SYNTHESES AND EVALUATION OF SOME NITROGEN HETEROCYCLES AS BENZODIAZEPINE RECEPTOR LIGANDS

A thesis submitted for the degree of Doctor of Philosophy of the Australian National University by Peter W. Harrison

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Canberra
June 1995
To my parents
Certificate of originality

The work described in this thesis was carried out by the candidate at the Australian National University, except for that described in Chapter VII which was carried out at Swinburne University of Technology. Where the work of others was employed or quoted, appropriate references are given.

Peter W. Harrison

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I would like to sincerely thank my supervisor Dr G.B. Barlin for his advice, assistance and encouragement throughout the course of this work and the preparation of this thesis. I would also particularly like to express my gratitude to Dr L.P. Davies for his supervision of the in vitro binding assays reported in this thesis and useful comments regarding the content of Chapter I, and to Dr M.G. Wong for her supervision of the molecular modelling work, enjoyable collaborations, and advice in drafting Chapter VII. Thanks must also go to the other two members of my supervisory panel, Dr B. Glenn and Dr W.A.R. Armarego, and to Dr D.J. Brown, for their valuable comments and helpful discussions during the preparation of this thesis.

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Finally, grateful acknowledgement is made to the Australian National University for the award of a Postgraduate Research Scholarship.
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<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>BZ</td>
<td>Benzodiazepine</td>
</tr>
<tr>
<td>BZR</td>
<td>Benzodiazepine receptor</td>
</tr>
<tr>
<td>β-CCE</td>
<td>Ethyl β-carboline-3-carboxylate</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>Da</td>
<td>Daltons</td>
</tr>
<tr>
<td>DMCM</td>
<td>Methyl 4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulphoxide</td>
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<tr>
<td>GABA</td>
<td>γ-Aminobutyric acid</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-Hydroxytryptamine</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
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<tr>
<td>IUPAC</td>
<td>International union of pure and applied chemistry</td>
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<tr>
<td>MAOI</td>
<td>Monoamine oxidase inhibitor</td>
</tr>
<tr>
<td>MCPBA</td>
<td>meta-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PBR</td>
<td>Peripheral-type benzodiazepine receptor</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>TBPS</td>
<td>t-Butylbicyclophosphorothionate</td>
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<tr>
<td>THDOC</td>
<td>3α, 21-Dihydroxy-5α-pregnan-20-one</td>
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<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-α</td>
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Some substituted imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines have been synthesised and characterised using analytical and spectral techniques. These compounds have been evaluated as ligands at central benzodiazepine receptors (BZRs) and/or peripheral-type benzodiazepine receptors (PBRs) by the in vitro displacement of $[^3H]$diazepam binding from rat brain (BZR) and rat kidney (PBR) membrane preparations. Novel high affinity ligands at either the BZR or the PBR have been identified and used to study the steric and electronic requirements of the ligand binding sites of the two receptor types.

A series of 3-(benzamidomethyl and methoxy)imidazo[1,2-b]pyridazines has been synthesised with a number of methyl-, methoxy- or methylenedioxy-containing substituent groups in the 6- and/or 2-positions, and evaluated for BZR affinity. The relationship between the electronic and steric properties of the substituent groups of the compounds and their resultant in vitro BZR affinities have been discussed.

The steric constraints of the area of ligand-receptor interaction between the 6-position groups of imidazo[1,2-b]pyridazines and the BZR protein have been examined by the synthesis of a series of 6-alkoxy-2-aryl-3-benzamidomethylimidazo[1,2-b]-pyridazines containing linear 6-alkoxy groups of varying steric bulk, and the determination of their in vitro BZR affinities. A number of 6(7 and 8)-(chloro and methoxy)imidazo[1,2-a]pyridines has been synthesised and evaluated as BZR and PBR ligands to determine the relative effects of substitution in the 6-, 7- and 8-positions of the molecules on in vitro affinities at both receptor types.

Several imidazo[1,2-b]pyridazines have been synthesised containing sterically bulky 2-position groups, and the in vitro BZR and PBR affinities of the compounds have been reported. Certain compounds containing either a 2-styryl group (where the phenyl ring of the 2-styryl group is separated from the imidazo[1,2-b]pyridazine nucleus by a CH=CH moiety) or a 2-aryl group with a large t-butyl or cyclohexyl substituent in the para-position of the phenyl ring have been found to have very high, selective affinities for the PBR. It has therefore been concluded that the area of ligand-receptor interaction
between the 2-position groups of the imidazo[1,2-b]pyridazines and the BZR protein is subject to more restrictive steric constraints than the corresponding area of ligand-receptor interaction with the PBR protein.

The effect on BZR or PBR selectivity of substitution of the phenyl ring of the 3-benzamidomethyl group by chloro, methyl or nitro groups has been examined. For imidazo[1,2-b]pyridazines containing sterically bulky 2-aryl groups, the 3-(substituted benzamidomethyl) compounds had reduced PBR affinities compared to 3-benzamidomethyl analogues. In the case of imidazo[1,2-b]pyridazines with less bulky 2-aryl groups, however, substitution of the phenyl ring of the 3-benzamidomethyl groups was shown to increase PBR selectivity by reducing BZR affinity while maintaining high PBR affinity.

To attempt to rationalise the structure-activity relationships at the BZR and the PBR for the compounds reported in this thesis, a molecular modelling study using selected imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines has been undertaken. The compounds have been examined for areas of likely ligand-receptor interaction by considering the electronic, lipophilic and steric properties of the compounds and their substituent groups.

A pharmacophoric model of ligand-receptor interaction at the BZR for the imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines has been developed. This pharmacophore contains two points of possible hydrogen bonding interaction and two areas of lipophilic interaction between ligand and receptor, as well as three areas of steric hindrance, which if occupied by the ligand cause a substantial reduction in its BZR affinity. A PBR pharmacophore has also been defined, and this is characterised by two areas of hydrogen bonding and two areas of lipophilic ligand-receptor interaction, as for the BZR pharmacophore. Although the principal pharmacophoric points at the BZR and PBR are identical, however, only one area of steric hindrance has been identified at the PBR.
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CHAPTER I

Introduction

1.1 Historical background

1.1.1 Definitions of anxiety and anxiety disorders

Every human being experiences the emotion of anxiety at some stage in life. Anxiety can be described as a result of human response to stress. It is recognized, however, that at certain levels anxiety can become pathological. In 1895 Freud accurately outlined the features present in cases of extreme anxiety, or anxiety neurosis. These were "general irritability, anxious expectation, free-floating anxiety, anxiety attacks, nocturnal terror, vestige of the development of phobias, gastrointestinal disturbance, parasthesias and a tendency towards chronicity". In 1926 Freud made an additional contribution to distinguishing between "normal anxiety" as "anxiety about a known danger" and "neurotic anxiety" as "anxiety about a danger that has yet to be discovered". Pathological anxiety is a psychiatric disorder when the anxiety felt by the patient is not related to any possible or understandable threat.

The standard definition of anxiety today is not greatly different to that described by Freud a century ago. According to the Diagnostic and Statistical Manual DSM-IV-TR, anxiety "may be focused on an object, situation or activity, which is avoided (phobias), or may be unfocused (free-floating anxiety). It may be experienced in discrete periods with sudden onset and be accompanied by physical symptoms (panic attacks). When anxiety is focused on physical signs or symptoms and causes preoccupation with the fear or belief of having a disease, it is termed hypochondriasis".

Factors which differentiate between normal anxiety and anxiety requiring clinical treatment include persistent occurrence or recurrence of the emotion, a disproportionality between the anxiety felt and the situation causing or being perceived to cause it, inability of the individual to control or end the anxiety-producing situation, and detrimental social or physiological functioning of the individual as a result of the feelings of anxiety. Common physical symptoms accompanying feelings of anxiety are increased heart rate, sweating, dry mouth and palpitations. It is estimated that 2.4% of the population suffer from pathological anxiety at some stage in their lives, often with no
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apparent causative factor. Compounds with anxiolytic actions are therefore of widespread potential benefit to society in treating these disorders.

1-1.2 Early agents used to treat anxiety

The chemical structures of some early compounds used in the treatment of anxiety disorders are shown in Figure 1-1. The oldest and most commonly used drug with psychoactive effects is ethanol. It is estimated that over 65% of the adult population of the USA drink alcoholic beverages on a regular basis, with approximately 10% consuming at least 1 oz of ethanol per day. Ethanol has anxiolytic and sedative/hypnotic properties in man, though behavioural and electrophysiological studies have not yet led to a clear understanding of its neurochemical mechanism(s) of action.

Figure 1-1 Some early anxiolytic agents

![Chemical structures of early anxiolytic agents](image)

During the 1950's, following the synthesis of meprobamate in 1951, propanediol derivatives were commonly used in the treatment of anxiety. These drugs did not appear to cause significant motor or intellectual impairment at therapeutic doses, whilst having beneficial anxiolytic effects in anxious patients and no effect on non-anxious patients. In
this respect they were superior to the other commonly prescribed agents at that time, the barbiturates. The barbiturates possess anxiolytic effects in low sub-hypnotic doses. Pentobarbitone and other related barbiturates enhance the action of \( \gamma \)-aminobutyric acid (GABA), the major inhibitory neurotransmitter in mammalian brain, by binding to a specific site on the GABA receptor (which is associated with a trans-membrane chloride ion channel) and increasing the \( \text{Cl}^- \) flux through the ion channel. The barbiturates increase the time that the \( \text{Cl}^- \) channel stays open in response to GABA. The barbiturates have sedative side-effects and are now little used as they are addictive, potentiated by alcohol and have a low therapeutic index, making them very dangerous at high doses - 20 times the therapeutic dose is sufficient to kill the patient.

Monoamine Oxidase Inhibitors (MAOIs) such as phenelzine and tricyclic antidepressants such as imipramine are also useful in treating certain types of anxiety disorder, in particular panic attacks in agoraphobic patients. It has been reported that the anxiety can exist and be treated by these agents in the absence of any depressed mood in the patient, suggesting that anxiety disorder is entirely separate from endogenous depression. However, conflicting evidence has also shown that the most depressed patients are those that show the greatest reduction in agoraphobic symptoms following treatment with imipramine. These compounds can take up to 8 weeks to achieve therapeutic effects, though the patient can experience unpleasant side effects from the first days of treatment, and this problem can lead to patients refusing to continue with the treatment before any beneficial effects occur.

I-2 The introduction of the benzodiazepines

I-2.1 Historical

In 1957, the benzodiazepine (BZ) derivative Ro5-0690 (chlordiazepoxide) was found from animal studies to have hypnotic, sedative and anticonvulsant effects. Tested on human psychoneurotic patients, it was found to effectively reduce tension and anxiety, and this led to the initial introduction into clinical practice of chlordiazepoxide (Librium) in 1960 and diazepam (Valium) in 1963. Since then, many BZ derivatives have been synthesised and the BZs have become one of the most widely prescribed class of drugs in
the world, with over 70 million prescriptions written for the BZs in the USA alone in 1982. They are used as anxiolytics, and also as pre-medication for anaesthesia, in the treatment of muscle spasms, as anticonvulsants, as hypnotics and in the treatment of alcohol withdrawal. For many years the BZs have been the agents of choice in the treatment of anxiety as they produce anxiolytic effects with relatively mild side effects. They are favoured over the barbiturates as their abuse potential is much lower and their high therapeutic index means that it is almost impossible to commit suicide with BZs taken alone.

I-2.2 Chemical structure

The first clinically available BZ, chlordiazepoxide, was followed by more potent compounds such as diazepam and oxazepam. Figure I-2 shows the structures of a number of commercially available BZ derivatives. The BZs 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-position (e.g. chlordiazepoxide) or the isomeric 1,5-position (e.g. clobazam). A phenyl substituent (generally either unsubstituted or containing a fluoro or chloro group in the ortho-position) is commonly present at the 5-position of the molecule. The compounds usually contain an electronegative substituent at the 7-position, such as a chloro or nitro group. An additional feature is the presence of a 2-position group containing a proton acceptor, usually either an oxygen or a nitrogen atom. Incorporation of annulated ring systems at the 1,2-position has led to the synthesis of triazolobenzodiazepines (e.g. triazolam) or imidazobenzodiazepines (e.g. flumazenil). An enormous number of substituted 1,4- and 1,5-BZs have been synthesised and evaluated as potential ligands at the BZ receptor.
For several years after their introduction, the BZs were prescribed as clinically useful agents for the treatment of a number of central nervous system disorders without their physiological mechanism of action being understood. In 1977 evidence was published that suggested that the BZs bound specifically to a single binding site, located
on rat brain and peripheral tissue. The radiolabelled 1,4-BZ [3H]diazepam demonstrated specific, high affinity, saturable binding to a binding site in rat brain membranes and also bound (with slightly lower affinity) to receptors present in the kidney, liver and lung. Further studies identified a number of subtypes of this BZ binding site in different areas of the brain, distinguished by different affinities for a series of BZ receptor ligands. At certain locations in the brain, areas of receptors were present with a high affinity for the triazolopyridazino CL218,872 whereas in other areas of the brain the receptors demonstrated reduced affinity for CL218,872, and the two receptor subtypes were designated BZI and BZII respectively. A good correlation was observed between the affinities of various BZs for the [3H]diazepam binding site in the brain and behavioural effects in vivo, suggesting that the known anxiolytic, anticonvulsant and sedative effects of the BZs were a direct result of their binding to this site.

The pharmacological actions of the BZs were also thought to be related to the actions of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). The BZ and GABA receptors were considered to be two separate entities, though functionally coupled in some way. It was noted that GABA was able to increase the affinity of the BZs for their binding sites, consistent with the hypothesis that the BZ binding sites were a part of a supramolecular complex that included the GABA receptor and an associated Cl⁻ channel.

It is now generally accepted that the pharmacological actions of the BZs result from their mediation of GABA regulated Cl⁻ flux through neuronal membranes. GABA causes the Cl⁻ channel at neuronal cell membranes to open, leaving the cell hyperpolarised and less susceptible to other excitatory potentials. The GABA receptors can be subdivided into GABA_A and GABA_B receptor types and their structure and function have been reviewed recently by Kuriyama et al. The GABA_A receptor is coupled to a Cl⁻ channel to form a macromolecular complex that also contains the BZ binding site, whereas GABA_B receptors are pharmacologically distinct (resistant to blockade by the GABA_A antagonist bicuculline) and are not associated with Cl⁻ channels, nor do they contain BZ binding sites. This thesis is only concerned with
compounds that have affinity for the BZ binding site on the GABA_A/Cl^- channel complex, therefore the properties of the GABA_B receptor will not be discussed further.

The GABA_A/Cl^- channel complex is a ligand-gated ion channel with several major binding domains associated with it. These include sites for GABA, BZs, barbiturates, picrotoxin and the anaesthetic steroids. The BZs bind to the BZ receptor associated with the GABA_A receptor complex (BZR), inducing a change in conformation of the complex so that the frequency of the Cl^- channel opening in response to GABA is increased. This is in contrast to the barbiturates which increase the time that the Cl^- channel stays open in response to GABA.

The BZs exhibit a continuum of pharmacological properties at the BZR, ranging from agonists to antagonists and inverse agonists, as described by Haefely and Fryer. In general, an agonist can be defined as a compound that is able to alter the function of a receptor as a result of binding to it, whereas an antagonist can be defined as a compound that binds to a receptor but does not alter its function in any way.

Full agonists at the BZR are defined as ligands that bind to the BZR and allosterically enhance the binding of GABA to its receptor complex. This results in a full pharmacological response over the complete agonist profile of activity, which is described as full intrinsic efficacy.

Full antagonists are defined as ligands that bind with high affinity to the BZR but do not have any ability to modulate the binding of GABA to its receptor complex. They are able to block the pharmacological effects of both agonist and inverse agonist ligands, but do not possess any pharmacological effects when administered on their own, and are therefore classed as having zero intrinsic efficacy.

Full inverse agonists are compounds that, after binding to the BZR, cause a reduction in the binding of GABA to its receptor complex. This results in a pharmacological response over the full inverse agonist profile of activity that is the opposite to that observed for full agonists, described as negative intrinsic efficacy.

Most ligands that bind to the BZR, however, exhibit less than either full positive or full negative intrinsic efficacy, and are classed as partial agonists or inverse agonists. Their relative intrinsic efficacies are likely to influence their pharmacological effects.
Agonists at the BZR have anxiolytic, anticonvulsant, sedative and muscle relaxant properties and conversely BZR inverse agonists have anxiogenic (anxiety inducing) and proconvulsant activities in vivo. It has been suggested that as BZRs in different brain areas may have slightly different properties, a greater percentage receptor occupancy may be required to obtain a full pharmacological response for sedative and muscle-relaxant properties compared with anxiolytic and anticonvulsant properties. The possibility would therefore exist for a partial agonist, for example, to demonstrate anxiolytic effects without sedation.

I-2.4 Structure of the GABA$_A$/Cl$^-$ channel complex

Advances in molecular biology in recent years have led to a greater understanding of the structure and composition of the GABA$_A$ receptor and the associated BZR binding domain. The GABA$_A$ receptor is now known to be a member of a gene superfamily of ligand-gated ion channels, with a molecular weight of approximately 275 kDa, and consisting of a combination of a number of $\alpha$, $\beta$, $\gamma$, $\delta$ and $\rho$ polypeptide subunits. To date a number of isoforms of these subunits ($\alpha_{1-6}$, $\beta_{1-3}$, $\gamma_{1-3}$, $\delta$ and $\rho_{1-2}$) have been cloned from mammalian brain. The heterogeneity of the GABA$_A$/Cl$^-$ channel complex and the associated BZR has recently been reviewed by Lüddens et al.

Recombinant receptors have been generated by co-expression of $\alpha$- and $\beta$-subunits and these receptors have shown some of the pharmacological properties of the native GABA$_A$ receptors, with the formation of a GABA-gated Cl$^-$ ion channel which is subject to potentiation by barbiturates and inhibition by bicuculline. However, these recombinant receptors did not show BZ potentiation, and the co-expression of an additional $\gamma$-subunit was found to be required to produce GABA$_A$ receptors that possessed high affinity binding sites and electrophysiological responses to BZs.

The previously known multiplicity of BZ binding sites present in the brain, characterised by different affinities for different BZ receptor ligands, and termed BZI and BZII receptors, is related to this heterogeneity of the GABA$_A$ receptor complex. The most common form of the GABA$_A$ receptor complex in the mammalian brain is formed from the subunit composition $\alpha_1\beta_x\gamma_2$ and this is thought to correspond to the receptor
complex demonstrating BZI pharmacology which is particularly abundant in the mammalian cortex.29-31

The BZ pharmacology of the recombinant receptors has been shown to be dependent on the isoform of the α-subunit expressed. Receptors demonstrating BZI-type pharmacology have been generated from the subunit composition α₁β₁γ₂ whereas receptors containing the subunit composition α₅β₂γ₂, α₂β₃γ₂ and α₃β₃γ₂ exhibit some aspects of BZII-type pharmacology.32 Receptors formed from the subunit composition α₁β₁γ₂ and α₅β₁γ₂ showed a potentiation of GABA responses by the benzodiazepine agonist flunitrazepam, whereas the β-carboline inverse agonist, methyl-4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate, decreased GABA-activated currents in receptors containing the α₁β₁γ₂ subunits and increased them in receptors composed of the α₅β₁γ₂ subunits.33 A different class of receptors found in rat cerebellum have been found to contain major populations of the subunit composition α₆γ₂ and α₆δ.34 The α₁ subunit can therefore be said to confer benzodiazepine pharmacology previously defined as BZI, the α₂ and α₃ subunits lead to BZII characteristics, the α₅ show some aspects of BZII pharmacology and the α₆ subunit results in the formation of a receptor showing neither BZI nor BZII characteristics.

The β-subunits have not been thought to be of major importance in determining the benzodiazepine pharmacology of the recombinant receptors, and to be principally required to form the functional characteristics of the Cl⁻ channel. However, the β-variant has been found to confer subtle differences in the properties of the receptors, with a β₃ subunit being required for [³H]Ro 15-4513 binding to α₅β₃γ₂ and α₅β₃γ₃ receptors.35 Similarly, the γ-subunit is thought to be required to form the binding pocket on the BZ binding domain, with the γ₂ variant forming the major population in vivo and the role of γ₁ and γ₃ variants being less clear.35

Exchange of specific amino acids in the amino acid sequence of the receptor subunits can result in an alteration in their BZ pharmacology. A substitution of a single glycine for glutamic acid in the α₃ subunit amino acid sequence resulted in a 10 fold increase in affinity for several BZ receptor ligands in GABA_A receptors containing these modified α₃β₂γ₂ subunits.36 A single histidine residue in a certain position of the amino
acid sequences of $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunits (replaced by an arginine in the $\alpha_6$ subunits) has been found to be necessary for the binding of BZ agonists and antagonists. Replacement of an arginine by glutamine in $\alpha_6$ subunits led to different sensitivities to diazepam in mutant $\alpha_6\beta_2\gamma_2$ receptors compared to the non-mutated form. Four amino acid alterations in the sequence of the $\alpha_6$ subunit conferred increased affinity for diazepam in receptors expressed using the altered $\alpha_6\beta_2\gamma_2$ subunits. The presence of an aspartate molecule at a specific position in the amino acid sequence of $\beta_2$ and $\beta_3$ subunits has been shown to explain the 300-fold increase in sensitivity of $\alpha_1\beta_2\gamma_2$ and $\alpha_1\beta_3\gamma_2$ receptors over $\alpha_1\beta_1\gamma_2$ receptors to the anticonvulsant lorazepam. Particular amino acids may interact directly with BZR ligands and their structural properties define regions of the BZ binding domain on the receptor.

1.2.5 The benzodiazepine pharmacophore

An examination of the structural and electronic features of, initially, the 1,4-BZs and later, other non-BZ ligands at the BZR, has led to the definition of a pharmacophore for the BZRs. A pharmacophore is defined as the three-dimensional arrangement of certain atoms or functional groups of a molecule (rather than the entire molecular structure) that interacts with a receptor site and hence stabilises the binding of the molecule to the receptor and effects a biological response. The definition of the BZR pharmacophore enables the features present on the ligands that are actively involved in binding to the BZR to be identified, and this in turn allows the design and synthesis of new compounds containing these features to be evaluated as potential BZR ligands.

When the X-ray crystal structure of diazepam was first reported and compared to the X-ray crystal structure of diphenylhydantoin (a non-BZ anticonvulsant) the authors suggested that the carbonyl oxygen and N-4 atoms of diazepam may be of some relevance in causing the biological effects of the molecule. Following the later discovery of the BZR associated with the GABA$_A$/Cl$^-$ channel complex, structure-activity relationship studies of the BZs attempted to correlate the structural features of the molecules with their BZR affinities and pharmacological profiles, with particular
emphasis on the determination of the features that may confer agonist, antagonist or inverse agonist properties.

These studies identified a number of potential hydrogen bonding sites and electron rich aromatic areas on the ligands as involved in binding at the active site of the BZR. Fryer stated that the carbonyl oxygen and fused aromatic A-ring of diazepam were thought to be essential features for binding to the BZR, and that the efficacy of a series of 1,4-BZs was related to the distance between a hydrogen bond accepting group (the carbonyl oxygen in the case of diazepam) and the centre of the A-ring. This model was revised at a later date, to include the requirement for at least two hydrogen bond accepting groups for antagonist or inverse agonist ligands.

Other studies have attempted to correlate the affinities and efficacies of both BZ and non-BZ ligands at the BZR and also to account for possible differences in the requirements for binding to the BZI / BZII receptor subtypes, leading to the description of models including up to nine points of pharmacophoric interaction. The number of pharmacophoric points that are essential for binding to the BZR and the number of other points that are simply useful in providing further interaction and stabilisation with the receptor binding site has been debated. Some reports suggested that the BZR could be envisaged as consisting of different zones and that the efficacy of the BZR ligands resulted from the particular zones of the receptor with which the ligands interacted.

Cook et al. have attempted to specifically define the agonist and antagonist pharmacophore at the BZR. The agonist pharmacophore was reported to require two hydrogen bond donating sites on the receptor protein located at a distance of 6.5 Å from each other, with three areas of lipophilic ligand-receptor interaction (with occupation by the ligand of at least two of these areas as being essential for agonist activity) and with an area of steric hindrance, which if occupied by the ligand would lead to a reduction in affinity for the BZR. This pharmacophore model was used to synthesise a novel β-carboline possessing high affinity at the BZR and an anticonvulsant / anxiolytic profile in vivo. Further studies with benzo-fused benzodiazepines enabled the dimensions of the area of negative steric interaction to be defined and also the identification of a second
area of negative steric interaction between the areas of lipophilic interaction on the receptor protein.\textsuperscript{52}

It is proposed that agonist and inverse agonist ligands bind to the same general region of the BZR, but that the specific pharmacophores are different. The agonist pharmacophore contains two hydrogen bond donating sites (which interact with appropriately positioned hydrogen bond accepting groups on the ligand) and two or three areas of lipophilic interaction, whereas the inverse agonist pharmacophore contains one hydrogen bond donating site, one hydrogen bond accepting site and one area of lipophilic interaction.\textsuperscript{53} The areas of negative steric interaction are common to both pharmacophores supporting the hypothesis that both classes of ligands bind to slightly different areas of the same BZR. The antagonist pharmacophore is described as being similar to the inverse agonist pharmacophore, with the electronic effects of substituent groups on the ligands influencing their efficacy.\textsuperscript{54}

The known heterogeneity of the BZR subtypes (eg BZI / BZII) leads to the possibility that the BZ ligand binding domains on the different subtypes may not have identical structural and electronic requirements. Approximately 25\% of the BZR in the rodent cerebellum is insensitive to the classical 1,4-BZs such as diazepam and flunitrazepam, but some imidazobenzodiazepines bind with high affinity, and these ligands for the diazepam-insensitive BZR may be involved in the mediation of the behavioural effects of alcohol.\textsuperscript{55} It has been found that the steric and electronic properties of substituent groups at certain positions on the imidazobenzodiazepine molecules do indeed confer selectivity for the diazepam sensitive / insensitive BZR.\textsuperscript{55}

Further advances in molecular biology and computational techniques are likely to lead to a greater understanding of the various BZR subtypes present in mammalian brain using the computer generated simulation of their properties, and those of BZR ligands. It may be that description of pharmacophores at the different BZR subtypes will eventually be possible and that this will assist in the design and synthesis of selective ligands at these BZR subtypes with specific biological effects \textit{in vivo}. As discussed in Chapter 1-2.4, the determination of amino acid sequences for various subunits of the GABA\textsubscript{A} receptor has revealed that particular amino acids at certain points in the sequence have a major
influence on the affinity of the GABA_A/Cl^- channel complex and associated BZR for BZR ligands. Further information pertaining to the three-dimensional structure of the protein itself, as deduced from its amino acid sequence, and of the structural and electronic properties of the receptor in the region around specific amino acids found to be of importance in conferring BZR ligand affinity to the receptor, may allow the features of the biologically active ligand binding site of the BZR to be elucidated in more detail.

1-2.6 Side effects of benzodiazepine treatment

The BZs have been widely prescribed in the treatment of anxiety disorders due to their clinical efficacy and moderate side effects. There has been an increasing awareness in recent years, however, that the BZs have been over-prescribed and that some patients do experience quite serious side effects, particularly resulting from long term BZ treatment.56,57

The major side effects that have been observed include sedation, psychological and physical dependence, withdrawal symptoms (including seizures, tremors and depression), possible potentiation of the effects of alcohol, and adverse reactions to BZs in organs such as the liver, circulatory system and the gastrointestinal system. Patients with additional psychotic symptoms can experience paradoxical effects following BZ treatment including increased aggression and hostility and it is thought that the BZs weaken or remove the stimuli that normally inhibit this behaviour. There is also a possibility that the BZs increase the risk of congenital abnormalities if taken during pregnancy. An effect similar to fetal alcohol syndrome and linked to regular maternal use of BZs during pregnancy has been reported,58 though this has been disputed and criticised for failing to specifically identify BZs over other possible agents in causing this effect.59

1-2.7 The development of new agents for the treatment of CNS disorders

Since the identification of a specific BZ binding site associated with the GABA_A/Cl^- channel complex, a number of classes of non-BZ compounds have been
found to bind with high affinity to the BZR. This has led to the development of new agents useful in the treatment of anxiety and sleep disorders, with a reduction in the side-effects observed with BZ treatment. The major classes of these compounds are discussed below.

### i. The β-carbolines

In 1980 Nielsen and Braestrup reported that ethyl β-carboline-3-carboxylate (β-CCE) bound to the BZR with a higher affinity than diazepam, and that β-CCE bound preferentially to cerebellar rather than hippocampal BZR sites. The pharmacological effects of β-CCE were to reverse or to inhibit the normal effects of BZ compounds and another analogue of β-CCE, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), demonstrated proconvulsant and anxiogenic behavioural effects. DMCM has also been shown (at saturating doses) to interact with a second site on the GABA_A/Cl^- channel complex separate to those for the BZs, barbiturates and neurosteroids. A large number of β-carboline compounds (Figure I-3) have now been synthesised and pharmacologically evaluated, with a broad spectrum of effects from agonist (ZK 93423) to antagonist (ZK 93426) and inverse agonist (β-CCE and DMCM) at the BZR. The compound isopropyl 6-benzylxoy-4-methoxymethyl-β-carboline-3-carboxylate (abecarnil) has been developed as a novel agent demonstrating anxiolytic and anticonvulsant effects.

**Figure I-3 Chemical structures of some β-carbolines**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-CCE</td>
<td>CO_2Et</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>DMCM</td>
<td>CO_2Me</td>
<td>Et</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
</tr>
<tr>
<td>ZK93423</td>
<td>CO_2Et</td>
<td>CH_2OMe</td>
<td>H</td>
<td>OCH_2Ph</td>
<td>H</td>
</tr>
<tr>
<td>ZK93426</td>
<td>CO_2Et</td>
<td>Me</td>
<td>OPr^t</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Abecarnil</td>
<td>CO_2Pr^t</td>
<td>CH_2OMe</td>
<td>H</td>
<td>OCH_2Ph</td>
<td>H</td>
</tr>
</tbody>
</table>
Abecarnil has promise as a clinically useful drug as it has shown effective anxiolytic properties in animal models of anxiety, with an absence of the withdrawal symptoms and development of tolerance seen after BZ treatment.\textsuperscript{65-68} Abecarnil has been classified as a partial agonist at the BZR and it has been suggested that this accounts for its selective anxiolytic profile with reduced potential for dependence and tolerance, however it has also been shown that some aspects of the \textit{in vitro} binding of abecarnil to the BZR are more consistent with full agonist properties.\textsuperscript{69} A possible explanation for these conflicting observations is that abecarnil binds with high affinity to specific BZ receptor subtypes and could thus be more accurately classified as a selective agonist. It has been reported that abecarnil may show a selective affinity for BZ receptors containing \( \alpha_1 \) subunits, though a link between this and reduced dependence potential has not been proven.\textsuperscript{70}

Recent reports of clinical trials of abecarnil with humans have demonstrated that the drug does have a useful profile of anxiolytic activity at moderate doses, with sedation and unpleasant "next day" effects occurring at higher doses.\textsuperscript{71} Due to these unpleasant effects if the drug is taken in excess, it has been suggested that abecarnil has a lower potential for abuse, and would be particularly useful as an anxiolytic for patients with a history of BZ-abuse.

\textbf{ii. The cyclopyrrolones} Cyclopyrrolones (Figure I-4) have been found to displace the binding of 1,4-BZs from the BZR, with zopiclone and suriclone being the most extensively evaluated. Both compounds demonstrate a pharmacological profile that is similar to that of the BZs and do not demonstrate selectivity between BZI and BZII receptor subtypes.\textsuperscript{72,73} Zopiclone and suriclone are not thought to interact directly with the BZR, but rather to bind to another site on the GABA\(_A\)/Cl\(^-\) channel that is allosterically linked to the BZ binding site.\textsuperscript{73-75}

Zopiclone has become accepted as an alternative to BZ hypnotics whereas suriclone has been shown in clinical studies to possess anxiolytic effects.\textsuperscript{76,77} Neither compound has been shown to induce dependence in animal studies, a potential advantage over BZ treatment.\textsuperscript{78} Other cyclopyrrolone derivatives RP 59037 and RP 60503 have been found to demonstrate partial agonist effects at their recognition site on the
GABA<sub>A</sub>/Cl<sup>-</sup> channel complex, with anxiolytic behavioural effects without causing significant sedation.79,80

**Figure I-4 Chemical structures of some cyclopyrrolones**

iii. **The imidazopyridines** The imidazopyridines zolpidem and alpidem (Figure I-5) belong to another class of BZR ligands. Zolpidem displaces the binding of [³H]diazepam more potently from cerebellar rather than hippocampal membranes, indicating that it may bind preferentially to BZI receptor subtypes, as the ratio of BZI / BZII receptors is greater in the cerebellum than the hippocampus.81,82 Alpidem also binds selectively to the BZI receptor and in addition possesses a high affinity for the peripheral BZ binding site.83
Zolpidem has highly selective sedating properties and is generally used as a hypnotic, whereas alpidem has been shown in clinical trials in humans to demonstrate anxiolytic effects in anxious patients comparable to those of the BZs, but with fewer withdrawal symptoms and a lesser tendency to induce dependence.\textsuperscript{84-87} It has been suggested that the hypnoselectivity of zolpidem is related to its selective binding to the BZI receptor.\textsuperscript{88,89} Studies of the distribution of zolpidem and triazolam (a typical BZ hypnotic) in the rodent brain, however, have provided evidence consistent with the possibility that the lower affinity binding of zolpidem to BZII receptor subtypes is also an important factor in inducing hypnotic effects.\textsuperscript{90}

The different behavioural profiles of alpidem and zolpidem, both selective BZI ligands, are not consistent with the hypothesis that it is solely BZI selectivity that confers hypnotic effects \textit{in vivo}. It may be that the affinity of alpidem for the peripheral BZ binding site leads to some of its observed anxiolytic effects, or that zolpidem and alpidem bind specifically to separate BZ receptor subtypes not differentiated in the BZI / BZII classification.

\textbf{iv. 5-HT receptor ligands} In 1979, a clinical trial with 9-4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl-8-azaspiro[4,5]decane-7,9-dione hydrochloride (buspirone) revealed that it showed promise as an anxiolytic and antidepressant agent.\textsuperscript{91} Buspirone (Figure I-6) is not structurally related to the BZ class of compounds and does not bind to the BZR. Although buspirone shows anxiolytic effects in humans, it is not characterised as an
anxiolytic in some animal models of anxiety, presumably because these models have been
principally developed for the characterisation of BZR ligands.92

Figure I-6 Some 5-HT ligands

![Chemical structures of buspirone, ipsapirone, odansetron, and granisetron](image)

It is thought that the anxiolytic effects of buspirone are due to its effect on 5-hydroxytryptamine (5-HT) neurones and in particular the selective interaction of buspirone with 5-HT1A receptors.93,94 At low doses buspirone has been found to demonstrate anxiolytic effects without the muscle relaxant, anticonvulsant and sedative effects of the BZs and also to alleviate some of the symptoms of BZ withdrawal, however at higher doses some anxiogenic effects have been observed in animal experiments, though this does not detract from its clinical efficacy as an anxiolytic at a more moderate dosage.95,96 Animal studies using chronic administration of buspirone have identified alterations in 5-HT and dopamine levels, and modifications in the properties of the BZR, as resulting from this treatment though the relative importance of these effects is unclear.94,97

Ipsapirone (Figure I-6) is another 5-HT1A agonist that has been found to have anxiolytic effects without directly altering GABA neurotransmission, with the effects blocked by the administration of 5-HT1A antagonists.98 Further evidence for the specific
involvement of 5-HT$_{1A}$ receptors in the behavioural effects of ipsapirone has come from the observation that the 5-HT$_{1C}$ / 5-HT$_{2}$ antagonist ritanserin is not effective in reversing or modifying these effects and it is thought that both presynaptic and postsynaptic 5-HT$_{1A}$ receptors are involved.99-101

Compounds with specific affinity for the 5-HT$_{3}$ receptor subtype have also been found to have potential anxiolytic activity. Behavioural studies using animal models of anxiety have demonstrated that 5-HT$_{3}$ antagonists such as GR38032F (odansetron), BRL43694 (granisetron) and ICS205-930 exhibit anxiolytic effects under certain experimental conditions.101-105 Conflicting evidence has also been published, however, cautioning that the classification of 5-HT$_{3}$ antagonists as anxiolytics may be premature.106 There is also contradictory evidence relating to the possible role of 5-HT$_{3}$ agents in the treatment of BZ withdrawal. Some studies have presented evidence stating that 5-HT$_{3}$ antagonists attenuate the symptoms of BZ withdrawal 107-109 whereas others have reported that they potentiate the BZ withdrawal symptoms.110,111 It appears likely that 5-HT$_{3}$ receptors do mediate certain behavioural effects though their mechanism of action is uncertain. Clinical studies may provide further information regarding the usefulness of these agents in humans.

v. CCK receptor ligands  Cholecystokinin (CCK) is a neuropeptide that is present in the intestine and the brain. The properties of CCK receptors and ligands have been reviewed by Harro et al.112, and Woodruff and Hughes.113 Two subtypes of CCK receptor have been identified, classified as CCK$_A$ (found mainly in pancreatic and intestinal tissue) and CCK$_B$ (found in the brain). Cloning experiments have indicated that there is a 48% identity in the amino acid sequences of CCK$_A$ and CCK$_B$ receptors and that this is in the expected range for receptors belonging to the same family.114 The chemical structures of some CCK receptor ligands are shown in Figure I-7.
There is considerable experimental evidence supporting a role for CCK<sub>B</sub> receptors in anxiety. The main facets of this evidence are (a) selective CCK<sub>B</sub> antagonists PD134308 (CI-988) and PD135158 have been shown to demonstrate potent anxiolytic effects in rodent models of anxiety without the sedative, anticonvulsant and alcohol potentiating effects or withdrawal symptoms of the BZs.115-117 (b) the CCK<sub>B</sub> agonist CCK-4 (a shortened tetrapeptide form of CCK) has been found to be a potent anxiogenic agent in humans, and clinical studies have shown that administration of CCK-4 can induce panic attacks in patients suffering from anxiety disorders.118,119 and (c) in vivo tests have found that CCK<sub>A</sub> antagonists are devoid of anxiolytic effects.120,121

Further evidence supporting an underlying role of CCK<sub>B</sub> receptors in behavioural states has recently been published. Certain CCK<sub>B</sub> antagonists including CI-988 have been observed to induce antidepressant effects in rodents, with the implication that the
use of these compounds clinically could be extended from the treatment of panic disorders to depressive illnesses.\textsuperscript{122,123} A number of peptide and non-peptide ligands at both CCK\textsubscript{A} and CCK\textsubscript{B} receptors have been synthesised and this has led to the development of structure-activity relationships and the definition of pharmacophores at both receptor sites. For CCK\textsubscript{B} antagonists, at least one site of interaction with a hydrophobic pocket on the receptor has been found to be essential for high affinity binding to the receptor.\textsuperscript{124-126}

In conclusion, the identification, pharmacological characterisation and further study of anxiolytic compounds that do not interact with the BZR should lead to a broader understanding of the neurochemical processes and mechanisms underlying anxiety, with the possibility that this may allow the design and synthesis of other novel therapeutic agents. Furthermore, it is possible that advances in the understanding of the structure and amino acid composition of the BZR, and thus of ligand-receptor interaction at the BZR, may allow the development of novel compounds with selective hypnotic, sedative or anxiolytic actions. Both these areas of research have the potential to identify new anxiolytic agents that overcome the principal drawback of many of the BZs, that their therapeutically useful properties are negated to a certain extent by unwanted side effects.

I-3. The peripheral-type benzodiazepine receptor (PBR)

I-3.1 Structure and location

The BZ binding sites identified on peripheral tissues have been found to be pharmacologically distinct from the BZRs associated with the GABA\textsubscript{A}/Cl\textsuperscript{-} channel complex. Mitochondrial fractions from tissues such as rat kidney, liver and lung demonstrate [\textsuperscript{3}H]diazepam binding ability, and Ro5-4864 (4'-chlorodiazepam) is extremely active in displacing the binding of [\textsuperscript{3}H]diazepam from these fractions, whereas it is almost inactive in displacing it from rat brain membranes.\textsuperscript{12} Although these so-called peripheral-type BZ binding sites have been found in a number of specific locations in peripheral tissues including the lung, the loop of Henle and distal convoluted tubule of
the kidney, the cortex of the adrenal gland, the testosterone producing Leydig cells of the testes, the uterus, and the heart, they are also located centrally in the brain, particularly in the olfactory bulb and pineal gland.

The peripheral-type BZ receptors (PBRs) are characterised by high affinity for the 1,4-BZ ligand Ro5-4864 and the isoquinoline carboxamide PK11195 (Figure I-8). Unlike the central BZ binding sites, however, the PBR binding sites in the brain do not appear to be allosterically coupled to other binding sites for GABA, barbiturates and picrotoxin, or chloride anions. Considerable evidence has accumulated to support an association between the PBR and the mitochondrial outer membrane.

Autoradiographic studies have indicated that there is a correlation between the density of [3H]PK11195 binding sites and the number of mitochondria in different organs, and a co-localisation of these sites and the mitochondrial enzymes cytochrome oxidase and monoamine oxidase.

Figure I-8 Selective PBR ligands

The structure, function and location of the PBR has been reviewed by Verma and Snyder, Gavish et al. and Parola et al. An initial molecular weight determination of the PBR led to an estimate of the apparent molecular weight of the [3H]diazepam binding site in rat kidney of 34000 daltons (34 kDa). Further studies using PBR ligands PK11195 and PK14105 (a photoaffinity probe that is a p-fluoronitrophenyl
analogue of PK11195 and that covalently labels PBR sites following UV radiation) showed that the PK14105 binding sites were associated with a 17 kDa polypeptide.\textsuperscript{142}

More recently, the PBR has been identified as a complex that consists of three protein subunits of 18, 30 and 32 kDa, with the PK11195 binding site associated with the 18 kDa subunit and the Ro5-4864 binding site associated with the 30 and 32 kDa subunits.\textsuperscript{143} The 32 kDa subunit has been identified as a voltage dependent anion channel and the 30 kDa subunit as an adenine nucleotide carrier.\textsuperscript{143} Other workers have presented evidence suggesting that Ro5-4864 and PK11195 bind to overlapping (but not identical) sites on the 18 kDa subunit.\textsuperscript{144} Although Ro5-4864 and PK11195 appear to interact with different areas of the PBR, there is an allosteric interaction between them as the binding of both classes of molecules is competitive at low (nanomolar) concentrations.

Binding studies using PBR preparations from a number of species (human, rodent, bovine and piscine) have demonstrated that high affinity binding of PK11195 to the PBR is observed across species, whereas that of 1,4-BZs such as Ro5-4864 is species-dependent.\textsuperscript{145-148} This suggests that the 18 kDa subunit of the PBR confers the minimum requirements for PBR ligand binding, with different amino acid sequences of the other subunits accounting for the species differences observed.\textsuperscript{149} Cloning studies have provided further evidence that the functional PBR consists of a complex of a number of proteins with the PK11195 binding site present on the 18 kDa subunit and the BZ site requiring co-expression of the 18 kDa subunit and a 34 kDa voltage dependent anion channel subunit.\textsuperscript{150}

\subsection*{1.3.2 PBR ligands}

As mentioned in Chapter I-3.1, the ligands initially found to specifically bind to the PBR were Ro5-4864, the 4' -chlorophenyl analogue of diazepam, and PK11195, an isoquinoline compound. Thermodynamic analysis of the binding properties of these ligands to the PBR using a technique developed to characterise ligands at the \(\beta\)-adrenergic receptors (where binding of agonists was enthalpy driven and binding of antagonists entropy driven) has indicated that Ro5-4864 may have agonist or partial
agonist properties and that PK11195 may act as an antagonist at the PBR. Modulation of the PBR by arachidonate and diethylpyrocarbonate has differing effects on the affinity of the PBR for Ro5-4864 and diazepam compared to PK11195, which is consistent with the possibility that they may interact with different conformations or sites of the receptor and hence possess different efficacies. Diazepam binds to both the BZR and the PBR, though its affinity for the BZR is approximately 15 times greater than that for the PBR.

