nM) bound more strongly than 3-benzamidomethyl-6-chloro-2-(4′-tolyl)imidazo[1,2-*b*]-pyridazine (**II.6b**; IC_{50} 18 nM in Chapter II).

The binding results for all other imidazo[1,2-*a*]-pyridine compounds (**VI.3,4** and **5**) listed in Table VI-3 revealed that they were less active than the corresponding imidazo[1,2-*b*]pyridazines. This difference was greatest between 3-benzamidomethyl-2-phenyl-6-phenylthio[1,2-*a*]-pyridine (**VI.5g**; 37% displacement at 1000 nM) and the corresponding 3-benzamidomethyl-2-phenyl-6-phenylthio-imidazo[1,2-*b*]pyridazine (**II.6g**; IC_{50} 9 nM in Chapter II); a similar situation was also apparent between 3-acet-amidomethyl-2-phenyl-6-phenylthioimidazo[1,2-*a*]pyridine (**VI.5f**; IC_{50} 455 nM) and the corresponding 3-acetamido-methyl-2-phenyl-6-phenylthioimidazo[1,2-*b*]pyridazine (**II.5f**; IC_{50} 24 nM in Chapter II); and also between 3-benzamidomethyl-6-propylthio-2-(4′-tolyl)imidazo[1,2-*a*]-pyridine (**VI.5e**; IC_{50} 155 nM) and 3-benzamidomethyl-6-propylthio-2-(4′-tolyl)imidazo[1,2-*b*]pyridazine (**VI.8a**; IC_{50} 24 nM).

Amongst the imidazo[1,2-*b*]pyridazines (**VI.7** and **8**), the 2-(4′-tolyl and 3′,4′-methylenedioxyphenyl)-6-propylthioimidazo[1,2-*b*]pyridazines (**VI.7a** and **b**; 53% displacement at 1000 nM and IC_{50} 254 nM, respectively) were more active than the corresponding 6-methylthio analogues (**II.3f** and **g**; 35 and 40% displacement at 1000 nM, respectively, in Chapter II) but in the 3-benzamidomethyl

derivatives, the situation was reversed and 3-benzamidomethyl-6-methylthio-2-(4′-tolyl and 3′,4′-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazines (**II.6d** and **e**; IC_{50} values 7 and 2 nM, respectively in Chapter II) bound more strongly than the corresponding 6-propylthio compounds (**VI.8a** and **b**; IC_{50} values 24 and 8 nM, respectively).

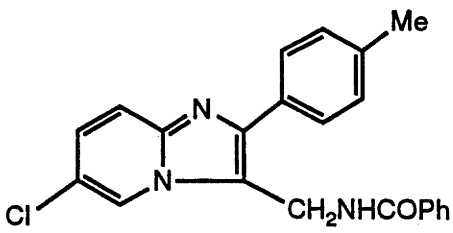
A comparison of 6-methylthio-2-(3′,4′-methylenedioxyphenyl)-3-(β-naphthamidomethyl)imidazo[1,2-*b*]pyridazine (**VI.8c**, IC_{50} 6 nM) with its 3-benzamidomethyl analogue (**II.6e**; IC_{50} 2 nM, in Chapter II) revealed that the (β-naphthamidomethyl) group was less effective than the 3-benzamidomethyl substituent.

The effect on biological activity of changing the position of nitrogen atom from 5 to 7 or 8 and a comparison of the results for the corresponding derivatives of the triazabicyclic systems revealed that the biological activities were in the order imidazo[1,2-*b*]pyridazines >> imidazo[1,2-*a*]pyrimidines $\gtrsim$ imidazo[1,2-*a*]pyrazines. This was shown clearly by the data in Fig. VI-2 and the binding data for 6-chloro-3-methoxy-2-(4′-tolyl)imidazo[1,2-*b*]-pyridazine (GBLD 173),[162] and corresponding imidazo[1,2-*a*]-pyrimidine (**VI.11c**) and imidazo[1,2-*a*]pyrazine (**IV.14**) which gave IC_{50} 148 nM, and 18% displacement at 3000 nM, and 10% displacement at 1000 nM, respectively.

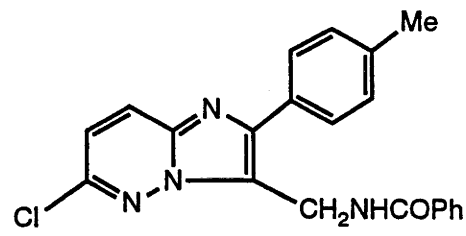
As in the series of imidazo[1,2-*b*]pyridazines (see Chapter II and III), the 3-acetamidomethyl group in 3-acetamidomethyl-6-chloro-2-(4′-tolyl)imidazo[1,2-*a*]-

pyrimidine (**VI.11a**, IC_{50} 742 nM) was less effective than the corresponding 3-benzamidomethyl substituent in compound (**VI.11b**; IC_{50} 504 nM).

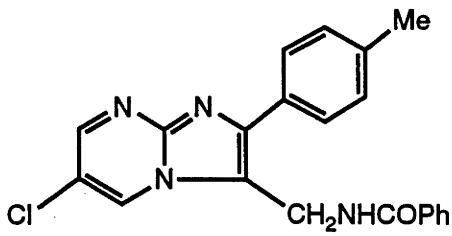
The results indicated that when N-5 of the pyridazine ring was removed (as in the imidazo[1,2-a]pyridines), a decrease of binding activity was generally observed, and insertion of a nitrogen atom at the 7- or 8-position, as in compounds (**VI.16**; 6% displacement at 1000 nM and **VI.11b**, IC_{50} 504 nM), resulted in decreased binding.



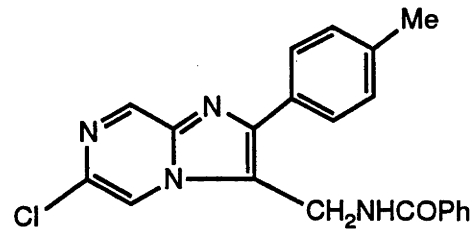
VI.5a, IC_{50} 11nM



II.6b, IC_{50} 18 nM



VI.11b, IC_{50} 504 nM



VI.16, 6 % displacement at 1000nM

Fig.VI-2

VI-5 Experimental

The general procedure, and experimental details for the [^{3}H]diazepam binding assay, are described in Chapter II-5.3, but samples for analysis were dried at 60-100°/0.1 mmHg for 3-20 h unless otherwise specified.

6-Chloro-2-(4′-tolyl)imidazo[1,2-a]pyridine (VI.4a)

A mixture of 5-chloropyridin-2-amine (0.5 g) and α-bromo-4-methylacetophenone[232] (0.836 g) in ethanol (10 ml) was refluxed with stirring in an oil bath at 90-95° for 2 h. Sodium hydrogen carbonate (0.12 g) was added and the refluxing continued for 14 h. The product was recrystallised from ethanol and subjected to t.l.c. [alumina; chloroform, light petroleum, 2:1, developed twice] to give the *title compound*, (0.347 g) m.p. 228-229° (Found, for a sample dried at 75°/710 mmHg for 5 h: C, 69.2; H, 4.4; N, 11.4. $C_{14}H_{11}ClN_2$ requires C, 69.3; H, 4.6; N, 11.5%). ^{1}H n.m.r.: δ 2.39, s, Me; 7.01, dd, $J_{7,8}$ 9 Hz, $J_{5,7}$ 2 Hz, H 7; 7.23, dd, $J_{2',3'}$ 8.5 Hz, $J_{3',5'}$ 3 Hz, H 3′,5′; 7.56, d, J 9 Hz, H 8; 7.78, s, H 3; 7.83, d, J 8.5 Hz, H 2′,6′; 8.14, bs, H 5.

6-Benzamidomethyl-6-chloro-2-(4′-tolyl)imidazo-[1,2-a]pyridine (VI.5a)

6-Chloro-2-(4′-tolyl)imidazo[1,2-*a*]pyridine (0.1 g) was added to a solution of *N*-(hydroxymethyl)benzamide[216] (0.067 g) in acetic acid (1.0 ml) containing sulphuric acid

(0.1 ml) and the mixture refluxed with stirring in an oil bath at 120° for 5 h. The acetic acid was evaporated under reduced pressure, the residue diluted with water and adjusted with aqueous ammonia to pH 10, and extracted with chloroform. The solid obtained was subjected to t.l.c. (alumina; chloroform, developed twice) and the product (0.054 g) in the band at higher R_F was recrystallised from toluene to give white crystals of the *title compound* (0.030 g), m.p. 225-226° (Found, for a sample dried at 110°/710 mmHg for 15 h: C, 70.3; H, 4.5; N, 10.9. $C_{22}H_{18}ClN_3O$ requires C, 70.3; H, 4.8; N, 11.2%). ^1H n.m.r.: δ 2.40, s, Me; 5.11, d, J 5.5 Hz, CH_2N; 6.6, bs, NH; 7.11-8.40, complex, ArH.

5-Methoxypyridin-2-amine

This compound[272] was prepared from pyridin-3-ol through 2-bromopyridin-3-ol,[272] 2-bromo-3-methoxy-pyridine,[273] and 2-bromo-3-methoxy-6-nitropyridine.[272]

An attempt to prepare the *title compound* by heating 5-iodopyridin-2-amine with sodium methoxide in the presence of copper powder at 150° for 22 h gave a low yield of impure product.

5-Methylthiopyridin-2-amine

A solution of sodium hydroxide (3.0 g) in water (30 ml) was saturated with methanethiol, 5-iodopyridin-2-amine[270] (4.0 g) and copper powder (1.17 g) were added and the mixture heated in a screw-top Teflon-lined stainless steel reaction vessel at 150° for 15 h. The contents were

extracted with chloroform to give the crude product (2.553 g). A sample was recrystallised from a mixture of benzene and light petroleum to give the *title compound*, m.p. 68-70° (Found, for a sample dried at 25°/0.1 mmHg for 5 h: C, 51.7; H, 6.0; N, 20.2. $C_6H_8N_2S$ requires C, 51.4; H, 5.8; N, 20.0%). ^{1}H n.m.r.: δ 2.40, s, MeS; 4.23, br, NH_2; 6.47, d, J 9.5 Hz, H 3; 7.50, dd, $J_{3,4}$ 9.5 Hz, $J_{4,6}$ 2 Hz, H 4; 8.13, bs, H 6.

5-Propylthiopyridin-2-amine

This compound was prepared from 5-iodopyridin-2-amine[270] and sodium propanethiolate by a procedure similar to that described for the methylthio analogue. The yield of crude product was 90%.