Figure 1-9 Various PBR ligands
Naturally occurring substances have been found to displace the binding of $[^3H]Ro5-4864$ and PK11195. Porphyrins such as hemin and protoporphyrin IX have demonstrated nanomolar affinities for the PBR and are thought to act as endogenous ligands at the PBR binding site.\textsuperscript{153} These porphyrins selectively bind to the PBR (with \textit{ca} 1000-fold stronger binding to the PBR than the central BZR), and their PBR affinity is related to the number of carboxylic acid groups present, with dicarboxylic acid porphyrins binding with nanomolar affinity to the PBR and tetracarboxylic acid porphyrins demonstrating considerably weaker micromolar affinity.\textsuperscript{154}

Other heterocyclic compounds have been synthesised and evaluated as ligands for the PBR. A number of pyrazoloisoquinoline, pyrroloquinolinone and azeepinoquinolinone derivatives have been shown to have affinity for the PBR.\textsuperscript{155-158} The isoquinoline carboxamide derivatives PK14105 and AHN683 have been developed as fluorescent ligands to label the PBR sites in tissue preparations.\textsuperscript{159} Diethylidithiocarbamate (DDC) and disulfuram (a dimer of DDC) have been found to competitively inhibit $[^3H]PK11195$ and $[^3H]Ro5-4864$ binding in the kidney with micromolar affinity.\textsuperscript{160} The syntheses of series of both 2-arylindole-3-acetamides and arylpyrrolobenzothiazepines have identified several compounds that bind to the PBR with nanomolar affinities, and have enabled details of the requirements for high affinity binding to the PBR to be ascertained.\textsuperscript{161,162}

\subsection*{I-3.3 Function of the PBR}

Initial studies of BZ binding \textit{in vitro} and \textit{in vivo} suggested possible PBR involvement in numerous biological processes. Induced hypertension in rats resulted in an increase of $[^3H]$flunitrazepam binding in the kidney, whereas the central BZR was unaffected.\textsuperscript{163} Flunitrazepam, Ro5-4864 and diazepam have been found to increase the rate of melanogenesis in melanoma cells, while differentiation of Friend erythroleukemia cells is also induced by PBR ligands.\textsuperscript{164,165} It is uncertain, however, whether PBR affinity is directly responsible for these effects or whether the structural features of the ligands that confer PBR specificity are also favourable features for additional kinds of biological interaction via different mechanisms. Evidence suggesting cardiovascular, hormonal, behavioural and neuroprotective effects of the PBRs and their possible roles in
steroidogenesis (resulting from their mitochondrial location) and their accumulation in tumour cells are discussed below.

i. Cardiovascular effects  Following the discovery of high densities of PBR binding sites in the left and right ventricles of the heart, PBR ligands have been shown to exert some cardivascular effects. Ro5-4864 has demonstrated an ability to decrease the duration of action potential and contractility of papillary muscle from guinea pig heart preparations and PK11195 protects dogs from induced coronary arrhythmias. An examination of a variety of PBR and BZR ligands with other compounds, however, has not demonstrated an unequivocal association between PBR affinity and coronary effects, suggesting that other effects not mediated by the PBR may influence heart activity.

ii. Hormonal effects  A variety of alterations in PBR density have been observed as a result of stress though it is difficult to identify consistent trends. Rodent studies simulating stress through surgical procedures resulted in an PBR up-regulation in the brain and kidney 1-3 days after surgery, whereas stress induced by food deprivation resulted in a decrease in PBR density in the adrenal gland, kidney and heart. Treatment of rodents with antidepressants resulted in down-regulation of adrenal PBR sites and up-regulation of hepatic PBR sites. Alterations of PBR densities following consumption of ethanol have been demonstrated as chronic ethanol administration to rats resulted in down-regulation of PBR in the olfactory bulb of the brain, and up-regulation in the liver, heart and testes.

iii. Behavioural effects  A number of behavioural effects have been observed following administration of PBR ligands, though the mechanism of the PBR alteration of behavioural states remains uncertain. The compound Ro5-4864 demonstrates potent convulsant activity, which has been shown to be unaffected by PK11195. Rodent studies using conflict paradigms have assigned proconflict properties to Ro5-4864. It is possible to block this effect with PK11195, consistent with a PBR involvement with the behaviour studied.

The inconsistency of the effects of PBR ligands on rodent convulsant and conflict behaviour has led to the hypothesis that Ro5-4864 may bind to a novel binding site that is coupled to both the GABA<sub>A</sub>/Cl<sup>-</sup> channel complex and a [<sup>35</sup>S] t-butylbicyclo-
phosphorothionate (TBPS) binding site.\textsuperscript{176} TBPS is a bicyclic cage compound with convulsant effects, that is thought to bind directly to the Cl\textsuperscript{−} channel of the GABA\textsubscript{A}/Cl\textsuperscript{−} channel complex and sterically hinder the entry of Cl\textsuperscript{−} ions into the ion channel itself. It is therefore likely that any alteration in TBPS affinity for its binding site would have direct behavioural effects \textit{in vivo}.

Studies involving a comparison of the effects of Ro5-4864 and FG7142 (an anxiogenic β-carboline with selective BZR affinity) showed that both compounds demonstrated different behavioural effects on rats and that PK11195 could antagonise the effects of Ro5-4864.\textsuperscript{177} This implies that the behavioural effects of FG7142 are a result of its specific affinity for the BZR and that the effects of Ro5-4864 result from interaction with the PBR only. Chronic treatment of rats with Ro5-4864 and PK11195 showed that both ligands produced only minor behavioural effects, with similar effects following withdrawal for both ligands.\textsuperscript{178} This is not consistent with the general classification of Ro5-4864 as an agonist and PK11195 as an antagonist at the PBR, and Rägo \textit{et al.}\textsuperscript{178} have hypothesised that the effects of Ro5-4864 involve modulation of the GABA\textsubscript{A}/Cl\textsuperscript{−} channel via a binding site that is coupled to the binding site for the cage convulsant [\textsuperscript{35}S]TBPS whereas PK11195 has partial agonist properties at the PBR.

Electrophysiological studies on rat cerebellar neurons have also indicated that Ro5-4864 may induce different responses resulting from binding to both the PBR and another separate site, with a Ro5-4864 induced depression of neuron activity being assigned to PBR affinity (due to suppression of this effect by PK11195) and an excitation observed in about half the neurons examined being assigned to modulation of ion flux through the GABA\textsubscript{A}/Cl\textsuperscript{−} channel complex.\textsuperscript{179} Further behavioural studies comparing the effects of the selective PBR ligand FGIN-1-27, anticonvulsant neurosteroid THDOC and anxiolytic imidazopyridine alpidem (a ligand with affinity for both the BZR and PBR) have shown that the anticonvulsant effects of FGIN-1-27 and alpidem were blocked by PK11195 whereas it had no effect on THDOC. Additionally, in animal tests of anxiety, all three compounds demonstrated anxiolytic effects, but the effects of alpidem were partially reversed by administration of the BZR antagonist flumazenil whereas the anxiolytic effects of FGIN-1-27 and THDOC were flumazenil insensitive.\textsuperscript{180} This
evidence suggests a PBR involvement with the in vivo effects observed, with the effects of alpidem resulting from a combination of its PBR and BZR affinity. A clinical pilot study has shown that PK11195 may have therapeutic uses as an anxiolytic in humans.181

iv. Neuroprotective effects An association has been reported between PBR density in the brain and areas of brain injury. Areas of the brain that have been damaged, whether by experimentally induced lesions or by states such as stroke or multiple sclerosis, have been found to contain increased PBR densities, possibly via a mechanism mediated by the cytokines IL-1 and TNF-α.182,183 The increased binding of the PBR ligands [3H]PK11195 and [3H]PK14105 to these areas have enabled these radioligands to be used as markers for brain damage.184 A series of PBR ligands, including PK11195 and PK14105, have also been patented as agents for the treatment of CNS damage. It has been reported that the PBR ligands both reduce the damage caused by the CNS injury and help healing after the CNS injury has occurred.185

v. Biochemical effects on cell respiration The association of a BZ binding site with cell mitochondria has led to attempts to characterise an interaction between the PBR and mitochondrial processes. The PBR ligand AHN086 has been shown to inhibit respiration stimulated by ADP in heart, kidney and liver mitochondria, with the heart mitochondria being the most sensitive in this respect.186

vi. Steroidogenesis effects The PBRs have been found to be connected with cellular steroidogenesis processes in adrenocortical and Leydig cell systems. The first step in the synthesis of steroids hormones is the conversion of cholesterol to pregnenolone, catalysed by the enzyme P450scc, though the rate limiting step in the steroidogenesis process is not this reaction but the initial transport of cholesterol to the inner mitochondrial membrane.187,188 It has been found that PK11195 stimulates the production of steroids by increasing this rate of cholesterol transport from the outer to the inner mitochondrial membranes of adrenocortical and Leydig cells.189-191

Further evidence for the association of the PBR with the steroid biosynthesis process has been demonstrated by the antagonism of flunitrazepam (a BZ with nanomolar affinity for the PBR) of hormone-stimulated steroidogenesis.192 This hypothesis has been tested by a computer generated three-dimensional model of the
PBR, built using knowledge of the amino acid sequence of human, murine and bovine PBR. The computer model of the PBR enabled five trans-membrane helical structures to be identified, and it was found that a molecule of cholesterol could be transported through the channel formed by these trans-membrane helices and that the length of the helices was less than the distance across the mitochondrial membrane, supporting evidence that the PBR is located on the outer mitochondrial membrane and is directly involved in the transport of cholesterol across mitochondrial membranes.

It may be that the ability of PBR ligands to increase the rate of steroid biosynthesis in mitochondria results in a modulation of the CNS GABA_A/Cl⁻ channel complex due to increased steroid binding to a site on this complex, which then contributes to the anticonvulsant and anxiolytic effects observed. It has also been found that the amnesic effects on rodents of dizocilpine (an N-methyl-D-aspartate [NMDA] receptor antagonist) were antagonised by FGIN-1-27, Ro5-4864 and pregnenolone sulphate and this effect has been correlated to the ability of these compounds to increase the accumulation of the neurosteroid allopregnanolone, thus providing further evidence that an alteration of neurosteroid concentrations can induce behavioural effects.

vii. Tumour cell association

Administration of [³H]PK11195 to rats with brain tumours has shown that there is an accumulation of [³H]PK11195 binding sites on the tumours, an effect that is blocked by pre-administration of non radio-labelled PK11195, but not the centrally selective BZ ligand clonazepam. It is now known that there are high concentrations of PBRs in tumours of the brain, gastrointestinal tract, ovary and prostate. This has led to the development and use of specific PBR ligands as probes to assist with the imaging and diagnosis of tumours in humans.

The density of PBR binding sites in several types of human brain tumours has been found to increase as the malignancy of the tumour increases, indicating that PBR ligands possess properties that make them useful and effective agents for the imaging of tumours. PK11195 has been selected as a useful ligand for PET (Positron Emission Tomography) labelling of brain tumours, fulfilling the requirements of high selectivity and affinity for the tumour sites, penetration of the blood-brain barrier and safety in use. The high PBR density in tumours associated with other areas of the body such as
the prostate and ovary may also lead to the development of PBR ligands as imaging agents for these tumour sites.

To review, the PBR appears to be involved in a number of different biological effects. Often the effects observed are a result of indirect rather than direct modulation by the PBR. Further research into the structure of the PBR itself, the development of other selective PBR ligands, and the mechanism of action of these ligands should allow more details of the physiological and biochemical role of the PBR to be elucidated.

I-4 Syntheses, chemical, physical and biological properties of some imidazo[1,2-b]-pyridazines

I-4.1 Structure and nomenclature

The imidazo[1,2-b]pyridazine ring system (Figure I-10) consists of fused imidazole and pyridazine rings with a common bridgehead nitrogen atom. It is a 10 \( \pi \) electron system; the singly bound nitrogen atom has \( \pi \)-excessive properties and the doubly bound ring nitrogens have \( \pi \)-deficient characteristics. The numbering of the ring positions, in accordance with current IUPAC rules, is also shown in Figure I-10.

Figure I-10 Numbering of the ring positions of imidazo[1,2-b]pyridazine

I-4.2 Syntheses

The first reported synthesis of imidazo[1,2-b]pyridazines was published by Yoneda et al.\textsuperscript{201} in 1964. Since then the chemistry of the imidazo[1,2-b]pyridazines has been extensively examined and many possible biological applications of the molecules have been explored. The syntheses, chemical and physical properties of this heterocyclic system have been reviewed by Tišler and Stanovnik,\textsuperscript{202} and also by Maury.\textsuperscript{203}
The most convenient route for the synthesis of the imidazo[1,2-b]pyridazines is the condensation of a 3-aminopyridazine with an α-halogenocarbonyl compound. Substituent groups present in the 6-position of the 3-aminopyridazine, and in the α-halogenocarbonyl compound, allow the synthesis of a variety of (6, 3 and 2)-substituted imidazo[1,2-b]pyridazines as outlined in Figure I-11. Initial publications described the use of 6-(chloro or alkyl)pyridazin-3-amine and bromoacetaldehyde or α-bromoacetophenone to afford the appropriate 6-substituted or 6,2-disubstituted imidazo[1,2-b]pyridazines respectively. This was extended to the reactions of 6-(chloro, methyl and methoxy)pyridazin-3-amine with bromoacetone, chloroacetone, α-bromoacetophenone, ethyl 2-bromoacetoacetate, ethyl bromopyruvate or ethyl 2-chloroacetoacetate to give the corresponding 6,2-disubstituted or 6,3,2-trisubstituted imidazo[1,2-b]pyridazines.

Figure I-11

The mechanism of this ring closure reaction with pyridazin-3-amine and an α-halogenocarbonyl compound is thought to be a two step process, firstly involving the formation of a mixture of N-1 and N-2 quaternized compounds and secondly involving the cyclisation of the N-2 quaternized isomer only to form the imidazo[1,2-b]pyridazine (Figure I-12). The yield of the final product is controlled by the proportion of the N-2 quaternized intermediate that is formed in the mixture of N-1 and N-2 quaternized isomers.
Barlin and co-workers reported the condensation of 4-methylaminopyridazin-3-amine with pyruvaldehyde dimethyl acetal in methanolic hydrogen chloride, resulting in the formation of 3-methoxy-2-methyl-8-methylaminolimidazo[1,2-b]pyridazine (Figure I-13). The structure of the product was confirmed by X-ray analysis. Other condensations were also carried out using the α,β-dicarbonyl compound phenylglyoxal with pyridazin-3-amines which led to the formation of 2-phenylimidazo[1,2-b]pyridazin-3(5H)-ones (Figure I-14). These products were stable in the solid state and had low solubility in organic solvents. The imidazo[1,2-b]pyridazin-3(5H)-ones underwent O-methylation when treated with diazomethane to give the corresponding 3-methoxy compounds, and with refluxing acetic anhydride formed the 3-acetoxy product.

Figure I-13
Another synthetic route leading to the formation of the imidazo[1,2-b]pyridazines is the valence isomerisation of tetrazolo[1,5-b]pyridazines. The conversion of 6-dimethoxyethylaminotetrazolo[1,5-b]pyridazine to 6-azidoimidazo[1,2-b]pyridazine in the presence of polyphosphoric acid was observed, resulting from the induced elimination of the fused tetrazolo ring, and is shown below in Figure I-15.

**Figure I-15**

I-4.3 Chemical reactivities

The parent unsubstituted imidazo[1,2-b]pyridazine ($pK_a$ of 4.4 in water at 20°C) has been found to be a weaker base than imidazole. 3,6-Disubstituted imidazo[1,2-b]pyridazines have also been found to be less basic than analogous 3,6-disubstituted pyridazines. Electron density calculations for the related imidazo[1,2-a]pyridine system have predicted that position-3 of the molecule is likely to be the most favourable to electrophilic substitution, and this has also been verified experimentally for the imidazo[1,2-b]pyridazines. For example, bromination of imidazo[1,2-b]pyridazine and 6-chloro-2-methylimidazo[1,2-b]pyridazine with bromine in acetic acid or $N$-bromo-succinimide led to the formation of 3-bromo- and 3-bromo-6-chloro-2-methyl-
imidazo[1,2-b]pyridazine respectively. Controlled catalytic hydrogenation of 3-bromo-6-chloroimidazo[1,2-b]pyridazine was found to remove the 3-bromo substituent before the 6-chloro group.

Halogeno substituents at the 6-position of the molecule, however, were found to be the most reactive to nucleophilic displacement. The reaction of 6-chloroimidazo[1,2-b]pyridazine with hydrazine hydrate proceeded to give the 6-hydrazino analogue. The 6-chloro group has also been replaced in a nucleophilic substitution with HO⁻ or MeS⁻ ions. This was achieved by heating 6-chloroimidazo[1,2-b]pyridazine with KOH in ethanol at 170°C or NaSMe in DMF at 105°C respectively.

Molecular Orbital calculations for imidazo[1,2-b]pyridazines have indicated that the N-1 nitrogen is the most basic in the molecule and therefore that quaternization would be most likely to take place at that position. This is also consistent with the earlier conclusion reached by Armarego from the measurement of ionisation constants and UV spectral measurements. Methylene studies on 2-phenylimidazo[1,2-b]pyrazin-6(5H)-one with methyl iodide under reflux conditions have shown that methylation may occur initially at either oxygen or at N-5 to give 6-methoxy-2-phenylimidazo[1,2-b]pyridazine and 5-methyl-2-phenylimidazo[1,2-b]pyrazin-6(5H)-one respectively (Figure I-16). The latter compound when heated at 160°C underwent methylation at N-1 to give 1,5-dimethyl-2-phenylimidazo[1,2-b]pyrazin-6(5H)-on-4-ium iodide.

**Figure I-16**
The treatment of imidazo[1,2-b]pyridazine with an oxidising agent has been shown to afford the \( \text{N-oxide of the imidazo[1,2-b]pyridazine}.^{217} \) Thus, imidazo[1,2-b]-pyridazine in the presence of 85% hydrogen peroxide in polyphosphoric acid below 40°C formed the 5-oxide in moderate yield (Figure I-17). The 6-(chloro and methoxy)-imidazo[1,2-b]pyridazines, however, underwent degradation rather than \( \text{N-oxidation} \) under the same conditions. Imidazo[1,2-b]pyridazine 1-oxides have been formed by the reaction of oxazolo[3,2-b]pyridazinium perchlorates with hydroxylamine, as shown in Figure I-18.\(^{218} \) The 1-oxides can be converted to the corresponding imidazo[1,2-b]-pyridazines by heating with phosphorus trichloride in chloroform. This has led to the synthesis of novel 6,7-disubstituted imidazo[1,2-b]pyridazines. A mechanism has been suggested for the formation of the \( \text{N-oxides} \) from the oxazolo[3,2-b]pyridazinium perchlorates involving nucleophilic attack by hydroxylamine at the C-8 position and a series of cyclisation and rearrangement reactions.\(^{219} \)

\[ \text{Figure I-17} \]

\[ \text{Figure I-18} \]

The imidazo[1,2-b]pyridazines have also been used as starting materials for the syntheses of other tricyclic systems (Figure I-19). The reaction of 6-hydrazino-imidazo[1,2-b]pyridazine with dimethoxymethyl acetate leads to the formation of imidazo[1,2-b]-s-triazolo[3,4-f]pyridazine whereas treatment with nitrous acid gave 6-azidoimidazo[1,2-b]pyridazine which exists in tautomeric equilibrium with the
imidazo[1,2-b]tetrazolo-[5,1-f]pyridazine system. 6-Hydrazinoimidazo[1,2-b]-pyridazine has also been shown to generate the 1H-imidazo[1',2':2,3]pyridazino[6,1-c]-as-triazine system via the reaction of 6-(α-carbethoxyethylidenehydrzino)imidazo[1,2-b]pyridazine with polyphosphoric acid (Figure I-20).

Figure I-19

![Chemical structure](image)

Figure I-20

![Chemical structure](image)

Unexpected 1,3-dipolar cycloaddition reactions have been reported with 6-substituted imidazo[1,2-b]pyridazines. The reactions between the imidazo[1,2-b]-pyridazines and 2-diazopropane were found to proceed as a 1,3-dipolar cycloaddition of the 2-diazopropane across the C7-C8 double bond of the imidazo[1,2-b]pyridazine followed by the loss of H₂ to generate the stable 9H-imidazo[1,2-b]pyrazolo[4,3-d]-pyridazine product (Figure I-21). Irradiation of the pyrazolopyridazines results in the loss of molecular nitrogen and, depending on the solvent used, the formation of either an 8-isopropenylimidazo[1,2-b]pyridazine or the methylethyl analogue. Similar 1,3-
dipolar cycloadditions have also been found to occur in other aromatic 10\pi-electron heterocyclic systems with a bridgehead nitrogen atom.\textsuperscript{224}

The reduction of imidazo[1,2-\textit{b}]pyridazines has been reported with sodium borohydride.\textsuperscript{225} A mechanism has been proposed for the reaction on the basis of isotopic labelling and n.m.r. spectral studies. This mechanism (Figure I-22) involves the sodium borohydride reduction of the C7-C8 bond before the reduction of the C6-N5 bond resulting in the formation of the 5,6,7,8-tetrahydro derivative.
Some imidazo[1,2-b]pyridazine derivatives form stable complexes with bromine and these complexes have been found to be useful brominating agents for a variety of other organic compounds, in some cases offering an improvement over other brominating agents such as N-bromosuccinimide, bromine and pyridine hydrobromide perbromide. The compounds are formed by the reaction of imidazo[1,2-b]pyridazine or 6-chloroimidazo[1,2-b]pyridazine with bromine in acetic acid (Figure I-23). Initially the electrophilic substitution of bromine at the 3-position of the molecules occurs, resulting in the formation of the 3-bromo hydrobromide salts of the starting materials, which are then converted to the imidazo[1,2-b]pyridazine-bromine complexes by the addition of excess bromine. The final bromine complexes have been found to brominate other organic compounds smoothly in equimolar amounts, either at room temperature or after gentle heating.
I-4.4 Biological properties

Shortly after the initial synthesis of the imidazo[1,2-\textit{b}]pyridazines a number of biological activities and potential applications of the compounds were identified. Nitta, Yoneda and Otaka patented a number of 2,6-disubstituted imidazo[1,2-\textit{b}]pyridazines as analgesics, sedatives and antispasmodics.\textsuperscript{227-230} In another early report, Almirante \textit{et al.}\textsuperscript{231} described imidazo[1,2-\textit{b}]pyridazines as possessing antipyretic, hypothermal and anticonvulsant properties. Over the past 2 decades imidazo[1,2-\textit{b}]pyridazines have been patented as agents with antibiotic,\textsuperscript{232-237} pesticidal,\textsuperscript{238} antidepressant,\textsuperscript{239} anticonvulsant,\textsuperscript{240} cardiovascular,\textsuperscript{241,242} antihypertensive,\textsuperscript{243} antitumour,\textsuperscript{244} and antiasthmatic properties.\textsuperscript{245-249} They have also shown affinity for M1 muscarinic\textsuperscript{250} and angiotensin II\textsuperscript{251,252} receptors, been used as cyanine dyes,\textsuperscript{253} and as treatment for foot rot, liver lesions and hemorrhagic colitis in animals.\textsuperscript{254,255} Details have been published of the synthesis and testing of cephalosporins containing (imidazo[1,2-\textit{b}]-pyrazinium-1-yl)methyl substituent groups (Figure 1-24) which have demonstrated potent antibacterial activity.\textsuperscript{213,256} Other imidazo[1,2-\textit{b}]pyridazine carbamates have been synthesised as potential analogues of known antifilarial agents though they have not be shown to have significant antifilarial activity,\textsuperscript{257,258} while the imidazo[1,2-\textit{b}]pyridazine carbamate (1069C) (Figure 1-24) has been developed as a novel synthetic antitumour agent.\textsuperscript{259}

Figure 1-24

\begin{center}
\begin{chemfig}
\drawnode{H2N}
X
\drawnode{S}
\drawnode{NOR_t1}
\drawnode{C=O}
\drawnode{NH}
\drawnode{\text{COO}^-}
\drawnode{\text{OMe}}
\drawnode{\text{OMe}}
\drawnode{\text{MeO}}
\drawnode{\text{MeO}}
\drawnode{\text{N}}
\drawnode{\text{N}}
\drawnode{\text{NHCO}_2\text{Me}}
\drawnode{\text{1069 C}}
\drawnode{\text{Cephalosporin derivative}}
\drawnode{\text{Antibacterial activity}}
\drawnode{\text{Antitumour activity}}
\end{chemfig}
\end{center}
Present Work

In the present work a number of imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines have been synthesised and evaluated as potential ligands at the BZR, as determined by in vitro displacement of [3H] diazepam binding to rat brain membranes. Compounds which showed low affinity for the BZR have also been tested to determine their affinity for the PBR by in vitro displacement of [3H] diazepam binding to rat kidney membranes.

The major aims of this work were (a) to find novel high affinity ligands at the BZR which may ultimately be superior to the traditional BZ compounds in the treatment of CNS disorders, by having more specific pharmacological actions with fewer of the undesirable side effects of the BZs, (b) to identify novel and specific ligands at the PBR with possible applications that are principally in the areas of tumour imaging, steroidogenesis, and treatment of CNS trauma or brain injury, and (c) to vary the substituent groups present on the imidazo[1,2-b]pyridazine or imidazo[1,2-a]pyridine nucleus to allow the active ligand binding site at either the BZR or PBR to be probed and hence to determine the pharmacophoric features of the molecules that are required for, or that inhibit, binding to either the BZR or PBR.

The work reported in this thesis contains the syntheses and pharmacological evaluation of imidazo[1,2-b]pyridazines or related imidazo[1,2-a]pyridines. The compounds have been made by two principal synthetic routes. The first of these is the condensation of the appropriately substituted pyridazin-3-amine (or pyridin-2-amine) with glyoxals followed by methylation to generate the relevant 3-methoxy compounds. The second is the condensation of the substituted pyridazin-3-amines (or pyridin-2-amines) with α-bromoacetyl compounds to give the 3-unsubstituted imidazo[1,2-b]pyridazines (or imidazo[1,2-a]pyridines) which were then subject to electrophilic substitution at the 3-position by N-hydroxymethylbenzamides.

The three principal aims (a), (b) and (c) of this project outlined above have been addressed in the following manner.

(a) A series of imidazo[1,2-b]pyridazines with high affinity for the BZR has been synthesised, and the effect of a variation in the number of potential hydrogen bonding
sites in the molecules on BZR affinity has been examined by the syntheses of a number of polymethoxy or methylenedioxy derivatives of these compounds. The effect of increased steric bulk in a number of 6-alkoxyimidazo[1,2-b]pyridazines on affinity for the BZR has been explored.

(b) A series of 6(7 and 8)-substituted imidazo[1,2-a]pyridines have been synthesised to compare the effect on affinity at both the BZR and the PBR of substitution in these positions. Imidazo[1,2-b]pyridazines have also been synthesised with bulkier 2-styryl and 2-aryl groups, and variously substituted benzamidomethyl groups in the 3-position. This has allowed the identification of a number of specific ligands for the PBR.

(c) The reported effects of various substituent groups in the 2-, 3-, 6-, 7- and 8-positions of the imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines on BZR and/or PBR affinity have been used to direct the molecular modelling study described in the final chapter of this thesis. Certain ligands with high affinity for either the BZR or PBR have been selected for conformational analyses and superimposition with diazepam or Ro5-4864. This has led to the identification of a number of likely points of pharmacophoric interaction with the ligands and the receptor binding site. Volume analyses have shown that the BZR and PBR have different steric requirements, though the fundamental electronic and lipophilic features required for ligand binding are common to both.
CHAPTER II

Syntheses and RZR affinities of some 6-(alkylthio and chloro)-3-aryls-2-methoxy, unsubstituted and benzaquinone-3(4H)-imidaazol-1,2-bipyridazines containing methoxy, methylsulfoxide and methyl substituents

II.4. Introduction

The known possible biological applications of the imidaazol(1,2-b)pyridazines have been discussed in Chapter 1.4.4. Prior to the work reported here, many substituted imidaazol(1,2-b)pyridazines have been prepared by Barin and co-workers [199,200] and used for their ability to displace [3H]-diazepam binding from the BZR in rat brain membrane preparations, leading to the identification of compounds with a range of affinities for the BZR and prompting the search for other novel substituted imidaazol(1,2-b)pyridazines that may be of value as potential therapeutic agents.

For a ligand to induce a biological response at a receptor such as the BZR, the interactions between ligand and receptor must be strong enough to allow the ligand to remain bound to the receptor site for the time that is required to induce the biological response. Short-range hydrogen bonding interactions between ligated and receptor may be of major importance in stabilizing the binding of the ligand to the receptor protein in a number of biological systems [273,274]. It is commonly for hydrogen bond donor groups (typically NH, SH and OH) on the receptor protein to interact with hydrogen bond acceptor groups (typically N, O, S and F) on the ligand. The effect of the presence of oxygen-containing substituent groups on imidaazol(1,2-b)pyridazines, which would have potential hydrogen bonding ability with any appropriately positioned hydrogen bond donor groups on the receptor protein, on affinity for the BZR was therefore examined in this initial investigation.

In this chapter the syntheses of 6-methoxy unsubstituted and benzaquinone-3(4H)-imidaazol(1,2-b)pyridazines with methoxy, methylsulfoxide and methyl groups present in various 6-alkylthiobenzoyl-2-aryl sulfoximines are reported. The compounds have been characterized by 1H n.m.r. and mass spectra and elemental analysis. The abilities of the compounds to displace [3H]-diazepam binding from an brain membrane have been determined and the results of these in vivo assays are reported here. The
CHAPTER II Syntheses and BZR affinities of some 6-(alkylthio and chloro)-2-aryl-3-(methoxy, unsubstituted and benzamidomethyl)imidazo[1,2-b]pyridazines containing methoxy, methylenedioxy and methyl substituents

II-1 Introduction

The known possible biological applications of the imidazo[1,2-b]pyridazines have been discussed in Chapter 1-4.4. Prior to the work reported here, many substituted imidazo[1,2-b]pyridazines have been prepared by Barlin and co-workers and tested for their ability to displace [3H]diazepam binding from the BZR in rat brain membrane preparations, leading to the identification of compounds with a range of affinities for the BZR and prompting the search for other novel substituted imidazo[1,2-b]pyridazines that may be of value as potential therapeutic agents.

For a ligand to induce a biological response from a receptor such as the BZR, the interactions between ligand and receptor must be strong enough to allow the ligand to remain bound to the receptor site for the time that is required to induce the biological response. Short range hydrogen bonding interactions between ligand and receptor are known to be of major importance in stabilising the binding of the ligand to the receptor protein in a number of biological systems. It is common for hydrogen bond donor groups (typically OH, SH and NH) on the receptor protein to interact with hydrogen bond acceptor groups (typically N, O, S and F) on the ligand. The effect of the presence of oxygen-containing substituent groups on imidazo[1,2-b]pyridazines, which would have potential hydrogen bonding ability with any appropriately positioned hydrogen bond donor groups on the receptor protein, on affinity for the BZR was therefore examined in this initial investigation.

In this chapter the syntheses of 3-(methoxy, unsubstituted and benzamidomethyl)imidazo[1,2-b]pyridazines with methoxy, methylenedioxy and methyl groups present in various 6-benzylthio and 2-aryl substituents are reported. The compounds have been characterised by 1H n.m.r. and mass spectra and elemental analyses. The abilities of the compounds to displace [3H]diazepam binding from rat brain membranes have been determined and the results of these in vitro assays are reported here. The
effects of structural modifications to the imidazo[1,2-b]pyridazines on their affinities for the BZR are described and factors that appear to be either beneficial or detrimental to binding to the BZR are discussed.

The experimental details of the syntheses, characterisation and in vitro ligand binding assays of the compounds described above are reported in the Experimental Section of this chapter.

II-2 Syntheses

The initial stage in the preparation of the compounds reported in this work was the synthesis of the appropriately substituted pyridazin-3-amine (Scheme II-1). This was achieved from 6-chloropyridazin-3-amine (II.1) by nucleophilic displacement of the 6-chloro group. In this way, 6-(methylthio, ethylthio and propylthio)pyridazin-3-amine (II.2a-c) were generated by reaction of 6-chloropyridazin-3-amine with the relevant alkanethiolate as described previously by Barlin and Ireland.²⁷⁵ The preparation of the 6-(variously substituted benzylthio)pyridazin-3-amines was a two step process, consisting of the preparation of 6-aminopyridazin-3-thiol (II.5)²⁷⁶ followed by the reaction of this compound with the relevant benzyl chlorides (II.6a-d) to afford the corresponding pyridazin-3-amines (II.7a-d).

The 6-substituted pyridazin-3-amines were condensed with either substituted glyoxals or α-bromoacetophenones to afford the ring closed products which were then modified further (Schemes II-2, II-3). Reaction of the 6-substituted pyridazin-3-amines (II.1), (II.2a) and (II.7a-d) with the appropriately substituted glyoxals led to the formation of the relevant 2-arylimidazo[1,2-b]pyridazin-3(5H)-ones (II.10), (II.14) and (II.8) respectively which were methylated directly with ethereal diazomethane in a variation of known procedures²⁷⁷ to give the 3-methoxyimidazo[1,2-b]pyridazine derivatives (II.11), (II.15a-g) and (II.9a-e) by O-methylation, in yields of 8-61 %. The 6-substituted pyridazin-3-amines (II.2) also reacted with appropriately substituted α-bromoacetophenones (using a general method initially applied by Yoneda et al.)²⁰¹ to generate the corresponding 3-unsubstituted imidazo[1,2-b]pyridazines (II.12a-p) in yields in the range 60-93 %. These compounds underwent electrophilic substitution at
Scheme II-1

Reagents: (i) RSH, 1 M NaOH, 130-140°C (ii) Ac₂O, reflux (iii) (H₂N)₂CS, EtOH, reflux (iv) 1 M NaOH, reflux (v) 1 M NaOH, EtOH, 20°C

<table>
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<th>II.6 and II.7</th>
<th>R</th>
</tr>
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<td>(OMe)₂ (2,3)</td>
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<td>b</td>
<td>Et</td>
<td>b</td>
<td>(OMe)₂ (3,4)</td>
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<tr>
<td>c</td>
<td>Pr</td>
<td>c</td>
<td>(OMe)₃ (3,4,5)</td>
</tr>
<tr>
<td>d</td>
<td></td>
<td>d</td>
<td>(3,4-OCH₂O)</td>
</tr>
</tbody>
</table>

Scheme II-2

Reagents: (i) R₃C₆H₂-CO-COH, conc HCl, EtOH, reflux (ii) CH₂N₂, Et₂O, 0°C

<table>
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<th>R</th>
<th>R₁</th>
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<td>b</td>
<td>(OMe)₂ (3,4)</td>
<td>Me-p</td>
</tr>
<tr>
<td>c</td>
<td>(OMe)₂ (3,4)</td>
<td>(3,4-OCH₂O)</td>
</tr>
<tr>
<td>d</td>
<td>(OMe)₃ (3,4,5)</td>
<td>(3,4-OCH₂O)</td>
</tr>
<tr>
<td>e</td>
<td>(3,4-OCH₂O)</td>
<td>(3,4-OCH₂O)</td>
</tr>
</tbody>
</table>
Scheme II-3

Reagents: (i) RSH, 1 M NaOH, 130-140°C (ii) R1C6HxCOCOH, cone HCl, EtOH, reflux (iii) CH2N2, Et2O, 0°C (iv) R1C6HxCOCH2Br, NaHCO3, EtOH, reflux (v) PhCONHCH2OH, cone H2SO4, AcOH, 100-120°C.
the 3-position, by heating with N-hydroxymethylbenzamide at 120°C in acetic acid in the presence of a catalytic amount of concentrated sulphuric acid to afford the 6-alkylthio-2-aryl-3-benzamidomethylimidazo[1,2-b]pyridazines (II.13a-j). Barlin et al. have previously reported the syntheses of related compounds by similar methods. The final products were purified by chromatography and recrystallisation and gave yields of 6-80%.

II-3 Physical Properties

II-3.1 ¹H n.m.r. Spectra

The novel compounds described in this work were examined by ¹H n.m.r. spectroscopy at 90 MHz as outlined in the Experimental Section. The ¹H n.m.r. spectrum of the parent unsubstituted imidazo[1,2-b]pyridazine (see Figure I-10, p30 for the IUPAC numbering of the ring positions) has been reported. The protons at the 2- and 3-positions gave rise to an AB quartet, with chemical shifts (in CDCl₃) of δ 7.79 and 7.99 respectively, with a coupling constant J₂,₃ of 1.0 Hz. In other imidazo[1,2-b]-pyridazine systems with multiple substituents in the 6-, 7- and 8-positions, the H-3 signal (assigned as such due to its disappearance following electrophilic substitution at the 3-position) is always observed downfield from the H-2 signal, and the value of the coupling constant J₂,₃ is maintained at 1.0 Hz. The protons at positions 6, 7 and 8 of imidazo[1,2-b]pyridazine appear as an ABX system, with chemical shifts of δ 8.30, 7.00 and 7.95 respectively, and coupling constants of magnitude 4.5 Hz (J₆,₇), 10.0 Hz (J₇,₈) and 2.0 Hz (J₆,₈). A long range coupling between the 3- and 8-position protons is also apparent, with a value J₃,₈ of 0.8 Hz.

Some ¹H n.m.r. spectral data for the compounds presented here are given for compounds (II.9, II.11 and II.15) in Table II-1, for (II.12) in Table II-2 and for (II.13) in Table II-3. An examination of the data for the 3-methoxyimidazo[1,2-b]pyridazines (Table II-1) shows that the signal from the 3-methoxy group is a characteristic singlet at δ 4.08 - 4.18. The 3-methoxy signal appears downfield of other methoxy signals (from methoxy groups in either the 2-aryl or 6-benzylthio substituents), as the protons of the 3-methoxy group are in areas of slightly lower electron density and are therefore less
shielded. The chemical shift of the 3-methoxy protons appears to be relatively consistent in various 2-(methoxy or polymethoxy)phenyl substituted compounds. A slight upfield shift of the 3-methoxy signal was observed, however, when the 2-phenyl substituent contained a methoxy group in the ortho position, as in (II.15a) and (II.15d).

The protons at the 7- and 8-position of the imidazo[1,2-b]pyridazines give rise to an AB quartet, with the H-7 proton more shielded than the H-8 proton, as defined for the parent unsubstituted imidazo[1,2-b]pyridazine. The H-7 and H-8 signals appear in the range δ 6.74 - 6.95 and δ 7.59 - 7.89 respectively, with the value of the coupling constant J_{7,8} of 9.5 Hz. The aromatic protons on the 2-aryl and 6-benzylthio substituents appear as a complex, though with some symmetrically substituted groups it is possible to assign the signals in more detail, as recorded in the Experimental Section. Compounds containing the 6-benzylthio group show a characteristic singlet (δ 4.40-4.53) assigned to the CH₂S protons.

The ^1H n.m.r. spectra for the 3-unsubstituted imidazo[1,2-b]pyridazines (Table II-2) show a characteristic downfield singlet in the range δ 8.03 - 8.48 corresponding to the proton at the 3-position. The chemical shift of this H-3 signal was affected by the substituents present in the 2-aryl group, with o-methoxyphenyl substituents (II.12a and 12g) causing a downfield shift relative to the m-methoxyphenyl substituents (II.12b, 12e and 12h), while the corresponding p-methoxyphenyl compounds (II.12c, 12f and 12i) were characterised by n.m.r. spectra showing the H-3 signal shifted further upfield. The compounds synthesised with other 2-aryl groups did not show a significant variation of the chemical shift of the H-3 signal, though the presence of a 2-(3,4-methylenedioxy-phenyl) group resulted in a slight upfield shift of the H-3 signal relative to other 2-(methyl, dimethyl and trimethoxy)phenyl analogues.
Table II-1 Some $^1$H n.m.r. spectral data (δ)$^A$ for 6-(alkylthio and chloro)-2-aryl-3-methoxyimidazo[1,2-b]pyridazines

![Chemical Structure](image)

<table>
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<th>Formula.</th>
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<th>H-8</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>II.11</td>
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<td>4.14</td>
<td>6.95</td>
<td>7.77</td>
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<tr>
<td>15a</td>
<td>SMe</td>
<td>OMe-°</td>
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<td>7.63</td>
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<td>15b</td>
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<td>OMe-°</td>
<td>4.18</td>
<td>6.85</td>
<td>7.72</td>
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<td>15c</td>
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<td>OMe-°</td>
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</tr>
<tr>
<td>15d</td>
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<td>15f</td>
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<td>(OMe)$_3$ (3,4,5)</td>
<td>4.18</td>
<td>6.83</td>
<td>7.67</td>
</tr>
<tr>
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<td>4.13</td>
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</tr>
<tr>
<td>9b</td>
<td>(OMe)$_2$ (3,4)</td>
<td>Me-°</td>
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<td>7.64</td>
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<td>(OMe)$_2$ (3,4)</td>
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<td>(3,4-OCH$_2$O)</td>
<td>4.11</td>
<td>6.74</td>
<td>7.59</td>
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</table>

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

B: Complex signal
Table II-2 Some $^1$H n.m.r. spectral data ($\delta$)\(^A\) for 6-alkythio-2-aryl-3-unsubstituted imidazo[1,2-b]pyridazines

![Diagram of 6-alkythio-2-aryl-3-unsubstituted imidazo[1,2-b]pyridazines]

<table>
<thead>
<tr>
<th>Formula No.</th>
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<th>H-7</th>
<th>H-8</th>
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<td>OMe-m</td>
<td>8.15</td>
<td>6.83</td>
<td>7.72</td>
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<tr>
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<td>OMe-p</td>
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<td>8.03</td>
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\(^A\): Reported as parts per million ($\delta$) downfield from tetramethylsilane (TMS) as internal standard in deuterochloroform.

B: Complex signal

The H-7 and H-8 protons for all 3-unsubstituted compounds with the exception of (II.12m) appear in the range $\delta$ 6.81-6.87 and $\delta$ 7.61-7.81 respectively, with a coupling constant $J_{7,8}$ of 9.5 Hz as observed and reported previously for other related compounds.\(^{267}\) Compound (II.12m) was an exception to this trend with H-7 and H-8 proton signals downfield from these values, at $\delta$ 7.15 and 8.42 respectively.
Table II-3 Some $^1$H n.m.r. spectral data (δ)$^A$ for 6-alkylthio-2-aryl-3-benzamidomethylimidazo[1,2-b]pyridazines

![Formula Image]

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>R</th>
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</tr>
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<td>(Me)$_2$ (3,4)</td>
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<td>6.80</td>
<td>7.59</td>
</tr>
<tr>
<td>13g</td>
<td>SEt</td>
<td>(Me)$_2$ (3,4)</td>
<td>5.18</td>
<td>6.74</td>
<td>B</td>
</tr>
<tr>
<td>13h</td>
<td>SPr</td>
<td>(Me)$_2$ (3,4)</td>
<td>5.19</td>
<td>6.79</td>
<td>7.59</td>
</tr>
<tr>
<td>13i</td>
<td>SEt</td>
<td>(3,4-OCH$_2$O)</td>
<td>5.17</td>
<td>6.83</td>
<td>7.61</td>
</tr>
<tr>
<td>13j</td>
<td>SPr</td>
<td>(3,4-OCH$_2$O)</td>
<td>5.17</td>
<td>6.86</td>
<td>7.63</td>
</tr>
</tbody>
</table>

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

B: Complex signal

Replacement of the proton at the 3-position of imidazo[1,2-b]pyridazines by a benzamidomethyl group gave more complex spectra arising from the additional phenyl ring. The $^1$H n.m.r. spectra of these compounds (Table II-3) were characterised by a doublet at δ 5.17-5.23 resulting from the benzamidomethyl CH$_2$ protons coupled to the neighbouring NH proton, with a coupling constant $J = 5.5$ Hz. The H-7 protons were assigned chemical shift values δ 6.74-6.89, and the H-8 protons (where it was possible to separate them from complex signals) appeared in the range δ 7.59-7.69, again with characteristic coupling constants $J_{7,8}$ of 9.5 Hz.
II-3.2 Mass spectra

The mass spectra of several imidazo[1,2-b]pyridazines with explanations postulated for the fragmentation patterns observed have been reported previously.\textsuperscript{279} It has been suggested that the mechanism of the fragmentation of the 5-membered ring of the imidazo[1,2-b]pyridazines (Scheme II-4) results from an initial loss of HCN followed by rearrangement and a further loss of either N\textsubscript{2} or HCN, with the eventual formation of the [C\textsubscript{4}H\textsubscript{4}]\textsuperscript{+} and [C\textsubscript{4}H\textsubscript{2}N]\textsuperscript{+} species. This mechanism, however, is for compounds unsubstituted in the 2- and 3-positions.

The mass spectrum of 3-methoxy-2-(4-methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.15c) shows a molecular ion peak at \textit{m/z} 301, with other peaks at \textit{m/z} 286 and 258, presumably corresponding to loss of a CH\textsubscript{3} group and then further loss of N\textsubscript{2} from the molecular ion. At the other end of the mass spectrum, a peak is apparent at \textit{m/z} 52 which could result from the [C\textsubscript{4}H\textsubscript{4}]\textsuperscript{+} species. The mass spectrum of 3-benzamidomethyl-2-(4-methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.13a) does not show a similar fragmentation pattern, as major peaks occur at \textit{m/z} 404 (molecular ion) and 299 (molecular ion - COC\textsubscript{6}H\textsubscript{5}). Peaks were also apparent at the other end of the spectrum at \textit{m/z} 77 and 105 which may be due to [C\textsubscript{6}H\textsubscript{5}]\textsuperscript{+} and [COC\textsubscript{6}H\textsubscript{5}]\textsuperscript{+} ions formed as a result of the fragmentation process.

II-4 \textit{In vitro} binding studies

The discovery of a single, saturable, benzodiazepine binding site on rat brain membranes, initially characterised by high affinity for diazepam and other 1,4-benzodiazepines, and the close correlation observed between the \textit{in vitro} affinity of a range of benodiazepines for this receptor site and their \textit{in vivo} anxiolytic and anticonvulsant activity has provided a straightforward method to screen potential BZR ligands.\textsuperscript{12,15,280} Determination of the affinity of new compounds for the BZR by the \textit{in vitro} displacement of [\textsuperscript{3}H] benzodiazepines can provide some indication of the \textit{in vivo} properties that the new compounds may possess.\textsuperscript{281}
The most commonly used $[^3H]$ benzodiazepines for these assays (see Figure I-2, p5 for chemical structures) are $[^3H]$ diazepam and $[^3H]$ flunitrazepam which are high affinity ligands for both the BZR and PBR. The closely related analogue $[^3H]$ clonazepam (a selective ligand for the BZR) is also frequently used for in vitro binding studies. The selective in vitro binding of ligands such as the triazolopyridazine CL 218,872 to rat brain membrane preparations from different areas of the brain has led to the discovery of subtypes of the BZR, initially labelled BZI and BZII, as described in Chapter I-2.3. In the work reported in this thesis, the affinity of imidazo[1,2-b]-
II-4.1 Biochemical characteristics of diazepam binding to the BZR

Pharmacological studies have shown that the binding of diazepam to the BZR in rat brain cortical preparations takes approximately 15 minutes to equilibrate and is both temperature dependent, with the maximum amount bound at 4°C, and pH dependent, with conditions optimum for binding in the range pH 7.0-7.4. The dissociation constant $K_D$ (i.e. half maximal binding, and defined as the ratio $k_1/k_{-1}$ where $k_1$ and $k_{-1}$ are the rate constants of association and dissociation respectively) of diazepam was found to occur at approximately 3 nM. This is in good agreement with the calculated inhibition constant ($K_i$) of diazepam of approximately 6 nM, determined by the ability of unlabelled diazepam to displace the binding of [3H] diazepam from the BZR.

The *in vitro* assays have the major advantage that they require only small sub-milligram quantities of test compound to determine the affinity of the compound for the BZR. Results of *in vitro* studies are not subject to the complications such as bio-availability and metabolism that affect *in vivo* studies, and therefore are of benefit in allowing direct comparison between ligands and assisting in the development of structure-activity relationships and in directing the future synthesis of other drug candidates. The main disadvantage of the *in vitro* assays is that the binding kinetics of ligands at the BZR observed at the assay temperatures of 0-4°C may not reflect the binding kinetics of the same compounds *in vivo* at 37°C. This does not detract, however, from the utility of the *in vitro* assays as preliminary screens to assess the BZR affinities of new compounds.

II-4.2 Results of *in vitro* testing

The *in vitro* affinity for the BZR of the imidazo[1,2-b]pyridazines reported here was measured by the ability to displace [3H] diazepam binding from rat brain membrane preparations as outlined in Chapter II-4.1. The experimental details of the assay conditions are described in Chapter II-5.3. The results are presented in Tables II-4 and
II-5 as either IC\textsubscript{50} values (defined as the concentration of the test compound found to displace 50\% of specific [\textsuperscript{3}H] diazepam binding to the BZR in the assay) or the percentage displacement of specific [\textsuperscript{3}H]diazepam binding at a single test compound concentration of 1000 nM. The IC\textsubscript{50} values of the compounds at the BZR are related to the inhibition constant (K\textsubscript{i}) and dissociation constant (K\textsubscript{D}) of [\textsuperscript{3}H] diazepam binding at the BZR complex by the equation K\textsubscript{i} = IC\textsubscript{50} / (1 + [Diaz]/K\textsubscript{D} where [Diaz] = concentration of [\textsuperscript{3}H] diazepam (0.7 ± 0.05 nM) and K\textsubscript{D} = 3.5 ± 0.1 nM. Unlabelled diazepam was used as control and 5\% DMSO in buffer as blank in the assays. Some data by other workers are included for comparative purposes, with appropriate footnotes.

II-4.3 Discussion of results

An examination of the results in Table II-4 for the 3-methoxy compounds (II.15 and II.9) shows that the compounds demonstrate a considerable range of affinities for the BZR.

The 3-methoxy-6-methylthio compounds (II.15a-f) generally showed low affinities for the BZR, with less than 50\% displacement of [\textsuperscript{3}H] diazepam binding at 1000 nM though (II.15g) gave 72\% displacement at 1000 nM. The 2-(o-, m-, or p-methoxyphenyl) substituents in compounds (II.15a-c) did not lead to an increase in BZR affinity when compared to the 2-phenyl analogue (II.15h). The corresponding 2-(polymethoxyphenyl) compounds (II.15d-f) showed a further decrease in BZR affinity. The 2-(3,4-methylenedioxyphenyl) compounds (II.15g) and (II.11), however, displayed higher affinities for the BZR than their 2-phenyl analogues (II.15h) and (II.11a) regardless of the nature of the 6-position substituent.
Table II-4 Results of displacement of $[^3]$H diazepam binding from rat brain membrane preparations by some 3-methoxyimidazo[1,2-b]pyridazines

<table>
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<tr>
<th>Formula No.</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>R&lt;sub&gt;3&lt;/sub&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;(nM) (or %) displacement at 1000 nM)&lt;sup&gt;A&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>II.15a</td>
<td>SMe</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;OMe-o</td>
<td>(23%)</td>
</tr>
<tr>
<td>15b</td>
<td>SMe</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;OMe-m</td>
<td>(46%)</td>
</tr>
<tr>
<td>15c</td>
<td>SMe</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;OMe-p</td>
<td>(45%)</td>
</tr>
<tr>
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<td>SMe</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;2&lt;/sub&gt; (2,4)</td>
<td>(12%)</td>
</tr>
<tr>
<td>15e</td>
<td>SMe</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;2&lt;/sub&gt; (3,4)</td>
<td>(8%)</td>
</tr>
<tr>
<td>15f</td>
<td>SMe</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;3&lt;/sub&gt; (3,4,5)</td>
<td>(5%)</td>
</tr>
<tr>
<td>15g</td>
<td>SMe</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>(72%)</td>
</tr>
<tr>
<td>15h&lt;sup&gt;B&lt;/sup&gt;</td>
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<td>OMe</td>
<td>Ph</td>
<td>884</td>
</tr>
<tr>
<td>11</td>
<td>Cl</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>84</td>
</tr>
<tr>
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<td>Cl</td>
<td>OMe</td>
<td>Ph</td>
<td>772</td>
</tr>
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<td>9a</td>
<td>SCH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;2&lt;/sub&gt; (2,3)</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;Me-p</td>
<td>91</td>
</tr>
<tr>
<td>9b</td>
<td>SCH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;2&lt;/sub&gt; (3,4)</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;Me-p</td>
<td>102</td>
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<tr>
<td>9h&lt;sup&gt;D&lt;/sup&gt;</td>
<td>SCH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;2&lt;/sub&gt; (2,3)</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>57</td>
</tr>
<tr>
<td>9c</td>
<td>SCH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;2&lt;/sub&gt; (3,4)</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>40</td>
</tr>
<tr>
<td>9d</td>
<td>SCH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;2&lt;/sub&gt;(OMe)&lt;sub&gt;3&lt;/sub&gt; (3,4,5)</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>183</td>
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<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>1</td>
</tr>
<tr>
<td>9f&lt;sup&gt;E&lt;/sup&gt;</td>
<td>SCH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;OMe-o</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;2&lt;/sub&gt; (3,4)</td>
<td>(34%)</td>
</tr>
<tr>
<td>9g&lt;sup&gt;F&lt;/sup&gt;</td>
<td>SCH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;OMe-m</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;(OMe)&lt;sub&gt;2&lt;/sub&gt; (3,4)</td>
<td>(47%)</td>
</tr>
<tr>
<td>9h&lt;sup&gt;F&lt;/sup&gt;</td>
<td>SCH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;OMe-m</td>
<td>OMe</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;Me-p</td>
<td>17</td>
</tr>
</tbody>
</table>

A: IC<sub>50</sub> values (or percentage displacement at a single ligand concentration of 1000 nM) in the presence of 100 µM γ-aminobutyric acid (see Experimental Section for details)

B: Ref 267
C: Ref 260
D: Prepared by Mr S.J. Ireland
E: Prepared by Mr R.A. Davis
F: Ref 263
Table II-5 Results of displacement of $[^3H]$ diazepam binding from rat brain membrane preparations by some 3-(unsubstituted or benzamidomethyl) imidazo[1,2-b]pyridazines

![Chemical structure](image_url)

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>$IC_{50}$ (nM) (or % displacement at 1000 nM)$^A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>II.12r^{B}</td>
<td>SMe</td>
<td>H</td>
<td>Ph</td>
<td>(21%)</td>
</tr>
<tr>
<td>12a</td>
<td>SMe</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$o$</td>
<td>(0%)</td>
</tr>
<tr>
<td>12b</td>
<td>SMe</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$m$</td>
<td>(18%)</td>
</tr>
<tr>
<td>12c</td>
<td>SMe</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>(47%)</td>
</tr>
<tr>
<td>12d</td>
<td>SEt</td>
<td>H</td>
<td>Ph</td>
<td>(51%)</td>
</tr>
<tr>
<td>12e</td>
<td>SEt</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$m$</td>
<td>(38%)</td>
</tr>
<tr>
<td>12f</td>
<td>SEt</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>(28%)</td>
</tr>
<tr>
<td>12g</td>
<td>SPr</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$o$</td>
<td>(12%)</td>
</tr>
<tr>
<td>12h</td>
<td>SPr</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$m$</td>
<td>(33%)</td>
</tr>
<tr>
<td>12i</td>
<td>SPr</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>(19%)</td>
</tr>
<tr>
<td>12j</td>
<td>SPr</td>
<td>H</td>
<td>C$_6$H$_2$(OMe)$_3$ (3,4,5)</td>
<td>(14%)</td>
</tr>
<tr>
<td>12s^{B}</td>
<td>SMe</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>(35%)</td>
</tr>
<tr>
<td>12k</td>
<td>SEt</td>
<td>H</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>(57%)</td>
</tr>
<tr>
<td>12l</td>
<td>SMe</td>
<td>H</td>
<td>C$_6$H$_3$(Me)$_2$ (3,4)</td>
<td>(6%)</td>
</tr>
<tr>
<td>12m</td>
<td>SEt</td>
<td>H</td>
<td>C$_6$H$_3$(Me)$_2$ (3,4)</td>
<td>(18%)</td>
</tr>
<tr>
<td>12n</td>
<td>SPr</td>
<td>H</td>
<td>C$_6$H$_3$(Me)$_2$ (3,4)</td>
<td>(16%)</td>
</tr>
<tr>
<td>12q^{B}</td>
<td>SMe</td>
<td>H</td>
<td>C$_6$H$_3$(3,4-OCH$_2$O)</td>
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</tr>
<tr>
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<td>C$_6$H$_3$(3,4-OCH$_2$O)</td>
<td>(69%)</td>
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<td>12p</td>
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<td>H</td>
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<td>(82%)</td>
</tr>
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<td>SMe</td>
<td>CH$_2$NHCOPh</td>
<td>Ph</td>
<td>20</td>
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<tr>
<td>13a</td>
<td>SMe</td>
<td>CH$_2$NHCOPh</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>17</td>
</tr>
<tr>
<td>13b</td>
<td>SEt</td>
<td>CH$_2$NHCOPh</td>
<td>Ph</td>
<td>20</td>
</tr>
<tr>
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<td>SEt</td>
<td>CH$_2$NHCOPh</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>27</td>
</tr>
<tr>
<td>13d</td>
<td>SPr</td>
<td>CH$_2$NHCOPh</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>57</td>
</tr>
<tr>
<td>13k^{B}</td>
<td>SMe</td>
<td>CH$_2$NHCOPh</td>
<td>C$_6$H$_4$OMe-$p$</td>
<td>7</td>
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<td>13e</td>
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<td>C$_6$H$_4$OMe-$p$</td>
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$^A$ Values represent the % displacement at 1000 nM. All compounds were tested at 100 nM in the presence of 400 nM diazepam.
Table II-5  Continued

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<th>Formula No.</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>IC₅₀ (nM) (or % displacement at 1000 nM)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>13f</td>
<td>SMe</td>
<td>CH₂NHCOPh</td>
<td>C₆H₃(Me)₂(3,4)</td>
<td>14</td>
</tr>
<tr>
<td>13g</td>
<td>SEt</td>
<td>CH₂NHCOPh</td>
<td>C₆H₃(Me)₂(3,4)</td>
<td>25</td>
</tr>
<tr>
<td>13h</td>
<td>SPr</td>
<td>CH₂NHCOPh</td>
<td>C₆H₃(Me)₂(3,4)</td>
<td>39</td>
</tr>
<tr>
<td>13i</td>
<td>SMe</td>
<td>CH₂NHCOPh</td>
<td>C₆H₃(3,4-OCH₂O)</td>
<td>2</td>
</tr>
<tr>
<td>13j</td>
<td>SEt</td>
<td>CH₂NHCOPh</td>
<td>C₆H₃(3,4-OCH₂O)</td>
<td>4</td>
</tr>
</tbody>
</table>

A: IC₅₀ values (or percentage displacement at 1000 nM) in the presence of 100 µM γ-aminobutyric acid (see Experimental Section for details)

B: Ref 263

The results where the 2-(3,4-methylenedioxyphenyl) substituent leads to a clear improvement in binding affinity when compared to analogous compounds containing a 2-phenyl group indicates that hydrogen bonding interactions of substituent groups (present on the 2-phenyl ring) are of importance in stabilising the binding of the compounds to the BZR. The hydrogen bonding capability of the 2-(3,4-methylenedioxyphenyl) group is greater than that of the 2-(o-, m- or p-methoxyphenyl) groups and this may explain the increased BZR affinities of compounds containing this substituent. It appears likely, however, that steric factors must also be considered and that the area of the binding site of the BZR that interacts with the groups at the 2-position is subject to quite restrictive steric constraints because replacement of the 2-(3,4-methylenedioxyphenyl) group in (II.15g) with a 2-(3,4-dimethoxyphenyl) group, as in (II.15e) results in a considerable reduction in affinity for the BZR. The 2-(3,4,5-trimethoxyphenyl) compound (II.15f) shows a further reduction in BZR affinity, consistent with this trend. Electronic and other factors may play some part in this effect, but it appears that the increased steric bulk of the polymethoxy over methylenedioxy substitution of the 2-phenyl ring is the major cause of this reduction in affinity.
The 3-methoxy-6-(substituted benzylthio) compounds (II.9a-i) were considerably more potent ligands at the BZR than the 6-methylthio analogues, indicating that the additional phenyl ring of the 6-benzylthio compounds was able to increase BZR affinity, possibly by occupying an area of lipophilic interaction at the BZR binding site. The BZR affinity of the compounds was sensitive to substitution in the phenyl rings of the substituent groups in both the 6- and 2-positions.

Those compounds which contained the 2-(3,4-methylendioxyphenyl) group (II.9c,d,e,h) again showed high affinities for the BZR and a comparison with analagous 2-(p-methylphenyl) compounds, (II.9h) with (II.9a), and (II.9c) with (II.9b), showed that (II.9h) and (II.9c) bound to the BZR approximately twice as strongly as (II.9a) and (II.9b). The effect of the 2-(3,4-dimethoxyphenyl) substituent was to reduce the affinity of the compounds for the BZR. This was demonstrated by a comparison of the relative affinities of (II.9g) and the 2-(p-methylphenyl) analogue (II.9i) which shows that the binding of (II.9g) is approximately 50 times weaker than (II.9i). In this series of 6-benzylthio compounds (II.9), as with the 6-(methylthio and chloro) analogues (II.15) and (II.11), the 2-(3,4-methylenedioxyphenyl) group conferred the highest affinity for the BZR.

Substitution of the phenyl ring of the 6-benzylthio compounds followed a similar pattern to that observed in aryl groups at the 2-position. The affinities of the 6-(dimethoxybenzylthio) compounds (II.9a and 9b) for the BZR were lower than that for the 6-(m-methoxybenzylthio) analogue (II.9i). This indicated that the effect of steric repulsion was greater than that of increased potential hydrogen bonding interaction at the BZR binding site. A comparison of the BZR affinities of compounds (II.9c,d,e,h) all containing the 2-(3,4-methylenedioxyphenyl) group showed that the 6-(trimethoxybenzylthio) compound (II.9d) had the lowest BZR affinity (IC$_{50}$ 183 nM), and the 6-(dimethoxybenzylthio) analogues (II.9h) and (II.9c) were characterised by ca 3-4.5 times greater affinity for the BZR. The 6-(3,4-methylenedioxybenzylthio) compound (II.9e) showed dramatically improved BZR affinity (IC$_{50}$ 1 nM). The very high BZR affinity of (II.9e) suggests that hydrogen bonding interactions are also of importance between ligand and receptor in the vicinity of the phenyl ring of the 6-benzylthio group,
but the increased steric bulk of the 6-(polymethoxybenzylthio) substituents leads to a reduction in BZR affinity.

The results of the ligand binding studies for the 3-(unsubstituted and benzamidomethyl)imidazo[1,2-b]pyridazines are shown in Table II-5. None of the 3-unsubstituted compounds (II.12a-n) gave rise to potent displacement of [3H] diazepam binding at 1000nM, with likely IC50 values in the micromolar region. Compounds (II.12o) and (II.12p), however, bind with slightly higher affinity to the BZR.

Substitution of the 2-phenyl ring appeared to have some variability on the resultant BZR affinity of the substituted molecules. Compounds with 2-(o-methoxyphenyl) substituents showed lower BZR affinity than their 2-(m- or p-methoxyphenyl) analogues, as illustrated when the affinity of (II.12a) is compared with (II.12b) and (II.12c), and similarly when (II.12g) is compared with (II.12h) and (II.12i). This may be a steric effect with the o-methoxy group interacting with the imidazo[1,2-b]pyridazine nucleus and altering the conformation of the 2-(o-methoxyphenyl) group relative to the m- and p-methoxy isomers. Replacement of a 2-(p-methylphenyl) group with a 2-(3,4-dimethylphenyl) substituent resulted in a reduction in affinity for the BZR as shown by the difference in binding for (II.12s) vs. (II.12l) and (II.12k) vs. (II.12m), indicating that negative steric interactions are beginning to dominate electronic effects. Compounds with the 2-(3,4-methylenedioxyphenyl) group present (II.12o-q) showed increased BZR affinity as described for the other imidazo[1,2-b]pyridazines reported in this chapter.

The size of the 6-alkylthio group in the 3-unsubstituted imidazo[1,2-b]-pyridazines did not seem to be of major importance in determining the BZR affinity of the compounds as no consistent trends were observed with the increased size of the 6-position substituents from methylthio to propylthio.

The 3-benzamidomethyl compounds synthesised all showed nanomolar affinity for the BZR, indicating that certain properties of the 3-benzamidomethyl group confer high affinity for the BZR binding site. The effects of substituent groups on the 2-phenyl ring on BZR affinity were beneficial in the case of 2-(p-methylphenyl) and 2-(3,4-methylenedioxyphenyl) groups as in, for example, (II.13e) and (II.13i) respectively, and detrimental in the case of 2-(p-methoxyphenyl) and 2-(3,4-dimethylphenyl) analogues
It was again noted that compounds containing the 2-(3,4-methylenedioxyphenyl) group demonstrated the highest affinities for the BZR.

It is possible to attribute these results to the steric and hydrogen bonding properties of the substituents. The 2-(p-methoxyphenyl) and 2-(3,4-dimethylphenyl) groups are detrimental to binding compared to the 2-(p-methylphenyl) analogues due to their greater size and therefore negative interaction with the receptor protein. The hydrogen bonding ability of the 2-(p-methoxyphenyl) group is not sufficient to stabilise binding to the receptor. In the case of the 2-(3,4-methylenedioxyphenyl) group, however, the greater hydrogen bonding ability resulting from the presence of two oxygen atoms is sufficient to lead to increased BZR affinity in molecules containing this substituent, despite the slightly greater steric bulk.

In contrast to the 3-unsubstituted imidazo[1,2-b]pyridazines, the BZR affinity of the 3-benzamidomethyl compounds was dependent on the size of the 6-alkylthio group, with the compounds binding less strongly at the BZR as the 6-position group was altered from methylthio to ethylthio to propylthio, as in (II.13a), (II.13c) and (II.13d) respectively.

To review the BZR affinities of the imidazo[1,2-b]pyridazines reported in this chapter, a number of factors appear to be of importance. Substitution of the 2-phenyl group, independent of the other 3- and 6-position groups, leads to increased BZR affinity in the case of 2-(3,4-methylenedioxyphenyl) and, where reported, 2-(p-methylphenyl) substituent groups whereas other 2-(dimethyl, methoxy or polymethoxy)phenyl groups result in decreased affinity for the BZR. Compounds (II.9) and (II.13), containing two phenyl groups as part of their chemical structure, are considerably more potent ligands for the BZR than (II.11, 12, and 15) where only one phenyl group is present. This indicates two areas of interaction between electron-rich aromatic areas of the BZR ligands and the active binding site on the receptor.

If all the compounds bind to the BZR in a similar orientation, regardless of the 3-position substituent present (with direct overlap of the imidazo[1,2-b]pyridazine nucleus and the 2-position group) then the biologically active conformations of (II.9) and (II.13), and hence spatial positions of the phenyl groups present in substituents at the
6- and 3-positions respectively, become of interest. As an aside, this would also provide a possible explanation for the consistent pattern of variations of BZR affinities observed with the different 2-aryl groups regardless of other substituent groups on the molecule (as these 2-position groups would occupy the same area of ligand-receptor interaction). For the 3-benzamidomethyl compounds, the larger 6-position propylthio groups decrease BZR affinity, suggesting that they occupy a receptor area of negative steric interaction. If this is indeed the case then the 6-benzylthio group of the compounds (II.9) must adopt a conformation avoiding this sterically disfavoured area. Alternatively, the 6-benzylthio compounds could adopt a different orientation altogether in the BZR binding site. These possibilities will be discussed in more detail in the molecular modelling studies in Chapter VII.

The increased BZR affinities of compounds containing 3,4-methylenedioxy substituents in phenyl rings in 2- and 6-position substituent groups suggests that additional hydrogen bonding interactions do stabilise the binding of these compounds to the receptor. These areas of hydrogen bonding interaction between ligand and receptor, however, are subject to steric constraints, with bulkier substituent groups reducing BZR affinity regardless of their potential hydrogen bonding ability.

In the next chapter, the effect of increasing steric bulk in the 6-position substituent group on the BZR affinity of a series of substituted imidazo[1,2-b]pyridazines will be examined in more detail.

II-5 Experimental

II-5.1 General Topics

(i) Melting points (m.p.s) were taken in open 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°C with a Jeol FX90Q fourier-transform spectrometer with tetramethylsilane (in CDCl$_3$, unless otherwise stated) as an internal standard ($\delta$ 0.00 ppm). Data are presented in the following order: chemical shift (ppm) relative to tetramethylsilane; multiplicity; coupling constant (J) in Hz; assignment (if appropriate). The following
abbreviations are adopted: s (singlet); d (doublet); t (triplet); dd (doublet of doublets). Exchangeable protons were identified by their disappearance following addition of deuterium oxide.

(iii) Low resolution electron impact mass spectra were recorded at 70 eV on an Incas data system attached to a VG-Micromass 7070 double focusing mass spectrometer. Data were presented in the following order: m/z value; relative intensity as a percentage of the base peak.

(iv) Microanalyses were performed by the Australian National University Analytical Services Unit. Solids for analysis were dried at 80-100°C and 710 mmHg for 3-24 h unless otherwise specified.

(v) Analytical thin layer chromatography (t.l.c.) was performed on alumina plates pre-coated with Merck Kieselgel 60 F_{254} or Merck aluminium oxide 60 F_{254} neutral (type E) of 0.25 mm thickness. Preparative thin layer chromatography was performed on glass plates pre-coated with Merck aluminium oxide 60 F_{254} (type E) of 1.5 mm thickness.

(vi) Ethereal diazomethane was prepared from nitrosomethylurea according to literature procedures.\(^{284}\)

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

(viii) Where full experimental details are not recorded, percentage yields are given in brackets as appropriate.

(ix) The light petroleum used as a solvent had b.p. 60-80°C unless otherwise specified.

### II-5.2 Synthetic work

The following pyridazin-3-amines and related intermediates were synthesised according to literature procedures and characterised using \(^1\)H n.m.r. spectroscopy:

- 6-methylthiopyridazin-3-amine,\(^{285}\)
- 6-(ethyldithio and propylthio)pyridazin-3-amine,\(^{275}\)
- 3,4,5-trimethoxybenzylchloride,\(^{286}\)
- 3',4'-dimethoxy and 3',4'-methylenedioxy)benzylthiopyridazin-3-amine.\(^{287}\)
The substituted glyoxals 2'-methoxyphenylglyoxal, \textsuperscript{288,289} 3'-methoxyphenylglyoxal, \textsuperscript{290} 4'-methoxyphenylglyoxal, \textsuperscript{291} 2',4'-dimethoxyphenylglyoxal, \textsuperscript{292} 3',4'-dimethoxyphenylglyoxal, \textsuperscript{293} 3',4',5'-trimethoxyphenylglyoxal, \textsuperscript{294} 3',4'-methylenedioxyphenylglyoxal ethanolate, \textsuperscript{266} and 4'-methylphenylglyoxal \textsuperscript{295} were also prepared as described in the appropriate literature references.

Similarly, \( \alpha \)-bromo-4'-methoxyacetophenone, \textsuperscript{296} \( \alpha \)-bromo-3',4',5'-trimethoxyacetophenone, \textsuperscript{297} \( \alpha \)-bromo-(4'-methyl and 3',4'-dimethyl)acetophenone, \textsuperscript{298} \( \alpha \)-bromo-3',4'-methylenedioxyacetophenone, \textsuperscript{299} and \( N \)-hydroxymethylbenzamide \textsuperscript{300} were prepared and characterised by \( \textsuperscript{1} \text{H} \) n.m.r. spectroscopy.

\textbf{6-Chloro-3-methoxy-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (II.11)}

A mixture of 6-chloropyridazin-3-amine (II.11) (0.13 g), 3,4-methylenedioxyphenylglyoxal ethanolate (0.20 g), ethanol (10.0 ml) and concentrated hydrochloric acid (0.07 ml) was refluxed with stirring in an oil bath for 14 h. After cooling, water (20 ml) was added and the red precipitate (0.15 g) was filtered off. This precipitate was stirred with excess ethereal diazomethane at 0°C and then at 20°C overnight. The remaining diazomethane and ether were then evaporated and the product subjected to t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum gave the title compound (0.08 g, 27%), m.p. 198-199°C (Found: C, 55.2; H, 3.3; N, 13.8. \( \text{C}_{14}\text{H}_{10}\text{ClN}_{3}\text{O}_{3} \) requires C, 55.4; H, 3.3; N, 13.8%). \( \textsuperscript{1} \text{H} \) n.m.r.: \( \delta \) 4.14, s, 3'-OCH\(_2\)O; 6.01, s, OCH\(_2\)O; 6.92, d, J 9 Hz, H 6'(2'); 6.95, d, J 9 Hz, H 7; 7.63, s, H 2'(6'); 7.67, d, J 9 Hz, H 5'; 7.77, d, J 9 Hz, H 8. Mass spectrum (e.i.) \( m/z \) 305, 303 (M, 20%, 50%), 260 (100), 147 (35).

\textbf{3-Methoxy-2-(2'-methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.15a)}

and related compounds

2'-Methoxyphenylglyoxal (0.48 g) was added to a solution of 6-methylthiopyridazin-3-amine (II.2a) (0.14 g), ethanol (10 ml) and concentrated hydrochloric acid (0.07 ml) and the mixture was refluxed in an oil bath for 14 h. After chilling, the orange-red solid (0.54
g) was filtered off and stirred with excess ethereal diazomethane at 0°C for 2 h and then at 20°C for 16 h. The ether was evaporated, the residue subjected to t.l.c. (alumina; chloroform / light petroleum, 3:1), and the yellow oily product (0.12 g, 40%) recrystallised from light petroleum to give the title compound (0.067 g), m.p. 120-122°C (Found: C, 59.5; H, 5.2; N, 13.8. C₁₅H₁₅N₃O₂S requires C, 59.8; H, 5.0; N, 13.9%).

1H n.m.r.: δ 2.65, s, MeS; 3.88, s, 2’-OMe; 4.08, s, 3-OMe; 6.76, d, J 9.5 Hz, H 7; 6.95-7.66, complex, H 3’,4’,5’,6’; 7.63, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 302 (M+1, 8%), 301 (M, 48), 258 (100).

In a similar manner the following compounds were prepared from 6-methylthiopyridazin-3-amine (II.2a) and the appropriately substituted glyoxals.

3-Methoxy-2-(3’-methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.15b), (40%), m.p. 134-136°C, after t.l.c. (alumina; chloroform / light petroleum, 2:1) and recrystallisation from light petroleum (Found: C, 59.6; H, 5.0; N, 13.9. C₁₅H₁₅N₃O₂S requires C, 59.8; H, 5.0; N, 13.9%). 1H n.m.r.: δ 2.67, s, MeS; 3.91, s, 3’-OMe; 4.18, s, 3-OMe; 6.85, d, J 9.5 Hz, H 7; 6.80-7.75, complex, H 2’,4’,5’,6’; 7.72, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 302 (M+1, 9%), 301 (M, 52), 258 (100).

3-Methoxy-2-(4’-methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.15c), (33%), m.p. 97-99°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found, for a sample dried at 45°C / 0.1 mmHg for 6 h: C, 60.2; H, 4.9; N, 13.8. C₁₅H₁₅N₃O₂S requires C, 59.8; H, 5.0; N, 13.9%). 1H n.m.r.: δ 2.66, s, MeS; 3.85, s, 4’-OMe; 4.15, s, 3-OMe; 6.78, d, J 9.5 Hz, H 7; 6.90-8.11, complex, H 2’,3’,5’,6’; 7.61, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 302 (M+1, 9%), 301 (M, 50), 286 (21), 258 (100).
2-(2',4'-Dimethoxyphenyl)-3-methoxy-6-methylthioimidazo[1,2-b]pyridazine (II.15d), (61%), m.p. 91-92°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from light petroleum (Found, for a sample dried at 50°C / 0.1 mm Hg for 6 h: C, 58.0; H, 5.4; N, 12.4. C_{16}H_{17}N_{3}O_{3}S requires C, 58.0; H, 5.2; N, 12.7%). 1H n.m.r.: δ 2.66, s, MeS; 3.86, s, 3.88, s, 2,4-(OMe)_2; 4.08, s, 3-OMe; 6.46-6.67 and 7.50-7.61, complex, H 3',5',6'; 6.79, d, J 9.5 Hz, H 7; 7.70, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 332 (M+1, 9%), 331 (M, 50), 288 (100).

2-(3',4'-Dimethoxyphenyl)-3-methoxy-6-methylthioimidazo[1,2-b]pyridazine (II.15e), (61%), m.p. 111-113°C after t.l.c. (alumina; chloroform / light petroleum, 2:1) and recrystallisation from light petroleum (Found: C, 57.8; H, 5.2; N, 12.5. C_{16}H_{17}N_{3}O_{3}S requires C, 58.0; H, 5.2; N, 12.7%). 1H n.m.r.: δ 2.66, s, MeS; 3.93, s, 4.00, s, 3',4'-(OMe)_2; 4.16, s, 3-OMe; 6.79, d, J 9.5 Hz, H 7; 6.91-7.73, complex, H 2',5',6'; 7.61, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 332 (M+1, 11%), 331 (M, 61), 316 (21), 288 (100).

3-Methoxy-6-methylthio-2-(3',4',5'-trimethoxyphenyl)imidazo[1,2-b]pyridazine (II.15f), (20%), m.p. 123-125°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from light petroleum (Found, for a sample dried at 50°C / 0.1 mm Hg for 6 h: C, 56.5; H, 5.3; N, 11.5. C_{17}H_{19}N_{3}O_{4}S requires C, 56.5; H, 5.3; N, 11.6%). 1H n.m.r.: δ 2.67, s, MeS; 3.90, s, 4'-OMe; 3.97, s, 3',5'- (OMe)_2; 4.18, s, 3-OMe; 6.83, d, J 9.5 Hz, H 7; 7.43, s, H 2',6'; 7.67, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 362 (M+1, 12%), 361 (M, 63), 346 (25), 318 (100).

3-Methoxy-2-(3',4'-methylenedioxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.15g), (25%), m.p. 193-194°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 55.8; H, 4.1; N, 13.1. C_{15}H_{13}N_{3}O_{3}S. 0.4 H_{2}O requires C, 55.9; H, 4.3; N, 13.0%). 1H n.m.r.: δ 2.66, s, MeS; 4.15, s, 3-OMe; 6.00, s, OCH_2O; 6.79, d, J 9.5 Hz, H 7; 6.85-7.71, complex,
H 2',5',6'; 7.60, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 316 (M+1, 24%), 315 (M, 72), 272 (100).

6-(3',4',5'-Trimethoxybenzylthio)pyridazin-3-amine (II.7c)

A solution of 6-aminopyridazine-3-thiol (0.64 g) in 1 M sodium hydroxide (30.0 ml) was shaken with 3,4,5-trimethoxybenzyl chloride (1.08 g) and ethanol (30.0 ml) for 1.5 h. The mixture was then extracted with chloroform and the product (97%) recrystallised from toluene to give the title compound (44%), m.p. 148-150°C. (Found: C, 54.6; H, 5.6; N, 13.4. C_{14}H_{17}N_{3}O_{3}S requires C, 54.7; H, 5.6; N, 13.7%). \(^{1}\)H n.m.r.: 3.83, s, (OMe)\(_{3}\); 4.41, s, CH\(_{2}\)S; 6.62, s, H 2',6'; 6.71, d, J 9.5 Hz, H 4(5); 7.08, d, J 9.5 Hz, H 5(4).

3-Methoxy-2-(3',4'-methylenedioxyphenyl)-6-(3'',4'',5''-trimethoxybenzylthio)imidazo[1,2-b]pyridazine (II.9d) and related compounds

3',4'-Methylenedioxyphenylglyoxal ethanolate (0.10 g) in ethanol (8.0 ml) was added to a solution of 6-(3',4',5'-trimethoxybenzylthio)pyridazin-3-amine (II.7c) (0.15 g) in ethanol (2.0 ml) containing concentrated hydrochloric acid (0.07 ml) and the mixture was refluxed overnight. After cooling, water (20.0 ml) was added and the solvent evaporated to give a red / yellow oil. This oil was directly stirred with excess ethereal diazomethane at 0°C for 2 h, and then at 20°C overnight. The volatiles were removed and the oily residue was subjected to t.l.c. (alumina; chloroform / light petroleum, 4:1). The product (0.02 g, 8%) was recrystallised from hexane and gave yellow crystals of the title compound, m.p. 149-151°C (Found, for a sample dried at 100°C / 720 mmHg for 6 h: C, 59.5; H, 4.8; N, 8.7. C\(_{24}\)H\(_{13}\)N\(_{3}\)O\(_{6}\)S requires C, 59.9; H, 4.8; N, 8.7%). \(^{1}\)H n.m.r.: 3.83, s, 4"-OMe; 3.87, s, 3'',5''-(OMe)\(_{2}\); 4.13, s, 3-OMe; 4.43, s, CH\(_{2}\)S; 6.01, s, OCH\(_{2}\)O, 6.74, s, H 2",6"; 6.81, d, J 9.5 Hz, H 7; 6.96, s, H 2"; 7.61-7.70, complex, H 5',6'; 7.69, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 481 (M, 20%), 421 (40), 291 (60), 151 (100).
In a similar manner from 6-(substituted benzylthio)pyridazin-3-amines (II.7) and appropriately substituted phenylglyoxals the following compounds were prepared.

6-(2',3'-Dimethoxyphenyl)-3-methoxy-2-(p-methyl)imidazo[1,2-b]pyridazine (II.9a), (38%), m.p. 80-81°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from hexane (Found, for a sample dried at 50°C / 0.1 mmHg for 6 h: C, 65.8; H, 5.6; N, 10.0. \( \text{C}_{23}\text{H}_{23}\text{N}_{3}\text{O}_{3}\text{S} \) requires C, 65.5; H, 5.5; N, 10.0%). \(^1\)H n.m.r.: \( \delta \) 2.40, s, 4''-Me; 3.87, s, 3.94, s, 2',3'-(0Me)\(_2\); 4.13, s, 3-0Me; 4.53, s, CH\(_2\)S; 6.74-7.09, complex, H 7,4',5',6'; 7.29, d, J 8 Hz, H 2'',6''(3'',5''); 7.89, d, J 9.5 Hz, H 8; 8.03, d, J 8 Hz, H 3'',5''(2'',6''). Mass spectrum (e.i.) \( m/z \) 421 (M, 100%), 378 (85), 183 (55), 136 (52), 91 (50).

6-(3',4'-Dimethoxyphenyl)-3-methoxy-2-(p-methylphenyl)imidazo[1,2-b]pyridazine (II.9b), (33%), m.p. 134-136°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from hexane (Found: C, 65.7; H, 5.5; N, 10.0. \( \text{C}_{23}\text{H}_{23}\text{N}_{3}\text{O}_{3}\text{S} \) requires C, 65.5; H, 5.5; N, 10.0%). \(^1\)H n.m.r.: \( \delta \) 2.40, s, 4''-Me; 3.86, s, 3.89, s, 3',4'-(0Me)\(_2\); 4.14, s, 3-0Me; 4.55, s, CH\(_2\)S; 6.77, ct, J 9.5 Hz, H 7; 6.76-8.06, complex, H 2',5',6',2'',5'',6''; 7.64, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) \( m/z \) 421 (M, 45%), 151 (100).

6-(3',4'-Dimethoxybenzylthio)-3-methoxy-2-(3'',4''-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (II.9c), (22%), m.p.123-125°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from cyclohexane (Found: C, 61.1; H, 4.7; N, 9.1. \( \text{C}_{23}\text{H}_{21}\text{N}_{3}\text{O}_{5}\text{S} \) requires C, 61.2; H, 4.7; N, 9.3%). \(^1\)H n.m.r.: \( \delta \) 3.86, s, 3.89, s, 3',4'-(0Me)\(_2\); 4.14, s, 3-0Me; 4.45, s, CH\(_2\)S; 6.01, s, OCH\(_2\)O, 6.75-7.72, complex, H 2',5',6',2'',5'',6''; 6.80, d, J 9.5 Hz, H 7; 7.67, d, J 9.5 Hz, H 8.

3-Methoxy-6-(3',4''-methylenedioxybenzylthio)-2-(3'',4''-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (II.9e), (23%), m.p. 111-112°C, after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum (b.p. 40-60°C) (Found: C, 60.5;
H, 3.8; N, 9.4. C_{22}H_{17}N_{3}O_{5}S requires C, 60.7; H, 3.9; N, 9.6%. \textsuperscript{1}H n.m.r.: \delta 4.11, s, 3-OMe; 4.40, s, CH_{2}S; 5.93, s, 5.99, s, (OCH_{2}O)_{2}; 6.74, d, J 9.5 Hz, H 7; 6.78-7.69, complex, H 2',5',6';2'',5'';6''; 7.59, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 435 (M, 70%), 392 (50), 135 (100).

\textbf{α-Bromo-(2' and 3')-methoxyacetophenones}

Bromination of 2'- and 3'-methoxyacetophenones in anhydrous ether in the presence of aluminium chloride by the procedure of Cowper and Davidson \textsuperscript{301} for the preparation of α-bromoacetophenone gave the required compounds as follows:

2'-methoxy: \textsuperscript{1}H n.m.r. (CDCl_{3}); \delta 3.95, s, OMe; 4.60, s, CH_{2}; 6.95-7.80, complex, ArH.

3'-methoxy: \textsuperscript{1}H n.m.r. (CDCl_{3}); \delta 3.87, s, OMe; 4.44, s, CH_{2} 7.00-7.53, complex, ArH.

\textbf{2-(4'-Methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.12c) and related compounds}

A mixture of 6-methylthiopyridazin-3-amine (II.2a) (0.28 g) and α-bromo-4'-methoxyacetophenone (0.46 g) in ethanol (20.0 ml) was refluxed for 2 h, then sodium hydrogen carbonate (0.16 g) was added and the refluxing was continued for 4 h. The ethanol was removed \textit{in vacuo} and the residue extracted with chloroform, the extract was washed with water, dried (Na_{2}SO_{4}), and the solvent evaporated. The product was recrystallised from ethanol and gave the \textit{title compound} (0.32 g; 60%), m.p. 162-164°C (Found: C, 61.7; H, 4.8; N, 15.2. C_{14}H_{13}N_{3}OS requires C, 62.0; H, 4.8; N, 15.5). \textsuperscript{1}H n.m.r.: \delta 2.62, s, MeS; 3.86, s, 4'-OMe; 6.87, d, J 9.5 Hz, H 7; 6.98, d, J 9 Hz, H 3';5'(2',6'); 7.75, d, J 9.5 Hz, H 8; 7.86, d, J 9 Hz, H 2',6'(3',5'); 8.07, s, H 3. Mass spectrum (e.i.) m/z 271 (M,100%), 224 (50), 119 (35).

In a similar manner the following compounds were prepared from 6-alkylthiopyridazin-3-amines (II.2) and the appropriately substituted α-bromoacetophenones.
2-(2'-Methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.12a), (63%), m.p. 118-120°C, after recrystallisation from ethanol (Found: C, 61.9; H, 4.9; N, 15.7. C_{14}H_{13}N_{3}OS requires C, 62.0; H, 4.8; N, 15.5%). \textsuperscript{1}H n.m.r.: \& 2.64, s, MeS; 4.01, s, 2'-OMe; 6.86, d, J 9.5 Hz, H 7; 6.92-7.60 and 8.31-8.41, complex, H 3',4',5',6'; 7.76, d, J 9.5 Hz, H 8; 8.48, s, H 3. Mass spectrum (e.i.) m/z 271 (M, 100%), 224 (30), 194 (33), 166 (25), 91 (20).