It was purified by t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallised from a mixture of benzene and light petroleum to give the *title compound*, m.p. 48-50° (Found, for a sample dried at 25°/0.1 mmHg for 18 h: C, 56.6; H, 7.2; N, 16.3. $C_8H_{12}N_2S$ requires C, 57.1; H, 7.2; N, 16.5%). ^{1}H n.m.r.: δ 0.98, t, J 7 Hz, Me; 1.54, complex, Me*CH*$_2$; 2.70, t, J 7 Hz, *CH*$_2$S, 4.28, br, NH_2; 6.45, d, J 9 Hz, H 3; 7.51, dd, $J_{3,4}$ 9 Hz, $J_{4,6}$ 2 Hz, H 4; 8.15, bs, H 6.

5-Phenylthiopyridin-2-amine

This compound was prepared from 5-iodopyridin-2-amine and sodium thiophenolate as described by Bochis *et al.*[271] It had m.p. 124-125° (lit.,[271] 123-125°). ^{1}H n.m.r.: δ

5.4, br, NH_2; 6.52, d, J 9 Hz, H 3; 7.17, s, Ph; 7.55, dd, $J_{3,4}$ 9 Hz $J_{4,6}$ 2 Hz, H 4; 8.21, bs, H 6.

3,6-Dimethoxy-2-(4'-tolyl)imidazo[1,2-a]pyridine (VI.3b)

A mixture of 5-methoxypyridin-2-amine (0.461 g), 4'-tolylglyoxal[281] (0.8 g), ethanol (10 ml) and concentrated hydrochloric acid (0.9 ml) was refluxed with stirring in an oil bath at 90° for 16 h. The mixture was cooled and the precipitate was filtered off and washed with ether to give a yellow solid (0.421 g). This solid was stirred overnight with excess ethereal diazomethane at 0°. The solvent was evaporated and the oil was subjected to t.l.c. (alumina; benzene) to give an oil (0.062 g) which crystallised from light petroleum to give the *title compound*, m.p. 105-107° (Found: C, 71.8; H, 6.0; N, 10.5. $C_{16}H_{16}N_2O_2$ requires C, 71.6; H, 6.0; N, 10.4%). ^1H n.m.r.: δ 2.40, s, MeC; 3.86, s, 6-OMe; 3.95, s, 3-OMe; 6.87-8.00, complex, ArH.

6-Methoxy-2-(3',4'-methylenedioxyphenyl)imidazo-[1,2-a]pyridine (VI.4c)

A mixture of 5-methoxypyridin-2-amine[272] (0.6 g) and α-bromo-3,4-methylenedioxyacetophenone[228] (1.207 g) in ethanol (20 ml) was refluxed for 2 h. Sodium hydrogen carbonate (0.5 g) was added and the refluxing continued for 5 h. The solvent was evaporated, the residue diluted with water and the solid (1.264 g) filtered off and recrystallised from cyclohexane to give the *title compound*, m.p. 182-183° (Found: C, 67.2; H, 4.6; N, 10.2.

$C_{15}H_{12}N_2O_3$ requires C, 67.2; H, 4.5; N, 10.4%). ^{1}H n.m.r.: δ 3.83, s, MeO; 5.99, s, OCH_2O; 6.81-7.70, complex, ArH.

3-Benzamidomethyl-6-methoxy-2-(3′,4′-methylenedioxyphenyl)imidazo[1,2-a]pyridine (VI.5c)

A mixture of 6-methoxy-2-(3′,4′-methylenedioxyphenyl)imidazo[1,2-*a*]pyridine (0.051 g), *N*-hydroxymethylbenzamide (0.038 g), acetic acid (1.0 ml) and concentrated sulphuric acid (2 drops) was refluxed with stirring at 120° for 14 h. The mixture was diluted with water, adjusted with aqueous ammonia to pH 10 and extracted with chloroform. The extract gave an oil which was subjected to t.l.c. (alumina; chloroform) and recrystallised from toluene to give the *title compound* (0.029 g), m.p. 233-235° (Found: C, 68.9; H, 4.6; N, 10.2. $C_{23}H_{19}N_3O_4$ requires C, 68.8; H, 4.8; N, 10.5%). ^{1}H n.m.r.: δ 3.80, s, MeO; 5.10, d, J 5.5 Hz, CH_2N; 5.98, s, OCH_2O; 6.73-7.90, complex, ArH.

2-Phenyl-6-phenylthioimidazo[1,2-a]pyridine (VI.4f)

A mixture of 5-phenylthiopyridin-2-amine (1.882 g) and α-bromoacetophenone (1.86 g) in ethanol (12 ml) was refluxed in an oil bath at 90° for 2 h. After cooling, sodium hydrogen carbonate (0.80 g) was added and the refluxing continued for 4 h, followed by dilution with water. The solid was filtered off, washed with ether and then recrystallised from ethanol to give the *title compound* (0.480 g), m.p. 164-165° (Found: C, 75.3; H, 4.7; N, 9.2. $C_{19}H_{14}N_2S$ requires C, 75.5; H, 4.7; N, 9.3%). ^{1}H n.m.r.: δ 7.14-8.29, complex, ArH.

3-Acetamidomethyl-2-phenyl-6-phenylthioimidazo[1,2-a]-pyridine (VI.5f)

2-Phenyl-5-phenylthioimidazo[1,2-*a*]pyridine (0.1 g) and *N*-(hydroxymethyl)acetamide[216] (0.184 g) in acetic acid (2.0 ml) with concentrated sulphuric acid (0.2 ml) were heated in an oil bath at 120° for 14 h, and then the acetic acid was evaporated under reduced pressure. The residue was diluted with water, adjusted to pH 10 with aqueous ammonia and extracted with chloroform. The extract gave an oil which was subjected to t.l.c. (alumina; chloroform, light petroleum, 3:1) and the product (0.067 g) at lower R_F was recrystallised from light petroleum to give the *title compound*, m.p. 192-194° (Found: C, 70.9; H, 4.9; N, 11.1. $C_{22}H_{19}N_3OS$ requires C, 70.7; H, 5.1; N, 11.3%). ^{1}H n.m.r.: δ 2.01, s, MeCO; 4.85, d, CH_2N; 7.26-8.45, complex, ArH.

The following imidazo[1,2-*a*]pyridines were prepared by procedures similar to those above:

6-Methoxy-2-(4'-tolyl)imidazo[1,2-a]pyridine (VI.4b) (42%) as yellow crystals, m.p. 129-131° [after t.l.c. (alumina; chloroform, light petroleum, 1:1) and recrystallisation from light petroleum] (Found: C, 75.6; H, 5.9; N, 11.8. $C_{15}H_{14}N_2O$ requires C, 75.6; H, 5.9; N, 11.8%). ^{1}H n.m.r.: δ 2.38, s, MeC; 3.82, s, MeO; 6.95, dd, J 9.5 Hz, $J_{5,7}$ 2.5 Hz, H 7; 7.22, d, 7.83, d, H 2',3',5',6'; 7.52, d, J 9.5 Hz, H 8; 7.64, d, J 2.5 Hz, H 5, 7.77, s, H3.

3-Benzamidomethyl-6-methoxy-2-(4′-tolyl)imidazo[1,2-a]-pyridine (VI.5b) (27%) as white crystals, m.p. 215-217° [after t.l.c. (alumina; chloroform) and recrystallisation from benzene] (Found: C, 74.7; H, 6.0; N, 11.5. $C_{23}H_{21}N_3O_2$ requires C, 74.4; H, 5.7; N, 11.3%). ^{1}H n.m.r.: δ 2.33, s, MeC; 3.76, s, MeO; 5.06, d, J 5.5 Hz, CH_2N; 6.68-8.21, complex, ArH.

6-Methylthio-2-(4′-tolyl)imidazo[1,2-a]pyridine (VI.4d) (41%) as yellow crystals, m.p. 117-119° (after column chromatography over alumina in a mixture of chloroform and light petroleum (1:1) and recrystallisation from light petroleum) (Found, for a sample dried at 25°/0.1 mmHg for 5 h: C, 70.5, H, 5.8; N, 11.0. $C_{15}H_{14}N_2S$ requires C, 70.8; H, 5.6; N, 11.0%). ^{1}H n.m.r.: δ 2.40, s, MeC; 2.49, s, MeS; 7.18, d, J 9 Hz, H 7; 7.24, d, J 8 Hz, H 3′,5′; 7.59, d, J 9 Hz, H 8; 7.79, s, H 3; 7.84, d, J 8 Hz, H 2′,6′; 8.10, s, H 5.

3-Benzamidomethyl-6-methylthio-2-(4′-tolyl)imidazo-[1,2-a]pyridine (VI.5d) (63%), m.p. 224-226° [after t.l.c. (alumina; chloroform) and recrystallisation from benzene] (Found: C, 71.1; H, 5.5; N, 10.8. $C_{23}H_{21}N_3OS$ requires C, 71.3; H, 5.5; N, 10.8%). ^{1}H n.m.r.: δ 2.38, s, MeC; 2.48, s, MeS; 5.12, d, J 5.5 Hz, CH_2N; 6.77. bs, NH; 7.09-8.40, complex, ArH.

6-Propylthio-2-(4'-tolyl)imidazo[1,2-a]pyridine (VI.4e) (44%), m.p. 85-87° [after chromatography over a column of alumina in a mixture of chloroform and light petroleum (1:1) and recrystallisation from light petroleum] (Found, for a sample dried at 25°/0.1 mmHg for 5 h: C, 72.6; H, 6.7; N, 10.1. $C_{17}H_{18}N_2S$ requires C, 72.3; H, 6.4; N, 9.9%). ^{1}H n.m.r.: δ 1.01, t, J 7 Hz, *Me*CH_2; 1.60, complex, Me*C*H_2; 2.40, s, MeC; 2.83, t, J 7 Hz, *C*H_2S; 7.14-8.19, complex, ArH.

3-Benzamidomethyl-6-propylthio-2-(4'-tolyl)imidazo-[1,2-a]pyridine (VI.5e) (41%) as white crystals, m.p. 182-184° [after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of benzene and light petroleum] (Found: C, 71.8; H, 6.2; N, 9.8. $C_{25}H_{25}N_3OS$ requires C, 72.3; H, 6.1; N, 10.1%). ^{1}H n.m.r.: δ 0.94, t, J 7 Hz, *Me*CH_2; 1.54, complex, Me*C*H_2; 2.34, s, MeC; 2.79, t, J 7 Hz, CH_2S; 5.04, d, J 5.5 Hz, CH_2N; 7.04-8.16, complex, ArH.