2-(3'-Methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.12b), (85%), m.p. 92-94°C, after recrystallisation from light petroleum (Found, for a sample dried at 70°C / 0.1 mmHg for 6 h: C, 61.9; H, 4.9; N, 15.5. C_{14}H_{13}N_{3}OS requires C, 62.0; H, 4.8; N, 15.5%). \textsuperscript{1}H n.m.r.: \& 2.64, s, MeS; 3.90, s, 3'-OMe; 6.86, d, J 9.5 Hz, H 7; 6.81-7.65, complex, H 2',4',5',6'; 7.72, d, J 9.5 Hz, H 8; 8.16, s, H 3. Mass spectrum (e.i.) m/z 271 (M,100%), 224 (30), 141 (30).

6-Ethylthio-2-phenylimidazo[1,2-b]pyridazine (II.12d), (92%), m.p. 111-113°C, after recrystallisation from light petroleum (Found: C, 66.0; H, 5.1; N, 16.4. C_{14}H_{13}N_{3}S requires C, 65.9 H, 5.1; N, 16.5%). \textsuperscript{1}H n.m.r.: \& 1.45, t, J 7.5 Hz, Me; 3.22, q, J 7.5 Hz, CH_{2}Me; 6.83, d, J 9.5 Hz, H 7; 7.30-8.00, complex, Ph; 7.71, d, J 9.5 Hz, H 8; 8.15, s, H 3. Mass spectrum (e.i.) m/z 255 (M, 100%), 222 (35), 194 (65), 89 (30).

6-Ethylthio-2-(3'-methoxyphenyl)imidazo[1,2-b]pyridazine (II.12e), (93%), m.p. 109-111°C, (from light petroleum) (Found, for a sample dried at 70°C / 0.1 mmHg for 6 h: C, 62.9; H, 5.3; N, 14.9. C_{15}H_{15}N_{3}OS requires C, 63.1 H, 5.3; N, 14.7%). \textsuperscript{1}H n.m.r.: \& 1.45, t, J 7.5 Hz, CH_{3}; 3.22, q, J 7.5 Hz, CH_{2}CH_{3}; 3.90, s, 3'-OMe; 6.83, d, J 9.5 Hz, H 7; 6.94-7.56, complex, H 2',4',5',6'; 7.72, d, J 9.5 Hz, H 8; 8.15, s, H 3. Mass spectrum (e.i.) m/z 285 (M, 100%), 224 (35), 181 (20), 96 (20).
6-Ethylthio-2-(4'-methoxyphenyl)imidazo[1,2-b]pyridazine (II.12f), (81%).
m.p. 137-139°C, (from ethanol) (Found: C, 63.4; H, 5.4; N, 14.6. C_{15}H_{15}N_{3}OS
requires C, 63.1 H, 5.3; N, 14.7%). ¹H n.m.r.: δ 1.45, t, J 7.5 Hz, Me; 3.22, q, J 7.5 Hz,
CH₂Me; 3.86, s, 4'-OMe; 6.81, d, J 9.5 Hz, H 7; 6.98, d, J 9 Hz, H 3',5'(2',6'); 7.69, d, J 9.5 Hz, H 8; 7.87, d, J 9 Hz, H 2',6'(3',5'); 8.07, s, H 3. Mass spectrum (e.i.) m/z 285
(M, 100%), 224 (60), 119 (45).

2-(2'-Methoxyphenyl)-6-propylthioimidazo[1,2-b]pyridazine (II.12g), (90%).
m.p. 120-122°C, after recrystallisation from a mixture of acetone and light petroleum
(Found: C, 63.9; H, 5.5; N, 13.8. C_{16}H_{17}N_{3}OS requires C, 64.2; H, 5.7; N, 14.0%). ¹H n.m.r.: δ 1.10, t, J 7.5 Hz, CH₃CH₂CH₂; 1.83, q, J 7.5 Hz, CH₃CH₂CH₂; 3.21, t, J 7.5 Hz, CH₃CH₂CH₂; 4.01, s, 2'-OMe; 6.81-7.44 and 8.31-8.41, complex, H 7,3',4',5',
6'; 7.81, d, J 9.5 Hz, H 8; 8.44, s, H 3.

2-(3'-Methoxyphenyl)-6-propylthioimidazo[1,2-b]pyridazine (II.12h), (60%).
m.p. 68-70°C, after recrystallisation from light petroleum (Found, for a sample dried at
30°C / 1 mmHg for 6 h: C, 64.2; H, 5.9; N, 14.2. C_{16}H_{17}N_{3}OS requires C, 64.2; H,
5.7; N, 14.0%). ¹H n.m.r.: δ 1.09, t, J 7.5 Hz, CH₃CH₂CH₂; 1.83, q, J 7.5 Hz,
CH₃CH₂CH₂; 3.21, t, J 7.5 Hz, CH₃CH₂CH₂; 3.90, s, 3'-OMe; 6.87, d, J 9.5 Hz, H 7;
6.94-7.57, complex, H 2',4',5',6'; 7.77, d, J 9.5 Hz, H 8; 8.14, s, H 3.

2-(4'-Methoxyphenyl)-6-propylthioimidazo[1,2-b]pyridazine (II.12i) (63%).
m.p. 138-140°C, after recrystallisation from ethanol (Found: C, 64.3; H, 5.8; N, 13.8.
C_{16}H_{17}N_{3}OS requires C, 64.2; H, 5.7; N, 14.0%). ¹H n.m.r.: δ 1.09, t, J 7.5 Hz,
CH₃CH₂CH₂; 1.82, q, J 7.5 Hz, CH₃CH₂CH₂; 3.19, t, J 7.5 Hz, CH₃CH₂CH₂; 3.86,
s, 4'-OMe; 6.85, d, J 9.5 Hz, H 7; 6.98, d, J 9 Hz, H 3',5'(2',6'); 7.74, d, J 9.5 Hz, H 8;
7.88, d, J 9 Hz, H 2',6'(3',5'); 8.07, s, H 3.
6-Propylthio-2-(3',4',5'-trimethoxyphenyl)imidazo[1,2-b]pyridazine (II.12j), (63%), m.p. 126-127°C, after recrystallisation from light petroleum (Found: C, 60.1; H, 5.8; N, 11.5. C₁₈H₂₁N₃O₃S requires C, 60.1; H, 5.9; N, 11.7%). ¹H n.m.r.: δ 1.09, t, J 7.5 Hz, CH₃CH₂CH₂; 1.82, q, J 7.5 Hz, CH₃CH₂CH₂; 3.19, t, J 7.5 Hz, CH₃CH₂CH₂; 3.89, s, 4'-OMe; 3.96, s, 3',5'-(OMe)₂; 6.86, d, J 9.5 Hz, H 7; 7.19, s, H 2',6'; 7.74, d, J 9.5 Hz, H 8; 8.12, s, H 3.

6-Ethylthio-2-(p-methylphenyl)imidazo[1,2-b]pyridazine (II.12k), (76%), m.p. 100-103°C, after recrystallisation from a mixture of acetone and light petroleum (Found, for a sample dried at 60°C / 1 mmHg for 6 h: C, 67.0; H, 5.5; N, 15.7. C₁₆H₁₇N₃S requires C, 66.9; H, 5.6; N, 15.6%). ¹H n.m.r.: δ 1.44, t, J 7.5 Hz, CH₃CH₂; 2.39, s, 4'-Me; 3.21, q, J 7.5 Hz, CH₃CH₂; 6.81, d, J 9.5 Hz, H 7; 7.25, d, J 9Hz, H 3',5'(2',6'); 7.70, d, J 9.5 Hz, H 8; 7.83, d, J 9 Hz, H 2',6'(3',5'); 8.11, s, H 3.

2-(3',4'-Dimethylphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.12l), (89%), m.p. 158-160°C, after recrystallisation from a mixture of acetone and light petroleum (Found: C, 66.6; H, 5.6; N, 15.7. C₁₅H₁₅N₃S requires C, 66.9; H, 5.6; N, 15.6%). ¹H n.m.r.: δ 2.30, s, 2.33, s, 3',4'-Me₂; 2.61, s, MeS; 6.83, d, J 9.5 Hz, H 7; 7.14-7.69, complex, H 2',5',6'; 7.69, d, J 9.5 Hz, H 8; 8.12, s, H 3.

2-(3',4'-Dimethylphenyl)-6-ethylthioimidazo[1,2-b]pyridazine (II.12m), (84%), m.p. 85-88°C, after recrystallisation from a mixture of acetone and light petroleum (Found, for a sample dried at 55°C / 1 mmHg for 5 h: C, 68.0; H, 6.0; N, 14.9. C₁₆H₁₇N₃S requires C, 67.8; H, 6.0; N, 14.8%). ¹H n.m.r.: δ 1.48, t, J 7.5 Hz, CH₃CH₂; 2.32, s, 2.36, s, 3',4'-Me₂; 3.26, q, J 7.5 Hz, CH₃CH₂; 7.15, d, J 9.5 Hz, H 7; 7.28-7.81, complex, H 2',5',6'; 8.09, s, H 3; 8.42, d, J 9.5 Hz, H 8.

2-(3',4'-Dimethylphenyl)-6-propylthioimidazo[1,2-b]pyridazine (II.12n), (93%), m.p. 99-102°C, after recrystallisation from a mixture of toluene and light petroleum and from light petroleum (Found, for a sample dried at 40°C / 1 mmHg for 6 h: C, 68.7; H,
6-Ethylthio-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (II.12o). (98%), m.p. 224-226°C, after recrystallisation from ethanol (Found: C, 60.4; H, 4.4; N, 14.0. C15H13N3O2S requires C, 60.2; H, 4.4; N, 14.0%). ¹H n.m.r.: δ 1.44, t, J 7.5 Hz, CH3CH2; 3.21, q, J 7.5 Hz, CH3CH2; 6.00, s, OCH2O; 6.82, d, J 9.5 Hz, H 7; 6.83-7.50, complex, H 2',5',6'; 7.68, d, J 9.5 Hz, H 8; 8.03, s, H 3.

2-(3',4'-Methylenedioxyphenyl)-6-propylthioimidazo[1,2-b]pyridazine (II.12p). (64%), m.p. 115-117°C, after recrystallisation from light petroleum (Found: C, 60.8; H, 4.7; N, 13.3. C16H15N3O2S requires C, 61.3; H, 4.8; N, 13.4%). ¹H n.m.r.: δ 1.09, t, J 7.5 Hz, CH3CH2CH2; 1.81, q, J 7.5 Hz, CH3CH2CH2; 3.18, t, J 7.5 Hz, CH3CH2CH2; 6.00, s, OCH2O; 6.82, d, J 7.5 Hz, H 7; 6.83-7.50, complex, H 2',5',6'; 7.68, d, J 9.5 Hz, H 8; 8.03, s, H 3.

3-Benzamidomethyl-2-(4'-methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.13a)

A mixture of N-(hydroxymethyl)benzamide(0.13 g), glacial acetic acid (5.0 ml) and concentrated sulphuric acid (0.09 ml) was heated in an oil bath at 50°C for 15 min, then 2-(4-methoxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.12c) (0.16 g) was added and the mixture refluxed at 120°C for 24 h. The acetic acid was removed in vacuo, the residue diluted with water, adjusted with aqueous ammonia to pH 10, and extracted with chloroform. The extract was washed with water, dried (Na2SO4) and solvent evaporated to give a waxy solid which was subjected to t.l.c. (alumina; chloroform / light petroleum, 2:1). The fluorescent band at Rf 0.4 was extracted with chloroform and the product recrystallised from toluene to give the title compound (0.14 g, 59%), m.p. 210-212°C (Found: C, 65.1; H, 5.0; N, 13.6. C22H20N4O2S requires C, 65.3; H, 5.0; N, 13.9. ¹H
n.m.r.: δ 2.61, s, MeS; 3.85, s, 4'-OMe; 5.23, d, J 5.5 Hz, CH₂N; 6.89, d, J 9.5 Hz, H 7; 7.03-7.89, complex, H 2',3',5',6' and Ph; 7.69, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 404 (M, 6%), 299 (40), 105 (100), 77 (71)

In a similar manner the following compounds were obtained from the relevant 3-unsubstituted imidazo[1,2-b]pyridazines (II.12a-p) described previously.

3-Benzamidomethyl-6-ethylthio-2-phenylimidazo[1,2-b]pyridazine (11.13b), (74%), m.p. 206-208°C, after t.l.c. (alumina; chloroform / light petroleum, 2:1) and recrystallisation from toluene (Found: C, 68.4; H, 5.1; N, 14.3. C₂₂H₂₀N₄O₂S requires C, 68.0; H, 5.2; N, 14.4%). ¹H n.m.r.: δ 1.40, t, J 7.5 Hz, Me; 3.19, q, J 7.5 Hz, CH₂Me; 5.22, d, J 5.5 Hz, CH₂N; 6.85, d, J 9.5 Hz, H 7; 7.38-7.92, complex, 2 x Ph; 7.66, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 388 (M, 5%), 283 (53), 105 (100), 77 (68).

3-Benzamidomethyl-6-ethylthio-2-(4'-methoxyphenyl)imidazo[1,2-b]pyridazine (11.13c), (39%), m.p. 180-181°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 65.0; H, 5.4; N, 13.0. C₂₃H₂₂N₄O₂S.0.4H₂O requires C, 64.9; H, 5.4; N, 13.2%). ¹H n.m.r.: δ 1.41, t, J 7.5 Hz, Me; 3.19, q, J 7.5 Hz, CH₂Me; 3.84, s, 4'-OMe; 5.20, d, J 5.5 Hz, CH₂N; 6.85, d, J 9.5 Hz, H 7; 6.98, d, J 9 Hz, H 3',5'(2',6'); 7.40-7.87, complex, Ph; 7.66, d, J 9.5 Hz, H 8; 7.82, d, J 9 Hz, H 2',6'(3',5'). Mass spectrum (e.i.) m/z 419 (M+1, 2%), 418 (M, 9) , 313 (39), 105 (100), 77 (52).

3-Benzamidomethyl-2-(4'-methoxyphenyl)-6-propylthioimidazo[1,2-b]pyridazine (II.13d), (23%), m.p. 173-174°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 66.3; H, 5.6; N, 12.8. C₂₄H₂₄N₄O₂S requires C, 66.6; H, 5.6; N, 13.0%). ¹H n.m.r.: δ 1.00, t, J 7.5 Hz, CH₃CH₂CH₂; 1.78, q, J 7.5 Hz, CH₃CH₂CH₂; 3.15, t, J 7.5 Hz, CH₃CH₂CH₂; 3.83, s, 4'-OMe; 5.18, d, J 5.5 Hz, CH₂N; 6.82, d, J 9.5 Hz, H 7; 6.95, d, J 9Hz, H 3',5'(2',6'); 7.39-7.91, complex,
H 8,2',6'(3',5') and Ph. Mass spectrum (e.i.) m/z 433 (M+1, 3%), 432 (M, 9), 327 (36), 105 (100), 77 (9).

3-Benzamidomethyl-6-ethylthio-2-(p-methylphenyl)imidazo[1,2-b]pyridazine (II.13e), (40%), m.p. 206-208°C, after recrystallisation from ethanol (Found: C, 68.4; H, 5.8; N, 13.8. C23H22N4OS requires C, 68.6; H, 5.5; N, 13.9%). 1H n.m.r.: δ 1.41, t, J 7.5 Hz, CH3CH2; 2.40, s, 4'-Me; 3.20, q, J 7.5 Hz, CH3CH2; 5.23, d, J 5.5 Hz, CH2N; 6.87, d, J 9.5 Hz, H 7; 7.23-7.84, complex, H 2',3',5',6' and Ph; 7.76, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 402 (M, 5%), 297 (46), 105 (100), 77 (61).

3-Benzamidomethyl-2-(3',4'-dimethylphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.13f), (12%), m.p. 218-220°C, after t.l.c. (alumina; chloroform / light petroleum, 1:1) and recrystallisation from toluene (Found: C, 68.0; H, 5.6; N, 13.7. C23H22N4OS requires C, 68.6; H, 5.5; N, 13.9%). 1H n.m.r.: δ 2.27, s, 3',4'-Me2; 2.56, s, CH3S; 5.20, d, J 5.5 Hz, CH2N; 6.80, d, J 9.5 Hz, H 7; 7.10-7.89, complex, H 2',5',6' and Ph, 7.59, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 402 (M, 7%), 298(10), 297 (52), 105 (100), 77 (7).

3-Benzamidomethyl-2-(3',4'-dimethylphenyl)-6-ethylthioimidazo[1,2-b]pyridazine (II.13g), (44%), m.p. 185-187°C, after t.l.c. (alumina; chloroform / light petroleum, 2:1) and recrystallisation from toluene (Found: C, 68.8; H, 5.8; N, 13.4. C24H24N4OS requires C, 69.2; H, 5.8; N, 13.5%). 1H n.m.r.: δ 1.38, t, J 7.5 Hz, CH3CH2; 2.24, s, 2.27, s, 3',4'-Me2; 3.15, q, J 7.5 Hz, CH3CH2; 5.18, d, J 5.5 Hz, CH2N; 6.74, d, J 9.5 Hz, H 7; 7.06-8.00, complex, H 8,2',5',6' and Ph. Mass spectrum (e.i.) m/z 416 (M, 8%), 312(9), 311 (46), 105 (100), 77 (53).

3-Benzamidomethyl-2-(3',4'-dimethylphenyl)-6-propylthioimidazo[1,2-b]pyridazine (II.13h), (80%), m.p. 177-179°C, after t.l.c. (alumina; chloroform / light petroleum, 2:1) and recrystallisation from toluene (Found: C, 69.8; H, 6.2; N, 13.0. C25H26N4OS requires C, 69.7; H, 6.1; N, 13.0%). 1H n.m.r.: δ 0.99, t, J 7.5 Hz.
\[ \text{CH}_3\text{CH}_2\text{CH}_2; 1.77, \text{q}, J 7.5 \text{ Hz}, \text{CH}_3\text{CH}_2\text{CH}_2; 2.27, \text{s}, 3',4'-\text{Me}_2; 3.14, \text{t}, J 7.5 \text{ Hz}, \text{CH}_3\text{CH}_2\text{CH}_2; 5.19, \text{d}, J 5.5 \text{ Hz}, \text{CH}_2\text{N}; 6.79, \text{d}, J 9.5 \text{ Hz}, \text{H 7}; 6.97-7.92, \text{complex, H 2',5',6' and Ph}; 7.59, \text{d}, J 9.5 \text{ Hz}, \text{H 8}. \] Mass spectrum (e.i.) \text{m/z} 430 (M, 6%), 326(8), 325 (41), 105 (100), 77 (54).

3-Benzamidomethyl-6-ethylthio-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-b]-pyridazine (II.13i), (6%), m.p. 230-232°C, after t.l.c. (alumina; chloroform / light petroleum, 2:1) and recrystallisation from toluene (Found: C, 64.2; H, 4.7; N, 12.7. \( \text{C}_{23}\text{H}_{20}\text{N}_4\text{O}_3\text{S} \) requires C, 63.9; H, 4.7; N, 13.0%). \text{H n.m.r.: } \delta 1.39, \text{t}, J 7.5 \text{ Hz}, \text{CH}_3\text{CH}_2; 3.18, \text{q}, J 7.5 \text{ Hz}, \text{CH}_3\text{CH}_2; 5.17, \text{d}, J 5.5 \text{ Hz}, \text{CH}_2\text{N}; 5.99, \text{s}, \text{OCH}_2\text{O}; 6.83, \text{d}, J 9.5 \text{ Hz}, \text{H 7}; 6.81-7.90, \text{complex, H 2',5',6' and Ph}; 7.61, \text{d}, J 9.5 \text{ Hz}, \text{H 8}. \] Mass spectrum (e.i.) \text{m/z} 432 (M, 8%), 328(6), 327 (34), 105 (100), 77 (66).

3-Benzamidomethyl-2-(3',4'-methylenedioxyphenyl)-6-propylthioimidazo[1,2-b]-pyridazine (II.13j) (74%), m.p. 200-201°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 64.6; H, 4.8; N, 12.5. \( \text{C}_{24}\text{H}_{22}\text{N}_4\text{O}_3\text{S} \) requires C, 64.6; H, 5.0; N, 12.5%). \text{H n.m.r.: } \delta 1.00, \text{t}, J 7.5 \text{ Hz}, \text{CH}_3\text{CH}_2\text{CH}_2; 1.78, \text{d}, J 7.5 \text{ Hz}, \text{CH}_3\text{CH}_2\text{CH}_2; 3.16, \text{t}, J 7.5 \text{ Hz}, \text{CH}_3\text{CH}_2\text{CH}_2; 5.17, \text{d}, J 5.5 \text{ Hz}, \text{CH}_2\text{N}; 6.00, \text{s}, \text{OCH}_2\text{O}; 6.86, \text{d}, J 9.5 \text{ Hz}, \text{H 7}; 6.83-7.87, \text{complex, H 2',5',6' and Ph}; 7.63, \text{d}, J 9.5 \text{ Hz}, \text{H 8}.

II-5.3 [\text{3}\text{H}]\text{Diazepam in vitro BZR binding assay}

Young adult male Wistar rats were decapitated and their brains removed and immediately placed on ice. The washed synaptosomal membranes used in the \text{in vitro} assay were prepared from P2 pellets, in accordance with a previously published procedure.\textsuperscript{302} The pellets were washed by repeated centrifugation and resuspension in Tris-citrate buffer and were then stored frozen until use. On the day of assay, the membrane preparations were thawed, washed once by suspension in ice-cold distilled water and centrifugation at 17000 rpm for 30 minutes, then the supernatant liquid was decanted and the pellet resuspended in 50 mM Tris-HCl buffer, pH 7.4 at 2°C.
The receptor binding assay contained aliquots of the rat brain membrane suspension (approximately 0.8 mg protein), various concentrations of the test compounds, 100 µM GABA (to stimulate ligand binding to the BZR in the rat brain membranes) and [3H] diazepam (86.6 Ci / mmol, 0.70 ± 0.05 nM final concentration) in a final volume of 2 ml of Tris-HCl buffer. The assays were incubated with the tritiated diazepam on ice at 0-4°C for 35 minutes. Nonspecific binding was determined in separate tubes by the addition of a large excess (10 µM) of unlabelled diazepam. The test compounds were initially tested at a single concentration, and those compounds found to demonstrate significant displacement of [3H] diazepam binding were then tested at a later date at four separate concentrations. The membranes were collected after the incubation period by filtration under vacuum on glass-fibre filters (Whatman GF/B, 2.5cm) and washed with 12 ml of ice-cold buffer. Filters were placed in scintillation vials with 1.2 ml of toluene/Triton X-100 scintillation fluid and bound radioactivity was determined using an LKB-Wallac Rack Beta II Liquid Scintillation Counter.

The test compounds were initially dissolved in dimethyl sulphoxide (DMSO) to give 4 mM stock solutions which were then diluted with buffer (or DMSO/buffer) and immediately added to the assay tubes. For the determination of IC50 values (the concentration of test compound causing 50% displacement of [3H] diazepam binding to the rat brain membranes under the standard assay conditions) the imidazo[1,2-b]-pyridazines were tested at four separate concentrations (spaced logarithmically eg 10, 30, 100, 300 nM) and within each experiment all assays were performed in triplicate. For each concentration of test compounds, the results were calculated as the percentage displacement of specific binding, where specific binding was taken as the amount of radioactive diazepam bound in control tubes (without inhibitor ligand) minus the amount bound in the presence of excess unlabelled diazepam. The IC50 values were calculated for each test compound using computer assisted log-logit analysis (Mathcad 2.52, Mathsoft Inc.), and the correlation coefficient of the lines of best fit to log-logit curves was not less than 0.95.
CHAPTER III

Synthesis and BZR affinities of some 6-(alkylthio and chloro)-2-aryl-
benzimidazoles, benzamidomethyl and o-fluorobenzamidomethyl-6-aryl-1,2,3-
triazoles

INTRODUCTION

Studies that have been developed for the BZR pharmacophore (discussed in
Chapter II) generally require a number of centres for hydrogen bonding and lipophilic
interactions between ligand and receptor. Areas of steric hindrance have also been
identified, which if occupied by a ligand result in a decrease in its BZR activity.

In the work reported here a series of imidazo[1,2-b]pyridazines containing linearly
stereogenic groups of varying size from methylen to ethoxycarbonyl has been synthesised.
The introduction of the 5- and 3-positions of the molecules were accomplished through the
formation of an amide bond to the benzamidomethyl group. This allowed the steric requirements of the BZR ligand binding	
site to be realised. The 6-position group of the compound was kept constant.

In this chapter, the preparation of some 6-(alkylthio and chloro)-2-aryl-
benzimidazoles, benzamidomethyl and o-fluorobenzamidomethyl-2-aryl-
imidazoles (1-3) is described. The 3-(o-fluorobenzamidomethyl) compounds were synthesised as other
derivatives containing this substituent showed promising results in in vivo studies with antidepressive activity, whereas isomeric compounds with the same
structure had no antidepressive activity. Further work has been performed on these compounds. The compounds
reported here have been characterised by 1H n.m.r. and mass spectra and elemental analyses
and results of in vitro BZR binding assays involving these compounds are reported and
discussed. Where appropriate, structure-activity relationships are identified. Finally the
experimental details of the preparation of the compounds are reported.

RESULTS AND DISCUSSION

The compounds reported in this work were synthesised from the well-studied
reactive pyridazin-3-amine (Scheme III-1). The 6-ArHNCOOC(ethylamino) and
o-fluorobenzamidomethyl 6-chloropyridazin-3-amine (III-1) by nucleophilic displacement of the
chloro group by alkylation ions. In this way 6-alkylthio and 6-alkylarylsubstituted
CHAPTER III  Syntheses and BZR affinities of some 6-(alkoxy and chloro)-2-aryl-3-(unsubstituted, benzamidomethyl and o-fluorobenzamidomethyl)imidazo[1,2-b]-pyridazazines

III-1 Introduction

Models that have been developed for the BZR pharmacophore (discussed in Chapter I-2.5) generally require a number of centres for hydrogen bonding and lipophilic interaction between ligand and receptor. Areas of steric hindrance have also been defined, which if occupied by a ligand result in a decrease in its BZR affinity.

In the work reported here a series of imidazo[1,2-b]pyridazines containing linear 6-alkoxy groups of varying size from methoxy to ethoxyethoxy has been synthesised. The substituents in the 3- and 2-positions of the molecules were maintained constant so that any changes in BZR affinity could be directly related to the changes in the properties of the 6-position group. This allowed the steric requirements of the BZR ligand binding site in the vicinity of the 6-position group of the compounds to be examined.

In this chapter the preparation of some 6-(alkylthio and chloro)-3-(unsubstituted, benzamidomethyl and o-fluorobenzamidomethyl)-2-arylimidazo[1,2-b]pyridazines are reported. The 3-(o-fluorobenzamidomethyl) compounds were synthesised as other compounds containing this substituent showed promising results in in vivo tests with rats for anxiolytic activity, whereas isomeric compounds were less active. The compounds prepared have been characterised by $^1$H n.m.r. and mass spectra and elemental analyses. The results of in vitro BZR binding assays involving the compounds are reported and discussed. Where appropriate, structure-activity relationships are identified. Finally the experimental details of the preparation of the compounds are recorded.

III-2 Syntheses

The compounds reported in this work were synthesised from the appropriately substituted pyridazin-3-amines (Scheme III-1). The 6-alkoxypyridazin-3-amines were prepared from 6-chloropyridazin-3-amine (III.1) by nucleophilic displacement of the 6-chloro group by alkoxide ions. In this way 6-(methoxy and ethoxy)pyridazin-3-amine
Scheme III-1

Reagents: (i) NaOR, 130-150°C (ii) BrCH₂COC₆H₄Cl-p, NaHCO₃, EtOH, 100°C (iii) BrCH₂COC₆H₄Rᵢ, NaHCO₃, EtOH, 100°C (iv) R₂C₆H₄CONHCH₂OH, conc H₂SO₄, AcOH, 100-120°C

III.6 R R₁ R₂
a Me OCH₂O (3,4) H
b Me Cl-p H
C Me H F-o
d Me Me-p F-o
e Me OCH₂O (3,4) F-o
f Et H H
G Et Me-p H
h Et OCH₂O (3,4) H
I Et Cl-p H
j Et H F-o
k Et Me-p F-o
l Et OCH₂O (3,4) F-o
m Pr H H
n Pr Me-p F-o
o Pr OCH₂O (3,4) H
p Pr Cl-p H
q Pr H F-o
r Pr Me-p F-o
s CH₂CH₂OMe H H
t CH₂CH₂OMe Me-p H
u CH₂CH₂OMe H H
(III.2a,b) were synthesised with properties consistent with those reported in the literature.\textsuperscript{304} The novel compounds 6-(propoxy, 2-methoxyethoxy and 2-ethoxyethoxy)pyridazin-3-amine (III.2c-e) were similarly prepared by Mr S.J. Ireland.

The 6-(chloro and alkoxy)pyridazin-3-amines (III.1 and 2) were condensed with substituted α-bromoacetophenones to form the 6-(chloro and alkoxy)-2-arylimidazo[1,2-b]pyridazines (III.3 and III.4a-m respectively) in yields of 24-69% using a modification of literature procedures.\textsuperscript{201} Compounds (III.3) and (III.4a-m) then underwent electrophilic substitution at the 3-position by heating with N-hydroxymethylbenzamide or o-fluoro-N-hydroxymethylbenzamide to afford the corresponding 3-(benzamidomethyl and o-fluorobenzamidomethyl)imidazo[1,2-b]pyridazines (III.5) and (III.6a-u) using reaction conditions previously reported for other similar compounds.\textsuperscript{267} The final products were purified by chromatography and recrystallisation in yields of 19-84%.

### III-3 Physical properties

The novel compounds synthesised in this chapter were examined by $^1$H n.m.r. spectroscopy at 90 MHz as recorded in the Experimental Section. Some $^1$H n.m.r. spectral data for compounds (III.3 and 4) and (III.5 and 6) are shown in Tables III-1 and III-2 respectively.

The results in Table III-1 for the 3-unsubstituted imidazo[1,2-b]pyridazines show that the $^1$H n.m.r. spectra of these compounds were characterised by a downfield singlet ($\delta$ 7.91-8.19) corresponding to the H-3 signal. For the 6-alkoxy-3-unsubstituted compounds (III.4a-m) this signal was in the range $\delta$ 7.91-8.02. The chemical shift of the H-3 signal was affected by the substituent present in the 2-aryl group by a modest, though consistent, degree. For example, for the 6-ethoxy-3-unsubstituted compounds (III.4a-f) $p$-methyl and $p$-chloro substitution of the 2-aryl group led to a small upfield shift of the H-3 signal when compared to the 2-phenyl analogue (III.4d and III.4f vs. III.4c), and (3,4-methylenedioxyphenyl) substitution of the 2-aryl group led to a greater upfield shift of the H-3 signal of ca $\delta$ 0.1 when compared to the 2-phenyl compound (III.4e vs. III.4c).
Table III-1 Some \textsuperscript{1}H n.m.r. spectral data (δ)\textsuperscript{A} for 6-alkoxy-2-aryl-3-unsubstituted imidazo[1,2-b]pyridazines

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R</th>
<th>R\textsubscript{1}</th>
<th>H-3</th>
<th>H-7</th>
<th>H-8</th>
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<td>OPr</td>
<td>H</td>
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<td>B</td>
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<td>6.66</td>
<td>7.79</td>
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<td>OPr</td>
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<td>7.91</td>
<td>6.65</td>
<td>7.76</td>
</tr>
<tr>
<td>4j</td>
<td>OPr</td>
<td>Cl-\textsubscript{p}</td>
<td>7.98</td>
<td>6.67</td>
<td>7.76</td>
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<td>6.74</td>
<td>B</td>
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<td>6.72</td>
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<td>H</td>
<td>8.02</td>
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A: Reported as parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard in deuterochloroform.
B: Complex signal

The chemical shift of the H-3 signal for the 6-alkoxy-3-unsubstituted imidazo[1,2-b]pyridazines was upfield relative to the 6-alkylthio analogues (II.12) reported in Section II-3.1. The 6-chloro-3-unsubstituted compound (III.3) gave an H-3 signal (δ 8.19) that was downfield relative to the H-3 signal of the 6-(methoxy, ethoxy and propoxy) analogues (III.4b,f,j).
Table III-2 Some $^1$H n.m.r. spectral data ($\delta$)$^A$ for 6-(alkoxy and chloro)-2-aryl-3-
(benzamidomethyl and substituted benzamidomethyl)imidazo[1,2-\(b\)]pyridazines

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<td>6.72</td>
<td>7.82</td>
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<td>6e</td>
<td>OMe</td>
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<td>7.82</td>
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<td>H</td>
<td>5.16</td>
<td>6.72</td>
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<td></td>
</tr>
</tbody>
</table>

$^A$: Reported as parts per million ($\delta$) downfield from tetramethylsilane (TMS) as internal standard in
deuterochloroform.

$^B$: complex signal
The protons at the 7- and 8-position of the 6-alkoxy-3-unsubstituted imidazo[1,2-b]pyridazines (III.4) gave rise to an AB quartet, with signals in the range δ 6.64-6.74 and δ 7.76-7.80 corresponding to H-7 and H-8 respectively. Again the signals for the 6-chloro-3-unsubstituted compound (III.3) were shifted downfield relative to the 6-alkoxy analogues, with H-7 and H-8 signals at δ 7.06 and δ 7.89 respectively. In all cases the value of the coupling constant $J_{7,8}$ was 9.5 Hz.

The 3-(benzamidomethyl and o-fluorobenzamidomethyl) compounds (III.5) and (III.6) gave more complex spectra than the 3-unsubstituted analogues (III.3) and (III.4). The $^1$H n.m.r. spectra of the 6-(alkoxy and chloro)-3-benzamidomethylimidazo[1,2-b]-pyridazines (III.5, III.6a,b,f-i,m-p,s-u) were characterised by a doublet (δ 5.13-5.20, $J = 5.5$ Hz) resulting from the CH$_2$ protons of the benzamidomethyl group coupled to the neighbouring NH proton. The o-fluorobenzamidomethyl analogues (III.6c-e,j-l,q,r) gave a characteristic doublet of doublets (δ 5.18-5.24, $J = 5.5$ Hz, $J = 1.5$ Hz) from the benzamidomethyl CH$_2$ protons, with coupling to the NH protons ($J = 5.5$ Hz) and long range coupling to the o-F atom on the phenyl group ($J = 1.5$ Hz). The H-7 and H-8 signals appeared in the ranges δ 6.53-7.11 and δ 7.68-7.84 respectively, as AB quartets with coupling constants $J_{7,8}$ of 9.5 Hz.

The mass spectra for the 3-unsubstituted compounds (III.3) and (III.4) showed a signal for the molecular ion but did not give any consistent fragmentation patterns. The 3-(benzamidomethyl and o-fluorobenzamidomethyl compounds (III.5) and (III.6) generally showed peaks corresponding to the molecular ion and (molecular ion - COPh), with other peaks and intensities varying depending on the other substituent groups present on the molecule.

**III.4 *In vitro* binding studies**

The compounds reported in this chapter were evaluated for BZR affinity by displacement of $[^3]$Hdiazepam from rat brain membranes using assay conditions described in Chapter II-5.3.
III-4.1 Results of in vitro testing

The results of the in vitro binding assays are shown in Tables III-3 and III-4 as either IC$_{50}$ values or percentage displacement of [$^3$H]diazepam binding at a single concentration of 1000 nM. Where relevant, some additional data by other workers are incorporated for comparative purposes, with appropriate footnotes.

Table III-3 Results of displacement of [$^3$H]diazepam binding from rat brain membrane preparations by some 6-(alkoxy and chloro)-2-aryl-3-unsubstituted imidazo[1,2-b]pyridazines

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>R</th>
<th>R$_1$</th>
<th>Percentage inhibition at 1000 nM$^A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>III.3</td>
<td>Cl</td>
<td>Cl-p</td>
<td>(38%)</td>
</tr>
<tr>
<td>4a</td>
<td>OMe</td>
<td>OCH$_2$O (3,4)</td>
<td>(54%)</td>
</tr>
<tr>
<td>4b</td>
<td>OMe</td>
<td>Cl-p</td>
<td>(46%)</td>
</tr>
<tr>
<td>4c</td>
<td>OEt</td>
<td>H</td>
<td>(19%)</td>
</tr>
<tr>
<td>4d</td>
<td>OEt</td>
<td>Me-p</td>
<td>(25%)</td>
</tr>
<tr>
<td>4e</td>
<td>OEt</td>
<td>OCH$_2$O (3,4)</td>
<td>(54%)</td>
</tr>
<tr>
<td>4f</td>
<td>OEt</td>
<td>Cl-p</td>
<td>(35%)</td>
</tr>
<tr>
<td>4g</td>
<td>OPr</td>
<td>H</td>
<td>(26%)</td>
</tr>
<tr>
<td>4h</td>
<td>OPr</td>
<td>Me-p</td>
<td>(41%)</td>
</tr>
<tr>
<td>4i</td>
<td>OPr</td>
<td>OCH$_2$O (3,4)</td>
<td>(22%)</td>
</tr>
<tr>
<td>4j</td>
<td>OPr</td>
<td>Cl-p</td>
<td>(22%)</td>
</tr>
<tr>
<td>4k</td>
<td>OCH$_2$CH$_2$OMe</td>
<td>H</td>
<td>(5%)</td>
</tr>
<tr>
<td>4l</td>
<td>OCH$_2$CH$_2$OMe</td>
<td>Me-p</td>
<td>(16%)</td>
</tr>
<tr>
<td>4m</td>
<td>OCH$_2$CH$_2$OEt</td>
<td>H</td>
<td>(23%)</td>
</tr>
</tbody>
</table>

$^A$: Percentage displacement at 1000 nM of specific binding of [$^3$H]diazepam in the presence of 100 µM γ-aminobutyric acid (see Chapter II-5.3, p75 for details)
Table III-4  Results of displacement of \(^{3}H\) diazepam binding from rat brain membrane preparations by some 3-(benzamidomethyl or o-fluoro-benzamidomethyl)imidazo[1,2-b]pyridazines

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>R</th>
<th>R(_1)</th>
<th>R(_2)</th>
<th>IC(_{50}) (nM) (or % displacement at 1000 nM)(^A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III.5</td>
<td>Cl</td>
<td>Cl-p</td>
<td>H</td>
<td>26</td>
</tr>
<tr>
<td>6v(^B)</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>79</td>
</tr>
<tr>
<td>6w(^B)</td>
<td>OMe</td>
<td>Me-p</td>
<td>H</td>
<td>23</td>
</tr>
<tr>
<td>6a</td>
<td>OMe</td>
<td>OCH(_2)O (3,4)</td>
<td>H</td>
<td>7</td>
</tr>
<tr>
<td>6b</td>
<td>OMe</td>
<td>Cl-p</td>
<td>H</td>
<td>29</td>
</tr>
<tr>
<td>6c</td>
<td>OMe</td>
<td>H</td>
<td>F-o</td>
<td>139</td>
</tr>
<tr>
<td>6d</td>
<td>OMe</td>
<td>Me-p</td>
<td>F-o</td>
<td>21</td>
</tr>
<tr>
<td>6e</td>
<td>OMe</td>
<td>OCH(_2)O (3,4)</td>
<td>F-o</td>
<td>14</td>
</tr>
<tr>
<td>6f</td>
<td>OEt</td>
<td>H</td>
<td>H</td>
<td>185</td>
</tr>
<tr>
<td>6g</td>
<td>OEt</td>
<td>Me-p</td>
<td>H</td>
<td>35</td>
</tr>
<tr>
<td>6h</td>
<td>OEt</td>
<td>OCH(_2)O (3,4)</td>
<td>H</td>
<td>25</td>
</tr>
<tr>
<td>6i</td>
<td>OEt</td>
<td>Cl-p</td>
<td>H</td>
<td>64</td>
</tr>
<tr>
<td>6j</td>
<td>OEt</td>
<td>H</td>
<td>F-o</td>
<td>208</td>
</tr>
<tr>
<td>6k</td>
<td>OEt</td>
<td>Me-p</td>
<td>F-o</td>
<td>51</td>
</tr>
<tr>
<td>6l</td>
<td>OEt</td>
<td>OCH(_2)O (3,4)</td>
<td>F-o</td>
<td>31</td>
</tr>
<tr>
<td>6m</td>
<td>OPr</td>
<td>H</td>
<td>H</td>
<td>(73%)</td>
</tr>
<tr>
<td>6n</td>
<td>OPr</td>
<td>Me-p</td>
<td>H</td>
<td>181</td>
</tr>
<tr>
<td>6o</td>
<td>OPr</td>
<td>OCH(_2)O (3,4)</td>
<td>H</td>
<td>116</td>
</tr>
<tr>
<td>6p</td>
<td>OPr</td>
<td>Cl-p</td>
<td>H</td>
<td>(58%)</td>
</tr>
<tr>
<td>6q</td>
<td>OPr</td>
<td>H</td>
<td>F-o</td>
<td>238</td>
</tr>
<tr>
<td>6r</td>
<td>OPr</td>
<td>Me-p</td>
<td>F-o</td>
<td>143</td>
</tr>
</tbody>
</table>
Table III-4 Continued

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>R</th>
<th>R1</th>
<th>R2</th>
<th>IC$_{50}$ (nM) (or % displacement at 1000 nM)$^A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6s</td>
<td>OCH$_2$CH$_2$OMe</td>
<td>H</td>
<td>H</td>
<td>(44%)</td>
</tr>
<tr>
<td>6t</td>
<td>OCH$_2$CH$_2$OMe</td>
<td>Me-p</td>
<td>H</td>
<td>318</td>
</tr>
<tr>
<td>6u</td>
<td>OCH$_2$CH$_2$OEt</td>
<td>H</td>
<td>H</td>
<td>(47%)</td>
</tr>
</tbody>
</table>

A: IC$_{50}$ values (or percentage displacement at 1000 nM) in the presence of 100 µM γ-aminobutyric acid (see Chapter II-5.3, p75 for details)
B: Ref 267

II.4.2 Discussion of results

The results of the in vitro binding studies for the 3-unsubstituted imidazo[1,2-b]-pyridazines reported in this chapter are shown in Table III-3. The 6-(alkoxy and chloro)-3-unsubstituted compounds (III.3) and (III.4a-m) did not have strong affinities for the BZR, with likely IC$_{50}$ values in the micromolar region.

It was difficult to identify consistent relationships between the structural features of these compounds and their resultant BZR affinities. For example, increasing the size of the 6-alkoxy group in the 2-phenylimidazo[1,2-b]pyridazines (III.4c,g,k,m), containing 6-(ethoxy, propoxy, 2-methoxyethoxy and 2-ethoxyethoxy) substituents respectively, led to displacements of [3H]diazepam binding of 19, 26, 5 and 16% at 1000 nM. A similar comparison of a series of 2-(p-chlorophenyl) compounds (III.4b,f,j), however, showed a consistent decrease in BZR affinity when the 6-alkoxy group was increased in size from methoxy to ethoxy to propoxy. The low overall BZR affinities of the 3-unsubstituted compounds examined here and their lack of consistent structure-activity relationships suggests that they do not possess sufficient structural or electronic features to stabilise binding to the active ligand binding site of the BZR.

The 3-(benzamidomethyl and o-fluorobenzamidomethyl) compounds reported here showed a considerable range of BZR affinities as shown in Table III-4. Many compounds had IC$_{50}$ values in the nanomolar region, corresponding to a very high...
affinity for the BZR, whereas others were less potent BZR ligands, with less than 50% displacement of \([^3]H\)diazepam binding at 1000 nM.

Compounds containing the 3-(o-fluorobenzamidomethyl) substituent generally showed slightly lower BZR affinities than the 3-benzamidomethyl analogues. Similar trends relating to BZR affinity were observed, however, in varying 6- and 2-position groups independent of 3-(benzamidomethyl or o-fluorobenzamidomethyl) substitution.