3-Methoxy-6-phenylthio-2-(4'-tolyl)imidazo[1,2-a]pyridine (VI.3c) (10%), m.p. 109-110° [after t.l.c. (alumina; benzene) and recrystallisation from light petroleum] (Found: C, 72.5; H, 5.6; N, 7.9. $C_{21}H_{18}N_2OS$ requires C, 72.8; H, 5.2; N, 8.1%). ^{1}H n.m.r.: δ 2.40, s, MeC; 3.95, s, MeO; 7.05-8.15, complex, ArH.

6-Benzamidomethyl-2-phenyl-6-phenylthioimidazo-[1,2-a]pyridine (VI.5g) (40%), as colourless crystals, m.p. 174-176° [after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of benzene and cyclohexane] (Found: C, 74.8; H, 5.1; N, 9.5. $C_{27}H_{21}N_3OS$ requires C, 74.5; H, 4.9; N, 9.6%). ^{1}H n.m.r.: δ 5.08, d, J 5.5 Hz, CH_2N; 6.76, br, NH; 7.12-8.42, complex, ArH.

6-Phenylthio-2-(4'-tolyl)imidazo[1,2-a]pyridine (VI.4g) (33%), m.p. 151-152° (after recrystallisation from ethanol) (Found: C, 75.7; H, 5.1; N, 8.7. $C_{20}H_{16}N_2S$ requires C, 75.9; H, 5.1; N, 8.9%). ^{1}H n.m.r.: δ 2.40, s, MeC; 7.14-8.27, complex, ArH.

3-Acetamidomethyl-6-phenylthio-2-(4'-tolyl)imidazo-[1,2-a]pyridine (VI.5h) (36%), m.p. 161-163° [after t.l.c. (alumina; chloroform, light petroleum, 3:1) and recrystallisation from light petroleum] (Found: C, 69.5; H, 5.8; N, 10.2. $C_{23}H_{21}N_3OS$ 0.5 H_2O requires C, 69.6; H, 5.6; N, 10.6%). ^{1}H n.m.r.: δ 2.02, s, MeCO; 2.39, s, MeC; 4.86, d, J 5.5 Hz, CH_2N; 6.09, br, NH; 7.70-8.37, complex, ArH.

3-Benzamidomethyl-6-phenylthio-2-(4'-tolyl)imidazo-[1,2-a]pyridine (VI.5i) (41%), m.p. 196-198° [after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of benzene and cyclohexane] (Found: C, 75.2; H, 5.4; N,

9.3. $C_{28}H_{23}N_3OS$ requires C, 74.8; H, 5.2; N, 9.3%). ^1H n.m.r.: δ 2.38, s, MeC; 5.08, d, J 5.5 Hz, CH_2N; 6.80, bs, NH; 7.15-8.40, complex, ArH.

Imidazo[1,2,-a]pyrimidines

6-Chloro-2-(4′-tolyl)imidazo[1,2-a]pyrimidine (VI.10)

A mixture of 5-chloropyrimidin-2-amine[276] (0.5 g) and α-bromo-4-methylacetophenone[232] (1.3 g), in ethanol (20 ml) was refluxed in an oil bath for 2 h, sodium hydrogen carbonate (0.51 g) added and the refluxing continued for 20 h. The mixture was diluted with water and extracted with chloroform and the product from the extract applied in benzene to a column of alumina which was then eluted with chloroform. The material eluted first was recrystallised from ethanol to give the *title compound* (0.142 g), m.p. 287-289° (Found, for a sample dried at 105°/0.1 mmHg for 3 h: C, 64.0; H, 4.0; N, 17.1. $C_{13}H_{10}ClN_3$ requires C, 64.1; H, 4.1; N, 17.2%). ^1H n.m.r.: δ 2.40, s, MeC; 7.26, d, 7.90, d, J 8 Hz, H 2′,3′,5′,6′; 7.76, s, H 3; 8.43, s, H 5,7.

3-Benzamidomethyl-6-chloro-2-(4′-tolyl)imidazo[1,2-a]-pyrimidine (VI.11b)

A mixture of 6-chloro-2-(4′-tolyl)imidazo[1,2-*a*]-pyrimidine (0.070 g), *N*-(hydroxymethyl)benzamide (0.1 g), acetic acid (1.5 ml) and concentrated sulphuric acid (3 drops) was refluxed in an oil bath at 120° for 15 h.

The acetic acid was evaporated under reduced pressure, the residue diluted with water, adjusted with aqueous ammonia to pH 10 and extracted with chloroform. After evaporation of the solvent the residue was subjected to t.l.c. (alumina; chloroform, developed twice) and the yellow solid (0.068 g) was recrystallised from toluene to give white crystals of the *title compound* (0.020 g), m.p. 252-254° (Found: C, 67.1; H, 4.9; N, 14.6. $C_{21}H_{17}ClN_4O$ requires C, 66.9; H, 4.5; N, 14.9%). ^{1}H n.m.r.: δ 2.27, s, MeC; 4.94, d, J 5.5 Hz, CH_2N; 6.80-8.48, complex, ArH.

3-Acetamidomethyl-6-chloro-2-(4′-tolyl)imidazo[1,2-a]-pyrimidine (VI.11a) was prepared as described above for the 3-benzamidomethyl analogue. The product (20%) was subjected to t.l.c. (alumina; chloroform) and recrystallised from benzene to give the *title compound*, m.p. 250-251° (Found: C, 61.3; H, 5.1; N, 17.5. $C_{16}H_{15}ClN_4O$ requires C, 61.0; H, 4.8; N, 17.8%). ^{1}H n.m.r.: δ 2.21, s, MeCO; 2.38, s, MeC; 4.74, d, J 5.5 Hz, CH_2N; 7.09, d, 7.37, d, J 8 Hz, H 2′,3′,5′,6′; 8.36, d, 8.51, d, J 3 Hz, H 5,7.

Pyrazines and imidazo[1,2-a]pyrazines

5-Chloropyrazin-2-amine

This compound was prepared from lumazine through 3-aminopyrazine-2-carboxylic acid,[277] methyl 3-aminopyrazine-2-carboxylate,[278] methyl 3-amino-6-chloropyrazine-2-

carboxylate[274] and 3-amino-6-chloropyrazine-2-carboxylic acid.[280] It had m.p. 129-131° (lit.,[280] 129-130°).

Attempted chlorination of pyrazin-2-amine with N-chlorosuccinamide

Pyrazin-2-amine (1.9 g) and *N*-chlorosuccinamide (2.7 g) in chloroform (40 ml) were refluxed at 90-92°C for 50 min. The mixture was cooled at 20° and evaporated to dryness to give an oil. This oil was diluted with acetone and the solid filtered off. The filtrate was evaporated and the residue subjected to column chromatography in chloroform over alumina. The main fraction (1.618 g) was dissolved in benzene and on standing gave a crystalline solid (0.250 g). This solid (0.150 g) was subjected to t.l.c. (alumina; benzene) and gave 5-chloropyrazin-2-amine (0.054 g), m.p. 134-136° (from a mixture of benzene and light petroleum) (lit.[282] m.p. 130-132°), ^{1}H n.m.r.: δ 7.76, d, $J_{3,6}$ 1 Hz, H3; 8.01, d, 1 Hz, H6; 3-chloropyrazin-2-amine (0.011 g) m.p. 167-168° (from light petroleum), (lit.[282] m.p. 167-168°) ^{1}H n.m.r.: δ 7.71, d, $J_{5,6}$ 2.5 Hz, H6; 7.93, d, 2.5 Hz, H5; and a dichloropyrazin-3-amine (0.006 g) (from light petroleum), m.p. 131-132° (lit. 140-141° [283], 140-142° [284]), ^{1}H n.m.r.: δ 7.96, s.

6-Chloro-3-methoxy-2-(4'-tolyl)imidazo[1,2-a]pyrazine (VI.14)

A solution of 5-chloropyrazin-2-amine (0.13 g), 4-tolylglyoxal hydrate[261,281] (0.21 g), ethanol (2.0 ml) and concentrated hydrochloric acid (0.3 ml) was refluxed with

stirring in an oil bath at 90° for 14 h. The ethanol was evaporated under reduced pressure and the residue evaporated with benzene. The oily residue was dissolved in a little methanol and stirred with excess ethereal diazomethane in ice overnight. After evaporation of the solvents, the residue was diluted with chloroform, washed with water and the chloroform evaporated to give an oil. This was subjected to t.l.c. (alumina; chloroform and then alumina; benzene) to give a light yellow solid (0.024 g) which was recrystallised from light petroleum and gave the *title compound* (0.010 g), m.p. 179-181° (Found: C, 61.1; H, 4.7; N, 15.5. $C_{14}H_{12}ClN_3O$ requires C, 61.4; H, 4.4; N, 15.4%). ^1H n.m.r.: δ 2.42, s, MeC; 4.00, s, MeO; 7.31, d, J 8 Hz, H 3′,5′; 7.97, d, 8 Hz, H 2′,6′; 7.97, s, H 7; 8.78, s, H 5.

6-Chloro-2-(4′-tolyl)imidazo[1,2-a]pyrazine (VI.15)

A mixture of 5-chloropyrazin-2-amine (0.305 g) and α-bromo-4-methylacetophenone[232] (0.543 g) in ethanol (5 ml) was refluxed for 2 h, sodium hydrogen carbonate (0.543 g) was added and the refluxing continued for 6 h. The solvent was evaporated and the residue extracted with chloroform and subjected to t.l.c. (alumina; chloroform). A solid (0.2 g) was obtained which was recrystallised from cyclohexane to give the *title compound* (0.070 g), m.p. 197-200° (Found: C, 64.4; H, 4.0; N, 17.1. $C_{13}H_{10}ClN_3$ requires C, 64.1; H, 4.1; N, 17.2%). ^1H n.m.r.: δ 2.41, s, MeC; 7.29, d, J 7 Hz, H 3′,5′; 7.88, d, J 7 Hz, $H_{2',6'}$; 7.91, s, H 3; 8.14, s, H 8; 8.90, s, H 5.

3-Benzamidomethyl-6-chloro-2-(4′-tolyl)imidazo[1,2-a]-pyrazine (VI.16)

A mixture of *N*-(hydroxymethyl)benzamide (0.050 g), 6-chloro-2-(4′-tolyl)imidazo[1,2-*a*]pyrazine (0.036 g) in acetic acid (1.0 ml) with concentrated sulphuric acid (3 drops) was refluxed with stirring in an oil bath at 120° for 14 h. The acetic acid was evaporated under reduced pressure, the residue diluted with water (10 ml), adjusted with aqueous ammonia to pH 10 and extracted with chloroform. The product was subjected to t.l.c. (alumina; chloroform) and recrystallised from ethanol to give the *title compound* (0.017 g), m.p. 270-272° (Found: C, 67.2; H, 4.6; N, 14.8. $C_{21}H_{17}ClN_4O$ requires C, 66.9; H, 4.6; N, 14.9%). ^{1}H n.m.r.: δ 2.44, s, MeC; 5.15, d, J 5.5 Hz, CH_2N; 6.44, br, NH; 7.25-7.85, complex, Ph and H 2′,3′,5′,6′; 8.54, bs, H 7; 8.89, bs, H 5.

Imidazo[1,2-b]pyridazines

6-Propylthio-2-(4′-tolyl)imidazo[1,2-b]pyridazine (VI.7a)

A mixture of 6-propylthiopyridazin-3-amine[161] (0.3 g) and α-bromo-4-methylacetophenone[232] (0.376 g) in ethanol (4.5 ml) was refluxed with stirring for 2 h. Sodium hydrogen carbonate (0.17 g) was added and the refluxing continued for 4 h. The mixture was cooled and the precipitate was filtered off and washed with water. It was recrystallized from light petroleum to give crystals of the

title compound (0.325 g), m.p. 126-128° (Found: C, 68.0; H, 6.2; N, 14.6. $C_{16}H_{17}N_3S$ requires C, 67.8; H, 6.1, N, 14.8%) ^{1}H n.m.r.: δ 1.09, t, J 7 Hz, *Me*CH_2; 1.78, complex, Me*C*H_2; 2.39, s, MeC; 3.18, t, J 7 Hz, *C*H_2S; 6.82, d, J 9 Hz, H7; 7.25, d J 7 Hz, H3′,5′; 7.70, d, J 9 Hz, H8; 7.83, d, J 7 Hz, H2′,6′; 8.11, s, H3.

2-(3′,4′-Methylenedioxyphenyl)-6-propylthioimidazo-[1,2-a]pyridazine (VI.7b) (87%), was prepared in a similar manner from 6-propylthiopyridazin-3-amine and α-bromo-3′,4′-methylenedioxyacetophenone.[228] It had m.p. 120-122° (after recrystallisation from ethanol) (Found: C, 61.0; H, 4.9; N, 13.2. $C_{16}H_{15}N_3O_2S$ requires C, 61.3; H, 4.8; N, 13.4%). ^{1}H n.m.r.: δ 1.09, t, J 7 Hz, *Me*CH_2; 1.78, complex, Me*C*H_2; 3.19, t, J 7 Hz, *C*H_2S; 6.01, s, OCH_2O; 6.77-8.04, complex, ArH.

In a similar manner the following compounds were prepared:

3-Benzamidomethyl-2-(3′,4′-methylenedioxyphenyl)-6-propylthioimidazo[1,2-a]pyridazine (VI.8b) (23%), m.p. 200-202° [after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene] (Found: C, 64.5; H, 4.7; N, 12.4. $C_{24}H_{22}N_4O_3S$ requires C, 64.6; H, 5.0; N, 12.5%). ^{1}H n.m.r.: δ 1.00, t, 7 Hz, *Me*CH_2; 1.72, complex, Me*C*H_2; 3.13, t, J 7 Hz, *C*H_2S; 5.13, d, J 5.5 Hz, CH_2N; 5.98, s, OCH_2O; 6.74-7.96, complex, ArH.

3-Benzamidomethyl-6-propylthio-2-(4′-tolyl)imidazo-[1,2-b]pyridazine (VI.8a) (58%) as colourless crystals, m.p. 202-203° [after t.l.c. (alumina; chloroform, light petroleum, 2:1) and recrystallisation from toluene] (Found: C, 69.0; H, 5.9; N, 13.8. $C_{24}H_{24}N_4OS$ requires C, 69.2; H, 5.8; N, 13.4%). ^{1}H n.m.r.: δ 0.98, t, J 7 Hz, *Me*CH_2; 1.72, complex, MeCH_2; 2.36, s, MeC; 3.11, t, J 7 Hz, CH_2S; 5.13, d, J 5.5 Hz, CH_2N; 6.68-7.99, complex, ArH.

2-(3′,4′-Methylemedioxyphenyl)-6-methylthio-3-β-napthamidomethylimidazo[1,2-b]pyridazine (VI.8c)

6-Methylthio-2-(3′,4′-methylenedioxyphenyl)imidazo-[1,2-*b*]pyridazine (0.11 g) was added to a solution of *N*-(hydroxymethyl)-β-naphthamide [0.092 g, m.p. 139-142° (^{1}H n.m.r.: δ 5.04, d, J 6 Hz, CH_2; 7.50-833, complex, ArH), prepared from β-naphthamide, m.p. 195-197° (lit.[285] m.p. 195°) by heating with aqueous formaldehyde] in glacial acetic acid (3.0 ml) containing sulphuric acid (0.1 ml) and the mixture was refluxed with stirring in an oil bath at 120° for 5 h. The mixture was cooled to 20° and the residue was diluted with water (60 ml), adjusted with aqueous ammonia to pH 10 and the mixture extracted with chloroform to give a mixture of some solid and oil (0.246 g). This crude product was subjected to t.l.c. (alumina; chloroform) and gave the *title compound* (0.117 g), m.p. 222-224° (toluene) (Found: C, 66.9; H, 4.2; N, 12.0. $C_{26}H_{20}N_4O_3S$ requires C, 66.7; H, 4.3; N, 12.0%).

^{1}H n.m.r.: δ 2.58, s, MeS; 5.21, d, J 5.0 Hz, CH_2N; 5.97, s, OCH_2O; 6.70, d, J 9.5 Hz, H7; 6.79-8.43, complex, H2′,5′,6′,8 and naphthalenyl.

REFERENCES

REFERENCES

1. Breier, A., and Paul, S.M., in "*Handbook of Anxiety*" (Eds Roth, M., Noyes, R., and Burrows, G.D.) Vol. 1, p. 193 (Elsevier: Amsterdam 1988).
2. Malenka, R.C., Hamblin, M.W., and Barchas, J.D., in "*Basic Neurochemistry*" (Eds Sigel, G.J., Agranoff, B.W., Albers, R.W., and Molinoff, P.B.) p.886 (Raven Press: New York 1989).
3. Johnston, G., *Chem. Aust.*, 1990, **57**, 334.
4. Robins, L.N., Helzer, J.E., Weissman, M.M., Orvaschel, H., Gruenberg, E., Burk, J.D., and Regier, D.A., *Arch. Gen. Psychiatry*, 1984, **41**, 949
5. Myers, J.K., Weissman, M.M., Tischler, G.L., Holzer, C.E., Leaf, P.J., Orvaschel, H., Anthony, J.C., Boyd, J.H., Burke, J.D., Kramer, M., and Stoltzman, R., *Arch. Gen. Psychiatry*, 1984, **41**, 959.
6. Friedel, R.O., in "*Handbook of Anxiety*" (Eds Roth, M., Noyes, R., and Burrows, G.D.) Vol. 1, p. 385 (Elsevier: Amsterdam 1988).
7. Norman, T.R., Judd, F.K., Marriott, P.F., and Burrows, G.D., in "*Handbook of Anxiety*" (Eds Roth, M., Noyes, R., and Burrows, G.D.) Vol. 1, p. 355 (Elsevier: Amsterdam, 1988).
8. Ludwing, B.J., and Piech, E.C., *J. Am. Chem. Soc.*, 1951, **73**, 5779.
9. Berger, F.M., *J. Pharmacol. Exp. Ther.*, 1954, **112**, 413.
10. Ludwing, B.J., and Potterfield, J.R., *Advan. Pharmacol. Chemother.*, 1971, **9**, 173.

11. Byck, R., in "*The Pharmacological Basis of Therapeutics*" 5th ed. (Eds Goodman, L.S., and Gilman, A.) p. 188 (Macmillan: New York 1975).

12. Morren, H., Denayer, R., Trolin, S., Grivsky, E., Linz, R., Strubbe, H., Dony, G., and Marico, J., *Ind. Chim. Belg.*, 1954, **19**, 1176.

13. Surrey, A.R., Webb, W.G., and Gesler, R.M., *J. Am. Chem. Soc.*, 1958, **80**, 3469.

14. Sternbach, L.H., in "*The Benzodiazepines*" (Eds Garattini, S., Mussini, E., and Randall, L.D.) p.1 (Raven Press: New York 1973).

15. Sternbach, L.H., Randall, L.O., and Gustafson, S.R., in "*Psychopharmacological Agents*" (Ed. Gordon, M.) Vol. 1, p. 137 (Academic: New York 1964).

16. Sternbach, L.H., in "*Psychotropic Agents*" Part II, (Eds Hoffmeister, F., and Stille, G.) p .1 (Springer-Verlag: Berlin 1981).

17. Tallman, J.F., Paul, S.M., Skolnick, P., and Gallager, D.W., *Science*, 1980, **207**, 274.

18. Vellucci, S.V., and File, S.E., *Psychopharmacology*, 1979, **62**, 61.

19. Randall, L.O., Schallek, W., Sternbach, L.H., and Ning, R.-Y., in "*Psychopharmacological Agents*" (Ed. Gordon, M.) Vol. 3. p. 175 (Academic: New York 1974).

20. Sternbach, L.H., in "*Progress in Drug Research*" (Ed. Tucker, E.) Vol. 22, p. 229 (Birkhauser: Basel, 1978).

21. Sternbach, L.H., Randall, L.O., Banziger, R., and Lehr, H., in "*Drugs Affecting the Nervous System*" (Ed. Burger, A.) p. 237 (Dekker: New York 1968).

22. Hunkeler, W., Möhler, H., Piere, L., Polc, P., Bonetti, E.P., Cumin, R., Schaffner, R., and Haefely, W., *Nature*, 1981, **290**, 514.

23. Crippen, G.M., *Mol. Pharmacol.*, 1982, **22**, 11.

24. Crippen, G.M., *J. Med. Chem.*, 1979, **22**, 988.

25. Blair, T., and Webb, G.A., *J. Med. Chem.*, 1977, **20**, 1206.

26. Loew, G.H., Nienow, J.R., and Poulsen, M., *Mol. Pharmacol.*, 1984, **26**, 19.

27. Gilli, G., Borea, P.A., Bertolasi, V., and Sacerdoti, M., in "*Molecular Structure and Biological Activity*" (Eds Griffin, J.F., and Duax, W.L.) p. 259 (Elsevier: New York 1983).

28. Codding, P.W., and Muir, A.K.S., *Mol. Pharmacol.*, 1985, **28**, 178.

29. Sunjic, V., Lisini, A., Sega, A., Kovac, T., Kajfez, F., and Ruscic, B., *J. Heterocycl. Chem.*, 1979, **16**, 757.

30. Blount, J.F., Fryer, R.I., Gilman, N.W., and Todaro, L.J., *Mol. Pharmacol.*, 1983, **24**, 425.

31. Fryer, R.I., Gilman, N.W., Madison, V., and Walser, A., in "*Proc. VIIIth Int. Symp. Med. Chem.*" (Eds Daholom, R., and Nilsson, J.L.G.) Vol. 2, p. 265 (Swedish Pharmaceutical: Stockholm 1985).