The substituent group present in the 2-position of the molecules had a considerable effect on BZR affinity. The 3-(benzamidomethyl or o-fluorobenzamidomethyl) compounds containing a 2-(p-methylphenyl) group showed \(ca\) 4-7 times greater BZR affinity than the 2-phenyl analogues. The presence of a 2-(3,4-methylenedioxyphenyl) group was more beneficial still, as compounds containing this substituent demonstrated \(ca\) 7-10 times stronger affinity for the BZR than the 2-phenyl analogues. The 2-(p-chlorophenyl) substituent had inconsistent effects on BZR affinity, being beneficial relative to 2-phenyl analogues in (III.6b) and (III.6i) and detrimental in (III.6p).

An examination of the relationship between the size of the 6-alkoxy group and BZR affinity shows that there is a clear and consistent reduction in affinity for the BZR as the 6-alkoxy group is increased in size, with substituents at the 2- and 3-position remaining constant. For example, the series of 6-alkoxy-3-benzamidomethyl-2-phenylimidazo[1,2-b]pyridazines (III.6v,f,m,s,u) with 6-alkoxy substituents increasing in size from methoxy to 2'-ethoxymethoxy have IC\(_{50}\) values (or percentage displacement at 1000 nM) of 79 nM, 185 nM, (73%), (44%) and (47%) respectively. Similarly, the 6-alkoxy-3-benzamidomethyl-2-(p-methylphenyl)imidazo[1,2-b]pyridazines (III.6w,g,n,t) with 6-alkoxy groups varying in size from methoxy to 2'-methoxyethoxy have IC\(_{50}\) values of 23, 35, 181 and 318 nM respectively. These observations suggest that the bulkier 6-(propoxy, 2-methoxyethoxy and 2-ethoxyethoxy) groups are interacting with areas of steric hindrance at the ligand binding site of the BZR protein.

The BZR pharmacophore model developed by Cook et al.\(^{52}\) for agonists contains two hydrogen bond donor sites (H1 and H2) on the receptor protein and two or three areas of lipophilic interaction (L1, L2 and L3), whereas the pharmacophore for inverse
agonist ligands contains one hydrogen bond donor (H1) and one hydrogen bond acceptor site (A2) plus one area of lipophilic interaction (L1). In the case of the agonist pharmacophore, two areas of negative steric interaction between ligand and receptor (S1 and S2) have also been defined.

The BZR affinity of the series of 6-alkoxy-3-(benzamidomethyl or o-fluorobenzamidomethyl)-2arylimidazo[1,2-b]pyridazines reported here decreases as the steric bulk of the 6-alkoxy group increases. The compounds contain a number of hydrogen bond acceptor groups, which could interact with H1 and H2 of the receptor protein, and also possible areas of lipophilic interaction, in particular the phenyl rings of the 3-(benzamidomethyl or o-fluorobenzamidomethyl) and 2-aryl groups. These interactions between ligand and receptor will to a large extent determine the orientation of the ligand on the binding site of the BZR protein. Depending on the orientation that is adopted by the ligand at the binding site, the larger 6-alkoxy groups of the imidazo[1,2-b]pyridazines (III.6) could either interact with one of the areas already defined as S1 or S2 or with another region of negative steric interaction of the receptor protein not yet described. These possibilities will be examined in more detail in the molecular modelling studies in Chapter VII.

In the following chapter, the effect on BZR affinity of substitution at the 6-, 7- and 8-position of imidazo[1,2-b]pyridazines and related imidazo[1,2-a]pyridines will be examined to gain further information relating to the steric requirements of the ligand binding site of the BZR protein in this area.

III-5 Experimental

The details of general aspects of the experimental procedures are described in Section II-5.1, and the assay conditions for the in vitro [3H]diazepam binding studies are outlined in Section II-5.3.

6-Chloropyridazin-3-amine, 6-methoxypyridazin-3-amine, 6-ethoxypyridazin-3-amine, α-bromo-4-methylacetophenone, α-bromo-3,4-methylene-dioxyacetophenone, α-bromo-4-chloroacetophenone, 6-chloro-2-(p-chlorophenyl)imidazo[1,2-b]pyridazine, 6-methoxy-2-phenylimidazo[1,2-b]pyridazine.
6-methoxy-2-(p-methylphenyl)imidazo[1,2-b]pyridazine, N-hydroxymethylbenzamide, and o-fluoro-N-hydroxymethylbenzamide were prepared according to the relevant literature procedures and characterised using $^1$H n.m.r. spectroscopy. $\alpha$-Bromoacetophenone was purchased from commercial sources.

6-(Propoxy, 2-methoxyethoxy and 2-ethoxyethoxy)pyridazin-3-amine were prepared by Mr S.J. Ireland.

2-(p-Methylphenyl)-6-propoxyimidazo[1,2-b]pyridazine (III.4h) and related compounds

A mixture of 6-propoxypyridazin-3-amine (III.2c) (0.15 g), $\alpha$-bromo-4-methylacetophenone (0.21 g) and ethanol (10 ml) was refluxed for 3 h. Sodium hydrogen carbonate (0.084 g) was then added and the refluxing continued for 3 h. The solvent was evaporated and the residue extracted with chloroform, extract washed with water and dried (Na$_2$SO$_4$), and the chloroform evaporated to give an oil. The crude product was subjected to t.l.c. (alumina; chloroform / light petroleum, 3:1) and gave yellow crystals of the title compound (56%), m.p. 86-88°C (from light petroleum) (Found, for a sample dried at 50°C / 1 mmHg for 6 h: C, 71.8; H, 6.5; N, 15.8. C$_{16}$H$_{17}$N$_3$O requires C, 71.9; H, 6.4; N, 15.7%). $^1$H n.m.r.: $\delta$ 1.06, t, J 7 Hz, CH$_3$CH$_2$; 1.81, complex, CH$_3$CH$_2$; 2.39, MeC; 4.27, t, J 7 Hz, CH$_2$O; 6.66, d, J 9.5 Hz, H$_7$; 7.24, d, J 8 Hz, H$_2'$,6' (3',5'); 7.81, d, J 8 Hz, H$_3'$,5' (2',6'); 7.79, d, J 9.5 Hz, H$_8$; 7.99, s, H$_3$. Mass spectrum (e.i.) m/z 267.1 (M, 100%), 225 (100), 130 (13), 115 (22), 80 (14).

In a similar manner the following compounds were prepared from 6-(chloro and alkoxy)pyridazin-3-amines (III.1) and (III.2) and $\alpha$-bromoacetophenone, $\alpha$-bromo-4-methylacetophenone, $\alpha$-bromo-3,4-methylenedioxyacetophenone or $\alpha$-bromo-4-chloroacetophenone.

6-Methoxy-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (III.4a), (28%), m.p. 193-194°C after recrystallisation from toluene (Found: C, 62.8; H, 3.9; N,
15.4. $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_3$ requires C, 62.4; H, 4.1; N, 15.6%). $^1\text{H n.m.r.}$: $\delta$ 3.99, s, MeO; 6.00, s, OCH$_2$O; 6.67, d, J 9.5 Hz, H 7; 6.82-6.93, complex, 7.39-7.49, complex, H 2',5',6'; 7.79, d, J 9.5 Hz, H 8; 7.93, s, H 3. Mass spectrum (e.i.) $m/z$ 269 (M, 100%), 211 (10), 80 (14).

2-($p$-Chlorophenyl)-6-methoxyimidazo[1,2-b]pyridazine (III.4b), (69%), m.p. 177-179°C after recrystallisation from light petroleum (Found: C, 60.4; H, 3.8; N, 16.2. $\text{C}_{13}\text{H}_{10}\text{ClN}_3\text{O}$ requires C, 60.1; H, 3.9; N, 16.2%). $^1\text{H n.m.r.}$: $\delta$ 4.00, s, MeO; 6.68, d, J 9.5 Hz, H 7; 7.39, d, J 9 Hz, H 2',6'(3',5'); 7.77, d, J 9 Hz, H 8; 7.85, d, J 9 Hz, H 3',5'(2',6'); 8.02, s, H 3. Mass spectrum (e.i.) $m/z$ 259 (M, 100%), 216 (12), 80 (26).

6-Ethoxy-2-phenylimidazo[1,2-b]pyridazine (III.4c), (42%), m.p. 126-128°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from cyclohexane (Found: C, 70.1; H, 5.5; N, 17.6. $\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2$ requires C, 70.3; H, 5.5; N, 17.6%). $^1\text{H n.m.r.}$: $\delta$ 1.45, t, J 7 Hz, Me; 4.39, q, J 7 Hz, CH$_2$CH$_3$; 6.69, d, J 9.5 Hz, H 7, 7.29-8.00, complex, H 8 and Ph; 8.02, s, H 3. Mass spectrum (e.i.) $m/z$ 239 (M, 100%), 211 (75), 102 (34), 80 (16).

6-Ethoxy-2-($p$-methylphenyl)imidazo[1,2-b]pyridazine (III.4d), (48%), m.p. 139-140°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from cyclohexane (Found: C, 71.2; H, 5.9; N, 16.6. $\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}$ requires C, 71.1; H, 6.0; N, 16.6%). $^1\text{H n.m.r.}$: $\delta$ 1.44, t, J 7 Hz, CH$_3$CH$_2$; 2.38, s, MeC; 4.38, q, J 7 Hz, CH$_3$CH$_2$; 6.65, d, J 9.5 Hz, H 7; 7.39, d, J 9 Hz, H 2',6'(3',5'); 7.80, d, J 9.5 Hz, H 8; 7.85, d, J 9 Hz, H 3',5'(2',6'); 7.98, s, H 3. Mass spectrum (e.i.) $m/z$ 253 (M, 100%), 225 (62), 115 (20), 80 (14).

6-Ethoxy-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (III.4e), (59%), m.p. 169-171°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from cyclohexane (Found: C, 63.7; H, 4.6; N, 15.1. $\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}_3$
requires C, 63.6; H, 4.6; N, 14.8%). $^1$H n.m.r.: $\delta$ 1.44, t, J 7 Hz, CH$_3$; 4.37, q, J 7 Hz, CH$_3$CH$_2$; 5.99, s, OCH$_2$O; 6.64, d, J 9.5 Hz, H 7, 6.82-7.46, complex, H 2',5',6'; 7.76, d, J 9.5 Hz, H 8; 7.90, s, H 3. Mass spectrum (e.i.) m/z 283 (M, 100%), 255 (60), 197 (11), 128 (11), 80 (10).

2-(p-Chlorophenyl)-6-ethoxyimidazo[1,2-b]pyridazine (III.4f), (35%), m.p. 157-159°C, after recrystallisation from light petroleum (Found: C, 61.2; H, 4.3; N, 15.5. C$_{14}$H$_{12}$ClN$_3$O requires C, 61.4; H, 4.4; N, 15.4%). $^1$H n.m.r.: $\delta$ 1.45, t, J 7 Hz, CH$_3$CH$_2$; 4.38, q, J 7 Hz, CH$_3$CH$_2$; 6.67, d, J 9.5 Hz, H 7; 7.39, d, J 9 Hz, H 2',6' (3',5'); 7.77, d, J 9.5 Hz, H 8; 7.85, d, J 9 Hz, H 3',5'(2',6'); 7.99, s, H 3. Mass spectrum (e.i.) m/z 275, 273 (M, 40%, 100%), 245 (60), 136 (28), 80 (23).

2-Phenyl-6-propoxyimidazo[1,2-b]pyridazine (III.4g), (64%), m.p. 102-104°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found, for a sample dried at 80°C / 710 mmHg for 24 h: C, 71.0; H, 6.1; N, 16.8. C$_{15}$H$_{15}$N$_3$O requires C, 71.1; H, 6.0; N, 16.6%). $^1$H n.m.r.: $\delta$ 1.06, t, J 7 Hz, CH$_3$; 1.81, complex, CH$_3$CH$_2$; 4.27, t, J 7 Hz, CH$_2$O; 6.66, d, J 9.5 Hz, H 7; 7.29-7.97, complex, H 8 and Ph; 8.02, s, H 3. Mass spectrum (e.i.) m/z 253 (M, 82%), 211 (100), 102 (34).

2-(3',4'-Methylenedioxyphenyl)-6-propoxyimidazo[1,2-b]pyridazine (III.4i), (50%), m.p. 125-126°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 64.9; H, 5.1; N, 14.2. C$_{16}$H$_{15}$N$_3$O$_3$ requires C, 64.6; H, 5.1; N, 14.1%). $^1$H n.m.r.: $\delta$ 1.05, t, J 7 Hz, CH$_3$; 1.89, complex, CH$_3$CH$_2$; 4.27, t, J 7 Hz, CH$_2$O; 5.99, s, OCH$_2$O; 6.65, d, J 9.5 Hz, H 7; 6.87, d, J 9 Hz, 7.38-7.47, complex, H 2',5',6'; 7.76, d, J 9.5 Hz, H 8; 7.91, s, H 3. Mass spectrum (e.i.) m/z 297 (M, 100%), 255 (81), 225 (12), 80 (11).
2-(p-Chlorophenyl)-6-propoxyimidazo[1,2-b]pyridazine (III.4j), (47%),
m.p. 149-151°C, after recrystallisation from light petroleum (Found: C, 62.0; H, 5.0; N, 14.9. C₁₅H₁₄ClN₃O requires C, 62.6; H, 4.9; N, 14.6%). ¹H n.m.r.: δ 1.06, t, J 7 Hz, CH₃; 1.85, complex, CH₃CH₂; 4.27, t, J 7 Hz, CH₂O; 6.67, d, J 9.5 Hz, H 7; 7.39, d, J 8 Hz, H 2',6'(3',5'); 7.76, d, J 9.5 Hz, H 8; 7.84, d, J 8 Hz, H 3',5'(2',6'); 7.98, s, H 3. Mass spectrum (e.i.) m/z 289, 287 (M, 35%, 85%), 245 (100), 136 (25), 80 (13).

6-(2'-Methoxyethoxy)-2-phenylimidazo[1,2-b]pyridazine (III.4k), (45%),
m.p. 91-92°C, after recrystallisation from light petroleum (Found, for a sample dried at 50°C / 1 mmHg for 6 h: C, 66.6; H, 5.5; N, 15.4. C₁₅H₁₅N₃O₂ requires C, 66.9; H, 5.6; N, 15.6%). ¹H n.m.r.: δ 3.46, s, CH₃; 3.73-3.84, complex, CH₃OCH₂; 4.43-4.54, complex, CH₃OCH₂CH₂O; 6.74, d, J 9.5 Hz, H 7; 7.36-7.97, complex, H 8,3',4',5',6' and Ph; 8.02, s, H 3. Mass spectrum (e.i.) m/z 269 (M, 92%), 211 (100), 102 (28), 59 (48).

6-(2'-Methoxyethoxy)-2-(p-methylphenyl)imidazo[1,2-b]pyridazine (III.4l), (52%),
m.p. 118-120°C, after recrystallisation from light petroleum (Found: C, 67.5; H, 5.9; N, 14.7. C₁₆H₁₇N₃O₂ requires C, 67.8; H, 6.0; N, 14.8%). ¹H n.m.r.: δ 2.38, s, MeC; 3.46, s, MeO; 3.73-3.84, complex, CH₃OCH₂; 4.43-4.54, complex, CH₃OCH₂CH₂O; 6.72, d, J 9.5 Hz, H 7; 7.24, d, J 8 Hz, H 2",6"(3",5")"; 7.78, d, J 9.5 Hz, H 8; 7.81, J 8 Hz; H 3",5"(2",6")"; 7.99, s, H 3. Mass spectrum (e.i.) m/z 283 (M, 100%), 225 (94), 115 (16), 59 (42).

6-(2'-Ethoxyethoxy)-2-phenylimidazo[1,2-b]pyridazine (III.4m), (44%),
m.p. 76-78°C, after recrystallisation from light petroleum (Found, for a sample dried at 50°C / 1 mmHg for 6 h: C, 67.8; H, 6.0; N, 15.0. C₁₆H₁₇N₃O₂ requires C, 67.8; H, 6.0; N, 14.8%). ¹H n.m.r.: δ 1.26, t, J 7 Hz, CH₃; 3.61, q, J 7 Hz, CH₃CH₂; 3.77-3.88, complex, CH₃CH₂OCH₂; 4.43-4.59, complex, CH₃CH₂OCH₂CH₂; 6.73, d, J 9.5 Hz, H 7; 7.80, d, J 9.5 Hz, H 8; 7.85-8.00, complex, Ph; 8.02, s, H 3. Mass spectrum (e.i.) m/z 283 (M, 80%), 211 (100), 102 (23).
3-(o-Fluorobenzamidomethyl)-6-methoxy-2-phenylimidazo[1,2-b]pyridazine (III.6c) and related compounds

A mixture of o-fluoro-N-(hydroxymethyl)benzamide (0.12 g) in glacial acetic acid (6.0 ml) with concentrated sulphuric acid (0.11 ml) was heated at 50°C for 15 minutes, then 6-methoxy-2-phenylimidazo[1,2-b]pyridazine (0.16 g) was added and the mixture was heated under reflux in an oil bath at 120°C for 24 h. The acetic acid was removed in vacuo and the residue diluted with water, adjusted with aqueous ammonia to pH 10, and the product extracted into chloroform. The extract was washed with water, dried (Na₂SO₄), solvent evaporated and the residue subjected to t.l.c. (alumina; chloroform / light petroleum, 4:1) which gave the title compound (0.11 g, 42%), m.p. 200-201°C (from toluene or methanol) (Found: C, 66.6; H, 4.7; N, 14.7. C₂₁H₁₇FN₄O₂ requires C, 66.5; H, 4.7; N, 14.9%). ¹H n.m.r.: δ 4.07, s, MeO; 5.24, dd, J 5.5 Hz, J 1.5 Hz, CH₂N; 6.75, d, J 9.5 Hz, H 7; 7.84, d, J 9.5 Hz, H 8; 6.95-8.25, complex, H 3',4',5',6' and Ph. Mass spectrum (e.i.) m/z 376 (M, 11%), 253 (100), 123 (78), 95 (26).

In a similar manner from the 3-unsubstituted imidazo[1,2-b]pyridazines (III.3) and (III.4), and N-hydroxymethylbenzamide or o-fluoro-N-hydroxymethylbenzamide were prepared the following compounds.

3-Benzamidomethyl-6-chloro-2-(p-chlorophenyl)imidazo[1,2-b]pyridazine (III.5). (29%), m.p. 237-238°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1), and recrystallisation from toluene (Found: C, 60.6; H, 3.5; N, 14.1. C₂₀H₁₄N₄Cl₂O requires C, 60.5; H, 3.6; N, 14.1%) ¹H n.m.r.: δ 5.20, d, J 5.5 Hz, CH₂N; 7.11, d, J 9.5 Hz, H 7; 7.40-7.48 and 7.76-7.87, complex, Ph; 7.47, d, J 9 Hz, H 2',6'(3',5'); 7.78, d, J 9.5 Hz, H 8; 8.01, d, J 9 Hz, H 3',5'(2',6'). Mass spectrum (e.i.) m/z 398, 396 (M, 6%, 10%), 291 (100), 105 (40), 77 (25).

3-(o-Fluorobenzamidomethyl)-6-methoxy-2-(p-methylphenyl)imidazo[1,2-b]-pyridazine (III.6d). (43%), m.p. 197-199°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1), and recrystallisation from methanol (Found: C, 67.4; H, 4.8; N, 14.2.
C_{22}H_{19}FN_{4}O_{2} requires C, 67.7; H, 4.9; N, 14.4%. 1H n.m.r.: δ 2.40, s, MeC; 4.05, s, MeO; 5.22, dd, J 5.5 Hz, J 1.5 Hz, CH$_{2}$N; 6.72, d, J 9.5 Hz, H 7; 7.82, d, J 9.5 Hz, H 8; 6.94-8.25, complex, H 3',4',5',6',2'',3'',5'',6''. Mass spectrum (e.i.) m/z 390 (M, 32%), 267 (100), 123 (47), 95 (17).

3-Benzamidomethyl-6-methoxy-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (III.6a), (25%), m.p. 247-249°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1), and recrystallisation from toluene (Found: C, 65.7; H, 4.5; N, 13.9. C$_{22}$H$_{18}$N$_{4}$O$_{4}$ requires C, 65.7; H, 4.5; N, 13.9%). 1H n.m.r.: δ 4.01, s, MeO; 5.17, d, J 5.5 Hz, CH$_{2}$N; 6.00, s, OCH$_{2}$O; 6.72, d, J 9.5 Hz, H 7; 6.85-7.80, complex, H 2',5',6' and Ph; 7.81, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 402 (M, 31%), 297 (81), 267 (100), 123 (50), 105 (35), 77 (24).

3-(o-Fluorobenzamidomethyl)-6-methoxy-2-(3',4'-methylenedioxyphenyl)-imidazo[1,2-b]pyridazine (III.6e), (44%), m.p. 226-227°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1), and recrystallisation from methanol (Found: C, 62.5; H, 4.1; N, 12.9. C$_{22}$H$_{17}$FN$_{4}$O$_{4}$ requires C, 62.8; H, 4.1; N, 13.3%). 1H n.m.r.: δ 4.06, s, MeO; 5.20, dd, J 5.5 Hz, J 1.5 Hz, CH$_{2}$N; 6.01, s, OCH$_{2}$O; 6.74, d, J 9.5 Hz, H 7; 6.89-7.51 and 8.07-8.27, complex, H 3',4',5',6',2'',5'',6''; 7.82, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 420 (M, 12%), 390 (21), 297 (31), 267 (70), 120 (42), 84 (83), 49 (100).

3-Benzamidomethyl-2-(p-chlorophenyl)-6-methoxyimidazo[1,2-b]pyridazine (III.6b), (30%), m.p. 259-261°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 64.0; H, 4.1; N, 14.0. C$_{21}$H$_{17}$ClN$_{4}$O$_{2}$ requires C, 64.2; H, 4.4; N, 14.3%). 1H n.m.r.: δ 4.03, s, MeO; 5.18, d, J 5.5 Hz, CH$_{2}$N; 6.75, d, J 9.5 Hz, H 7; 7.44, d, J 9 Hz, H 2',6'(3',5'); 7.82, d, J 9.5 Hz, H 8; 7.88, d, J 9 Hz, H 3',5'(2',6'). Mass spectrum (e.i.) m/z 394, 392 (M, 8%, 23%), 287 (100), 105 (47), 77 (36).
3-Benzamidomethyl-6-ethoxy-2-phenylimidazo[1,2-b]pyridazine (III.6f), (46%), m.p. 183-185°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from toluene (Found: C, 71.1; H, 5.3; N, 15.0. \( \text{C}_{22}\text{H}_{20}\text{N}_{4}\text{O}_{2} \) requires C, 70.9; H, 5.4; N, 15.0%). \( ^1\text{H n.m.r.}: \delta 1.42, t, J 7\text{ Hz}, \text{CH}_3; 4.35, q, J 7\text{ Hz}, \text{CH}_3\text{CH}_2; 6.53, d, J 9.5\text{ Hz}, \text{H}_7; 7.34-7.46\text{ and }7.77-7.86,\text{ complex, }2\times \text{Ph}; 7.68, d, J 9.5\text{ Hz}, \text{H}_8.\) Mass spectrum (e.i.) \( m/\zeta \) 372 (M, 8%), 267 (100), 105 (83), 77 (80).

6-Ethoxy-3-(o-fluorobenzamidomethyl)-2-phenylimidazo[1,2-b]pyridazine (III.6j), (37%), m.p. 184-186°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from methanol (Found: C, 67.4; H, 4.8; N, 14.2. \( \text{C}_{22}\text{H}_{19}\text{FN}_{4}\text{O}_{2} \) requires C, 67.7; H, 4.9; N, 14.35%). \( ^1\text{H n.m.r.}: \delta 1.46, t, J 7\text{ Hz}, \text{CH}_3; 4.44, q, J 7\text{ Hz}, \text{CH}_3\text{CH}_2; 6.71, d, J 9.5\text{ Hz}, \text{H}_7; 6.96-7.49\text{ and }7.90-8.24,\text{ complex, }H 3',4',5',6'\text{ and Ph}; 7.81, d, J 9.5\text{ Hz}, \text{H}_8.\) Mass spectrum (e.i.) \( m/\zeta \) 390 (M, 11%), 267 (96), 123 (100), 95 (33).

3-Benzamidomethyl-6-ethoxy-2-(p-methylphenyl)imidazo[1,2-b]pyridazine (III.6g), (30%), m.p. 216-218°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from toluene (Found: C, 71.3; H, 5.9; N, 14.5. \( \text{C}_{23}\text{H}_{22}\text{N}_{4}\text{O}_{2} \) requires C, 71.5; H, 5.7; N, 14.5%). \( ^1\text{H n.m.r.}: \delta 1.41, t, J 7\text{ Hz}, \text{CH}_3\text{CH}_2; 2.36, s, \text{MeC}; 4.35, q, J 7\text{ Hz}, \text{CH}_3\text{CH}_2; 5.13, d, J 5.5\text{ Hz}, \text{CH}_2\text{N}; 6.60, d, J 9.5\text{ Hz}, \text{H}_7; 7.15-7.88,\text{ complex, }H 8,2'',3'',5'',6''\text{ and Ph.}\) Mass spectrum (e.i.) \( m/\zeta \) 386 (M, 17%), 381 (100), 105 (41), 84 (31), 49 (40).

6-Ethoxy-3-(o-fluorobenzamidomethyl)-2-(p-methylphenyl)imidazo[1,2-b]pyridazine (III.6k), (42%), m.p. 196-198°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from toluene (Found: C, 67.9; H, 5.3; N, 13.5. \( \text{C}_{23}\text{H}_{21}\text{FN}_{4}\text{O}_{2} \) requires C, 68.3; H, 5.2; N, 13.9%). \( ^1\text{H n.m.r.}: \delta 1.47, t, J 7\text{ Hz}, \text{CH}_3\text{CH}_2; 2.40, s, \text{MeC}; 4.44, q, J 7\text{ Hz}, \text{CH}_3\text{CH}_2; 5.20, dd, J 5.5\text{ Hz}. 1.5\text{ Hz}, \text{CH}_2\text{N}, 6.73, d, J 9.5\text{ Hz}, \text{H}_7; 6.95-8.25,\text{ complex, }H 3',4',5',6',2'',3'',5'',6''; 7.84, d, J 9.5\text{ Hz}, \text{H}_8.\) Mass spectrum (e.i.) \( m/\zeta \) 404 (M, 17%), 281 (89), 123 (100), 95 (29).
3-Benzamidomethyl-6-ethoxy-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-b]-pyridazine (III.6h), (20%), m.p. 252-254°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 65.9; H, 4.8; N, 13.1.
C_{23}H_{20}N_{4}O_{4} requires C, 66.3; H, 4.8; N, 13.5%). 1H n.m.r.: δ 1.44, t, J 7 Hz, CH_{3}CH_{2}; 4.40, q, J 7 Hz, CH_{3}CH_{2}; 5.16, d, J 5.5 Hz, CH_{2}N; 5.99, s, OCH_{2}O; 6.71, d, J 9.5 Hz, H7; 6.85-7.86, complex, H2',5',6' and Ph; 7.80, d J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 416 (M, 43%), 311 (100), 283 (18), 105 (39), 77 (21).

6-Ethoxy-3-(o-fluorobenzamidomethyl)-2-(3',4'-methylenedioxyphenyl)-imidazo[1,2-b]pyridazine (III.6l), (19%), m.p. 202-203°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from methanol (Found: C, 63.4; H, 4.3; N, 12.8. C_{23}H_{19}FN_{4}O_{4} requires C, 63.6; H, 4.4; N, 12.9%). 1H n.m.r.: δ 1.47, t, J 7 Hz, CH_{3}CH_{2}; 4.45, q, J 7 Hz, CH_{3}CH_{2}; 5.18, dd, J 5.5 Hz, 1.5 Hz, CH_{2}N, 6.00, s, OCH_{2}O; 6.72, d, J 9.5 Hz, H 7; 6.88-8.26, complex, H 3',4',5',6',2'',3'',5'',6''; 7.82, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 434 (M, 28%), 311 (72), 123 (100), 95 (29).

3-Benzamidomethyl-2-(p-chlorophenyl)-6-ethoxyimidazo[1,2-b]pyridazine (III.6i), (50%), m.p. 229-230°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 64.6; H, 4.7; N, 13.6. C_{22}H_{19}ClN_{4}O_{2} requires C, 64.9; H, 4.7; N, 13.8%). 1H n.m.r.: δ 1.45, t, J 7 Hz, CH_{3}CH_{2}; 4.39, q, J 7 Hz, CH_{3}CH_{2}; 5.14, d, J 5.5 Hz, CH_{2}N; 6.68, d, J 9.5 Hz, H 7; 7.00, br, NH; 7.34-7.80, complex, H 8,2',3',5',6' and Ph. Mass spectrum (e.i.) m/z 408, 406 (M, 10%, 26%), 301 (100), 273 (23), 105 (63), 77 (33).

3-Benzamidomethyl-2-phenyl-6-propoxyimidazo[1,2-b]pyridazine (III.6m), (30%), m.p. 177-179°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 71.2; H, 6.0; N, 14.4. C_{23}H_{22}N_{4}O_{2} requires C, 71.5; H, 5.7; N, 14.5%). 1H n.m.r.: δ 1.02, t, J 7 Hz, CH_{3}; 1.82, complex, CH_{3}CH_{2}; 4.26, t, J 7 Hz, CH_{2}O; 5.17, d, J 5.5 Hz, CH_{2}N; 6.66, d, J 9.5 Hz, H 7; 7.02,
br, NH; 7.35-7.90, H 8 and 2 x Ph. Mass spectrum (e.i.) m/z 386 (M, 23%), 281 (100), 257 (20), 239 (34), 105 (59), 77 (40).

3-(o-Fluorobenzamidomethyl)-2-phenyl-6-propoxyimidazo[1,2-b]pyridazine (III.6q), (54%), m.p. 185-187°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from toluene (Found: C, 68.4; H, 5.2; N, 14.0. C₂₃H₂₁FN₄O₂ requires C, 68.3; H, 5.2; N, 13.9%). ¹H n.m.r.: δ 1.06, t, J 7 Hz, CH₃; 1.87, complex, CH₃CH₂; 4.34, t, J 7 Hz, CH₂O; 5.22, dd, J 5.5 Hz, 1.5 Hz, CH₂N; 6.74, d, J 9.5 Hz, H 7; 6.95-7.57 and 7.92-8.26, complex, H 3',4',5',6' and Ph; 7.83, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 386 (M, 23%), 281 (100), 239 (24), 123 (48).

3-Benzamidomethyl-6-propoxy-2-(p-methylphenyl)imidazo[1,2-b]pyridazine (III.6n), (42%), m.p. 195-197°C, after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from toluene (Found: C, 71.9; H, 5.9; N, 13.8. C₂₄H₂₄N₄O₂ requires C, 72.0; H, 6.0; N, 14.0%). ¹H n.m.r.: δ 1.02, t, J 7 Hz, CH₃CH₂; 1.73, complex, CH₃CH₂; 2.38, s, CH₃C; 4.26, t, J 7 Hz, CH₂O; 5.17, d, J 5.5 Hz, CH₂N; 6.65, d, J 9.5 Hz, H 7; 7.01, br, NH; 7.18-7.87, complex, H 2",3",5",6" and Ph; 7.70, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 400 (M, 22%), 295 (100), 253 (22), 105 (32).

3-(o-Fluorobenzamidomethyl)-6-propoxy-2-(p-methylphenyl)imidazo[1,2-b]-pyridazine (III.6r), (84%), m.p. 180-181°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from methanol (Found: C, 68.8; H, 5.8; N, 13.6. C₂₄H₂₃FN₄O₂ requires C, 68.9; H, 5.5; N, 13.4%). ¹H n.m.r.: δ 1.05, t, J 7 Hz, CH₃CH₂; 1.86, complex, CH₃CH₂; 2.40, s, MeC; 4.33, t, J 7 Hz, CH₂O; 5.21, dd, J 5.5 Hz, 1.5 Hz, CH₂N; 6.72, d, J 9.5 Hz, H 7; 6.94-8.25, complex, H 8,3',4',5',6',2",3", 5". Mass spectrum (e.i.) m/z 418 (M, 36%), 295 (100), 253 (31), 123 (62).

3-Benzamidomethyl-2-(p-chlorophenyl)-6-propoxyimidazo[1,2-b]pyridazine (III.6p), (52%), m.p. 218-220°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 65.5; H, 5.0; N, 13.2. C₂₃H₂¹ClN₄O₂
requires C, 65.6; H, 5.0; N, 13.3%). $^1$H n.m.r.: $\delta$ 1.04, t, J 7 Hz, CH$_2$; 1.81, complex, CH$_3$CH$_2$; 4.28, t, J 7 Hz, CH$_2$O; 5.15, d, J 5.5 Hz, CH$_2$N; 6.71, d, J 9.5 Hz, H 7; 6.99, br, NH; 7.35-7.89, complex, H 8,2",3",5",6" and Ph. Mass spectrum (e.i.) m/z 422, 420 (M, 12%, 30%), 315 (100), 273 (30), 105 (53), 77 (22).

3-Benzamidomethyl-2-(3',4'-methylenedioxyphenyl)-6-propoximidazo[1,2-b]-pyridazine (III.6o), (36%), m.p. 219-220°C, after t.l.c. (aluina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 67.4; H, 5.2; N, 12.7. C$_{24}$H$_{22}$N$_4$O$_4$ requires C, 67.0; H, 5.2; N, 13.0%). $^1$H n.m.r.: $\delta$ 1.02, t, J 7 Hz, CH$_3$CH$_2$; 1.83, complex, CH$_3$CH$_2$; 4.27, t, J 7 Hz, CH$_2$O; 5.13, d, J 5.5 Hz, CH$_2$N; 5.98, s, OCH$_2$O; 6.67, d, J 9.5 Hz, H 7; 6.82-7.87, H 2',5',6' and Ph; 7.71, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 430 (M, 45%), 325 (100), 297 (39), 283 (27), 105 (59), 77 (30).

3-Benzamidomethyl-6-(2'-methoxyethoxy)-2-phenylimidazo[1,2-b]pyridazine (III.6s), (40%), m.p. 188-189°C, after t.l.c. (aluina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 68.4; H, 5.6; N, 13.9. C$_{23}$H$_{22}$N$_4$O$_3$ requires C, 68.6; H, 5.5; N, 13.9%). $^1$H n.m.r.: $\delta$ 3.40, s CH$_3$O, 3.68-3.79, complex, CH$_3$OCH$_2$; 4.45-4.52, complex, CH$_3$OCH$_2$CH$_2$; 5.17, d, J 5.5 Hz, CH$_2$N; 6.73, d, J 9.5 Hz, H 7; 6.95, br, NH; 7.34-7.50 and 7.67-7.89, complex, 2 x Ph; 7.72, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 402 (M, 21%), 297 (100), 239 (33), 105 (43), 77 (23).

3-Benzamidomethyl-6-(2'-methoxyethoxy)-2-(p-methylphenyl)imidazo[1,2-b]-pyridazine (III.6t), (36%), m.p. 187-189°C, after t.l.c. (aluina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 68.8; H, 5.7; N, 13.2. C$_{24}$H$_{24}$N$_4$O$_3$ requires C, 69.2; H, 5.8; N, 13.5%). $^1$H n.m.r.: $\delta$ 2.38, s CH$_3$C, 3.40, s CH$_3$O, 3.68-3.79, complex, CH$_3$OCH$_2$; 4.41-4.52, complex, CH$_3$OCH$_2$CH$_2$; 5.16, d, J 5.5 Hz, CH$_2$N; 6.72, d, J 9.5 Hz, H 7; 7.18-7.86, complex, H 2",3",5",6" and Ph; 7.72, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 416 (M, 17%), 311 (100), 253 (30), 105 (38), 77 (18).
3-Benzamidomethyl-6-(2'-ethoxyethoxy)-2-phenylimidazo[1,2-b]pyridazine (III.6u), (48%), m.p. 195-197°C, after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 68.9; H, 5.6; N, 13.3. C_{24}H_{24}N_{4}O_{3} requires C, 69.2; H, 5.8; N, 13.5%). \textsuperscript{1}H n.m.r.: \^d 1.22, t, J 7 Hz, CH_{3}; 3.55, q, J 7 Hz, CH_{3}CH_{2}; 3.73-3.83, complex, CH_{3}CH_{2}OCH_{2}; 4.41-4.51, complex, CH_{3}CH_{2}OCH_{2}CH_{2}O; 5.16, d, J 5.5 Hz, CH_{2}N; 6.72, d, J 9.5 Hz, H 7; 7.01, br, NH; 7.35-7.87, complex, H 8 and 2 x Ph. Mass spectrum (e.i.) m/z 416 (M, 21%), 325 (20), 311 (100), 239 (48), 105 (54), 77 (26).
CHAPTER IV

Synthesis and BZR and FBR affinities of some 2-aryl-4(1H)- and 8-
(thiophene- and methoxy)-3-(aminoethylated, benzamidomethyl and methyl-)
imidazo[1,2-a]pyridines

Introduction

Following the work presented in Chapter III (where the steric requirements of
the BZR ligand binding site in the vicinity of the groups at the 6-position of imidazo[1,2-
alpyridazine ligands were examined) it was decided to synthesise and examine
compounds containing substituent groups in the 7- and 8-positions. The determination of
the BZR affinities of these compounds would provide further information regarding the
steric constraints of the BZR ligand binding site. It was of interest to determine whether
the 7- and 8-substituted compounds would have decreased affinity for the BZR due to
unfavourable ligand / receptor interactions or whether BZR affinity due to beneficial
interaction with areas of the receptor inaccessible to 6-substituted analogues.

Imidazo[1,2-alpyridines were synthesised rather than imidazo[1,2-b]pyridazines
because the relevant substituted pyridine starting materials were readily available
whereas the corresponding pyridazines were not, and also because previous studies had
identified a close correlation between the BZR affinities of 3,5,6-trisubstituted
imidazo[1,2-alpyridines and analogous imidazo[1,2-b]pyridazines. This implies that
the 2-5 atom of the imidazo[1,2-alpyridazines does not play a major role in stabilising
the binding of these ligands to the BZR. It was therefore assumed that the results
gained in the BZR affinities of the imidazo[1,2-alpyridines reported here could also be
applied to the corresponding imidazo[1,2-b]pyridazines.

In order to effect a comparison between the results reported in this and other
chapters, compounds were synthesised with substituents in the 2- and 7-position similar
to those reported in Chapters II and III of this thesis. Variations in BZR affinity would
directly relate to the effects of the 8-7- or 8-position groups, suggesting that these substituents did not greatly alter the conformation of the 2- and 7-pyridine
 groups. Accordingly, compounds were synthesised containing 2-aryl and 7-
thiophene, benzamidomethyl and methoxy) groups. Previous results were extended...
CHAPTER IV Syntheses and BZR and PBR affinities of some 2-aryl-6(7 and 8)-(chloro and methoxy)-3-(unsubstituted, benzamidomethyl and methoxy)-imidazo[1,2-a]pyridines

IV-1 Introduction

Following the work presented in Chapter III (where the steric requirements of the BZR ligand binding site in the vicinity of the groups at the 6-position of imidazo[1,2-b]pyridazine ligands were examined) it was decided to synthesise and examine compounds containing substituent groups in the 7- and 8-positions. The determination of the BZR affinities of these compounds would provide further information regarding the steric constraints of the BZR ligand binding site. It was of interest to determine whether the (7 and 8)-substituted compounds would have decreased affinity for the BZR due to unfavourable ligand/receptor interactions or increased BZR affinity due to beneficial interaction with areas of the receptor inaccessible to 6-substituted analogues.

Imidazo[1,2-a]pyridines were synthesised rather than imidazo[1,2-b]pyridazines because the relevant substituted pyridine starting materials were readily available whereas the corresponding pyridazines were not, and also because previous studies had identified a close correlation between the BZR affinities of 2,3,6-trisubstituted imidazo[1,2-a]pyridines and analogous imidazo[1,2-b]pyridazines. This implies that the N-5 atom of the imidazo[1,2-b]pyridazines does not play a major role in stabilising the binding of these ligands to the BZR. It was therefore assumed that the trends observed in the BZR affinities of the imidazo[1,2-a]pyridines reported here could also be applied to the corresponding imidazo[1,2-b]pyridazines.

In order to effect a comparison between the results reported in this and other chapters, compounds were synthesised with substituents in the 2- and 3-positions similar to those reported in Chapters II and III of this thesis. Variations in BZR affinity could therefore be directly related to the effects of the 6-, 7- or 8-position groups, assuming that these substituents did not greatly alter the conformation of the 2- and 3-position groups. Accordingly, compounds were synthesised containing 2-aryl and 3-(unsubstituted, benzamidomethyl and methoxy) groups. Previous results with 6-(chloro
and methylthio) compounds in Chapter II indicated that a 3-benzamidomethyl group was a necessary requirement for high affinity binding to the BZR, whereas a 3-methoxy group led to less potent BZR ligands (in compounds containing 6-chloro and 6-methylthio groups). The syntheses and BZR affinities of the 6(7 and 8)-substituted compounds synthesised here provide additional data to determine whether this is also the situation with these compounds.

In this chapter the syntheses of 2-aryl-6(7 and 8)-(chloro and methoxy)-3-(unsubstituted, benzamidomethyl and methoxy)imidazo[1,2-a]pyridines are reported. The compounds have been characterised by the usual spectral and analytical techniques. The in vitro affinities of the compounds at the BZR have been determined. In vitro assays to determine the PBR affinities of these compounds (under assay conditions described in the Experimental Section of this chapter) have also been undertaken and the results of this work are given. The structure-activity relationships between the compounds and BZR and PBR affinity are discussed. The experimental details of the preparations of the compounds are recorded in Chapter IV-5.1.

IV-2 Syntheses

The starting materials required for the syntheses reported in this work were 5(4 and 3)-substituted pyridin-2-amines. The 5(4 and 3)-chloropyridin-2-amines (IV.4) were synthesised according to the appropriate literature procedures.\textsuperscript{308-310} 3-Methoxypyridin-2-amine (IV.3) was prepared by methylation of 2-nitropyridin-3-ol (IV.1) to 3-methoxy-2-nitropyridine (IV.2)\textsuperscript{311} which was then reduced to (IV.3) (Scheme IV-1). This technique was different to that previously reported for the syntheses of 5-methoxypyridin-2-amine,\textsuperscript{312} and 4-methoxypyridin-2-amine.\textsuperscript{309}

The 5(4 and 3)-substituted pyridin-2-amines were condensed with either substituted glyoxals or α-bromoacetophenones to form the imidazo[1,2-a]pyridines which were then subjected to further modification. (Scheme IV-2). The reaction of the 5(4 and 3)-substituted pyridazin-2-amines (IV.4a-c) and (IV.3) with the relevant substituted α-bromoacetophenones (under literature conditions reported for the syntheses of similar imidazo[1,2-b]pyridazines)\textsuperscript{201} afforded the appropriate 6(7 and 8)-
substituted imidazo[1,2-\(a\)]pyridines (IV.5a-h) and (IV.9a-c) respectively in yields of 39-99%. The attempted preparations of the corresponding 7-methoxyimidazo[1,2-\(a\)]pyridines from 4-methoxypyridin-2-amine 309 and substituted \(\alpha\)-bromoacetophenones under similar conditions to those described above, in our hands, were unsuccessful.

Electrophilic substitution at the 3-position of the 3-unsubstituted imidazo[1,2-\(a\)]pyridines (IV.5) and (IV.9) by treatment with N-hydroxymethylbenzamide \(2^6^7\) led to the formation of the 2-aryl-3-benzamidomethyl-6(7 and 8)-chloroimidazo[1,2-\(a\)]pyridines (IV.6a-h) and 2-aryl-3-benzamidomethyl-8-methoxyimidazo[1,2-\(a\)]pyridines (IV.10a-c) respectively in yields of 13-75%.

The 5(4 and 3)-chloropyridin-2-amines (IV-4a-c) when reacted with phenylglyoxal under literature conditions \(2^7^7\) formed 6(7 and 8)-chloro-3-hydroxy-2-phenyl-imidazo[1,2-\(a\)]pyridines (IV.7a-c), and O-methylation with ethereal diazomethane generated the corresponding 6(7 and 8)-chloro-3-methoxy-2-phenylimidazo[1,2-\(a\)]pyridines (IV.8a-c) in yields of 27-50%.

All compounds were purified by chromatography (where necessary) and by recrystallisation as outlined in Chapter IV-5.

### IV-3 Physical properties

Table IV-1 shows some \(^1\text{H}\) n.m.r. spectral data for the novel imidazo[1,2-\(a\)]pyridines reported in this chapter. The \(^1\text{H}\) n.m.r. spectra were more complex than those for analogous imidazo[1,2-\(b\)]pyridazines due to the presence of a signal resulting from
the proton at the 5-position. To assist in the interpretation of the spectra, 2-D COSY $^1$H n.m.r. spectra were obtained for (IV.5d,g) and (IV.10b).

Scheme IV-2

Reagents: (i) RC$_6$H$_x$COCH$_2$Br, NaHCO$_3$, EtOH, reflux (ii) PhCONHCH$_2$OH, cone H$_2$SO$_4$, AcOH, 100-120°C (iii) PhCOCH$_2$, cone HCl, EtOH, reflux (iv) CH$_2$N$_2$, Et$_2$O, 0°C

A The 2-D COSY n.m.r. spectra were run by Mrs P.T. Culnane at the Research School of Chemistry, Australian National University
Table IV-1 Some $^1$H n.m.r. spectral data (δ)\(^A\) for some 6-, 7- and 8-(chloro and methoxy)imidazo[1,2-a]pyridines

![Diagram of imidazo[1,2-a]pyridine structure](image)

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\(A\): Reported as parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard in deuterochloroform.

\(B\): Complex signal
Figure IV-1  2-D COSY $^1$H n.m.r. spectrum (with expansion of aromatic region) of 7-chloro-2-(p-methylphenyl)imidazo[1,2-a]pyridine (IV.5d)
Figure IV-2  2-D COSY $^1$H n.m.r. spectrum (with expansion of aromatic region) of 8-chloro-2-(p-methylphenyl)imidazo[1,2-a]pyridine (IV.5g)
Figure IV-3 2-D COSY $^1$H n.m.r. spectrum (with expansion of aromatic region) of
3-benzamidomethyl-8-methoxy-2-($p$-methylphenyl)imidazo[1,2-α]pyridine (IV.10b)

The signal at $\delta$ 8.09 demonstrates long-range coupling to the signal at $\delta$ 6.89. The signal at $\delta$ 8.64 shows coupling to the signal at $\delta$ 6.89. The aromatic proton of the 3-$p$-methylphenyl group
each coupled to the other. The $\delta$ 8.09 is the aromatic proton of the 3-$p$-methylphenyl group.

Figure IV-3 shows the 2-D COSY $^1$H n.m.r. spectrum for 3-benzamidomethyl-8-methoxy-2-($p$-methylphenyl)imidazo[1,2-α]pyridine (IV.10b). The signal at $\delta$ 8.09 was coupled to signals at $\delta$ 7.24 and $\delta$ 6.96. There appeared to be two signals superimposed on each other at $\delta$ 7.24 in a one-dimensional spectrum with both the signal at $\delta$ 6.71 and to another signal at $\delta$ 7.04. The signal at $\delta$ 8.09 was coupled to that at $\delta$ 7.24 only, and the signal at $\delta$ 6.96 was coupled to that at $\delta$ 7.04 only. These cross peaks were therefore assigned to $\delta$ 6.71 (H-6), $\delta$ 7.24 (H-7, aromatic proton from the 2-($p$-methylphenyl) group), $\delta$ 7.04 (H-5) and $\delta$ 6.96 (H-5).

The 2-D COSY $^1$H n.m.r. spectrum for 3-benzamidomethyl-8-methoxy-2-($p$-methylphenyl)imidazo[1,2-α]pyridine (IV.10b) allowed the assignment of the n.m.r. spectrum using similar methods. The signal at $\delta$ 8.64 (H-10) was the aromatic proton from the 2-($p$-methylphenyl) group. Coupling was also observed between the other signals at $\delta$ 7.41 and $\delta$ 7.43. It was assumed that these additional signals arose from protons in the 2-($p$-methylphenyl) group.

To provide an overview of the n.m.r. spectrum of the imidazo[1,2-α]pyridine (IV.10b), the signal at $\delta$ 8.09 was identified as a 5-$p$-methylphenyl group and (F1 7.44, 7.3, 7.8, 7.6, 7.7, 6.5, 6.8, 6.6, 6.3) and (F2 6.71, 7.8) the H-3 signal presented in a broad off-field singlet (2-H-3, 3-H-3) signal as a doublet of quartets.
The 2-D COSY $^1$H n.m.r. spectrum for 7-chloro-2-(p-methylphenyl)imidazo[1,2-$a$]pyridine (IV.5d) is shown in Figure IV-1. The signal at $\delta$ 6.80 shows coupling to the signal at $\delta$ 8.04 and minor long range coupling to the signal at $\delta$ 7.69. The signal at $\delta$ 7.69 demonstrates long range coupling with the signal at $\delta$ 6.80. The signal at $\delta$ 8.04 shows coupling to the signal at $\delta$ 6.80 only. Therefore the signals were assigned as $\delta$ 6.80 (H-6), $\delta$ 7.69 (H-8) and $\delta$ 8.04 (H-5). The signals at $\delta$ 7.24 and 7.82, each coupled to the other, resulted from the aromatic protons of the 2-(p-methylphenyl) group.

Figure IV-2 shows the 2-D COSY $^1$H n.m.r. spectrum for 8-chloro-2-(p-methylphenyl)imidazo[1,2-$a$]pyridine (IV.5g). The signal at $\delta$ 6.71 was coupled to signals at $\delta$ 7.24 and $\delta$ 8.06. There appeared to be two signals superimposed on each other at $\delta$ 7.24 as coupling was observed with both the signal at $\delta$ 6.71 and to another signal at $\delta$ 7.88. The signal at $\delta$ 7.88 was coupled to that at $\delta$ 7.24 only, and the signal at $\delta$ 8.06 was coupled to that at $\delta$ 6.71 only. The signals were therefore assigned as $\delta$ 6.71 (H-6), $\delta$ 7.24 [H-7 superimposed with two of the aromatic protons from the 2-(p-methylphenyl) group], $\delta$ 7.88 [the remaining two protons from the 2-(p-methylphenyl) group] and $\delta$ 8.06 (H-5).

The 2-D COSY $^1$H n.m.r. spectrum for 3-benzamidomethyl-8-methoxy-2-(p-methylphenyl)imidazo[1,2-$a$]pyridine (IV.10b) (Figure IV-3) allowed the assignment, using similar methods, of $\delta$ 6.42 (H-7), $\delta$ 6.60 (H-6), $\delta$ 7.85 (H-5) and $\delta$ 7.08 and $\delta$ 7.58 [the protons from the 2-(p-methylphenyl group)]. Coupling was also observed between other signals at $\delta$ 7.41 and $\delta$ 7.95 and it was assumed that these additional signals resulted from protons in the phenyl ring of the benzamidomethyl group.

To provide an overview of the $^1$H n.m.r. spectra of the imidazo[1,2-$a$]pyridines reported in this chapter, the signals from the protons at the 5-, 6-, 7- and 8-position (where present) were in the ranges $\delta$ 7.72-8.36, $\delta$ 6.65-6.75, $\delta$ 6.36-7.18 and $\delta$ 7.64-7.80 respectively. The H-3 signals from the 3-unsubstituted imidazo[1,2-$a$]pyridines were observed as singlets in the range $\delta$ 7.71-7.87.

For the 6-chloro compounds (IV.5a,b) and (IV.6a,b) the H-5 signal appeared as a broad downfield singlet ($\delta$ 8.17-8.36). The H-7 signal was a doublet of doublets.
(δ 7.15-7.18) with coupling to both H-8 (J7,8 = 9 Hz) and H-5 (J5,7 = 2 Hz). The H-8 signal appeared as a doublet (δ 7.64-7.67) with coupling to H-7 (J7,8 = 9 Hz) and no further long range coupling.

In the 7-chloro compounds (IV.5c-e) and (IV.6c-e) the H-5 signal was a downfield doublet (δ 8.01-8.36) with coupling to H-6 (J5,6 = 7 Hz). The H-6 signal appeared as a doublet of doublets, with coupling to both H-5 and H-8 (J5,6 = 7 Hz, J6,8 = 2 Hz). The H-8 signal was a broad singlet (δ 7.47-7.63).

The 8-chloro compounds (IV.5f-h) and (IV.6f-h) were characterised by H-5 signals that were broad doublets (δ 7.99-8.24) coupled to H-6 (J5,6 = 7 Hz). In some cases long range coupling to H-7 was also apparent (J5,7 = 2 Hz). The H-6 protons were coupled to both H-5 and H-7 (J = 7 Hz) and the signals appeared as triplets. The H-7 signals could not be separated from other complexes in the spectra of the 3-benzamidomethyl compounds (IV.6f-h), however in the 3-unsubstituted compounds (IV.5g,h) they appeared as doublets (δ 7.21-7.24) with coupling constants J6,7 of 7 Hz.

The 1H n.m.r. spectra of the 8-methoxy compounds (IV.9a-c) and (IV.10a-c) show H-6 and H-7 signals shifted upfield (δ 6.64-6.68 and δ 6.36-6.45 respectively) relative to the 8-chloro analogues, most probably as a result of increased shielding of these protons by the 8-methoxy group (with a greater electron-donating ability than the 8-chloro group), and the signals from the 8-methoxy group appear as characteristic singlets (δ 3.89-4.03).

The mass spectra of the 3-unsubstituted imidazo[1,2-a]pyridines (IV.5a,c,f) did not show any consistent fragmentation patterns. The 3-benzamidomethyl compounds (IV.6a,c,f), however, all showed fragment ion peaks at m/z 256 and 241, presumably corresponding to the loss of PhCO and PhCONH respectively from the molecular ion.

IV-4 In vitro binding studies

The BZR affinities of the compounds reported in this chapter were measured by in vitro displacement of [3H] diazepam binding from rat brain membranes under assay conditions outlined in Chapter II-5.3. The compounds were also evaluated for PBR
affinity by the *in vitro* displacement of $[3^H]$diazepam from rat kidney preparations, under assay conditions described below in the Experimental Section of this chapter.

**IV-4.1 Results of *in vitro* testing**

The results of the *in vitro* BZR and PBR binding assays are shown in Table IV-2 as either percentage displacement of $[3^H]$diazepam binding at a single concentration of 1000 nM, or IC$_{50}$ values. Additional data by other workers are incorporated, where appropriate, for comparison.

**IV-4.2 Discussion of results**

An examination of the BZR affinities of the imidazo[1,2-α]pyridines reported in this chapter allows some comparative effects of substitution in the 6-, 7- and 8-positions of the molecules to be identified.

None of the 3-unsubstituted compounds (IV.5) and (IV.9) showed high affinity for the BZR, with less than 50% displacement of $[3^H]$diazepam binding at 1000 nM. The 2-aryl-7(and 8)-(choro and methoxy)imidazo[1,2-α]pyridines had lower BZR affinities than the 6-chloro analogues. Substitution of the 2-phenyl groups of these compounds with p-methyl or (3,4-methylenedioxy) substituents led to a modest increase in BZR affinities when compared to analogous compounds containing a 2-phenyl group. For example, the 2-aryl-6(7 and 8)-chloroimidazo[1,2-α]pyridines (IV.5a,c and f) gave displacements of $[3^H]$diazepam at 1000 nM of 26, 0 and 0% respectively, whereas the values for the corresponding 2-(3,4-methylenedioxyphenyl) compounds (IV.5b,e and h) were 42, 21 and 20%.

The 3-methoxy compounds (IV.8) also showed low BZR affinities. As observed for the 3-unsubstituted compounds (IV.5), the 2-aryl-6-chloro-3-methoxyimidazo[1,2-α]pyridine (IV.8a) had higher affinity for the BZR than the 7(or 8)-chloro analogues (IV.8b) and (IV.8c). In a similar manner to the related 6-(alkoxy, alkylthio and chloro)-2-aryl-3-(unsubstituted and methoxy)imidazo[1,2-b]pyridazines described in Chapters II and III, the 2-aryl-6(7 and 8)-(chloro and methoxy)-3-(unsubstituted and methoxy)imidazo[1,2-α]pyridines do not appear to have the neccessary steric and
Table IV-2 Results of displacement of \([^3]H\)diazepam binding from rat brain (BZR) and kidney (PBR) membrane preparations by some 6(7 and 8)-(chloro and methoxy)imidazo[1,2-a]pyridines

![Chemical structure](image)

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<td>5d</td>
<td>7-Cl</td>
<td>H</td>
<td>Me-p</td>
<td>(10%) (44%)</td>
</tr>
<tr>
<td>5e</td>
<td>7-Cl</td>
<td>H</td>
<td>(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>(21%) (38%)</td>
</tr>
<tr>
<td>5f</td>
<td>8-Cl</td>
<td>H</td>
<td>H</td>
<td>(0%) (20%)</td>
</tr>
<tr>
<td>5g</td>
<td>8-Cl</td>
<td>H</td>
<td>Me-p</td>
<td>(41%) (13%)</td>
</tr>
<tr>
<td>5h</td>
<td>8-Cl</td>
<td>H</td>
<td>(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>(20%) (24%)</td>
</tr>
<tr>
<td>9a</td>
<td>8-OMe</td>
<td>H</td>
<td>H</td>
<td>(41%) (57%)</td>
</tr>
<tr>
<td>9b</td>
<td>8-OMe</td>
<td>H</td>
<td>Me-p</td>
<td>(16%) (17%)</td>
</tr>
<tr>
<td>9c</td>
<td>8-OMe</td>
<td>H</td>
<td>(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>(0.1%) (48%)</td>
</tr>
<tr>
<td>6a</td>
<td>6-Cl</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>H</td>
<td>47 (65%)</td>
</tr>
<tr>
<td>6b</td>
<td>6-Cl</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>Me-p</td>
<td>11 (73%)</td>
</tr>
<tr>
<td>6c</td>
<td>6-Cl</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>6d</td>
<td>7-Cl</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>H</td>
<td>58% (48%)</td>
</tr>
<tr>
<td>6e</td>
<td>7-Cl</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>79% (58%)</td>
</tr>
<tr>
<td>6f</td>
<td>8-Cl</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>H</td>
<td>12% (17%)</td>
</tr>
<tr>
<td>6g</td>
<td>8-Cl</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>Me-p</td>
<td>16% (85%)</td>
</tr>
<tr>
<td>6h</td>
<td>8-Cl</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>21% (37%)</td>
</tr>
<tr>
<td>10a</td>
<td>8-OMe</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>H</td>
<td>32% (13%)</td>
</tr>
<tr>
<td>10b</td>
<td>8-OMe</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>Me-p</td>
<td>24% (11%)</td>
</tr>
<tr>
<td>10c</td>
<td>8-OMe</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NHCOPh</td>
<td>(3,4-OCH&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>21% (37%)</td>
</tr>
</tbody>
</table>
### Table IV-2 Continued

<table>
<thead>
<tr>
<th>Formula</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>IC₅₀ (or % displacement at 1000 nM)²⁶⁹</th>
<th>BZR</th>
<th>PBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>6-Cl</td>
<td>OMe</td>
<td>H</td>
<td>(61%)</td>
<td>(24%)</td>
<td></td>
</tr>
<tr>
<td>8b</td>
<td>7-Cl</td>
<td>OMe</td>
<td>H</td>
<td>(0%)</td>
<td>(31%)</td>
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<tr>
<td>8c</td>
<td>8-Cl</td>
<td>OMe</td>
<td>H</td>
<td>(15%)</td>
<td>(36%)</td>
<td></td>
</tr>
</tbody>
</table>

A: IC₅₀ values (or percentage displacement at 1000 nM) in the presence of 100 µM γ-aminobutyric acid (GABA) for BZR assays (see Chapter II-5.3, p75) and in the absence of GABA for PBR assays (see Chapter IV-5.2, p122)

B: Ref 269

electronic properties for high affinity binding to the BZR. Substitution at the 7- and 8-position of the molecules with a chloro or methoxy group leads to a reduction in BZR affinity when compared to the 6-chloro analogues.

The BZR affinities of the 3-benzamidomethyl compounds (IV.6) and (IV.10) showed a considerable range, from very high affinity BZR ligands such as (IV.6b) (IC₅₀ 9 nM) to compounds with negligible affinity such as (IV.6f) (12% displacement of [³H]diazepam binding at 1000 nM).

The 2-aryl-3-benzamidomethyl-6-chloroimidazo[1,2-a]pyridines (IV.6a,b) and (IV.6i)²⁶⁹ showed high BZR affinities with IC₅₀ values of 9-47 nM. Compounds containing 2-(p-methylphenyl) and 2-(3,4-methylenedioxyphenyl) groups, (IV.6i) and (IV.6b) respectively, showed higher affinity for the BZR than the 2-phenyl analogue (IV.6a). These trends in BZR affinity are similar to the structure-activity relationships observed for 6-(alkoxy and alkylthio)-2-aryl-3-benzamidomethylimidazo[1,2-b]-pyridazines in Chapters II and III, consistent with the hypothesis that substituted imidazo[1,2-a]pyridines can be used as models for similarly substituted imidazo[1,2-b]-pyridazines when comparing structure-activity relationships with reference to BZR affinity.

The 2-aryl-3-benzamidomethyl-7(and 8)-(chloro and methoxy)imidazo[1,2-a]-pyridines (IV.6c-h) and (IV.10a-c) showed lower affinity for the BZR than the 6-chloro...
analogues (IV.6a,b,i). The 7-chloro compounds (IV.6c-e) (with percentage displacements of $[^3]H$ diazepam at 1000 nM of 58-79% which would lead to IC$_{50}$ values of approximately 800-200 nM) were characterised by BZR affinities that were approximately 20 times less potent than the 6-chloro isomers. The favourable effects on BZR affinity of substitution of the 2-aryl group by $p$-methyl or (3,4-methylenedioxy) substituents, however, were maintained. The 8-chloro compounds (IV.6f-h) were found to have dramatically lower BZR affinities than similarly substituted 6-chloro compounds, with displacement of $[^3]H$diazepam binding at 1000 nM in the range 12-16%. The 8-methoxy analogues (IV.10a-c) had slightly higher BZR affinities (21-32% displacement at 1000 nM) though they were still weak BZR ligands.

These results suggest that substitution in the 7- and 8-position of imidazo[1,2-a]-pyridines (and therefore by analogy substitution in these positions of imidazo[1,2-b]-pyridazines) is detrimental to BZR affinity, independent of the substituents in the 2- and 3-positions. This effect is particularly marked in compounds containing a 3-benzamidomethyl group, as 2-aryl-3-benzamidomethyl-6-chloroimidazo[1,2-a]pyridines are very high affinity BZR ligands, whereas 7-chloro and 8-(chloro and methoxy) analogues show a progressive reduction in affinity for the BZR.

The PBR affinities of the compounds generally follow similar trends to those observed in the BZR affinities, though there are some interesting exceptions. The 7(and 8)-(chloro and methoxy) substituents generally reduce PBR affinity relative to analogous molecules containing 6-chloro groups, regardless of the substituent groups present in the 2- and 3-position, mirroring the BZR structure-activity trends.

The 3-unsubstituted compounds (IV.5) and (IV.9) have higher affinity for the PBR than the BZR, with the exception of (IV.5g). The highest affinity PBR ligands in this series of compounds are (IV.5a,b) and (IV.9a) with percentage displacements of $[^3]H$diazepam binding at 1000 nM of 64, 79 and 57% respectively (cf. BZR affinities of 26, 42 and 41%). A different trend is observed in the 3-methoxy compounds (IV.8), where PBR affinity increases moderately in the 7(and 8)-chloro compounds (IV.8b) and (IV.8c) relative to the 6-chloro isomer (IV.8a).
The 3-benzamidomethyl-7(and 8)-chloro compounds generally show a reduction in PBR affinity relative to the 6-chloro analogues. The 2-aryl-3-benzamidomethyl-6-chloroimidazo[1,2-a]pyridines (IV.6a,b,i) have PBR affinities at 1000 nM of 64-73% displacement of [3H]diazepam, and the corresponding 7-choro compounds (IV.6c-e) have PBR affinities in the range of 44-58% displacement. The 8-chloro isomers gave unexpected results. Compounds (IV.6f) and (IV.6h) had PBR affinities of the expected magnitude (17 and 37% displacement respectively) whereas 3-benzamidomethyl-8-chloro-2-(p-methylphenyl)imidazo[1,2-a]pyridine (IV.6g) gave 85% displacement of [3H]diazepam binding of at 1000 nM. It is difficult to explain this last result on the basis of steric or electronic effects alone as (IV.6g) is similar structurally and electronically to (IV.6f) and (IV.6h), and it therefore appears that other unknown factors (such as an alteration in lipophilicity or pKa to a value that is particularly favourable for PBR affinity) stabilise the binding of this compound specifically to the PBR. The 8-methoxy analogues (IV.10a-c) have PBR affinities of 11-37% displacement, in the expected range.

To summarise the BZR and PBR structure-activity relationships observed in the imidazo[1,2-a]pyridines reported in this chapter, it can be stated that, with the exception of (IV.6g), chloro or methoxy substitution in the 7-and 8-positions of the molecules leads to a reduction in both BZR and PBR affinity when compared to corresponding 6-chloro analogues. The 7- and 8-position substituent groups do not seem to lead to favourable ligand-receptor interactions. This could be due to either steric or electronic repulsion between ligand and receptor, or other factors resulting from altered molecular properties of the 7(and 8)-substituted compounds relative to the 6-substituted isomers. For the 2-aryl-6-chloroimidazo[1,2-a]pyridines, the presence of a 3-benzamidomethyl group as in (IV.6) confers a higher BZR affinity, and greater selectivity for the BZR over the PBR when compared to 3-unsubstituted analogues (IV.5).

The compounds for which syntheses have been reported in this chapter have, in some cases, shown selective high affinity for the BZR. Only (IV.6g), however, has shown selective PBR affinity. In the following chapters, structural modifications will be
made to imidazo[1,2-b]pyridazines with the aim of identifying other selective high affinity PBR ligands.

**IV-5 Experimental**

General aspects of the experimental procedures are outlined in Chapter II-5.1. The details of the *in vitro* [3H]diazepam BZR binding studies are described in Chapter II-5.3.

**IV-5.1 Syntheses**

4-Chloropyridin-2-amine, 308, 309 3-chloropyridin-2-amine, 310 α-bromo-4-methylacetophenone, 306 α-bromo-3,4-methylenedioxyacetophenone, 299 and *N*-hydroxymethylbenzamide, 300 were synthesised according to literature procedures and characterised using 1H n.m.r. spectroscopy. 5-Chloropyridin-2-amine, α-bromoacetophenone and 2-nitropyridin-3-ol were purchased from commercial sources.

**6-Chloro-2-phenylimidazo[1,2-a]pyridine (IV.5a) and related compounds**

A mixture of 5-chloropyridin-2-amine (0.19 g), α-bromoacetophenone (0.30 g) and ethanol (15.0 ml) was refluxed for 3 h, then sodium hydrogen carbonate (0.13 g) was added and the refluxing continued for 3 h. The ethanol was evaporated under reduced pressure, the residue diluted with water, and the product extracted into chloroform and the extract dried (Na2SO4). The solvent was evaporated to give a brown solid (0.29 g, 85%), part of which was recrystallised from light petroleum to give the *title compound*, m.p. 201-202°C (Found: C, 68.1; H, 4.0; N, 12.1. C13H9ClN2 requires C, 68.3; H, 4.0; N, 12.3%). 1H n.m.r.: δ 7.17, dd, J7,8 9 Hz, J5,7 2 Hz, H 7; 7.34-7.60 and 7.89-7.98, complex, Ph; 7.67, d, J7,8 9 Hz, H 8; 7.87, s, H 3; 8.20, br s, H 5. Mass spectrum (e.i.) m/z 230, 228 (M, 40%, 100%), 112 (37), 89 (31), 76 (30), 63 (27).
In a similar manner the following compounds were prepared from 5(4 and 3)-chloropyridin-2-amines (IV.4) and α-bromoacetophenone, α-bromo-4-methylacetophenone or α-bromo-3,4-methylenedioxyacetophenone.

6-Chloro-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-a]pyridine (IV.5b), (88%), m.p. 207-208°C (from light petroleum) (Found: C, 61.4; H, 3.3; N, 10.2. C_{14}H_{9}ClN_{2}O_{2} requires C, 61.7; H, 3.3; N, 10.3%). \(^1\)H n.m.r.: δ 6.00, s, OCH\(_2\)O; 6.88, d, J 7 Hz, H 5'(6'); 7.18, dd, J\(_5\), J\(_7\) 9 Hz, J\(_5\), J\(_7\) 2 Hz, H 7; 7.41-7.53, complex, H 2',6'(5'); 7.64, d, J\(_7\), J 9 Hz., H 8; 7.74, s, H 3; 8.17, br s, H 5.

7-Chloro-2-phenylimidazo[1,2-a]pyridine (IV.5c), (99%), m.p. 180-181°C (from light petroleum) (Found: C, 67.9; H, 3.9; N, 12.1. C\(_{13}\)H\(_9\)ClN\(_2\) requires C, 68.3; H, 4.0; N, 12.3%). \(^1\)H n.m.r.: δ 6.77, dd, J\(_5\), J\(_6\) 7 Hz, J\(_6\), J\(_8\) 2 Hz, H 6; δ 7.35-7.45 and 7.85-7.97, complex, Ph; 7.63, br s, H 8; 7.81, s, H 3; 8.02, d, J\(_5\), J\(_6\) 7 Hz, H 5. Mass spectrum (e.i.) \(m/z\) 230, 228 (M, 40%, 100%), 112 (18).

7-Chloro-2-(p-methylphenyl)imidazo[1,2-a]pyridine (IV.5d), (75%), m.p. 201-203°C (from light petroleum) (Found: C, 69.0; H, 4.7; N, 11.3. 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; 6.80, dd, J\(_5\), J\(_6\) 7 Hz, J\(_6\), J\(_8\) 2 Hz, H 6; 7.24, d, J 8 Hz, H 2',6'(3',5'); 7.69, br s, H 8; 7.80, s, H 3; 7.82, d, J 8 Hz, H 3',5'(2',6'); 8.04, d, J\(_5\), J\(_6\) 7 Hz, H 5.

7-Chloro-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-a]pyridine (IV.5e), (98%), m.p. 195-196°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 61.8; H, 3.3; N, 10.2. C\(_{14}\)H\(_9\)ClN\(_2\)O\(_2\) requires C, 61.7; H, 3.3; N, 10.3%). \(^1\)H n.m.r.: δ 6.00, s, OCH\(_2\)O; 6.76, dd, J\(_5\), J\(_6\) 7 Hz, J\(_6\), J\(_8\) 2 Hz, H 6; 6.85-6.93 and 7.40-7.65, complex, H 2',5',6'; 7.60, br s, H 8; 7.71, s, H 3; 8.01, d, J\(_5\), J\(_6\) 7 Hz, H 5.
8-Chloro-2-phenylimidazo[1,2-a]pyridine (IV.Sf) (39%),
m.p. 98-100°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and
recrystallisation from light petroleum (Found, for a sample dried at 60°C / 1 mmHg for 6
h: C, 68.0; H, 3.9; N, 12.0. C_{13}H_{9}ClN_{2} requires C, 68.3; H, 4.0; N, 12.3%).
^1H n.m.r.: δ 6.71, t, J 7 Hz, H 6; 7.20-7.45, complex and 7.94-7.97, complex, H 7 and
Ph; 7.91, s, H 3; 8.06, dd, J_{5,6} 7 Hz, J_{5,7} 2 Hz, H 5. Mass spectrum (e.i.) m/z 230, 228
(M, 40%, 100%), 192 (15), 114 (20).

8-Chloro-2-\((\text{p-methylphenyl})\text{imidazo}[1,2-a]\text{pyridine (IV.Sg)}\) (81%),
m.p. 136-137°C (from light petroleum) (Found: C, 69.5; H, 4.6; N, 11.5.
C_{14}H_{11}ClN_{2} requires C, 69.3; H, 4.6; N, 11.5%). ^1H n.m.r.: δ 2.37, s, Me; 6.71, t, J 7
Hz, H 6; 7.24, d, J_{6,7} 7 Hz, H 7; 7.24, d, J 8 Hz, H 2',6'(3',5'); 7.88, s, H 3; 7.88, d, J 8
Hz, H 3',5'(2',6'); 8.06, dd, J_{5,6} 7 Hz, J_{5,7} 2 Hz, H 5.

8-Chloro-2-(3',4'-methylene dioxyphenyl)imidazo[1,2-a]pyridine (IV.Sh), (42%),
m.p. 172-173°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and
recrystallisation from light petroleum (Found: C, 61.7; H, 3.2; N, 10.1. C_{14}H_{9}ClN_{2}O_{2}
requires C, 61.7; H, 3.3; N, 10.3%). ^1H n.m.r.: δ 5.99, s, OCH_{2}O; 6.68, t, J 7 Hz, H 6;
6.86, d, J 9 Hz, H 6'(5'); 7.15-7.55, complex, H 2',5'(6'); 7.21, d, J_{6,7} 7 Hz, H 7; 7.77, s,
H 3; 8.02, br d, J_{5,6} 7 Hz, H 5.

3-Methoxy-2-nitropyridine (IV.2)
A mixture of 2-nitropyridin-3-ol (0.56 g), dimethyl sulphate (0.9 ml; 1.14 g) and
potassium carbonate (0.56 g) in acetone (40.0 ml) was refluxed for 2 h. The mixture
was then diluted with water and the product extracted into chloroform and gave an oil
which slowly crystallised. It was recrystallised from light petroleum to give the title
compound (0.38 g, 61%), m.p. 72-73°C (Found, for a sample dried at 30°C / 1 mmHg
for 6 h: C, 46.3; H, 3.5; N, 17.6. C_{6}H_{5}N_{2}O_{3} requires C, 46.8; H, 3.9; N, 18.2%).
^1H n.m.r.: δ 3.98, s, MeO; 7.51, s, 7.55, s, H 4.6; 8.09, t, J 2.5 Hz, H 5.
8-Methoxy-2-phenylimidazo[1,2-a]pyridine (IV.9a) and related compounds

A mixture of 3-methoxy-2-nitropyridine (IV.2) (0.15 g) in methanol (10.0 ml) with palladium-charcoal (10%) was shaken with hydrogen until uptake ceased. The catalyst was filtered off and the solvent evaporated to leave 3-methoxypyridin-2-amine (IV.3) as an oil (0.11 g) ($^1$H n.m.r.: δ 3.85, s, MeO; 6.92, m, H 4,5,6; 8.31, br s, NH$_2$).

This oil (IV.3) (0.11 g) and α-bromoacetophenone (0.18 g) in ethanol (10.0 ml) were refluxed for 3 h, sodium hydrogen carbonate (0.076 g) was added, and the mixture refluxed for 3 h. It was then diluted with water and the product extracted into chloroform and the oil so obtained was subjected to t.l.c. (alumina; chloroform / light petroleum, 3:1). The product (0.12 g, 55%) crystallised from light petroleum and gave the title compound, m.p. 106-108°C (Found, for a sample dried at 50°C / 1 mmHg for 6 h: C, 74.9; H, 5.8; N, 12.4. C$_{14}$H$_{12}$N$_2$O requires C, 75.0; H, 5.4; N, 12.5%). $^1$H n.m.r.: δ 4.01, s, MeO; 6.41, br d, J$_{6,7}$ 7 Hz, H 7; 6.64, t, J 7 Hz, H 6; 7.32-7.60, complex and 7.67-7.76, complex, and 7.94-8.06, complex, H 5 and Ph; 7.80, s, H 3.

The following compounds were prepared from 3-methoxypyridin-2-amine (IV.3) and α-bromo-4-methylacetophenone or α-bromo-3,4-methylenedioxyacetophenone in a similar manner.

8-Methoxy-2-(p-methylphenyl)imidazo[1,2-a]pyridine (IV.9b), (68%), m.p. 141-142°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 75.2; H, 5.8; N, 11.4. C$_{15}$H$_{14}$N$_2$O requires C, 75.6; H, 5.9; N, 11.8%). $^1$H n.m.r.: δ 2.37, s, Me; 4.02, s, MeO; 6.42, br d, J$_{6,7}$ 7 Hz, H 7; 6.65, t, J 7 Hz, H 6; 7.16-7.69, complex and 7.84-7.94, complex, H 5,2',3',5',6'; 7.78, s, H 3.

8-Methoxy-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-a]pyridine (IV.9c), (43%), m.p. 168-169°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from a mixture of acetone and cyclohexane (Found: C, 67.2; H, 4.8; N, 10.4. C$_{15}$H$_{12}$N$_2$O$_3$ requires C, 67.2; H, 4.5; N, 10.4%). $^1$H n.m.r.: δ 4.03, s, MeO;
5.98, s, OCH₂O; 6.43, br d, J₆,7 7 Hz, H 7; 6.66, t, J 7 Hz, H 6; 6.80-7.68, complex and 7.72-7.77, complex, H 5,2',5',6'; 7.71, s, H 3.

3-Benzamidomethyl-7-chloro-2-phenylimidazo[1,2-a]pyridine (IV.6c) and related compounds

A mixture of N-hydroxymethylbenzamide (0.11 g), glacial acetic acid (5.0 ml) and concentrated sulphuric acid (0.18 ml) was heated in an oil bath at 50°C for 15 min, then 7-chloro-2-phenylimidazo[1,2-a]pyridine (IV.6c) was added and the mixture refluxed at 120°C for 24 h. The acetic acid was removed in vacuo and the residue diluted with water (20 ml) and adjusted with aqueous ammonia to pH 10. The product was extracted into chloroform, and after washing with water the extract was dried (Na₂SO₄) and solvent removed to give an oil. This was subjected to t.l.c. (alumina; chloroform / light petroleum, 3:1) and gave the title compound (52%), as white crystals, m.p. 226-227°C (from toluene). (Found: C, 70.0; H, 4.4; N, 11.5. C₂₁H₁₆ClN₃O requires C, 69.7; H, 4.5; N, 11.6%). ¹H n.m.r.: δ 5.05, d, J 5.5 Hz, CH₂N; 6.74, dd, J 7 Hz, J 2 Hz, H 6; 7.27-7.45 and 7.50-7.91, complex, 2 x Ph, 7.47, br s, H 8; 8.18, br d, J₅,₆ 7 Hz, H 5. Mass spectrum (e.i.) m/z 363, 361 (M, 25%, 70%), 256 (60), 241 (100), 229 (40), 105 (50), 77 (44).

The following compounds were prepared by similar procedures from the 3-unsubstituted imidazo[1,2-a]pyridines (IV.5) and (IV.9) with N-hydroxymethylbenzamide.

3-Benzamidomethyl-6-chloro-2-phenylimidazo[1,2-a]pyridine (IV.6a), (55%), m.p. 237-238°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 69.9; H, 4.8; N, 11.8. C₂₁H₁₆ClN₃O requires C, 69.7; H, 4.5; N, 11.6%). ¹H n.m.r.: δ 5.09, d, J 5.5 Hz, CH₂N; 6.75, br, NH; 7.15, d; 17,8 9 Hz, J₅,7 2 Hz, H 7; 7.33-7.90, complex, H 8 and 2 x Ph; 8.36,br s, H 5. Mass spectrum (e.i.) m/z 363, 361 (M, 40%, 100%), 256 (50), 241 (82), 229 (37), 105 (47), 77 (37).
3-Benzamidomethyl-6-chloro-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-a]-pyridine (IV.6b), (21%), m.p. 227-228°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 65.1; H, 3.9; N, 10.3. \( \text{C}_{22}\text{H}_{16}\text{ClN}_{3}\text{O}_{3} \) requires C, 65.1; H, 4.0; N, 10.4%). \( ^1\text{H} \text{n.m.r.} : \delta \text{ 5.04, d, J 5.5 Hz, CH}_2\text{N;} \ 5.98, \text{s, OCH}_2\text{O; 6.74-7.97, complex, H 7,8,2',5',6' and Ph; 8.34, br s, H 5.} \)

3-Benzamidomethyl-7-chloro-2-(p-methylphenyl)imidazo[1,2-a]pyridine (IV.6d), (43%), m.p. 245-246°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 70.4; H, 5.1; N, 11.5. \( \text{C}_{22}\text{H}_{18}\text{ClN}_{3}\text{O} \) requires C, 70.3; H, 4.8; N, 11.2%). \( ^1\text{H} \text{n.m.r.} : \delta \text{ 2.35, s, Me; 5.06, ct, J 5.5 Hz, CH}_2\text{N;} \ 6.75, \text{dd, J 7 Hz, J 2 Hz, H 6; 7.07-7.92, complex, H 2',3',5',6' and Ph; 7.48, br s, H 8; 8.20, br d, J 5.6, 7 Hz, H 5.} \)

3-Benzamidomethyl-7-chloro-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-a]-pyridine (IV.6e), (75%), m.p. 260-261°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 64.6; H, 3.9; N, 10.1. \( \text{C}_{22}\text{H}_{16}\text{ClN}_{3}\text{O}_{3} \) requires C, 65.1; H, 4.0; N, 10.4%). \( ^1\text{H} \text{n.m.r.} : \delta \text{ 5.09, d, J 5.5 Hz, CH}_2\text{N;} \ 5.97, \text{s, OCH}_2\text{O; 6.73-7.93, complex, H 6,8,2',5',6' and Ph; 8.36, br d, J5.6, 7 Hz, H 5.} \)

3-Benzamidomethyl-8-chloro-2-phenylimidazo[1,2-a]pyridine (IV.6f), (50%), m.p. 226-228°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 69.9; H, 4.6; N, 11.4. \( \text{C}_{21}\text{H}_{16}\text{ClN}_{3}\text{O} \) requires C, 69.7; H, 4.5; N, 11.6%). \( ^1\text{H} \text{n.m.r.} : \delta \text{ 5.09, d, J 5.5 Hz, CH}_2\text{N;} \ 6.72, \text{t, J 7 Hz, H 6; 7.19-8.00, complex, H 7 and 2 x Ph; 8.24, br d, J5.6, 7 Hz, H 5. Mass spectrum (e.i.) m/z 363, 361 (M, 30%, 80%), 256 (65), 241 (100), 229 (65), 105 (66), 77 (55).} \)

3-Benzamidomethyl-8-chloro-2-(p-methylphenyl)imidazo[1,2-a]pyridine (IV.6g), (30%), m.p. 259-261°C after t.l.c. (alumina; chloroform / light petroleum) and recrystallisation from toluene. (Found: C, 70.8; H, 4.4; N, 11.5. \( \text{C}_{22}\text{H}_{18}\text{ClN}_{3}\text{O} \) requires C, 70.3; H, 4.8; N, 11.2%). \( ^1\text{H} \text{n.m.r.} : \delta \text{ 2.35, s, Me; 5.06, ct, J 5.5 Hz, CH}_2\text{N;} \ 6.75, \text{dd, J 7 Hz, J 2 Hz, H 6; 7.07-7.92, complex, H 2',3',5',6' and Ph; 7.48, br s, H 8; 8.20, br d, J 5.6, 7 Hz, H 5.} \)
requires C, 70.3; H, 4.8; N, 11.2%). $^1$H n.m.r.: $\delta$ 2.31, s, Me; 5.09, d, J 5.5 Hz, CH$_2$N; 6.73, t, J 7 Hz, H 6; 7.10-8.00, complex, H 7,2',3',5',6' and Ph; 8.24, br d, J$_{5,6}$ 7 Hz, H 5.

3-Benzamidomethyl-8-chloro-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-a]pyridine (IV.6h), (32%), m.p. 248-249°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 64.9; H, 4.0; N, 10.3. C$_{22}$H$_{16}$ClN$_3$O$_3$ requires C, 65.1; H, 4.0; N, 10.4%). $^1$H n.m.r.: $\delta$ 5.06, d, J 5.5 Hz, CH$_2$N; 5.92, s, OCH$_2$O; 6.59-7.98, complex, H 6,7,2',5',6' and Ph; 8.24, br d; J 7 Hz, H 5.

3-Benzamidomethyl-8-methoxy-2-(3',4'-methylenedioxyphenyl)imidazo[1,2-a]pyridine (IV.10c), (13%), m.p. 251-253°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene. (Found: C, 68.9; H, 4.8; N, 10.4. C$_{23}$H$_{19}$N$_3$O$_4$ requires C, 68.8; H, 4.8; N, 10.5%). $^1$H n.m.r.: $\delta$ 3.95, s, MeO; 5.06, d, J 5.5 Hz, CH$_2$N; 5.94, s, OCH$_2$O; 6.45, br d, J 7 Hz, H 7; 6.68, t, J 7 Hz, H 6; 7.06-7.99, complex, H 2',5',6' and Ph; 7.85, d, J$_{5,6}$ 7 Hz, H 5.
6-Chloro-3-methoxy-2-phenylimidazo[1,2-a]pyridine (IV.8a) and related compounds

A solution of 5-chloropyridin-2-amine (0.13 g), phenylglyoxal monohydrate (0.15 g), ethanol (10.0 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed with stirring in an oil bath at 100°C for 14 h. The solvent was evaporated and the residue stirred with ethereal diazomethane (from 1.03 g nitrosomethylurea) at 0°C for 2 h and then at 20°C overnight. The solvent and excess reagent were then evaporated and the residue subjected to t.l.c. (alumina; chloroform / light petroleum, 3:1). It gave the title compound (0.10 g, 38%) as a yellow oil. (Found, for a sample dried at 40°C / 1 mmHg for 6 h: C, 63.7; H, 4.2; N, 10.6. C\textsubscript{14}H\textsubscript{11}ClN\textsubscript{2}O. 0.05 CHCl\textsubscript{3} requires C, 63.8; H, 4.2; N, 10.6%). \textsuperscript{1}H n.m.r.: \(\delta\) 3.97, s, MeO; 7.03-8.11, complex, H 5,7,8 and Ph. Mass spectrum (e.i.) m/z 260, 258 (M, 12%, 34%), 217 (48), 215 (100), 112 (65), 84 (48).

The following compounds were prepared in a similar manner from 4(and 3)-chloropyridin-2-amines (IV.4b,c) and phenylglyoxal monohydrate.

7-Chloro-3-methoxy-2-phenylimidazo[1,2-a]pyridine (IV.8b), (27%), as an oil, after t.l.c. (alumina; chloroform / light petroleum, 3:1) (Found, for a sample dried at 40°C / 1 mmHg for 6 h: C, 64.5; H, 4.5; N, 10.4. C\textsubscript{14}H\textsubscript{11}ClN\textsubscript{2}O. 0.02 CHCl\textsubscript{3} requires C, 64.5; H, 4.3; N, 10.7%). \textsuperscript{1}H n.m.r.: \(\delta\) 3.95, s, MeO; 6.75-6.94, complex, 7.31-7.54, complex, and 7.84-8.09, complex, H 5,6,8 and Ph. Mass spectrum (e.i.) m/z 260, 258 (M, 15%, 35%), 217 (46), 215 (100), 112 (71), 76 (37).

8-Chloro-3-methoxy-2-phenylimidazo[1,2-a]pyridine (IV.8c), (50%), as an oil, after t.l.c. (alumina; chloroform / light petroleum, 1:1) (Found, for a sample dried at 35°C / 1 mmHg for 6 h: C, 64.2; H, 4.3; N, 10.3. C\textsubscript{14}H\textsubscript{11}ClN\textsubscript{2}O. 0.04 CHCl\textsubscript{3} requires C, 64.0; H, 4.2; N, 10.6%). \textsuperscript{1}H n.m.r.: \(\delta\) 3.96, s, MeO; 6.73, t, J 7 Hz, H 7; 7.15-8.10, complex, H 8 and Ph; 8.13, dd, J\textsubscript{7,8} 8 Hz, J\textsubscript{5,8} 1 Hz, H 8. Mass spectrum (e.i.) m/z 260, 258 (M, 15%, 37%), 217 (47), 215 (100), 112 (59), 76 (32).
IV-5.2 [³H]Diazepam in vitro PBR binding assay

The experimental conditions of the [³H]diazepam PBR assay have been previously described. Young adult male Wistar rats were decapitated and the kidneys removed. The kidneys were dissected free of fat and the kidney capsule was rinsed in ice-cold saline, blotted and weighed, and then chopped with scissors and homogenised using an 'Ultra-Turrax' in 16 volumes of ice-cold 0.32 M sucrose. The homogenate thus obtained was centrifuged at 17000 rpm for 30 minutes, then the supernatant liquid was decanted and the remaining pellet suspended in ice-cold distilled water. After 10 minutes, this suspension was centrifuged a second time and the pellet resuspended in 50 mM Tris-HCl buffer, pH 7.4. Finally this suspension was centrifuged and the pellet resuspended in Tris buffer and stored frozen. On the day of use, the suspension was thawed, recentrifuged and the pellet suspended in fresh Tris buffer.