32. Fryer, R.I., Cook, C., Gilman, N.W., and Walser, A., *Life Sci.*, 1986, **39**, 1947.

33. Fryer, R.I., in "*Comprehensive Medical Chemistry*" (Ed. Emmett, J.C.) Vol. 3, p. 539 (Pergamon: Oxford 1990).

34. Fryer, R.I., and Zi-Qiang, Gu., *Life Sci.*, 1990, **47**, 833.

35. Haefely, A., Kulcsar, A., Möhler, L., Pieri, L., Polc, P., and Schaffner, R., in "*Mechanism of Action of Benzodiazepines*", (Eds Costa, E., and Greengard, P.) p.131 (Raven: New York 1975).

36. Costa, E., Guidotti, A., Mao, C.C., and Suria, A., *Life Sci.*, 1975, **17**, 167.

37. Tallman, J.F., Thomas, J.W., and Gallager, D.W., *Nature*, 1978, **274**, 383.

38. Willow, M., and Johnston, G.A.R., *Neurosci. Lett.*, 1980, **18**, 323-327.

39. Davies, L.P., *Aust. Prescriber*, 1985, **8**, 23.

40. Sieghart, W., *Trends Pharmacol. Sci.*, 1989, **10**, 407.

41. Seighart, W., and Karohath, M., *Nature*, 1980, **286**, 285.

42. Haefely, W., Kyburz, E., Gerecke, M., and Möhler, H., in "*Advances in Drug Research*" (Ed. Testa, B.) Vol. 14, p. 166 (Academic: London 1985).

43. Haefely, W., *Neurosci. Lett.*, 1984, **47**, 201.

44. Schofield, P.R., Darlison, M.G., Fujita, N., Burt, D.R., Stephenson, F.A., Rodriguez, H., Rhee, L.M., Ramachandran, J., Reale, V., Glencorse, T.A., Seeburg, P.H., and Barnard, E.A., *Nature*, 1987, **328**, 221.

45. Pritchett, D.B., Sontheimer, H., Shivers, B.D., Ymer, S., Kettenmann, H., Schofield, P.R., and Seeburg, P.H., *Nature*, 1989, **338**, 582.

46. Birdsall, N.J.M., *Trends Pharmacol. Sci.*, 1989, **10**, 50.

47. Haefely, W., Martin, J.R., and Schoch, P., *Trends Pharmacol. Sci.*, 1990, **11**, 452.

48. Schofield, P.R., and Abbott, A., *Trends Pharmacol. Sci.*, 1989, **10**, 207.

49. Levitan, E.S., Schofield, P.R., Burt, D.R., Rhee, L.M., Wisden, W., Köhler, M., Fujita, N., Rodriguez, H.F., Stephenson, A., Darlison, M.G., Barnard, E.A., and Seeburg, P.H., *Nature*, 1988, **335**, 76.

50. Vellucci, S.V., and Webster, R.A., *Psychopharmacol.*, 1982, **78**, 256.

51. File, S.E., Lister, R.G., and Nutt, D.J., *Neuropharmacology*, 1982, **21**, 1033.

52. Nutt, D.J., Cowen, P.J., and Little, H.J., *Nature*, 1982, **295**, 436.

53. Vellucci, S.V., and Webster, R.A., *Europ. J. Pharmacol.*, 1983, **90**, 263.

54. Sangameswaran, L., Fales, H.M., Friedrich, P., and de Blas, A.L., *Proc. Nat. Acad. Sci.*, USA, 1986, **83**, 9236.

55. Wildmann, J., Möhler, H., Vetter, W., Ranalder, U., Schmidt, K., and Maurer, R., *J. Neurol. Transm.*, 1987, **70**, 383.

56. De Robertis, E., Pena, C., Paladini, A.C., and Medina, J.H., *Neurochem. Int.*, 1988, **13**, 1.

57. Medina, J.H., Pena, C., Piva, M., Paladini, A.C., and De Robertis, E., *Biochem. Biophys. Res. Commun.*, 1988, **152**, 534.

58. Unseld, E., Fischer, C., Rothemund, E., and Klotz, U., *Biochem. Pharmacol.*, 1990, **39**, 210.

59. De Blas, A.L., Park, D., and Friedrich, P., *Brain Res.*, 1987, **413**, 275.

60. Dencker, S.J., and Johansson, G., *Lancet*, 1990, **335**, 413.

61. Klotz, U., *Life Sci.*, 1991, **48**, 209.

62. Braestrup, C., Nielsen, M., and Olsen, C.E., *Proc. Nat. Acad. Sci.*, USA, 1980, **77**, 2288.

63. Lippa, A.S., Klepner, C.A., Yunger, L., Sano, M.C., Smith, W.V., and Beer, B., *Pharmacol. Biochem. Behav.*, 1978, **9**, 853.

64. Lippa, A.S., Jackson, D., Wennogle, L.P., Beer, B.A., and Meyerson, L.R., in "*Pharmacology of Benzodiazepines*" (Eds Usdin, E., Skolnick, P., Tallman, J.F., Greenblatt, D., and Paul, S.M.) p. 431 (Macmillan: New York 1982).

65. Klepner, C.A., Lippa, A.S., Benson, D.I., Sano, M.C., and Beer, B., *Pharmacol. Biochem. Behav.*, 1979, **11**, 457.

66. Bourguignon, J.J., Chambon, J.P., and Wermuth, C.G., Eur. Pat. EP 156734 (*Chem. Abstr.* 1986, **104**, 68878).

67. Biziere, K., Bourguignon, J.J., Chambon, J.P., Heaulme, M., Perio, A., Tebib, S., and Wermuth, C.G., *Br. J. Pharmacol.*, 1987, **90**, 183.

68. Yokoyama, N., Ritter, B., and Neubert, A.D., *J. Med. Chem.*, 1982, **25**, 337.

69. Czernik, A.J., Petrack, B., Kalinsky, H.J., Psychoyos, S., Cash, W.D., Tsai, C., Rinehart, R.K., Granat, F.R., Lovell, R.A., Brundish, D.E., and Wade, R., *Life Sci.*, 1982, **30**, 363.

70. Francis, J.E., Cash, W.D., Barbaz, B.S., Bernard, P.S., Lovell, R.A., Mazzenga, G.C., Friedmann, R.G., Hyun, J.L., Braunwalder, A.F., Loo, P.S., and Bennett, D.A., *J. Med. Chem.*, 1991, **34**, 281.

71. File, S.E., *Trends Neurosci.*, 1987, **10**, 461.

72. Pecknold, J.C., Familamiri, P., Chang, H., Wilson, R., Alarcia, J., and McClure, D.J., *Neuropsychopharmacol. Biol. Psychiatr.*, 1985, **9**, 639. (through File, S.E., *Trends Neurosci.*, 1987, **10**, 461).

73. Goa, K.L., and Ward, A., *Drugs*, 1986, **32**, 114.

74. *Buspiron: A New Introduction to the Treatment of Anxiety*. (Ed. Lader, M.) (RSM Services 1988) (through Green, S. *Trends Neurosci.*, 1991, **14**, 101).

75. Glaser, T., and Traber, J., *Eur. J. Pharmacol.*, 1983, **88**, 137.

76. Broekkamp, C.L.E., Berendsen, H.H.G., Jenck, F., and Van Delft, A.M.L., *Psychopathology*, 1989, **22**, (Suppl. 1) 2.

77. Jensen, L.H., Petersen, E.N., Honoré, T., and Drejer, J., in "*GABAergic Transmission and Anxiety*" (Eds Biggio, G., and Costa, E.) p. 79 (Raven: New York 1986).

78. Wettstein, J.G., and Spealman, R.D., *J. Pharmácol. Exp. Ther.*, 1987, **240**, 471.

79. Corda, M.G., Longoni, G.B., Mereu, G.P., and Biggio, G., *J. Neurochem.*, 1987, **48**, 1355.

80. Dorow, R., Duka, T., Holler, L., and Sauerbrey, N., *Brain Res. Bull.*, 1987, **19**, 319.

81. Stephens, D.N., Schneider, H.H., Kehr, W., Andrews, J.S., Rettig, K.-J., Turski, L., Schmiechen, R., Turner, J.D., Jensen, L.H., Petersen, E.N., Honore, T., and Hansen, J.B., *J. Pharmacol. Exp. Ther.*, 1990, **253**, 334.

82. Turski, L., Stephens, D.N., Jensen, L.H., Petersen, E.N., Meldrum, B.S., Patel, S., Hansen, J.B., Löscher, W., Schneider, H.H., and Schmiechen, R. *J., Pharmacol. Exp. Ther.*, 1990, **253**, 344.

83. Boast, C.A., Bernard, P.S., Barbaz, B.S., and Bergen, K.M., *Neuropharmacology*, 1983, **22**, 1511.

84. Möhler, H., Burkard, W.P., Keller, H.H., Richards, J.G., and Haefely, W., *J. Neurochem.*, 1981, **37**, 714.

85. Bonetti, E.P., Pieri, L., Cumin, R., Schaffner, R., Pieri, M., Gamzu, E.R., Müller, R.K.M., and Haefely, W., *Psychopharmacol.*, 1982, **78**, 8.

86. Kawasaki, K., Kodama, M., and Matsushita, A., *Eur. J. Pharmacol.*, 1984, **102**, 147.

87. Darragh, A., Lamba, R., O'Boyle, C., Kenny, M., and Brick, I., *Psychopharmacol.*, 1983, **80**, 192.

88. O'Boyle, C., Lambe, R., Darragh, A., Taffe, W., Brick, I., and Kenny, M., *Br. J. Anaesth.*, 1983, **55**, 349.

89. Scollo-Lavizzari, G., *Eur. Neurol.*, 1983, **22**, 7.

90. Haefely, W., and Polc, P., in "*Anxiolytic Neurochemical Behavioural and Clinical Perspectives*" (Eds Malick, J.B., Enna, S.J., and Yamamura, H.I.) p. 113 (Raven: New York 1983).