The PBR binding assay contained aliquots of the rat kidney membrane preparations (approximately 1 mg protein), various concentrations of the test compounds and [³H]diazepam (86.6 Ci / mmol, 0.70 ± 0.05 nM final concentration) in a final volume of 2 ml Tris-HCl buffer. Assays were performed in the absence of γ-amino-butyric acid as it does not stimulate BZ binding to the PBR. The assays were incubated with [³H]diazepam on ice at 0-4°C for 60 minutes. Nonspecific binding was determined in separate tubes by the addition of a large excess (10 µM) of unlabelled diazepam. After the incubation period the 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 toluene / Triron X-100 scintillation fluid and bound radioactivity was determined using conventional techniques.

Compounds were initially tested for their ability to displace specific [³H]diazepam binding from the PBR at a single concentration of 1000 nM, and for compounds showing high percentage displacement, IC₅₀ values were determined over 4 separate concentrations, with all assays within each experiment being performed in triplicate. The IC₅₀ values for the test compounds were calculated using log-logit
analysis (with the correlation coefficients of the lines of best fit to log-logit curves not less than 0.95) as described in Chapter II-5.3 for the BZR assays.
CHAPTER V  Syntheses and BZR and PBR affinities of some 6-alkylthio, phenylthio, benzylthio and pyridinylmethylthio-3-acyl-3-(methoxyethyl) and benzamidomethylthioimidazo[1,2-b]pyridazines

V-1 Introduction

In previous chapters, the structural requirements of the BZR have been examined principally by the synthesis of imidazo[1,2-b]pyridazine and imidazo[1,2-a]pyridine ligands with various substituents in the 9-, 7- and 8-positions. The compounds with high BZR affinity frequently also contained 3-aryl and 3-benzamidomethyl substituents. To provide further information relating to the structural and electronic requirements of other areas of the BZR ligand binding site, it was of interest to examine the effects on BZR affinity of structural modifications at the 3- and 5-positions of imidazo[1,2-b]pyridazines.

In this chapter, a series of 2-arylpyridazines has been synthesised, in which the phenyl ring of the 2-aryl substituent is separated by a rigid CH=CH group from the imidazo[1,2-b]pyridazine nucleus. This is in contrast to compounds synthesised in previous chapters where the phenyl ring of the 3-aryl substituent is directly connected to the imidazo[1,2-b]pyridazine nucleus. As it is thought that the phenyl ring of the 2-aryl groups is important in stabilising ligand binding at the BZR, possibly by interaction with a hydrophobic pocket on the receptor protein, the determination of BZR affinity of compounds containing 2-aryl groups (which are also conjugated to the triazadiazol 1,2-b pyridazine ring) is of interest as it could provide information regarding the static constraints of this hydrophobic pocket. The evaluation of the PBR affinities of these compounds would also indicate the similarities or otherwise, of the structural and electronic requirements for ligand binding for both types of BZ receptors at the central and peripheral sites.

The syntheses of 6-alkylthio, phenylthio, benzylthio and pyridinylmethylthio-3-acyl-3-(methoxyethyl) and benzamidomethylthioimidazo[1,2-b]pyridazines are described in this chapter. The compounds have been characterised by the usual spectral and analytical techniques, and details of their BZR and PBR affinities have been determined. Structural features shown to be either beneficial or detrimental to BZR and/or PBR affinity are
CHAPTER V Syntheses and BZR and PBR affinities of some 6-(alkylthio, phenylthio, benzylthio and pyridinylmethylthio)-2-styryl-3-(unsubstituted and benzamidomethyl)imidazo[1,2-b]pyridazines

V-1 Introduction

In previous chapters, the structural requirements of the BZR have been examined principally by the synthesis of imidazo[1,2-b]pyridazine and imidazo[1,2-a]pyridine ligands with various substituents in the 6-, 7- and 8-positions. The compounds with high BZR affinity frequently also contained 2-aryl and 3-benzamidomethyl substituents. To provide further information relating to the structural and electronic requirements of other areas of the BZR ligand binding site, it was of interest to examine the effects on BZR affinity of structural modifications at the 2- and 3-positions of imidazo[1,2-b]pyridazines.

In this chapter a series of 2-styrylimidazo[1,2-b]pyridazines has been synthesised, in which the phenyl ring of the 2-styryl substituent is separated by a rigid CH=CH group from the imidazo[1,2-b]pyridazine nucleus. This is in contrast to compounds synthesised in previous chapters where the phenyl ring of the 2-aryl substituents is directly connected to the imidazo[1,2-b]pyridazine nucleus. As it is thought that the phenyl ring of the 2-aryl groups is important in stabilising ligand binding to the BZR, possibly by interaction with a hydrophobic pocket on the receptor protein, the determination of BZR affinities of compounds containing 2-styryl groups (which are also conjugated to the imidazo[1,2-b]-pyridazine ring) is of interest as it could provide information regarding the steric constraints of this hydrophobic pocket. The evaluation of the PBR affinities of these compounds would also indicate the similarity, or otherwise, of the structural and electronic requirements for ligand binding for both types of BZ receptor at the central and peripheral sites.

The syntheses of 6-(alkylthio, phenylthio, benzylthio and pyridinylmethylthio)-2-styryl-3-(unsubstituted and benzamidomethyl)imidazo[1,2-b]pyridazines are described in this chapter. The compounds have been characterised by the usual spectral and analytical techniques, and their in vitro BZR and PBR affinities have been determined. Structural features shown to be either beneficial or detrimental to BZR and / or PBR affinity are
discussed. The experimental details of the syntheses of the compounds are recorded in Chapter V-5.

V-2 Syntheses

The compounds reported in this chapter were synthesised by the reaction of the appropriate 6-substituted pyridazin-3-amine (V.1) (prepared according to the relevant literature procedures as outlined in the Experimental Section of this chapter) with 1-bromo-4-phenylbut-3-en-2-one 314 to give the 2-styryl-6-substituted imidazo[1,2-b]-pyridazines (V.2) in yields of 21-70% (Scheme V-1).

The reaction of the imidazo[1,2-b]pyridazines (V.2a-d,f,i,j) with N-hydroxymethylbenzamide (under reaction conditions previously reported for the synthesis of other substituted imidazo[1,2-b]pyridazines)267 led to the formation of the corresponding 3-benzamidomethyl-2-styryl-6-substituted imidazo[1,2-b]pyridazines (V.3) by electrophilic displacement of the hydrogen atom in the 3-position, and gave yields of 18-74%. Compounds (V.2e,g,h) did not form the 3-benzamidomethyl products, in our hands, when treated similarly, and instead appeared to decompose. All compounds were purified by chromatographic techniques (as required) and by recrystallisation.

V-3 Physical properties

Some 1H n.m.r. spectral data for the imidazo[1,2-b]pyridazines reported in this chapter are presented in Table V-1.

The 3-unsubstituted imidazo[1,2-b]pyridazines (V.2) show a characteristic downfield singlet (δ 7.87-7.92) corresponding to the H-3 signal, and doublets corresponding to H-7 (δ 6.72-6.91) and H-8 (δ 7.67-7.75) with a coupling constant J7,8 of 9.5 Hz. The signal resulting from the CH2S protons of (V.2d-j) appeared as a singlet at δ 4.36-4.60.
Reagents: (i) BrCH₂COCH=CHPh, NaHCO₃, EtOH, reflux (ii) PhCONHCH₂OH, conc H₂SO₄, AcOH, 120°C

The 3-benzamidomethyl compounds (V.3) showed doublets at δ 6.75-6.91 and δ 7.63-7.70 corresponding to the signals from H-7 and H-8 respectively, with a coupling constant $J_{7,8}$ of 9.5 Hz. The CH₂ protons from the benzamidomethyl group gave a doublet (δ 4.90-5.19) resulting from coupling to the neighbouring NH group.

Compounds (V.3d-g) were also characterised by a singlet (δ 4.37-4.51) assigned as the signal from the CH₂S protons.

The mass spectra for the 3-unsubstituted imidazo[1,2-b]pyridazines (V.2) did not show any consistent fragmentation patterns and are described in the Experimental Section of this chapter. The mass spectra for the 3-benzamidomethyl compounds (V.3) showed peaks corresponding to the molecular ion and a number of fragment ions. Consistent fragmentation patterns were not observed, though peaks at $m/z$ 77 and 105, presumably corresponding to [C₆H₅]⁺ and [COC₆H₅]⁺ ions, were always present.
Table V-1 Some $^1$H n.m.r. spectral data (δ)\textsuperscript{A} for some 2-styrylimidazo[1,2-b]-pyridazines

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\textsuperscript{A} Reported as parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard in deuterochloroform.

B: Complex signal

It was neccessary to determine the configuration (cis or trans) of the hydrogen atoms of the styryl moiety of the 2-styrylimidazo[1,2-b]pyridazines reported in this chapter, as this would affect the orientation of the phenyl ring of the styryl group, and therefore the area of possible lipophilic ligand-receptor interaction. The X-ray crystal structure was determined by Dr. A.C. Willis at the Research School of Chemistry, Australian National University.
structure of (V.2a) was determined and the results are shown in Figures V-1 and V-2. These results show that (V.2a) existed as the trans isomer, and it was therefore assumed that all the compounds reported in this chapter adopted the trans configuration at the ligand binding site of the BZR and PBR.

V-4 In vitro binding studies

The BZR and PBR affinities of the compounds reported in this chapter were measured by the in vitro displacement of [3H]diazepam binding from rat brain and kidney membranes under the assay conditions described in Chapter II-5.3 and Chapter IV-5.2 respectively.

V-4.1 Results of in vitro testing

Table V-2 shows the results of the in vitro BZR and PBR binding assays as either the percentage displacement of [3H]diazepam binding at a single ligand concentration of 1000 nM, or IC50 values. Data by other workers are included, with appropriate footnotes, for comparative purposes.

V-4.2 Discussion of results

Table V-2 shows the in vitro BZR and PBR affinities of the compounds reported in this chapter. In general, the compounds bound weakly to the BZR and demonstrated PBR affinities ranging from zero to nanomolar IC50 values.

The 3-unsubstituted compounds (V.2) did not demonstrate high affinity for the BZR or PBR, as determined by the in vitro displacement of [3H]diazepam binding from these receptors. This is consistent with the results observed for other 3-unsubstituted imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines described in previous chapters.

A The X-ray crystal structure was determined by Dr A.C. Willis at the Research School of Chemistry, Australian National University.
Figure V-1 X-Ray crystal structure of 6-methylthio-2-styrylimidazo[1,2-b]pyridazine (V.2a)^A

^A Thermal ellipsoid diagram of the C_{15}H_{13}N_{3}S molecule with labelling of selected atoms. Ellipsoids show 50% probability levels except for hydrogen atoms which are drawn as circles of arbitrary small radius.
Figure V-2  Unit cell X-ray crystal structure of 6-methylthio-2-styrylimidazo[1,2-b]-pyridazine (V.2a)^A

^A Thermal ellipsoid diagram of the contents of the unit cell, projected along the b axis. Ellipsoids show 50% probability levels except for hydrogen atoms which are drawn as circles of arbitrary small radius.
Table V-2  Results of dispacement of [3H]diazepam binding from rat brain (BZR) and kidney (PBR) membrane preparations by some 2-styrylimidazo[1,2-b]-pyridazines

<table>
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A: IC_{50} values (or percentage displacement at 1000 nM) in the presence of 100 µM γ-aminobutyric acid (GABA) for BZR assays (see Chapter II-5.3, p75) and in the absence of GABA for PBR assays (see Chapter IV-5.2, p122)

B: Prepared by Dr N.W. Jacobsen

Compounds (V.2a-d), containing 6-(alkylthio, phenylthio and benzylthio) substituents all showed less than 50% displacement of [3H]diazepam binding from the
BZR, and zero displacement from the PBR. Conversely, the compounds (V.2e-g) containing 6-(substituted benzylthio) groups were found to bind more strongly to the PBR than to the BZR. This is most clearly illustrated in compound (V.2f), with 13\% displacement of [\textsuperscript{3}H]diazepam binding from the BZR, and 69\% displacement from the PBR, at 1000 nM. 6-Pyridinylmethylthio groups, as in (V.2h-j) did not confer consistent effects on BZR or PBR affinity, as (V.2h) has higher \textit{in vitro} affinity for the PBR than the BZR, (V.2i) has identical PBR and BZR affinities, and (V.2j) binds more strongly to the BZR than to the PBR.

The 3-benzamidomethyl compounds (V.3) demonstrated a considerable range of BZR and PBR affinities, depending on the substituent group at the 6-position. The 6-(alkylthio and phenylthio) compounds (V.3a-c) were found to have low affinity for both the BZR and the PBR. A direct comparison of the BZR affinities of 3-benzamidomethyl-6-methylthio-2-phenylimidazo[1,2-b]pyridazine (II.13I) (IC\textsubscript{50} 20 nM)\textsuperscript{263} with (V.3a) (17\% displacement of [\textsuperscript{3}H]diazepam binding at 1000 nM) illustrates that replacement of a 2-phenyl group with a 2-styryl substituent leads to a dramatic reduction in BZR affinity. The phenyl ring of the 2-styryl group does not appear to be favourably positioned for interaction with the BZR protein. If the aromatic phenyl ring of the 2-phenyl compound (II.13I) is interacting with a hydrophobic pocket or cleft in the BZR protein, it seems that the 2-styryl group is sterically too bulky to occupy this pocket, and therefore binding to the BZR is hindered.

In contrast to the 6-(alkylthio and alkoxy)-2-aryl-3-benzamidomethyl compounds (II.13) and (III.6), in which an increase in the size of the 6-position group led to a decrease in BZR affinity, a consistent relationship between the size of the group in the 6-position and BZR affinity was not observed in the 3-benzamidomethyl-2-styryl-6-substituted imidazo[1,2-b]pyridazines (V.3).

The 6-(benzylthio and m-nitrobenzylthio) compounds (V.3d) and (V.3e) showed negligible BZR affinity but bound strongly to the PBR, with IC\textsubscript{50} values of 115 nM and 10 nM respectively. The 6-chloro compound (V.3h) was also found to be a selective, high affinity PBR ligand, with very low BZR affinity and an IC\textsubscript{50} of 5 nM at the PBR. It was of interest that the PBR affinities of (V.3d) and (V.3e) were much greater than that
of the 6-phenylthio analogue (V.3c) (35% displacement at 1000 nM). It is possible that the 6-benzylthio group can adopt a conformation where the phenyl ring is orientated towards a different area of the PBR protein to that occupied by the phenyl ring of a 6-phenylthio substituent. This orientation could either be towards an area of favourable ligand/receptor interaction inaccessible to the 6-phenylthio group, or away from a region of unfavourable ligand/receptor interaction occupied by the 6-phenylthio group.

A comparison of the BZR affinities of (V.3d) (7% displacement at 1000 nM) and (V.3f) (IC₅₀ 68 nM) showed that the replacement of a 6-benzylthio substituent with a 6-(pyridin-3-yl)methylthio group increased BZR affinity greatly, with a corresponding 3-fold decrease in PBR affinity. The 6-(pyridin-4-yl)methylthio compound (V.3g) did not show high affinity for either the BZR or the PBR. The presence of a nitrogen atom in the β-position of the pyridinyl ring therefore appears to be of value in stabilising interactions specifically at the BZR.

To summarise, the 2-styryl compounds (V.2) and (V.3) show low affinity for the BZR, with the exception of (V.3f), whether unsubstituted at the 3-position or containing a 3-benzamidomethyl group. This indicates that the steric bulk of the 2-styryl group hinders binding to the BZR. PBR affinity, however, is not affected in the same way.

Amongst the 1,4-benzodiazepine class of compounds (see Chapter I, Figure I-2 for chemical structures), diazepam and flunitrazepam show high BZR and PBR affinity, whereas clonazepam and Ro5-4864 (the 4'-chloro analogue of diazepam) are BZR and PBR specific respectively. This is consistent with the hypothesis that the BZR and PBR pharmacophores are similar, with minor structural modifications of ligands conferring BZR and/or PBR selectivity.

In the case of the imidazo[1,2-b]pyridazine ligands reported here, structural modifications in the 6- and 2-position appear to influence PBR selectivity. The replacement in analogous compounds of a 2-phenyl group with a 2-styryl group leads to a considerable reduction in BZR affinity regardless of the nature of the 6-position group. Certain 3-benzamidomethyl-2-styryl-6-substituted imidazo[1,2-b]pyridazines, however, do bind with nanomolar affinity to the PBR. This suggests, assuming that the ligands adopt similar orientations at both the BZR and PBR, that the area of the receptor protein
interacting with the 2-position groups of the ligands is subject to more restrictive steric constraints at the BZR than at the PBR site.

Differences are also apparent in the steric and/or electronic requirements of the BZR and PBR proteins in the vicinity of the 6-position groups of the imidazo[1,2-b]pyridazine ligands. As mentioned previously in this discussion, compounds (V.3d) and (V.3e) bind with high affinity and selectivity to the PBR, while the BZR affinities of these compounds are reduced compared to analogous compounds (V.3a-c) with smaller 6-position groups. Their low BZR affinities cannot therefore only be ascribed to the effect of the 2-styryl group. It seems most probable that the ligand binding site on the PBR protein can accommodate the 6-(benzylthio or substituted benzylthio) group of 3-benzamidomethyl-6-(benzylthio or substituted benzylthio)-2-styrylimidazo[1,2-b]pyridazines, whereas the area of the BZR that interacts with this substituent cannot.

In the following chapter, the effects on BZR/PBR selectivity of other bulky groups in the 2-position and also of substitution of the phenyl ring of the 3-benzamidomethyl group of some imidazo[1,2-b]pyridazines will be examined.

V-5 Experimental

The general aspects of the experimental procedures are described in Chapter II-5.1. The details of the in vitro [3H]diazepam BZR assay are outlined in Chapter II-5.3 and those of the PBR assay in Chapter IV-5.2.

6-Chloropyridazin-3-amine,\(^{305}\) 6-methylthiopyridazin-3-amine,\(^{285}\) 6-(ethylthio and phenylthio)pyridazin-3-amine,\(^{275}\) 6-(benzylthio and 3-nitrobenzylthio)pyridazin-3-amine,\(^{262}\) 6-(3-methoxybenzylthio)pyridazin-3-amine,\(^{263}\) 6-(3,4-methylenedioxybenzylthio)pyridazin-3-amine,\(^{287}\) 6-(pyridin-2-yl, pyridin-3-yl and pyridin-4-yl)methylthiopyridazin-3-amine,\(^{270}\) and 1-bromo-4-phenylbut-3-en-2-one \(^{314}\) were synthesised according to literature procedures and characterised using \(^1\)H n.m.r. spectra.
6-Ethylthio-2-styrylimidazo[1,2-b]pyridazine (V.2b) and related compounds

6-Ethylthiopyridazin-3-amine (0.23 g) (V.1b) was dissolved in boiling ethanol (15.0 ml) under reflux, 1-bromo-4-phenyl-3-buten-2-one (0.90 g) was added and the mixture was refluxed for 3 h. Sodium hydrogen carbonate (0.13 g) was then added and the refluxing continued for 3 h. The ethanol was removed in vacuo and the residue extracted with chloroform, the extract was washed with water, dried (Na₂SO₄), and the solvent evaporated. The product was recrystallised from light petroleum and gave yellow crystals of the title compound (0.10 g, 24%), m.p. 87-88°C (Found, for a sample dried at 50°C / 1 mmHg for 6 h: C, 68.8; H, 5.3; N, 15.0. C₁₆H₁₅N₃S requires C, 68.3; H, 5.4; N, 14.9%). ¹H n.m.r.: δ 1.47, t, J 7.5 Hz, CH₂; 6.88, d, J 9.5 Hz, H 7; 7.14-7.60, complex, PhCH=CH; 7.73, d, J 9.5 Hz, H 8; 7.91, s, H 3.

In a similar manner, from 6-(variously substituted)pyridazin-3-amines (V.1) and 1-bromo-4-phenyl-3-buten-2-one the following compounds were prepared.

6-Methylthio-2-styrylimidazo[1,2-b]pyridazine (V.2a), (43%),
m.p. 160-162°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 67.6; H, 5.0; N, 15.7. C₁₅H₁₃N₃S requires C, 67.4; H, 4.9; N, 15.7%). ¹H n.m.r.: δ 2.64, s, MeS; 6.91, d, J 9.5 Hz, H 7; 7.11-7.60, complex, PhCH=CH; 7.73, d, J 9.5 Hz, H 8; 7.92, s, H 3. Mass spectrum (e.i.) m/z 267 (M, 98%), 266 (100), 219 (60), 142 (30), 115 (67).

6-Phenylthio-2-styrylimidazo[1,2-b]pyridazine (V.2c), (70%),
m.p. 192-193°C after recrystallisation from a mixture of ethanol and toluene (2 : 1) (Found: C, 72.5; H, 4.5; N, 12.7. C₂₀H₁₅N₃S requires C, 72.9; H, 4.6; N, 12.8%). ¹H n.m.r. δ 6.74, d, J 9.5 Hz, H 7; 7.23-7.62, complex, PhCH=CH and Ph; 7.67, d, J 9.5 Hz, H 8; 7.87, s, H 3.
6-Benzylthio-2-styrylimidazo[1,2-b]pyridazine (V.2d), (47%), m.p. 138-139°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from cyclohexane (Found: C, 73.5; H, 5.1; N, 12.1. C21H17N3S requires C, 73.4; H, 5.0; N, 12.2%). 1H n.m.r.: δ 4.43, s, CH2; 6.86, d, J 9.5 Hz, H 7; 7.03-7.79, complex, PhCH=CH and Ph; 7.73, d, J 9.5 Hz, H 8; 7.91, s, H 3. Mass spectrum (e.i.) m/z 434 (M, 100%), 310 (45), 266 (35), 220 (35), 115(35), 91 (95).

6-(3'-Methoxybenzylthio)-2-styrylimidazo[1,2-b]pyridazine (V.2e), (49%), m.p. 89-90°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found, for a sample dried at 60°C / 0.1 mmHg for 6 h: C, 71.0; H, 5.2; N, 11.0. C22H19N3OS requires C, 70.8; H, 5.1; N, 11.3%). 1H n.m.r.: δ 3.30, s, MeO; 4.40, s, CH2S; 6.86, d, J 9.5 Hz, H 7; 6.89-7.65, complex, PhCH=CH and H 2',4',5',6'; 7.74, d, J 9.5 Hz, H 8; 7.90, s, H 3. Mass spectrum (e.i.) m/z 373 (M, 100%), 340 (40), 220 (30), 121 (100), 91 (40).

6-(3'-Nitrobenzylthio)-2-styrylimidazo[1,2-b]pyridazine (V.2f), (21%), m.p. 118-120°C after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from cyclohexane (Found: C, 65.0; H, 4.0; N, 14.2. C21H16N4O2S requires C, 64.9; H, 4.2; N, 14.4%). 1H n.m.r.: δ 4.46, s, CH2; 6.80, d, J 9.5 Hz, H 7; 7.02-7.88, complex, PhC=CH, and H 8,5',6'; 7.90, s, H 3; 8.12, br d, J 8.5 Hz, H 4'; 8.39, t, J 2 Hz, H 2'.

6-(3',4'-Methylenedioxybenzylthio)-2-styrylimidazo[1,2-b]pyridazine (V.2g), (62%), m.p. 164-164°C (from ethanol) (Found: C, 68.5; H, 4.5; N, 10.8. C22H17N3O2S requires C, 68.2; H, 4.4; N, 10.8%). 1H n.m.r.: δ 4.36, s, CH2S; 5.95, s, OCH2O; 6.79-7.69, complex, PhCH=CH and H 7,2',5',6'; 7.75, d, J 9.5 Hz, H 8; 7.91, s, H 3. Mass spectrum (e.i.) m/z 387 (M, 35%), 135 (100).
6-(Pyridin-2'-ylmethylthio)-2-styrylimidazo[1,2-b]pyridazine (V.2h), (47%), m.p. 112-113°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 69.8; H, 4.6; N, 16.0. C_{20}H_{16}N_{4}S requires C, 69.7; H, 4.7; N, 16.3%). $^1$H n.m.r.: δ 4.60, s, CH$_2$S; 6.91, d, J 9.5 Hz, H 7; 7.01-7.69 and 8.56-8.64, complex, PhCH=CH and pyridinyl; 7.73, d, J 9.5 Hz, H 8; 7.90, s, H 3.

6-(Pyridin-3'-ylmethylthio)-2-styrylimidazo[1,2-b]pyridazine (V.2i), (29%), m.p. 113-115°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 69.5; H, 4.9; N, 16.4. C$_{20}$H$_{16}$N$_{4}$S requires C, 69.7; H, 4.7; N, 16.3%). $^1$H n.m.r.: δ 4.40, s, CH$_2$S; 6.72, d, J 9.5 Hz, H 7; 7.02-7.60 and 8.56-8.74, complex, PhCH=CH and pyridinyl; 7.69, d, J 9.5 Hz, H 8; 7.91, s, H 3.

6-(Pyridin-4'-ylmethylthio)-2-styrylimidazo[1,2-b]pyridazine (V.2j), (31%), m.p. 149-150°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from cyclohexane (Found: C, 70.0; H, 4.9; N, 16.4. C$_{20}$H$_{16}$N$_{4}$S requires C, 69.7; H, 4.7; N, 16.3%). $^1$H n.m.r.: δ 4.40, s, CH$_2$S; 6.85, d, J 9.5 Hz, H 7; 7.12-7.73 and 8.57-8.60, complex, PhCH=CH and pyridinyl; 7.71, d, J 9.5 Hz, H 8; 7.89, s, H 3.

3-Benzamidomethyl-6-phenylthio-2-styrylimidazo[1,2-b]pyridazine (V.3c) and related compounds

6-Phenylthio-2-styrylimidazo[1,2-b]pyridazine (V.2c) (0.13 g) was added to N-hydroxymethylbenzamide (0.06 g) in glacial acetic acid (3.0 ml) containing concentrated sulphuric acid (0.03 ml), and the mixture was heated with stirring in an oil bath at 120°C for 24 h. The acetic acid was evaporated under reduced pressure, the residue was diluted with water (5.0 ml), adjusted with aqueous ammonia to pH 10, and extracted with chloroform. The extract was washed with water, dried (Na$_2$SO$_4$) and solvent evaporated to give a yellow solid. This was subjected to t.l.c. (alumina;
chloroform / light petroleum, 3:1) and gave the title compound (13%), as yellow crystals
m.p. 201-203°C (from toluene). (Found: C, 72.7; H, 4.8; N, 12.0. C_{28}H_{22}N_{4}O_S
requires C, 72.7; H, 4.8; N, 12.1%). 1H n.m.r.: δ 4.94, d, J 5.5 Hz, CH₂; 6.85, d, J 9.5
Hz, H 7; 7.32-7.76, complex, PhCH=CH and 2 x Ph; 7.70, d, J 9.5 Hz, H 8. Mass
spectrum (e.i.) m/z 462 (M, 5%), 375 (25), 171 (18), 105 (100), 77 (93).

The following compounds were prepared in a similar manner from the 3-unsubstituted
imidazo[1,2-b]pyridazines (V.2) and N-hydroxymethylbenzamide.

3-Benzamidomethyl-6-methylthio-2-styrylimidazo[1,2-b]pyridazine (V.3a), (74%),
m.p. >265°C (from ethanol) (Found: C, 68.8; H, 4.8. C_{23}H_{20}N_{4}O_S requires C, 69.0;
H, 5.0%). 1H n.m.r.: δ 2.63, s, MeS; 5.19, d, J 5.5 Hz, CH₂; 6.91, d, J 9.5 Hz, H 7;
7.28-7.88, complex, PhCH=CH and 2 x Ph; 7.70, d, J 9.5 Hz, H 8. Mass spectrum (e.i.)
m/z 400 (M, 5%), 353 (1), 295 (31), 105 (92), 77 (100).

3-Benzamidomethyl-6-ethylthio-2-styrylimidazo[1,2-b]pyridazine(V.3b), (30%),
m.p. >270°C (from methanol) (Found: C, 68.4; H, 5.6; N, 13.6.

C_{24}H_{22}N_{4}O_S.0.4H_{2}O requires C, 68.3; H, 5.4; N, 13.3%). 1H n.m.r.: δ 1.41, t, J 7.5
Hz, Me; 3.18, q, J 7.5 Hz, CH₂Me; 5.15, d, J 5.5 Hz, CH₂N; 6.85, d, J 9.5 Hz, H 7;
7.27-7.92, complex, PhCH=CH and 2 x Ph; 7.63, d, J 9.5 Hz, H 8; 8.92, br, NH. Mass
spectrum (e.i.) m/z 414 (M, 5%), 353 (2), 309 (30), 105 (100), 77 (86).

3-Benzamidomethyl-6-benzylthio-2-styrylimidazo[1,2-b]pyridazine (V.3d), (26%),
m.p. 187-188°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and
recrystallisation from ethanol (Found: C, 72.4; H, 5.0; N, 11.8 C_{29}H_{24}N_{4}O_S requires
C, 73.1; H, 5.1; N, 11.8%). 1H n.m.r.: δ 4.37, s, CH₂S; 5.10, d, J 5.5 Hz, CH₂N; 6.75,
d, J 9.5 Hz, H 7; 7.20-7.91, complex, PhCH=CH, 2 x Ph and H 8. Mass spectrum (e.i.)
m/z 476 (M, 4%), 371 (17), 105 (100), 77 (73).
3-Benzamidomethyl-6-(3'-nitrobenzylthio)-2-styrylimidazo[1,2-b]pyridazine (V.3e), (18%), m.p. 218-222°C after recrystallisation from methanol (Found C, 66.7; H, 4.4; N, 13.3 C_{29}H_{23}N_{5}O_{3}S requires C, 66.8; H, 4.4; N, 13.4%). $^1$H n.m.r.: δ 4.49, s, CH$_2$S; 5.19, d, J 5.5 Hz, CH$_2$N; 6.90, d, J 9.5 Hz, H 7; 7.28-7.82, complex, PhCH=CH, Ph, H 8,5′,6′; 8.02, br d, J 8.5 Hz, H 4′; 8.39, t, J 2 Hz, H 2′. Mass spectrum (e.i.) m/z 521 (M, 2%), 504 (26), 105 (100), 77 (56).

3-Benzamidomethyl-6-(pyridin-3'-ylmethylthio)-2-styrylimidazo[1,2-b]pyridazine (V.3f), (36%), m.p. 175-176°C after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from toluene (Found C, 68.9; H, 4.7; N, 14.3 C$_{28}$H$_{23}$N$_{5}$OS.0.5H$_{2}$O requires C, 69.1; H, 5.0; N, 14.4%). $^1$H n.m.r.: δ 4.51, s, CH$_2$S; 4.90, d, J 5.5 Hz, CH$_2$N; 6.89, d, J 9.5 Hz, H 7; 7.17-8.53, complex, PhCH=CH, Ph, pyridinyl and H 8; 8.34, br, NH. Mass spectrum (e.i.) m/z 477 (M, 0.1%), 385 (16), 124 (9), 105 (100), 77 (42).

3-Benzamidomethyl-6-(pyridin-4'-ylmethylthio)-2-styrylimidazo[1,2-b]pyridazine (V.3g), (29%), m.p. 211-212°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from ethanol (Found C, 70.1; H, 4.9; N, 14.7 C$_{28}$H$_{23}$N$_{5}$OS requires C, 70.4; H, 4.9; N, 14.7%). $^1$H n.m.r.: δ 4.37, s, CH$_2$S; 5.12, d, J 5.5 Hz, CH$_2$N; 6.85, d, J 9.5 Hz, H 7; 7.21-7.57 and 7.70-8.49, complex, PhCH=CH, Ph and pyridinyl; 7.63, d, J 9.5 Hz, H 8; 8.34, br, NH. Mass spectrum (e.i.) m/z 477 (M, 0.6%), 105 (100), 77 (47).
CHAPTER VI: Syntheses and BZR and PBR affinities of some 3-p-(diethylene), cyclobutyl and 1-buty1phenyl-6-(halogeno, nitrobenzoy and methyl)-3-(unsubstituted, benzimidomethyl, unsubstituted benzimidomethyl and 1-naphthylimidomethyl)imidazo[1,2-b]pyridazines.

VI.1 Introduction

As a continuation of the studies reported in Chapter V, where certain 2-aryl-imidazo[1,2-b]pyridazines were found to be specific, high affinity PBR ligands, it was decided to synthesise and evaluate the BZR and PBR affinities of other compounds containing bulky 2-position groups. Imidazo[1,2-b]pyridazines were synthesised containing sterically bulky substituents in the para-position of the 2-phenyl ring to clarify whether these larger 2-aryl BZRs and PBRs contain similar effects on the affinities of the molecules for the BZR and PBR (where the phenyl ring itself was separated from the imidazo[1,2-b]pyridazine moiety by a CH2CH group). The effect on BZR and PBR affinities of substitution of the phenyl ring of the 3-benzimidomethyl group (an area of possible lipophilic interaction thought to be of importance in stabilizing the binding of these ligands in the receptor sites) was also examined.

In this chapter a series of imidazo[1,2-b]pyridazines containing 3-p-(diethylene), cyclobutyl and 1-buty1phenyl-6-(halogeno, nitrobenzoy and methyl)-3-(unsubstituted, benzimidomethyl, unsubstituted benzimidomethyl and 1-naphthylimidomethyl)imidazo[1,2-b]pyridazines have been synthesised. Two imidazo[1,2-b]pyridazine analogues have also been prepared for comparative purposes. The evaluation of the PBR and BZR affinities of these compounds provides further information relating to the steric constraints of the area of ligand-complex formation of these 3-position groups at the respective receptor sites.

Imidazo[1,2-b]pyridazines have also been synthesised with ethynyl, more and methyl substitution (to provide a range of possible steric and electronic effects) of the phenyl ring of the 3-benzimidomethyl group. The affinities of these substituents on BZR and PBR affinities have been examined. It was of particular interest to identify any correlations between the structural and electronic effects of the substituents of the phenyl ring of the 3-benzimidomethyl group and BZR or PBR selectivity, as it is known that diazepam (nanomolar BZR and PBR affinity) and p-chlorodiazepam (B.Z=4.664L...
CHAPTER VI Syntheses and BZR and PBR affinities of some 2-p-(chloro, cyclohexyl and t-butyl)phenyl-6-(halogeno, methoxy and methylthio)-3- (unsubstituted, benzamidomethyl, substituted benzamidomethyl and \( \beta \)-naphthamidomethyl)imidazo[1,2-b]pyridazines

VI-1 Introduction

In a continuation of the studies reported in Chapter V, where certain 2-styryl-imidazo[1,2-b]pyridazines were found to be specific, high affinity PBR ligands, it was decided to synthesise and evaluate the BZR and PBR affinities of other compounds containing bulky 2-position groups. Imidazo[1,2-b]pyridazines were synthesised containing sterically bulky substituents in the para-position of the 2-phenyl ring to clarify whether these larger 2-aryl groups conferred similar effects on the affinities of the molecules for the BZR and PBR as the 2-styryl groups (where the phenyl ring itself was separated from the imidazo[1,2-b]pyridazine moiety by a CH=CH group). The effect on BZR and PBR affinities of substitution of the phenyl ring of the 3-benzamidomethyl group (an area of possible lipophilic interaction thought to be of importance in stabilising the binding of these ligands to the receptor sites) was also examined.

In this chapter a series of imidazo[1,2-b]pyridazines containing 2-p-(chloro, cyclohexyl and t-butyl)phenyl substituents has been synthesised. Two imidazo[1,2-a]-pyridine analogues have also been prepared for comparative purposes. The evaluation of the PBR and BZR affinities of these compounds provides further information relating to the steric constraints of the area of ligand-receptor interaction of these 2-position groups at the respective receptor sites.

Imidazo[1,2-b]pyridazines have also been synthesised with chloro, nitro and methyl substitution (to provide a range of possible steric and electronic effects) of the phenyl ring of the 3-benzamidomethyl group. The effects of these substituents on BZR and PBR affinities have been examined. It was of particular interest to identify any correlations between the structural and electronic effects of the substituents of the phenyl ring of the 3-benzamidomethyl group and BZR or PBR selectivity, as it is known that diazepam (nanomolar BZR and PBR affinity) and p-chlorodiazepam (Ro5-4864),
(specific nanomolar PBR affinity) differ structurally only in the para-chloro substitution of the 5-phenyl ring.

The syntheses of 6-(chloro, fluoro, methoxy and methylthio)-2-p-(chloro, cyclohexyl and t-butyl)phenyl-3-( unsubstituted, benzamidomethyl, substituted benzamidomethyl and β-naphthamidomethyl)imidazo[1,2-b]pyridazines are reported in this chapter, with the additional syntheses of two imidazo[1,2-a]pyridine analogues. The usual spectral and analytical techniques have been used in the characterisation of the compounds. The in vitro BZR and PBR affinities of the compounds are recorded and the structural and / or electronic features influencing BZR and PBR affinity, and BZR or PBR selectivity, are discussed.

VI-2 Syntheses

The synthetic routes used to form the compounds reported in this chapter are shown in Scheme VI-1. The condensation of the relevant 6-substituted pyridazin-3-amines (VI.1) (prepared by literature procedures described in the Experimental Section of this chapter) with either α-bromo-4-t-butylacetophenone 303,316 or α-bromo-4-cyclohexylacetophenone 303,317 gave the 3-unsubstituted imidazo[1,2-b]pyridazines (VI.2) in yields of 41-65%. The imidazo[1,2-a]pyridine analogue (VI.5) was prepared by the reaction of 5-chloropyridin-2-amine (VI.4) with α-bromo-4-t-butylacetophenone in 39% yield. The preparation of 6-chloro-2-(p-chlorophenyl)imidazo[1,2-b]pyridazine (III.3) is described in Chapter III-2.

Electrophilic substitution at the 3-position of the 3-unsubstituted imidazo[1,2-b]-pyridazines (VI.2) and (III.3) and imidazo[1,2-a]pyridine (VI.5) by treatment with N-hydroxymethylbenzamide, substituted N-hydroxymethylbenzamides and N-hydroxy-methyl-β-naphthamide (prepared by literature procedures described in the Experimental Section of this chapter) resulted in the formation of the 6,3,2-trisubstituted imidazo[1,2-b]pyridazines (VI.3) and imidazo[1,2-a]pyridines (VI.6) in yields of 22-90%. All compounds were purified by chromatography (where necessary) and by recrystallisation as outlined in Chapter VI-5.
Scheme VI-1

![Chemical structures and reactions](image)

Reagents: (i) R2C6H4COCH2Br, NaHCO3, EtOH, reflux (ii) R2C6H4CONHCH2OH, cone H2SO4, AcOH, 120°C (iii) p-C(Me)3C6H4COCH2Br, NaHCO3, EtOH (iii) PhCONHCH2OH, cone H2SO4, AcOH, 120°C

<table>
<thead>
<tr>
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<th>VI.2 R1</th>
<th>VI.2 R2</th>
<th>VI.3 R1</th>
<th>VI.3 R2</th>
<th>VI.3 R3</th>
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<td>OMe</td>
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<td>c</td>
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<td>t</td>
<td>SMe</td>
<td>C6H11</td>
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A: Ref 303
VI-3 Physical properties

Table VI-1 shows some 1H n.m.r. spectral data for the 3-unsubstituted imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines reported in this chapter. These 1H n.m.r. spectra were characterised by a downfield singlet (δ 7.83-8.14) corresponding to the H-3 signal. The H-3 signal of the imidazo[1,2-a]pyridine (VI.5) was upfield (δ 7.83) relative to the imidazo[1,2-b]pyridazines (VI.2a-e) (δ 8.00-8.14). The H-7 and H-8 signals for the imidazo[1,2-b]pyridazines (VI.2a-e) appeared as doublets (J7,8 = 9.5 Hz) in the range (δ 6.67-6.86) and (δ 7.71-7.98) respectively. In the imidazo[1,2-a]-pyridine (VI.5) the H-7 signal appeared as a doublet of doublets at δ 7.18 with coupling to both H-8 and H-5 (J7,8 = 9.5 Hz ; J5,7 = 2 Hz), and the H-8 signal appeared as a doublet at δ 7.73, coupled to H-7 only (J7,8 = 9.5 Hz). A downfield doublet at δ 8.19 assigned to the H-5 proton (J5,7 = 2 Hz) was also apparent, in a manner similar to the 1H n.m.r. spectra of the 6-chloro-2-arylimidazo[1,2-a]pyridines (IV.5a,b) reported in Chapter IV-3.

Some 1H n.m.r. spectral data for the 3-substituted imidazo[1,2-b]pyridazines (VI.3) and imidazo[1,2-a]pyridines (VI.6) are shown in Table VI-2. The 1H n.m.r. spectra of the imidazo[1,2-b]pyridazines (VI.3a-t) showed doublets at δ 6.65-7.15 and δ 7.64-7.99 corresponding to the signals from H-7 and H-8 respectively, with a coupling constant J7,8 of 9.5 Hz. The H-7 signal from the imidazo[1,2-a]pyridine (VI.6) appeared at δ 7.15 as a doublet of doublets (J7,8 = 9.5 Hz ; J5,7 = 2 Hz) and the H-8 signal at δ 7.85 was a doublet (J7,8 = 9.5 Hz). The CH2 protons from the benzamidomethyl, substituted benzamidomethyl or β-naphthamidomethyl groups of compounds (VI.3) and (VI.6) gave rise to doublets (δ 5.12-5.25) with coupling (J = 5.5 Hz) to the neighbouring NH group.