91. Gardner, C.R., *Drug Dev. Res.*, 1988, **12**, 1 (through ref. 47).

92. Petrack, B., and Yokoyama, N., *Annu. Rep. Med. Chem.*, 1985, **20**, 19.

93. Gotfryd, M.A., *Clin. Neuropharmacol.*, 1984, Suppl. 1, 626.

94. Blanchard, J.C., and Julou, L., *J. Neurochem.*, 1983, **40**, 601.

95. Blanchard, J.C., Zundel, J.L., and Julou, L., *Biochem. Pharmacol.*, 1983, **32**, 3651.

96. Trifiletti, R.R., and Snyder, S.H., *Mol. Pharmacol.*, 1984, **26**, 458.

97. Morselli, P.L., *Pharmacopsychiatry*, 1990, **23**, 129.

98. Morton, S., and Lader, M., *Pharmacopsychiatry*, 1990, **23**, 120 (through ref. 47).

99. Langer, S.Z., *et al. Pharmacopsychiatry*, 1990, **23**, 103 (through ref. 47).

100. Zivkovic, B., *et al. Pharmacopsychiatry*, 1990, **23**, 108 (through ref. 47).

101. Clements-Jewery, S., Danswan, G., Gardner, C.R., Matharu, S.S., Murdoch, R., Tully, W.R., and Westwood, R., *J. Med. Chem.*, 1988, **31**, 1220.

102. Tully, W.R., Gardner, C.R., Gillespie, R.J., and Westwood, R., *J. Med. Chem.*, 1991, **34**, 2060.

103. Yoneda, F., Ohtaka, T., and Nitta, T., *Chem. Pharm. Bull.*, 1964, **12**, 1351.

104. Tisler, M., and Stanovnik, B., in "*Condensed Pyridazines Including Cinnolines and Phthalazines*" (Ed. Castle, R.N.) Vol. 27, p. 801-807 (Wiley: New York 1973).

105. Maury, G., in "*Special Topics in Heterocyclic Chemistry*" (Eds Weissberger, A., and Taylor, E.C.) p.179 (Wiley: New York 1977).

106. Armarego, W.L.F., *J. Chem. Soc.*, 1965, 2778.

107. Stanovnik, B., and Tisler, M., *Tetrahedron*, 1967, **23**, 387.

108. Kobe, J., Stanovnik, B., and Tisler, M., *Tetrahedron*, 1968, **24**, 239.

109. Stanovnik, B., and Tisler, M., *Tetrahedron Lett.*, 1966, **22**, 2403.

110. Lombardino, J.G., *J. Heterocycl. Chem.*, 1968, **5**, 35.

111. Pfizer, Chas., and Co. Inc., Brit. 1,135,893 (*Chem. Abstr.*, 1969, **70**, 57870).

112. Nitta, Y., Yoneda, F., and Otaka, T., Jpn. Pat 22,264 (*through Chem. Abstr.*, 1966, **64**, 3566).

113. Stanovnik, B., and Tisler, M., *Tetrahedron*, 1967, **23**, 2739.

114. Almirante, L., Polo, L., Mugnaini, A., Provinciali, E., Rugurli, P., Gamba, A., Olivi, A., and Murmann, W., *J. Med. Chem.*, 1966, **9**, 29.

115. Boehringer, C.F., and Soehne, G.m.b.H., Fr. Addn., 84,814 (*through Chem. Abstr.*, 1965, **63**, 4306).

116. Pollak, A., Stanovnik, B., and Tisler, M., *Tetrahedron*, 1968, **24**, 2623.

117. Ostroversnik, S., Stanovnik, B., and Tisler, M., *Croat. Chem. Acta*, 1969, **41**, 135.

118. Barlin, G.B., *J. Chem. Soc. Perkin. Trans.* **1**, 1976, 1424.

119. Barlin, G.B., Brown, I.L., Golic, L., and Kaucic, V., *Aust. J. Chem*, 1982, **35**, 423.

120. Barlin G.B., *Aust. J. Chem.*, 1986, **39**, 1803.

121. Barlin, G.B., Davies, L.P., and Ngu, M.M.L., *Aust. J. Chem.*, 1989, **42**, 1759.

122. Barlin, G.B., Davies, L.P., Ireland, S.J., Khoo, C.L.Y., and Nguyen, T.M.T., *Aust. J. Chem.*, 1990, **43**, 503.

123. Yoshiro, K., Teruo, K., and Kunio, M., *Chem. Pharm. Bull.*, 1971, **19**, 2106.

124. Podergajs, S., Stanovnik, B., and Tisler, M., *Synthesis*, 1984, 263.

125. Stanovnik, B., Tisler, M., Ceglar, M., and Bah, V., *J. Org. Chem.*, 1970, **35**, 1138.

126. Paudler, W.W., and Blewitt, H.L., *Tetrahedron*, 1965, **21**, 355.

127. Lombardino, J.G., *J. Org. Chem.*, 1965, **30**, 2403.

128. Albert, A., Goldacre, R., and Phillips, J., *J. Chem. Soc.*, 1948, 2240.

129 Pugmire, R.J., Smith, J.C., Grant, D.M., Stanovnik, B., and Tisler, M., *J. Heterocycl. Chem.*, 1976, **13**, 1057.

130. Pugmire, R.J., Smith, J.C., Grant, D.M., Stanovnik, B., Tisler, M., and Vercek, B., *J. Heterocycl. Chem.*, 1987, **24**, 805.

131. Stanovnik, B., and Tisler, M., *Croat. Chem. Acta*, 1968, **40**, 1.

132. Japelj, M., Stanovnik, B., and Tisler, M. *J., Heterocycl. Chem.*, 1969, **6**, 559.

133. Stanovnik, B., *Synthesis*, 1971, **8**, 424.

134. Furlan, M., Stanovnik, B., and Tisler, M., *J. Org. Chem.*, 1972, **37**, 2689.

135. Barlin, G.B., Brown, D.J., Kadunc, Z., Petric, A., Stanovnik, B., and Tisler, M., *Aust. J. Chem.*, 1983, **36**, 1215.

136. Schulenberg, J.W., and Archer, S., *Org. React.*, 1965, **14**, 24.

137. Kobe, J., Stanovnik, B., and Tisler, M., *Tetrahedron*, 1970, **26**, 3357.

138. Merslavic, M., Petric, A., Rozman, D., Stanovnik, B., and Tisler, M., *J. Heterocycl. Chem.*, 1989, **26**, 445.

139. Stanovnik, B., Kupper, M., Tisler, M., Leban, I., and Golic, L., *J. Chem. Soc., Chem. Commun.*, 1984, 268.

140. Huc, B., Furlan, B., Stanovnik, B., and Tisler, M., *J. Heterocycl. Chem.*, 1990, **27**, 2145.

141. Paudler, W.W., and Kuder, J.E., *J. Org. Chem.*, 1966, **31**, 809.

142. Rival, Y., Grassy, G., Taudou, A., and Ecalle, R., *Eur. J. Med. Chem.*, 1991, **26**, 13.

143. Sugiura, S., Inoue, S., and Goto, T., *Yakugaku Zasshi*, 1970, **90**, 423 (*Chem. Abstr.*, 1970, **73**, 45459).

144. Sugiura, S., Kakoi, H., Inoue, S., and Goto, T., *Yakugaku Zasshi*, 1970, **90**, 441 (*Chem. Abstr.*, 1970, **73**, 45462).

145. Sugiura, S., Inoue, S., and Goto, T., *Yakugaku Zasshi*, 1970, **90**, 707 (*Chem. Abstr.*, 1970, **73**, 98904).

146. Werbel, L.M., and Zamora, M.L., *J. Heterocycl. Chem.*, 1965, **2**, 287.

147. Gurret, P., Jacquier, R., and Maury, G., *J. Heterocycl. Chem.*, 1971, **8**, 643.

148. DePompei, M.F., and Paudler, W.W., *J. Heterocycl. Chem.*, 1975, **12**, 861.

149. Vercek, B., Stanovnik, B., and Tisler, M., *Heterocycles*, 1976, **4**, 943.

150. Bradac, J., Furek, Z., Janezic, D., Molan, S., Smerkolj, I., Stanovnik, B., Tisler, M., and Vercek, B., *J. Org. Chem.*, 1977, **42**, 4197.

151. Lumma, W.C., Eur. Pat. Appl. 13914 (*Chem. Abstr.*, 1981, **94**, 84167).

152. Goto, T., Inoue, S., and Sugiura, S., *Tetrahedron Lett.*, 1968, **36**, 3873.

153. Schvedov, V.I., Altukhova, L.B., Chernyshkova, L.A., and Grinev, A.N., *Khim-Farm. Zh.*, 1969, **3**, 15 (*Chem. Abstr.*, 1970, **72**, 66899).

154. Roe, A.M., *J. Chem. Soc.*, 1963, 2195.

155. George, P., Rossey, G., Depoortere, H., Mompon, B., Allen, J., and Wick, A., in "*Imidazopyridines in Sleep Disorders*" (Eds Sauvanet, J.P., Langer, S.Z., and Morselli, P.L.) p.11-23 (Raven: New York 1988).

156. Nitta, Y., Yoneda, F., and Otaka, T., Jpn Pats 22,265, 22,267 (*Chem. Abstr.*, 1966, **64**, 3566-3567).

157. Abignente, E., Arena, F., Deprariis, P., Montanaro, G., Rossi, F., Lampa, F., Giordano, L., Vacca, C., and Marmo, E., *Farmaco. Med. Sci.*, 1980, **35**, 654.

158. Bristol, J.A., and Lovey, R.G., US Pat. 4,464,372 (*Chem. Abstr.*, 1984, **101**, 171275).

159. Moran, D.B., Powell, D.W., and Albright, J.D., US Pat. 4,569,934 (*Chem. Abstr.*, 1987, **106**, 50237).

160. Meyer, H., Ingendoh, A., Garthoff, B., and Hirth, C., Ger. Offen. DE 3,542,661 (*Chem. Abstr.*, 1987, **107**, 115601).

161. Barlin, G.B., and Ireland, S.J., *Aust. J. Chem.*, 1987, **40**, 1491.

162. Barlin, G.B., Davies, L.P., and Ngu, M.M.L., *Aust. J. Chem.*, 1988, **41**, 1149.

163. Barlin, G.B., Davies, L.P., and Ngu, M.M.L., *Aust. J. Chem.*, 1988, **41**, 1735.

164. Barlin, G.B., Davies, L.P., Ireland, S.J., and Ngu, M.M.L., *Aust. J. Chem.*, 1989, **42**, 1133.

165. Barlin, G.B., Davies, L.P., Ireland, S.J., and Ngu, M.M.L., *Aust. J. Chem.*, 1989, **42**, 1735.

166. Barlin, G.B., Davies, L.P., and Ngu, M.M.L., *Aust. J. Chem.*, 1989, **42**, 1749.

167. Barlin, G.B., Davies, L.P., Ireland, S.J., Ngu, M.M.L., and Zhang, J., *Sci. Pharm.*, 1990, **58**, 173.

168. Alexander, C.A., Gregge, R.J., and Peet, N.P., US Pats 4,391,806 and 4,391,807 (*Chem. Abstr.*, 1983, **99**, 105270, 105271).

169. Heider, J., Austel, A., Hauel, N., Noll, K., Bombard, A., Van Meel, J., and Diederen, W., Ger. Offen, DE 3446812 (*Chem. Abstr.*, 1986, **105**, 153064).

170. Heider, J., Hauel, N., Austel, V., Noll, K., Bomhard, A., Van Meel, J., and Diedere, W., Ger. Offen., DE 3446778 (*Chem. Abstr.*, 1986, **105**, 172459).

171. Nishimura, T., Yoshimura, Y., Miyake, A., and Hashimoto, N., Eur. Pat. Appl. EP. 229,369 (*Chem. Abstr.*, 1987, **107**, 197941).

172. Kishimoto, S., Tominatsu, K., Miyake, A., and Yoshimura, Y., Eur. Pat. Appl. EP. 249,170 (*Chem. Abstr.*, 1988, **108**, 221494).

173. Miyake, A., Kawai, T., and Yoshimura, Y., Jpn. Kakai Tokkyo Koho. JP. 0140,489 (*Chem. Abstr.*, 1989, **111**, 114970).

174. Miyake, A., Kondo, M., and Eujino, M., PCT Int. Appl. WO 86 05,183 (*Chem. Abstr.*, 1988, **108**, 167195).

175. Miyake, A., Kondo, M., and Eujino, M., PCT Int. Appl. WO 86 05,184 (*Chem. Abstr.*, 1987, **106**, 101958).

176. Kishimoto, S., Tomimatsu, K., Miyake, A., Eur. Pat. Appl. EP 304858 (*Chem. Abstr.*, 1989, **111**, 114964).

177. Arya, V.P., Fernandes, F., and Sudersanam, V., *Indian. J. Chem.*, 1972, **10**, 598.

178. Tomcufcik, A.S., Izzo, P.T., and Fabio, P.F., Ger. Offen. DE 2,208,830 (*Chem. Abstr.*, 1972, **77**, 164737).

179. Maeda, Y., Mizuno, Y., Nakatani, A., and Yamano, M., Eur. Pat. Appl. EP 319,019 (*Chem. Abstr.*, 1989, **111**, 214,323).

180. Tomcufcik, A.S., and Wilkinson, R.G., US Pat. 3,711,613 (*Chem. Abstr.*, 1973, **78**, 115230).

181. Tomcufcik, A.S. Izzo, P.T., and Fabio, P.E., US. Pat. 3,905,974 (*Chem. Abstr.*, 1975, **84**, 4989).

182. American Cyanamid Co., Neth. Appl. 72 14242 (*Chem. Abstr.*, 1975, **83**, 28266).

183. Tomcufcik, A.S., Izzo, P.T., and Fabio, P.F., Can. 986,112 (*Chem. Abstr.*, 1976, **85**, 21419).

184. Jojima, T., *Sankyo Kenkyusho Nempo*, 1972, **24**, 121 (*Chem. Abstr.*, 1972, **78**, 159538).

185. Yasuo, I., Kazunari, O., Tatsuo, N., and Harutoshi, Y., Eur. Pat. Appl. EP 238,070 (*Chem. Abstr.*, 1988, **108**, 204632).

186. Tseng, C.P., Eur. Pat. Appl. EP. 244,166 (*Chem. Abstr.*, 1988, **108**, 186762).

187. Yoshikawa, H., and Yamawaki, T., Jpn. Kokai Tokkyo Koho JP 01207211 (*Chem. Abstr.*, 1990, **112**, 114195).

188. Tseng, C.P., US 4,838,925 (*Chem. Abstr.*, 1990, **112**, 7508).

189. Yoshikawa, H., and Yamawak, T., Jpn. Kokai Tokkyo Koho JP 0209805 (*Chem. Abstr.*, 1990, **113**, 128,082).

190. Yoshikawa, H., and Yamawak, T., Jpn. Kokai Tokkyo Koho JP 02,152,912 (*Chem. Abstr.*, 1990, **113**, 167390).

191. Yoshikawa, H., and Yamawak, T., Jpn. Kokai Tokkyo Koho JP 0222208 (*Chem. Abstr.*, 1990, **113**, 2048).

192. Nakahama, T,, Ota, K., Ito, S., Miki, H., and Ishido, Y., Jpn Kokai Tokkyo Koho JP 01316379 (*Chem. Abstr.*, 1990, **113**, 6345).

193. Roussel-UCLAF, *Fr. Demande* 2,311,026 (*Chem. Abstr.*, 1977, **87**, 102368).

194. Roussel-UCLAF, *Fr. Demande* 2,315,507 (*Chem. Abstr.*, 1977, **87**, 135376).

195. Hodgson, S.T., Eur. Pat. Appl. EP 305093 (*Chem. Abstr.*, 1989, **111**, 57746).

196. Sircar, Ila and Bristol, J.A., (Warner-Lambert Co.) US. 4734415 (*Chem. Abstr.*, 1989, **111**, 97259).

197. Perronnet, J., and Taliani, L., Ger. Offen. DE 2,432,357 (*Chem. Abstr.*, 1975, **83**, 10146).

198. Adam, H.E., US Pat. 4,060,614 (*Chem. Abstr.*, 1978, **88**, 99317).

199. Adam, A.E., US Pat. 4,061,751 (*Chem. Abstr.*, 1978, **88**, 58575).

200. Almirante, L., Polo, L., Mugnaini, A., Provinciali, E., Rugarli, P., Biancotti, A., Gamba, A., and Murmann, W., *J. Med. Chem.*, 1965, **8**, 305.

201. George, P., and Allen, J., Eur. Pat. Appl. EP 267111 (*Chem. Abstr.*, 1988, **109**, 149531).

202. George, P., Allen, J., and Jaurand, G., *Fr. Demande* FR 2612927 (*Chem. Abstr.*, 1989, **111**, 115178).

203. Allen, J., and George, P., *Fr. Demande* FR 2612928 (*Chem. Abstr.*, 1989, **111**, 174090).

204. Yamanaka, M., Miyake, K., Suda, S., Ohhara, H., and Ogawa, T., *Chem. Pharm. Bull.*, 1991, **39**, 1556.

205. Kosáry, J., Kasztreiner, E., Rablockzy, G., and Kürthy, M., *Eur. J. Med. Chem.*, 1989, **24**, 97-9.

206. Abignente, E., Arena, F., de Caprariis, P., and Parente, L., *Farmaco. Ed. Sci.*, 1975, **30**, 815-822.

207. Abignente, E., Arena, F., De Caprariis, P., Nuzetti, R., Marmo, E., Lampa, E., Rosatti, F., and Ottavo, R., *Farmaco. Ed. Sci.*, 1981, **36**, 61-80.

208. Baldwin, J.J., and Lumma, W.C., (Merck and Co. Inc.) Ger. Offen. 2820938, 1978; US Appl. 796,958, 1977.

209. Sablayrolles, C., Cros, G.H., Milhavet, J.C., Recheng, E., Chapat, J.-P., Boucard, M., Serrano, J.J., and McNeill, J.H., *J. Med. Chem.*, 1984, **27**, 206-212.

210. Kaminski, J.J., Hilbert, J.M., Pramanik, B.N., Solomon, D.M., Conn, D.J., Rizvi, R.K., Elliott, A.J., Guzik, H., Lovey, R.G., Donalski, M.S., Wong, S.-C., Puchalski, C., Gold, E.H., Long, J.F., Chiu, P.J.S., and McPhail, A.T., *J. Med. Chem.*, 1987, **30**, 2031.

211. Kaminski, J.J., Perkins, D.G., Frantz, J.D., Solomon, D.M., Elliott, A.J., Chiu, P.J.S., and Long, J.F., *J. Med. Chem.*, 1987, **30**, 2047.

212. Spitzer, W.A., Victor, F., Pollock, G.D., and Hayes, J.S., *J. Med. Chem.*, 1988, **31**, 1590.

213. George, P., and Giron, C., US Pat. 4,650,796 (*Chem. Abstr.*, 1987, **107**, 134308).

214. Bourguignon, J.J., Wermuth, C.G., and Worms, P., Eur. Pat. Appl. EP 306,408 (*Chem. Abstr.*, 1989, **111**, 97262).

215. Ledincer, D., and Hauser, C.R., *Org. Synth.*, 1960, **40**, 31.

216. Einhorn, A., Bischkopff, E., Ladish, C., Mauermayer, T., Schupp, G., Spröngerts, E., and Szelinski, B., *Justus Leibigs Ann. Chem.*, 1905, **343**, 207.

217. Braestrup, C., and Nielsen, M., in "*Handbook of Psychopharmacology*" (Eds Iversen, L.L., Iversen, S.D., and Synder, S.H.) Vol. 17, p.285 (Plenum: New York 1983).

218. Mackerer, C.R., Kockmen, R.L., Bierschenk, B.A., and Bremner, S.S., *J. Pharmacol. Exp. Ther.*, 1978, **206**, 405.

219. Möhler, H., and Okada, T., *Science*, 1977, **198**, 849.

220. Squires, R.F., and Braestrup, C., *Nature*, 1977, **266**, 732.

221. Möhler, H., and Okada, T., *Life Sci.*, 1977, **20**, 2101.

222. Lippa, A.S., Klepner, C.A., Benson, D.I., Critchett, D.J., Sano, M.C., and Beer, B., *Brain Res. Bull.*, **5** Suppl., 1980, **2**. 861.

223. Klepner, C.A., Lippa, A.S., Benson, D.J., Sano, M.C., and Beer, B., *Pharmacol. Biochem. Behav.*, 1979, **11**, 457.

224. Lippa, A.S., Critchett, D.J., Sano, M.C., Klepner, C.A., Greenblatt, E.N., Coupet, J., and Beer, B., *Pharmacol. Biochem. Behav.*, 1979, **10**, 831.

225. Braestrup, C., and Squires, R.F., *Eur. J. Pharm.*, 1978, **48**, 263.

226. Hansch, C., Leo, A., and Taft, R.W., *Chem. Rev.*, 1991, **91**, 165.

227. Vogel, A.I., "*A Text-book of Practical Organic Chemistry*", p.843 (Longmans, London 1948).

228. Drake, N.L., and Tuemmler, W.B., *J. Am. Chem. Soc.*, 1955, **77**, 1204.

229. Steck, E.A., Brundage, R.P., and Fletcher, L.T., *J. Am. Chem. Soc.*, 1954, **76**, 3225.

230. Horie, T., Kinjo, K., and Ueda, T., *Chem. Pharm. Bull.*, 1962, **10**, 580.

231. Clark, J.H., English, J.P., Jansen, G.R., Marson, H.W., Rogers, M.M., and Taft, W.E., *J. Am. Chem. Soc.*, 1958, **80**, 980.

232. Blank, B., DiTullio, N.W., Deviney, L., Roberts, J.T., and Saunders, H.L., *J. Med. Chem.*, 1975, **18**, 952.

233. Skerritt, J.N., Chow, S.C., Johnston, G.A.R., and Davies, L.P., *Neurosci. Lett.*, 1982, **34**, 63.

234. Allen, M.S., Hagen, T.J., Trudell, M.L., Codding, P.W., Skolnick, P., and Cook, J.M., *J. Med. Chem.*, 1988, **31**, 1854.

235. Martin, Y.C., in "*Quantitative Drug Design*" Medicinal Research Series, (Ed. Grunewald, G.L.) Vol. 8, p. 379 (Dekker: New York 1978).

236. Sarma, R.H., and Woronick, C.L., *Biochemistry*, 1972, **11**, 170.

237. Slothouwer, J.H., *Recl. Trav. Chim. Pays-Bas*, 1914, **33**, 324.

238. Schönenberger, H., Bindl, L., and Adam, A., *Arch. Pharm. (Weinheim, Ger.)*, 1973, **306**, 64.

239. Chechelska, B., *Rocz. Chem.*, 1956, **30**, 149 (*Chem. Abstr.*, 1957, **51**, 279).

240. Szabo, J., Fodor, L., Szucs, E., Bernath, G., and Sohar, P., *Pharmazie*, 1984, **39**, 426 (*Chem. Abstr.*, 1984, **101**, 230450).

241. Schönenberger, H., Petter, A., Kühling, V., and Bindl, L., *Arch. Pharm. (Weinheim, Ger.)*, 1976, **309**, 289.

242. Graf, R., *J. Prakt. Chem.*, 1933, **138**, 292.

243. Itai, T., and Nakashima, T., *Chem. Pharm. Bull.*, 1962, **10**, 936.

244. Lutz, R.E., Allison, R.K., Ashburn, G., Bailey, P.S., Clark, M.T., Codington, J.F., Deinet, A.J., Freek, J.A., Jordan, R.H., Leake, N.H., Martin, T.A., Nicodemus, K.C., Rowlett, R.J., Shearer, N.H., Smith, J.D., and Wilson, J.W., *J. Org. Chem.*, 1947, **12**, 617.

245. Cowper, R.M., and Davidson, L.H., *Org. Synth*, 1948, Coll. Vol. II, 480.

246. Dahn, H., Hauth, H., and Gold, H., *Helv. Chim. Acta*, 1963, **46**, 1000.

247. Bestmann, H.J., Klein, O., Göthlich, L., and Buckschewski, H., *Chem. Ber.*, 1963, **96**, 2259.

248. Musante, C., and Parrini, V., *Gazz. Chim. Ital.*, 1950, **80**, 868.

249. Grieve, W.S.M., and Hey, D.H., *J. Chem. Soc.*, 1933, 968.

250. Fuson, R.C., Gray, H., and Gouza, J.J., *J. Am. Chem. Soc.*, 1939, **61**, 1937.

251. Rubin, M., Paist, W.D., and Elderfield, R.C., *J. Org. Chem.*, 1941, **6**, 261.

252. Tiffany, B.D., Wright, J.B., Moffett, R.B., Heinzelman, R.V., Strube, R.E., Aspergren, B.D., Lincoln, E.H., and White, J.L., *J. Am. Chem. Soc.*, 1957, **79**, 1682.

253. Lohrisch, H.-J., Kopanski, L., Herrmann, R., Schmidt, H., and Steglich, W., *Liebigs, Ann. Chem.*, 1986, 177.

254. Bestmann, H.J., Seng, F., and Schulz, H., *Chem. Ber.*, 1963, **96**, 465.

255. Drake, N.L., and Bronitsky, J., *J. Am. Chem. Soc.*, 1930, **52**, 3715.

256. Boyer, J.H., and Straw, D., *J. Am. Chem. Soc.*, 1952, **74**, 4506.

257. Gaudry, M., and Marquet, A., *Org. Synth.* 1976, **55**, 24.

258. Thayer, F.K., *Org. Synth.*, 1932, Coll. Vol I, 12.

259. Mohr, E., *J. Prakt. Chem.*, 1905, **71**, 305.

260. Taylor, T.W.J., Callow, N.H., and Francis, C.R.W., *J. Chem. Soc.*, 1939, 257.

261. Riley, H.A., and Gray, A.R., *Org. Synth.*, 1948, Coll. Vol. II, 509.

262. Arbilla, S., Depoortere, H., George, P., and Langer, S.Z., *Naunyn-Schmiedeberg's Arch Pharmacol.*, 1985, **330**, 248.

263. George, P., Giron, C., and Froissant, J., *Fr. Demande* FR 2,593,181 (*Chem. Abstr.*, 1988, **108**, 131820).

264. George, P., Giron, C., and Froissant, J., *Fr. Demande* FR 2,593,818 (*Chem. Abstr.*, 1988, **108**, 131816).

265. George, P., and Allen, J., *Fr. Demande* FR 2,606,409 (*Chem. Abstr.*, 1989, *110*, 231625).

266. Kaplan, J.P., and George, P., *Eur. Pat. Appl*, EP 50,563 (*Chem. Abstr.*, 1982, **97**, 92280).

267. George, P., and Allen, J., *Fr. Demande* FR 2,581,646 (*Chem. Abstr.*, 1987, **107**, 59031).

268. Browne, L., and Shaw, K.J., in "*Annu. Rep. Med. Chem.*, 1991, **26**, 1.

269. Tully, W.R., British Pat. 2128989, 1984. (through ref. 102).

270. Magidson, O., and Menschikoff, G., *Ber. Dtsch. Chem. Ges.*, 1925, **58**, 113.

271. Bochis, R.J., Dybas, R.A., Eskola, P., Kulsa, P., Linn, B.O., Lusi, A., Meitzner, E.P., Milkowski, J., Mrozik, H., Olen, L.E., Peterson, L.H., Tolman, R.L., Wagner, A.F., Waksmunski, F.S., Egerton, J.R., and Ostlind, D.A., *J. Med. Chem.*, 1978, **21** , 235.

272. Clark, G.J., and Deady, L.W., *Aust. J. Chem.*, 1981, **34**, 927.

273. Nedenskov, P., Clauson-Kaas, N., Lei, J., Heide, H., Olsen, G., and Jansen, G., *Acta Chem. Scand.*, 1969, **23**, 1791.

274. Tschitschibabin, A.E., *Ber. Dtsch. Chem. Ges.*, 1925, **58**, 1704.

275. Deady, L.W., and Stanborough, M.S., *J. Heterocycl. Chem.*, 1979, **16**, 187.

276. Brown, D.J., and Waring, P., *Aust. J. Chem.*, 1973, **26**, 443.

277. Weijlard, J., Tishler, M., and Erickson, A.E., *J. Am. Chem. Soc.*, 1945, **67**, 802.

278. Ellingson, R.C., Henry, R.L., and McDonald, F.G., *J. Am. Chem. Soc.*, 1945, **67**, 1711.

279. Bicking, J.B., Mason, J.W., Woltersdorf, O.W., Jones, J.H., Kwong, S.F., Robb, C.M., and Cragoe, E.J., *J. Med. Chem.*, 1965, **8**, 638.

280. Palamidessi, G., and Bernardi, L., *J. Org. Chem.*, 1964, **29**, 2491.

281. Kimura, S., Miyaki, Y., and Goto, R., *Bull. Soc. Chim. Jpn.*, 1966, **39**, 1333.

282. Okada, S., Kosasayama, A., Konno, T., and Uchimaru, F., *Chem. Pharm. Bull.*, 1971, **19**, 1344.

283. Palamidessi, G., Bernardi, L., and Leone, A., *Farmaco. Ed. Sci.*, 1966, **21**, 805.

284. Bernardi, L., Larini, G., and Leone, A., Ger. 1,178,436 (*Chem. Abstr.*, 1965, **62**, 4039).

285. Derick, C.G., and Kamm, O., *J. Am. Chem. Soc.*, 1916, **38**, 400.

PUBLICATIONS

Publications

(Based on work described in this thesis)

1. Barlin, G.B., Davies, L.P., Ireland, S.J., Ngu, M.M.L., and Zhang, J.
"Synthesis of imidazo[1,2-*b*]pyridazines and studies of their activities in the central nervous system".
Sci. Pharm., 1990, **58**, 173-175.

Publications in Proof

2. Barlin, G.B., Davies, L.P., Ireland, S.J., Ngu, M.M.L., and Zhang, J.
"Imidazo[1,2-*b*]pyridazines. X
Syntheses and Central Nervous System Activities of Some 3-(Acetamido, benzamido, substituted benzamido or dimethylamino)methyl-2-(phenyl or substituted phenyl)-6-(halogeno, alkylthio, alkoxy, phenylthio, phenoxy, benzylthio or benzyloxy)imidazo[1,2-*a*]pyridazines."
Aust. J. Chem., 1992, Paper No. C/91118.

3. Barlin, G.B., Davies, L.P., Ireland, S.J. and Zhang, J.
"Imidazo[1,2-*b*]pyridazines. XI
Syntheses and Central Nervous System Activities of Some 6-(*N*-Benzyl-*N*-methylamino)-3-methoxy-2-phenyl(and substituted phenyl)imidazo[1,2-*b*]pyridazines".
Aust. J. Chem., 1992, Paper No. C/91182.

Accepted for Publication

4. Barlin, G.B., Davies, L.P., Ireland, S.J., Ngu, M.M.L., and Zhang, J.
"Imidazo[1,2-*b*]pyridazines. XII.
Syntheses and Central Nervous System Activities of Some Substituted Imidazo[1,2-*b*]pyridazines and Related Imidazo[1,2-*a*]pyridines, Imidazo[1,2-*a*]pyrimidines and Imidazo[1,2-*a*]pyrazines".
Aust. J. Chem., 1992, Paper No. C/91251.

Submitted for Publication

5. Barlin, G.B., Davies, L.P., Ireland, S.J., and Zhang, J.
"Imidazo[1,2-*b*]pyridazines. XIII
Syntheses and Central Nervous System Activities of Some 2-Benzyl(phenethyl, biphenyl-4′-yl, 6′-methylnaphthalen-2′-yl, t-butyl, and cyclohexyl)-3-methoxy(acylaminomethyl and dimethylaminomethyl)-6-(variously substituted)imidazo[1,2-*b*]pyridazines".
Aust. J. Chem.