The mass spectra of the compounds (VI.2), (VI.3), (VI.5) and (VI.6) did not show any consistent fragmentation patterns, as detailed in the Experimental Section of this chapter.
Table VI-1 Some $^1$H n.m.r. spectral data (δ)\(^A\) for some 2-p-(cyclohexyl and t-butyl)phenyl-3-unsubstituted imidazo[1,2-b]pyridazines and imidazo[1,2-a]-pyridines

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>X</th>
<th>R1</th>
<th>R2</th>
<th>H-3</th>
<th>H-7</th>
<th>H-8</th>
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<tr>
<td>VI.2a</td>
<td>N</td>
<td>F</td>
<td>C(Me)(_3)</td>
<td>8.12</td>
<td>6.86</td>
<td>7.98</td>
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<tr>
<td>2b</td>
<td>N</td>
<td>OMe</td>
<td>C(Me)(_3)</td>
<td>8.02</td>
<td>6.67</td>
<td>7.81</td>
</tr>
<tr>
<td>2c</td>
<td>N</td>
<td>OMe</td>
<td>C(_6)H(_11)</td>
<td>8.00</td>
<td>6.67</td>
<td>7.81</td>
</tr>
<tr>
<td>2d</td>
<td>N</td>
<td>SMe</td>
<td>C(Me)(_3)</td>
<td>8.14</td>
<td>6.82</td>
<td>7.71</td>
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<tr>
<td>2e</td>
<td>N</td>
<td>SMe</td>
<td>C(_6)H(_11)</td>
<td>8.12</td>
<td>6.85</td>
<td>7.73</td>
</tr>
<tr>
<td>5</td>
<td>CH</td>
<td>Cl</td>
<td>C(Me)(_3)</td>
<td>7.83</td>
<td>7.18</td>
<td>7.73</td>
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</table>

A: Reported as parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard in deuterchloroform.
B: Complex signal
Table VI-2 Some $^1$H n.m.r. spectral data ($\delta$)\textsuperscript{A} for some 3-(benzamidomethyl,
substituted benzamidomethyl and napth-2-amidomethyl)-2-$p$-(chloro, cyclohexyl
and t-butyl)phenylimidazo[1,2-\textit{b}]pyridazines and imidazo[1,2-\textit{a}]pyridines

\[
\begin{align*}
\text{Formula No.} & & \text{X} & \text{R}_1 & \text{R}_2 & \text{R}_3 & \text{CH}_2\text{N} & \text{H-7} & \text{H-8} \\
\text{VI.3a} & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{Cl-o} & 5.25 & 7.09 & 7.94 \\
3b & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{Cl-m} & 5.23 & 7.10 & 7.97 \\
3c & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{Me-p} & 5.23 & 7.08 & 7.92 \\
3d & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{Me-o} & 5.25 & 7.10 & 7.96 \\
3e & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{Me-M} & 5.24 & 7.10 & 7.94 \\
3f & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{Me-p} & 5.24 & 7.10 & 7.95 \\
3g & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{NO}_2-o & 5.24 & 7.06 & 7.92 \\
3h & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{NO}_2-m & 5.26 & 7.08 & 7.89 \\
3i & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{NO}_2-p & 5.27 & 7.10 & 7.94 \\
3j & & \text{N} & \text{Cl} & \text{C(Me)}_3 & \text{C}_4\text{H}_4(3,4) & 5.30 & 7.08 & 7.93 \\
3k & & \text{N} & \text{Cl} & \text{Cl} & \text{Cl-o} & 5.21 & 7.13 & 7.95 \\
3l & & \text{N} & \text{Cl} & \text{Cl} & \text{Cl-m} & 5.19 & 7.14 & 7.95 \\
3m & & \text{N} & \text{Cl} & \text{Cl} & \text{Cl-p} & 5.19 & 7.15 & 7.94 \\
3n & & \text{N} & \text{F} & \text{C(Me)}_3 & \text{H} & 5.20 & 6.87 & \text{b} \\
3o & & \text{N} & \text{F} & \text{C(Me)}_3 & \text{Cl-p} & 5.19 & 6.89 & 7.99 \\
3p & & \text{N} & \text{OMe} & \text{C(Me)}_3 & \text{H} & 5.21 & 6.68 & 7.77 \\
3q & & \text{N} & \text{OMe} & \text{C(Me)}_3 & \text{Cl-p} & 5.21 & 6.71 & 7.79 \\
3r & & \text{N} & \text{OMe} & \text{C}_6\text{H}_{11} & \text{H} & 5.19 & 6.65 & 7.73 \\
3s & & \text{N} & \text{SMe} & \text{C(Me)}_3 & \text{H} & 5.24 & 6.84 & 7.64 \\
3t & & \text{N} & \text{SMe} & \text{C}_6\text{H}_{11} & \text{H} & 5.27 & 6.91 & \text{b} \\
6 & & \text{CH} & \text{Cl} & \text{C(Me)}_3 & \text{H} & 5.12 & 7.15 & 7.85 \\
\end{align*}
\]

\text{A: Reported as parts per million (}$\delta$\text{) downfield from tetramethylsilane (TMS) as internal standard in
deuterochloroform.}  
\text{B: Complex signal}
VI-4 *In vitro* binding studies

The BZR and PBR affinities of the compounds reported in this chapter were measured by the *in vitro* displacement of [³H]diazepam binding from rat brain and kidney membrane preparations under the assay conditions described in Chapter II-5.3 and Chapter IV-5.2 respectively.

VI-4.1 Results of *in vitro* testing

Table VI-3 shows the results of the *in vitro* BZR and PBR binding assays as either the percentage displacement of [³H]diazepam binding at a single ligand concentration of 1000 nM or as IC₅₀ values (nM). Data from previous publications are included, with appropriate footnotes, for comparative purposes. For compounds (VI.2f,2g,3u,3v) the BZR affinities only had been determined, and the results of the determination of the PBR affinities of these compounds (carried out by Dr L.P. Davies) are reported here.

VI-4.2 Discussion of results

The results presented in Table VI-3 show that the compounds reported in this chapter show a range of BZR and PBR affinities (from zero to nanomolar IC₅₀ values) with several specific, nanomolar PBR ligands being identified.

The 3-unsubstituted imidazo[1,2-b]pyridazines (VI.2) showed very low *in vitro* affinities for both the BZR and the PBR (less than 30% displacement of [³H]diazepam binding at 1000 nM). These compounds did not possess the necessary properties for high affinity binding at either receptor site, as observed for the 3-unsubstituted compounds described in other chapters of this thesis. The imidazo[1,2-a]pyridine analogue (VI.5), however, unexpectedly proved to be a much stronger ligand at both receptor sites (47% displacement of [³H]diazepam binding at 1000 nM at the BZR, and a PBR IC₅₀ of 134 nM). The imidazo[1,2-b]pyridazine (VI.2f), to give a direct comparison, had zero displacement at the BZR and 4% displacement at the PBR.
Table VI-3 Results of displacement of $[^3]$H]diazepam binding from rat brain (BZR) and kidney (PBR) membrane preparations by some 3-(unsubstituted and substituted)-2-p-(chloro, cyclohexyl and t-butyl)phenylimidazo[1,2-b]pyridazines and imidazo[1,2-a]-pyridines

![Diagram](attachment:image.png)

<table>
<thead>
<tr>
<th>Formula X</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
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<th>BZR</th>
<th>PBR</th>
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<td>(0%)</td>
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<td>2b</td>
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<td>H</td>
<td>(14%)</td>
<td>(22%)</td>
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<td>2c</td>
<td>N</td>
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<td>C₆H₁₁</td>
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<td>(27%)</td>
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<td>2d</td>
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<td>(0%)</td>
<td>(4%)</td>
</tr>
<tr>
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<td>Cl</td>
<td>C₆H₁₁</td>
<td>H</td>
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<td>(3%)</td>
</tr>
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<td>Cl</td>
<td>C(Me)₃</td>
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<tr>
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<td>CH₂NHCOC₆H₄Cl-o</td>
<td>(30%)</td>
<td>(48%)</td>
</tr>
<tr>
<td>3b</td>
<td>N</td>
<td>Cl</td>
<td>C(Me)₃</td>
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<td>(11%)</td>
<td>(63%)</td>
</tr>
<tr>
<td>3c</td>
<td>N</td>
<td>Cl</td>
<td>C(Me)₃</td>
<td>CH₂NHCOC₆H₄Cl-p</td>
<td>(24%)</td>
<td>(68%)</td>
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<tr>
<td>3d</td>
<td>N</td>
<td>Cl</td>
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<td>(60%)</td>
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<tr>
<td>3e</td>
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<td>(85%)</td>
</tr>
<tr>
<td>3f</td>
<td>N</td>
<td>Cl</td>
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<td>(39%)</td>
</tr>
<tr>
<td>3g</td>
<td>N</td>
<td>Cl</td>
<td>C(Me)₃</td>
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<td>(16%)</td>
<td>(54%)</td>
</tr>
<tr>
<td>3h</td>
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<td>3i</td>
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<td>(50%)</td>
</tr>
<tr>
<td>3j</td>
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<td>Cl</td>
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<td>(66%)</td>
</tr>
<tr>
<td>VI.3k</td>
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<td>Cl</td>
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<td>20</td>
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<td>3m</td>
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<td>Cl</td>
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<td>CH₂NHCOC₆H₄Cl-p</td>
<td>401</td>
<td>10</td>
</tr>
<tr>
<td>III.5</td>
<td>N</td>
<td>Cl</td>
<td>Cl</td>
<td>CH₂NHCOPh</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td>VI.3nB</td>
<td>N</td>
<td>Cl</td>
<td>C(Me)₃</td>
<td>CH₂NHCOPh</td>
<td>(8%)</td>
<td>6</td>
</tr>
<tr>
<td>3nB</td>
<td>N</td>
<td>Cl</td>
<td>C₆H₁₁</td>
<td>CH₂NHCOPh</td>
<td>(12%)</td>
<td>24</td>
</tr>
<tr>
<td>3n</td>
<td>N</td>
<td>F</td>
<td>C(Me)₃</td>
<td>CH₂NHCOPh</td>
<td>(21%)</td>
<td>(43%)</td>
</tr>
<tr>
<td>3o</td>
<td>N</td>
<td>F</td>
<td>C(Me)₃</td>
<td>CH₂NHCOC₆H₄Cl-p</td>
<td>(14%)</td>
<td>(29%)</td>
</tr>
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</table>
This result is interesting as the previously observed approximate correlation between the BZR and PBR affinities of imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines clearly is not applicable in this case. The steric and electronic properties of the two compounds are similar and other factors appear to cause the unexpectedly high PBR affinity of (VI.5).

The 3-substituted imidazo[1,2-b]pyridazines (VI.3) showed much higher BZR and PBR affinities than their 3-unsubstituted analogues (VI.2). The 3-benzamidomethylimidazo[1,2-a]pyridine (VI.6), however, bound less strongly to both the BZR and the PBR than the 3-unsubstituted compound (VI.5). The binding affinities of these two imidazo[1,2-a]pyridines to the BZR and the PBR differ from the expected values based on comparisons with similarly substituted imidazo[1,2-b]pyridazines. It seems that factors other than steric or electronic effects, possibly differences in the pKₐ or lipophilicity of the imidazo[1,2-a]pyridines, are the cause of these observed differences.

Amongst the 6,3,2-trisubstituted imidazo[1,2-b]pyridazines, the general order of BZR affinities of the compounds with various 6-position groups (for directly comparable analogues with identical 3- and 2-position substituents) was 6-OMe > SMe > F > Cl, and the order of PBR affinities was 6-CI > OMe > SMe > F. Compounds (VI.3u) and

### Table VI-3 Continued

<table>
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<tr>
<th>Formula X</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>IC₅₀ (or % displacement at 1000 nM)ᴬ</th>
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<tr>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td>BZR</td>
</tr>
<tr>
<td>VI.3p</td>
<td>N</td>
<td>OMe</td>
<td>C(Me)₃</td>
<td>CH₂NHCOPh</td>
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<tr>
<td>3q</td>
<td>N</td>
<td>OMe</td>
<td>C(Me)₃</td>
<td>CH₂NHCOC₆H₄Cl-p</td>
</tr>
<tr>
<td>3r</td>
<td>N</td>
<td>OMe</td>
<td>C₆H₁₁</td>
<td>CH₂NHCOPh</td>
</tr>
<tr>
<td>3s</td>
<td>N</td>
<td>SMe</td>
<td>C(Me)₃</td>
<td>CH₂NHCOPh</td>
</tr>
<tr>
<td>3t</td>
<td>N</td>
<td>SMe</td>
<td>C₆H₁₁</td>
<td>CH₂NHCOPh</td>
</tr>
<tr>
<td>6</td>
<td>CH</td>
<td>Cl</td>
<td>C(Me)₃</td>
<td>CH₂NHCOPh</td>
</tr>
</tbody>
</table>

A: IC₅₀ values (nM) (or percentage displacement at 1000 nM) in the presence of 100 µM γ-aminobutyric acid (GABA) for BZR assays (see Chapter II-5.3, p75) and in the absence of GABA for PBR assays (see Chapter IV-5.2, p122)

B: Ref 303
(VI.3p), with 6-Cl and 6-OMe groups respectively, proved to be the most satisfactory PBR ligands, with high, selective affinity for the PBR site. The 6-SMe analogue (VI.3s) was also found to be a good PBR ligand, with *ca* 20-fold higher affinity for the PBR than the BZR, but the 6-F compounds (VI.3n) and (VI.3o) showed low affinities at both receptor sites.

Substitution of the phenyl ring of the 3-benzamidomethyl group had significant effects on BZR and PBR affinities. All the 3-(substituted benzamidomethyl) compounds (VI.3a-i) had slightly higher BZR affinities and lower PBR affinities than the 3-benzamidomethyl analogue (VI.3u).

The 3-(chlorobenzamidomethyl) compounds (VI.3a-c) had low BZR affinities (11-30% displacement of [3H]diazepam at 1000 nM) and modest PBR affinities (48-68% displacement at 1000 nM). Of the three isomers, the 3-(m- and p-chlorobenzamidomethyl) compounds (VI.3b,c) were the strongest binders at the PBR. The PBR affinity of (VI.3c) of 68% displacement at 1000 nM (the strongest of the three isomers), however, was still much less than that of the 3-benzamidomethyl analogue (VI.3u) (IC$_{50}$ 6 nM).

The 3-(nitrobenzamidomethyl and methylbenzamidomethyl)imidazo[1,2-b]-pyridazines (VI.3d-i) showed consistent effects on BZR and PBR affinities. From this series, the para-substituted benzamidomethyl compounds (VI.3f) and (VI.3i) had the highest BZR affinities. The meta-substituted benzamidomethyl compounds (VI.3e) and (VI.3h) bound more strongly to the PBR than their ortho- and para-substituted isomers. This effect was most significant in the 3-(nitrobenzamidomethyl) series, with (VI.3h) having a PBR IC$_{50}$ of 37 nM, compared with 54% displacement and 50% displacement at 1000 nM for (VI.3g) and (VI.3i) respectively.

The series of 6-chloro-3-(chlorobenzamidomethyl)-2-(p-chlorophenyl)-imidazo[1,2-b]-pyridazines (VI.3k-m) showed some interesting results. The 3-benzamidomethyl analogue (III.5) reported in Chapter III was found to have nanomolar affinity at both the BZR (IC$_{50}$ 26 nM) and the PBR (IC$_{50}$ 8 nM). Selectivity for the PBR, however, could be greatly improved by chloro-substitution of the phenyl ring of the 3-benzamidomethyl group. This was most effective in the 3-(o and p-chloro-
benzamidomethyl) compounds (VI.3k) and (VI.3m), with (BZR IC$_{50}$ 488 nM, PBR IC$_{50}$ 13 nM) and (BZR IC$_{50}$ 401 nM, PBR IC$_{50}$ 10 nM) respectively.

The 3-(β-naphthamidomethyl) compound (VI.3j) had zero affinity at the BZR and was a much weaker PBR ligand than the 3-benzamidomethyl analogue (VI.3u). Therefore increasing the area of possible lipophilic interaction in the vicinity of the 2-position groups of imidazo[1,2-b]pyridazines (with the additional fused phenyl ring of the β-naphthamidomethyl group) does not seem to result in increased affinity for the PBR.

The results for the 3-substituted benzamidomethyl compounds (VI.3a-i) and (VI.3k-m) suggest that substitution of the phenyl ring of the 3-benzamidomethyl group has particularly sensitive effects on PBR affinities and selectivities. In the case of the imidazo[1,2-b]pyridazines (VI.3a-i) containing bulky substituents in the 2-position, the meta-substitution by moderately sized functional groups (such as methyl and nitro) in the phenyl ring of the 3-benzamidomethyl group is favoured for PBR affinity. An electron-withdrawing substituent in this position appears to be the most beneficial to PBR affinity, as the 3-(m-nitrobenzamidomethyl) compound (VI.3h) has a considerably higher PBR affinity than (VI.3e). It should be stated, however, that the PBR affinities of the 3-(m-substituted benzamidomethyl) compounds (VI.3e) and (VI.3h) are still slightly lower than that of the 3-benzamidomethyl analogue (VI.3u). Therefore, in combination with large 2-position groups, imidazo[1,2-b]pyridazines containing simple 3-benzamidomethyl groups are the most potent PBR ligands.

The 3-(chlorobenzamidomethyl) compounds (VI.3k-m), also containing the smaller 2-(p-chlorophenyl) substituents, demonstrate useful effects on PBR selectivity. In the case of the 3-benzamidomethyl analogue (III.5) of these compounds, the compact 2-position group is no hindrance to BZR affinity, and nanomolar IC$_{50}$ values are obtained at both the BZR and the PBR. The 3-(chlorobenzamidomethyl) compounds (VI.3k-m) show increased selectivity for the PBR. The 3-(o and p-chlorobenzamidomethyl) compounds in particular show a marked decrease in BZR affinity while maintaining the nanomolar IC$_{50}$ values for the PBR, hence increasing considerably the PBR selectivity of the compounds.
The BZR affinities of the imidazo[1,2-b]pyridazines with the bulky 2-\((p\text{-cyclohexylphenyl}\) and \(p\text{-t-butylphenyl}\) groups are low (less than 60% displacement of \([3H]\text{diazepam binding at 1000 nM}\)). The PBR affinities of the compounds, however, are much higher, with the 2-(\(p\text{-t-butylphenyl}\)) compounds binding approximately 5 times more strongly to the 2-(\(p\text{-cyclohexylphenyl}\)) analogues. For example, (VI.3u) and (VI.3v) have PBR IC\(_{50}\)'s of 6 nM and 24 nM respectively, and (VI.3p) and (VI.3r) have respective PBR IC\(_{50}\) values of 32 nM and 155 nM. Both these 2-aryl groups can be accommodated at their area of interaction of the ligand binding site of the PBR. It seems that, although the 2-(\(p\text{-t-butylphenyl}\)) group can easily be accommodated at its site of ligand-receptor interaction of the PBR protein, the slightly bulkier 2-(\(p\text{-cyclohexylphenyl}\)) group may be approaching the steric limits of this area, resulting in lower PBR affinities. 3-Benzamidomethyl-6-chloro-2-(\(p\text{-cyclohexylphenyl}\))imidazo[1,2-b]pyridazine (VI.3v), however, has still proven to be a relatively selective, high affinity PBR ligand.

The results presented in this chapter have shown that the BZR and PBR affinities and selectivities of appropriately substituted imidazo[1,2-b]pyridazines can be altered by varying the substituent groups in both the 2- and 3-positions of the molecules. It is possible to conclude that increasing the steric bulk of substituent groups in the 2-position of the molecules reduces BZR affinity while maintaining high affinity binding to the PBR. In combination with bulky 2-position groups, imidazo[1,2-b]pyridazines with 3-(substituted benzamidomethyl) groups show a reduction in PBR affinities compared to the 3-benzamidomethyl analogues. The 3-(\(m\)-substituted benzamidomethyl) compounds (where the substituents are methyl or nitro) have noticeably higher PBR affinities than the 3-(\(o\) or \(p\)-substituted benzamidomethyl) isomers. In the case of compounds with the smaller 2-\(p\)-chlorophenyl group, where the 3-benzamidomethyl compound has high, non-selective nanomolar affinity for both the BZR and the PBR, \((o\) or \(p\))-chloro substitution of the phenyl ring of the 3-benzamidomethyl group significantly reduces the BZR affinities of the compounds while having little effect on their PBR affinities.
VI-5 Experimental

The general aspects of the experimental procedures are described in Chapter II-5.1. The procedure for the *in vitro* [3H]diazepam BZR assay is outlined in Chapter II-5.3 and that of the PBR assay in Chapter IV-5.2.

6-Fluoropyridazin-3-amine,277 6-methoxypyridazin-3-amine,304 6-methylthiopyridazin-3-amine,285 6-chloro-2-(4-t-butylphenyl)imidazo[1,2-b]pyridazine,303 α-bromo-4-t-buty lacetophenone,303,316 α-bromo-4-cyclohexylacetophenone,303,317 N-hydroxymethylbenzamide,300 (2 and 3)-chloro-N-hydroxymethylbenzamide,267 4-(chloro and methyl)-N-hydroxymethylbenzamide,318 N-hydroxymethyl-2-methyl benzamide,319 N-hydroxymethyl-3-methylbenzamide,320 N-hydroxymethyl-(2, 3 and 4)-nitrobenzamide,307 and N-hydroxymethyl-β-naphthamide 321 were prepared according to the relevant literature procedures and characterised using 1H n.m.r. spectroscopy.

5-Chloro-pyridin-2-amine was purchased from commercial sources.

2-(4-t-Butylphenyl)-6-fluoroimidazo[1,2-b]pyridazine (VI.2a) and related compounds

A mixture of 6-fluoropyridazin-3-amine (0.23 g), α-bromo-4-t-buty lacetophenone (0.51 g), and ethanol (15 ml) was refluxed for 3 h, sodium hydrogen carbonate (0.17 g) was added and the refluxing continued for 3 h. The ethanol was removed *in vacuo*, the residue extracted with chloroform, extract washed with water, and evaporated to give a brown solid which was recrystallised from a mixture of acetone and light petroleum to give a light grey solid (0.30 g; 56%). It was then subjected to t.l.c. (alumina; chloroform / light petroleum, 1:1) and recrystallised from light petroleum to give white crystals of the *title compound*, m.p. 213-214°C (Found: C, 71.1; H, 6.1; N, 15.5. C24H23FN4O requires C, 71.6; H, 5.8; N, 15.6%). 1H n.m.r.: δ 1.36, s, CMe3; 6.86 , d , J 9.5 Hz , H 7 ; 7.48 , d , J 8.5 Hz , H 3',5'(2',6'); 7.88, d, J 8.5 Hz, H 2',6'(3',5'); 7.98, d, J 9.5 Hz, H 8; 8.12, s, H 3. Mass spectrum (e.i.) *m/z* 269 (M, 45%), 254 (100), 113 (17).
In a similar manner, from 6-(variously substituted)pyridazin-3-amines (VI.1) and α-bromo-4-t-butylacetophenone and α-bromo-4-cyclohexylacetophenone were prepared the following compounds.

**2-(4'-t-Butylphenyl)-6-methoxyimidazo[1,2-b]pyridazine (VI.2b),** (55%), m.p. 126-128°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 71.8; H, 6.5; N, 14.7. C_{17}H_{19}N_{3}O requires C, 72.6; H, 6.8; N, 14.9%). 1H n.m.r.: 8 1.36, s, CMe3; 4.00, s, OMe; 6.67, d, J 9.5 Hz, H 7; 7.46, d, J 8.5 Hz, H 3',5'(2',6'); 7.81, d, J 9.5 Hz, H 8; 7.86, d, J 8.5 Hz, H 2',6'(3',5'); 8.02, s, H 3. Mass spectrum (e.i.) m/z 281 (M, 45%), 266 (100).

**2-(4'-Cyclohexylphenyl)-6-methoxyimidazo[1,2-b]pyridazine (VI.2c),** (52%), m.p. 194-196°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 73.4; H, 7.1; N, 13.4. C_{19}H_{21}N_{3}O requires C, 74.2; H, 6.9; N, 13.7%). 1H n.m.r.: 8 1.33-1.86, complex, cyclohexyl; 3.99, s, MeO; 6.67, d, J 9.5 Hz, H 7; 7.28, d, J 8 Hz, H 3',5'(2',6'); 7.81, d, J 9.5 Hz, H 8; 7.84, d, J 8 Hz, H 2',6'(3',5'); 8.00, s, H 3. Mass spectrum (e.i.) m/z 307 (M, 100%), 264 (65), 238 (40).

**2-(4'-t-Butylphenyl)-6-methylthioimidazo[1,2-b]pyridazine (VI.2d),** (41%), m.p. 160-162°C after t.l.c. (alumina; chloroform / light petroleum, 1:1) and recrystallisation from light petroleum (Found: C, 68.9; H, 6.8; N, 14.2. C_{17}H_{19}N_{3}S requires C, 68.7; H, 6.4; N, 14.1%). 1H n.m.r.: 8 1.36, s, CMe3; 2.61, s, MeS; 6.82, d, J 9.5 Hz, H 7; 7.46, d, J 9 Hz, H 3',5'(2',6'); 7.71, d, J 9.5 Hz, H 8; 7.88, d, J 9 Hz, H 2',6'(3',5'); 8.14, s, H 3.

**2-(4'-Cyclohexylphenyl)-6-methylthioimidazo[1,2-b]pyridazine (VI.2e),** (65%), m.p. 174-176°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from light petroleum (Found: C, 70.0 H, 6.3; N, 12.7. C_{19}H_{21}N_{3}O requires C, 70.6; H, 6.5; N, 13.0%). 1H n.m.r.: 8 1.33-1.85, complex, cyclohexyl; 2.61,
6-Chloro-3-(4′-chlorobenzamidomethyl)-2-(4′′-chlorophenyl)imidazo[1,2-b]-pyridazine (VI.3m) and related compounds

A mixture of 6-choro-2-(4-chlorophenyl)imidazo[1,2-b]pyridazine (III.3) (0.16 g), 4-chloro-N-hydroxymethylbenzamide (0.11 g), acetic acid (5.0 ml) and concentrated sulphuric acid (0.11 ml) was refluxed with stirring in an oil bath at 120°C for 14 h. The acetic acid was removed in vacuo, the residue diluted with water, adjusted to pH 10, extracted with chloroform, extract washed with water and dried (Na₂SO₄) and the solvent was evaporated to leave a solid. This solid was subjected to t.l.c. (alumina; chloroform / light petroleum, 3:1) and gave the title compound as white crystals (0.20 g, 77%), m.p. 236-237°C (from toluene). (Found: C, 55.9 H, 2.9; N, 12.7. C₂₀H₁₃Cl₃N₄O requires C, 55.6; H, 3.0; N, 13.0%). ¹H n.m.r.: δ 5.19; d, J 5.5 Hz, CH₂; 7.13, d, J 9.5 Hz, H 7; 7.04-8.04, complex, H 2',3',5',6',2'',3'',5'',6''; 7.94, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 430, 432 (M, 8%, 7%), 291 (100), 139 (30), 111 (30), 91 (55).

The following compounds were prepared in a similar manner from the 3-unsubstituted imidazo[1,2-b]pyridazines (VI.2) and (III.3), and the relevant substituted (or unsubstituted) N-hydroxymethylbenzamides or N-hydroxymethyl-β-naphthamide.

2-(4′-t-Butylphenyl)-6-chloro-3-(2′′-chlorobenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3a), (60%), m.p. 246-248°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 63.9; H, 5.1; N, 12.3. C₂₄H₂₂Cl₂N₄O requires C, 63.6; H, 4.9; N, 12.4%). ¹H n.m.r.: δ 1.37; s, CMe₃; 5.25, d, J 5.5 Hz, CH₂; 7.09, d, J 9.5 Hz, H 7; 7.30-8.02, complex, H 2',3',5',6',3'',4'',5'',6''; 7.94, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 454, 452 (M, 10%, 13%), 313 (100), 139 (30).
2-(4'-t-Butylphenyl)-6-chloro-3-(3''-chlorobenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3b), (74%), m.p. 262-263°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 63.4; H, 4.6; N, 11.9. C$_{24}$H$_{22}$Cl$_2$N$_4$O requires C, 63.6; H, 4.9; N, 12.4%). $^1$H n.m.r.: δ 1.36, s, CMe$_3$; 5.23; d, J 5.5 Hz, CH$_2$; 7.10, d, J 9.5 Hz, H 7; 7.28-7.97, complex, H 2',3',5',6',2'',4'',5'',6''; 7.97, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 454, 452 (M, 11%, 16%), 313 (100), 139 (35).

2-(4'-t-Butylphenyl)-6-chloro-3-(4''-chlorobenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3c), (26%), m.p. 248-250°C after t.l.c. (alumina; chloroform / light petroleum, 4:1) and recrystallisation from toluene (Found: C, 63.3; H, 4.7; N, 11.9. C$_{24}$H$_{22}$Cl$_2$N$_4$O requires C, 63.6; H, 4.9; N, 12.4%). $^1$H n.m.r.: δ 1.36, s, CMe$_3$; 5.23; d, J 5.5 Hz, CH$_2$; 7.08, d, J 9.5 Hz, H 7; 7.92, d, J 9.5 Hz, H 8; 7.38, d, J 9 Hz, 7.52, d, 9 Hz, 7.71, d, J 9 Hz, 7.93, d, J 9 Hz, H 2',6', 3',5', 2'',6'', 3'',5''. Mass spectrum (e.i.) m/z 454, 452 (M, 9%, 16%), 313 (100), 139 (27).

2-(4'-t-Butylphenyl)-6-chloro-3-(2''-methylbenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3d), (54%), m.p. 250-251°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 69.3; H, 6.0; N, 12.7. C$_{25}$H$_{25}$Cl$_2$N$_4$O requires C, 69.4; H, 5.8; N, 12.9%). $^1$H n.m.r.: δ 1.37, s, CMe$_3$; 2.45, s, 2''-Me; 5.25; d, J 5.5 Hz, CH$_2$; 7.10, d, J 9.5 Hz, H 7; 7.20-7.60, complex, H 2',3',5',6',3'',4'',5'',6''; 7.96, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 434, 432 (M, 3%, 9%), 313 (100), 119 (25), 91 (23).

2-(4'-t-Butylphenyl)-6-chloro-3-(3''-methylbenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3e), (38%), m.p. 226-227°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 69.4; H, 5.6; N, 12.6. C$_{25}$H$_{25}$Cl$_2$N$_4$O requires C, 69.4; H, 5.6; N, 12.6%). $^1$H n.m.r.: δ 1.36, s, CMe$_3$; 2.38, s, 3''-Me; 5.24; d, J 5.5 Hz, CH$_2$; 7.10, d, J 9.5 Hz, H 7; 7.32-8.02, complex.
2-(4'-t-Butylphenyl)-6-chloro-3-(4''-methylbenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3f), (54%), m.p. 247-248°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 69.0; H, 5.8; N, 13.1. C25H25ClN4O requires C, 69.4; H, 5.8; N, 12.9%). 1H n.m.r.: δ 1.36, s, CMe3; 2.38, s, 4''-Me; 5.24; d, J 5.5 Hz, CH2; 7.10, d, J 9.5 Hz, H 7; 7.49-8.03, complex, H 2',3',5',6',2'',3'',5'',6''; 7.95, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 434, 432 (M, 7%, 20%), 313 (100), 119 (40), 91 (28).

2-(4'-t-Butylphenyl)-6-chloro-3-(2''-nitrobenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3g), (36%), m.p. 260-262°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 61.7; H, 4.6; N, 14.6. C24H22ClN5O3 requires C, 62.1; H, 4.8; N, 15.1%). 1H n.m.r.: δ 1.37, s, CMe3; 5.24; d, J 5.5 Hz, CH2; 6.84, br, NH; 7.06, d, J 9.5 Hz, H 7; 7.50-8.18, complex, H 2',3',5',6',3'',4'',5'',6''; 7.92, d, J 9.5 Hz, H 8. Mass spectrum (e.i.) m/z 465, 463 (M, 3%, 8%), 446 (35), 311 (100), 295 (40).

2-(4'-t-Butylphenyl)-6-chloro-3-(3''-nitrobenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3h), (82%), m.p. 232-235°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 62.2; H, 4.8; N, 5.0. C24H22ClN5O3 requires C, 62.1; H, 4.8; N, 5.1%). 1H n.m.r.: δ 1.35, s, CMe3; 5.26; d, J 5.5 Hz, CH2; 7.08, d, J 9.5 Hz, H 7; 7.09-8.39, complex, H 2',3',5',6',4'',5'',6''; 7.89, d, J 9.5 Hz, H 8; 8.62, t, J 1.5 Hz, H 2''. Mass spectrum (e.i.) m/z 465, 463 (M, 7%, 22%), 313 (100), 150 (25).

2-(4'-t-Butylphenyl)-6-chloro-3-(4''-nitrobenzamidomethyl)imidazo[1,2-b]-pyridazine (VI.3i), (22%), m.p. 232-235°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 61.6; H, 4.7; N, 14.8.
C_{24}H_{22}ClN_{5}O_{3} \text{ requires } C, 62.1; H, 4.8; N, 15.1\%.

1H n.m.r.: \( \delta 1.37, \text{s, CMe3; 5.27; d, J 5.5 \text{ Hz, CH2; 7.10, d, J 9.5 \text{ Hz, H 7; 7.55, d, J 8.5 \text{ Hz, H 3',5'(2',6')}; 7.94, d, J 9.5 \text{ Hz, H 8; 7.87-8.00, complex, H 2',6'(3',5'), 2",6"; 8.28, d, J 9 \text{ Hz, H 3",5".} Mass}

spectrum (e.i.) } m/z 465, 463 (M, 6\%, 15\%), 313 (100), 150 (26).

2-(4'-t-Butylphenyl)-6-chloro-3-(2''-naphthamidomethyl)imidazo[1,2-b]pyridazine (VI.3j), (36\%), m.p. 255-256°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene) (Found: C, 71.2; H, 5.7; N, 11.8. C_{28}H_{25}ClN_{4}O requires C, 71.7; H, 5.4; N, 11.9\%). 1H n.m.r.: \( \delta 1.36, \text{s, CMe3; 5.30; d, J 5.5 \text{ Hz, CH2; 7.08, d, J 9.5 \text{ Hz, H 7; 7.47-8.04, complex, H 2',3',5',6',3",4",5",6",8"; 7.93, d, J 9.5 \text{ Hz, H 8; 8.30, br s, H 2".}}

6-Chloro-3-(2'-chlorobenzamidomethyl)-2-(4''-chlorophenyl)imidazo[1,2-b]-pyridazine (VI.3k), (68\%), m.p. 239-240°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 56.0; H, 2.9; N, 12.7. C_{20}H_{13}Cl_{3}N_{4}O requires C, 55.6; H, 3.0; N, 13.0\%). 1H n.m.r.: \( \delta 5.21; \text{J 5.5 Hz, CH2; 7.13, d, J 9.5 \text{ Hz, H 7; 7.18-7.40 and 7.67-7.80, complex, H 2',3',5',6'; 7.50, d, J 8.5 \text{ Hz, H 3",5"(2",6")}; 7.95, d, J 9.5 \text{ Hz, H 8; 8.02, d, J 8.5 \text{ Hz, H 2",6"(3",5")}. Mass}

spectrum (e.i.) } m/z 432, 430 (M, 8\%, 8\%), 291 (100), 139 (45), 111 (25).

6-Chloro-3-(3'-chlorobenzamidomethyl)-2-(4''-chlorophenyl)imidazo[1,2-b]-pyridazine (VI.3l), (35\%), m.p. 234-235°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 55.7; H, 3.0; N, 12.8. C_{20}H_{13}Cl_{3}N_{4}O requires C, 55.6; H, 3.0; N, 13.0\%). 1H n.m.r.: \( \delta 5.19; \text{d, J 5.5 Hz, CH2; 7.15, d, J 9.5 \text{ Hz, H 7; 7.30-8.04, complex, H 2',4',5',6',2",3",5",6"; 7.95, d, J 9.5 \text{ Hz, H 8. Mass}

spectrum (e.i.) } m/z 432, 430 (M, 10\%, 10\%), 291 (100), 139 (41), 111 (35).

3-Benzamidomethyl-2-(4'-t-butylphenyl)-6-fluorimidazo[1,2-b]pyridazine (VI.3n),
(50\%), m.p. 261-262°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and
recrystallisation from toluene) (Found: C, 71.4; H, 5.7; N, 13.8. C_{24}H_{23}FN_{4}O requires C, 71.6; H, 5.8; N, 13.9%). \( ^{1}\)H n.m.r.: \( \delta \) 1.36, s, CMe$_{3}$; 5.20; d, J 5.5 Hz, CH$_{2}$; 7.87, d, J 9.5 Hz, H 7; 7.05, br, NH; 7.38-8.09, complex, H 8,2',3',5',6' and Ph. Mass spectrum (e.i.) m/z 402 (M, 15%), 297 (100), 105 (36), 77 (25).

2-(4'-t-Butylphenyl)-3-(4''-chlorobenzamidomethyl)-6-fluoroimidazo[1,2-b]-pyridazine (VI.3o), (38%), m.p. 257-258°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 65.2; H, 4.7; N, 12.3. C$_{24}$H$_{22}$ClFN$_{4}$O requires C, 66.0; H, 5.1; N, 12.8%). \( ^{1}\)H n.m.r.: \( \delta \) 1.36, s, CMe$_{3}$; 5.19; d, J 5.5 Hz, CH$_{2}$; 6.89, d, J 9.5 Hz, H 7; 7.38, d, J 8.5 Hz, 7.53, d, J 8.5 Hz, 7.72, d, J 8.5 Hz, 7.92, d, J 8.5 Hz, H 2',6', 3',5', 2",6", 3",5"; 7.99, d, J 9.5 Hz, H 8.

3-Benzamidomethyl-2-(4'-t-butylphenyl)-6-methoxyimidazo[1,2-b]pyridazine (VI.3p), (24%), m.p. 215-217°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 72.7; H, 6.7; N, 13.2. C$_{25}$H$_{26}$N$_{4}$O$_{2}$ requires C, 72.4; H, 6.3; N, 13.5%). \( ^{1}\)H n.m.r.: \( \delta \) 1.35, s, CMe$_{3}$; 4.00, s, MeO; 5.21; d, J 5.5 Hz, CH$_{2}$; 6.68, d, J 9.5 Hz, H 7; 6.70, br, NH; 7.38-7.87, complex, H 2',3',5',6' and Ph; 7.77, d, J 9.5 Hz, H 8.

2-(4'-t-Butylphenyl)-3-(4''-chlorobenzamidomethyl)-6-methoxyimidazo[1,2-b]-pyridazine (VI.3q), (22%), m.p. 282-283°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 66.9; H, 5.6; N, 12.5. C$_{25}$H$_{25}$ClN$_{4}$O$_{2}$ requires C, 66.9; H, 5.6; N, 12.5%). \( ^{1}\)H n.m.r.: \( \delta \) 1.35, s, CMe$_{3}$; 4.00, s, MeO; 5.21, d, J 5.5 Hz, CH$_{2}$; 6.71, d, J 9.5 Hz, H 7; 6.83, br, NH; 7.32-7.80, complex, H 2',3',5',6',2",3",5",6"; 7.79, d, J 9.5 Hz, H 8.

3-Benzamidomethyl-2-(4'-cyclohexylphenyl)-6-methoxyimidazo[1,2-b]pyridazine (VI.3r), (45%), m.p. 192-193°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 73.8; H, 6.5; N, 12.5. C$_{27}$H$_{27}$N$_{4}$O$_{2}$ requires C, 73.8; H, 6.2; N, 12.7%). \( ^{1}\)H n.m.r.: \( \delta \) 1.32-1.85, complex, cyclohexyl; 3.98.
s, MeO; 5.19, d, J 5.5 Hz, CH₂; 6.65, d, J 9.5 Hz, H 7; 6.97, br, NH; 7.23-7.84, complex, H 2',3',5',6' and Ph; 7.73, d, J 9.5 Hz, H 8.

3-Benzamidomethyl-2-(4'-t-butylphenyl)-6-methylthioimidazo[1,2-b]pyridazine (VI.3s), (82%), m.p. 218-219°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 69.1; H, 6.0; N, 12.7. C₂₅H₂₆N₄OS requires C, 69.7; H, 6.1; N, 13.0%). ^H n.m.r.: δ 1.35, s, CMe₃; 2.58, s, MeS; 5.24, d, J 5.5 Hz, CH₂; 6.84, d, J 9.5 Hz, H 7; 6.99, br, NH; 7.37-7.52 and 7.73-7.86, complex, H 2',3',5',6' and Ph; 7.64, d, J 9.5 Hz, H 8.

3-Benzamidomethyl-2-(4'-cyclohexylphenyl)-6-methylthioimidazo[1,2-b]pyridazine (VI.3t), (39%), m.p. 224-226°C after t.l.c. (alumina; chloroform / light petroleum, 3:1) and recrystallisation from toluene (Found: C, 71.1; H, 5.8; N, 12.1. C₂₇H₂₇N₄OS requires C, 71.2; H, 6.0; N, 12.3%). ^H n.m.r.: δ 1.33-1.86, complex, cyclohexyl; 2.61, s, MeS; 5.27, d, J 5.5 Hz, CH₂; 6.91, d, J 9.5 Hz, H 7; 7.27-7.88, complex, H 8, 2',3',5',6' and Ph.

2-(4'-t-Butylphenyl)-6-chloroimidazo[1,2-a]pyridine (VI.5)
A mixture of 5-chloropyridin-2-amine (0.26 g) and α-bromo-4-t-butylacetophenone in ethanol (15 ml) was refluxed for 3 h, sodium hydrogen carbonate (0.17 g) was added and the refluxing was continued for 3 h. The ethanol was evaporated and the residue extracted with chloroform (60 ml) which was then washed with water (3 x 20 ml), dried (Na₂SO₄) and evaporated, and the product was recrystallised from a mixture of acetone and light petroleum to give the title compound (0.22 g; 39%), m.p. 169-170°C (Found: C, 71.4; H, 6.2; N, 9.9. C₁₇H₁₇ClN₂ requires C, 71.7; H, 6.0; N, 9.8%). ^H n.m.r.: δ 1.35, s, CMe₃; 7.18, dd, J 9.5 Hz, J 2 Hz, H 7; 7.47, d, J 9 Hz, H 2',6'(3',5'); 7.67, d, J 9.5 Hz, H 8; 7.83, s, H 3; 7.88, d, J 9 Hz, H 3',5'(2',6'); 8.19, d, J 2 Hz, H 5. Mass spectrum (e.i.) m/z 286, 284 (M, 20%, 50%), 269 (100), 121 (20).
3-Benzamidomethyl-2-(4'-t-butylphenyl)-6-chloroimidazo[1,2-a]pyridine (VI.6)

A mixture of 2-(4-t-butylphenyl)-6-chloroimidazo[1,2-a]pyridine (VI.5) (0.14 g), N-hydroxymethylbenzamide (0.076 g), acetic acid (5.0 ml) and concentrated sulphuric acid (0.09 ml) was refluxed with stirring in an oil bath at 120°C for 24 h. The acetic acid was evaporated, water (20 ml) added, the pH was adjusted to 10, the mixture extracted with chloroform, washed with water, dried (Na₂SO₄), and solvent evaporated to give an oil which crystallised. This product was applied in chloroform / methanol (3:1) to a t.l.c. plate (alumina) which was developed with a mixture of chloroform and light petroleum (3:1) and extraction with chloroform gave a white solid (0.19 g, 90%) which was recrystallised from toluene to give cream crystals of the title compound (0.045 g), m.p. 226-227°C (Found: C, 71.9; H, 5.7; N, 9.7. C₂₅H₂₄C₁N₃O requires C, 71.8; H, 5.8; N, 10.1%). ¹H n.m.r.: δ 1.35, s, CMe₃; 5.12, d, J 5.5 Hz, CH₂; 6.73, br, NH; 7.15, dd, J 9.5 Hz, J 2 Hz, H 7; 7.30-7.70, complex, H 2',3',5',6' and Ph; 7.85, dd, J 9.5 Hz, J 2 Hz, H 8; 8.40, br s, H 5.
CHAPTER VII Molecular Modelling Studies

VII.1 Introduction

The process of drug design has traditionally commenced with the biological screening of large numbers of novel compounds, leading to the discovery (often serendipitous or unexpected) of lead compounds with biological activities. It is common for several hundred or thousands analogues of these lead compounds to be synthesized and biologically screened. Quantitative structure-activity relationships may then be derived by considering the relationship between parameters such as the geometric, electronic, and lipophilic effects of substituent groups and the resultant biological activities of the molecules. This technique is therefore both time-consuming and expensive.

The tremendous advances in computational power in the past decade have assisted in the development of rational drug design. Rational drug design analyses the structural and electronic properties at the active binding site of a receptor for a small number of lead compounds of known biological activities, and identifies possible pharmacophoric points of ligand-receptor interaction. These are common to a large number of lead compounds, and can be used to make predictions about new compounds. Computational modelling and graphical facilities are integral parts of the rational drug design process allowing the calculation and visualization of suitable properties of novel compounds. The results of computational searches of databases of lead compounds can be displayed and compared, and the spatial arrangements of potential areas of the commonality in active sites may be identified. These can then be used to design new compounds that may bind to the receptor.

Compounds that have not yet been synthesized or biologically evaluated can be modeled and various properties computed with known compounds. These data can then influence and direct future synthetic strategies. New compounds can be generated using the related pharmacophoric properties that may bind to the receptor.

In this way, the number of drug candidates can be increased. Proposal no. 13223.
CHAPTER VII Molecular Modelling Studies

VII-1 Introduction

The process of drug design has traditionally commenced with the biological screening of large numbers of novel compounds, leading to the discovery (often serendipitous or unexpected) of lead compounds with biological activities. It is common for several hundred or thousand analogues of these lead compounds to be synthesised and biologically screened. Quantitative structure-activity relationships may then be derived by considering the relationship between parameters such as the geometric, electronic and lipophilic effects of substituent groups and the resultant biological activities of the molecules. This technique is therefore both time consuming and expensive.

The enormous advances in computational power in the past decade have assisted in the development of rational, or structure-based, drug design. Rational drug design analyses the structural and electronic properties at the active binding site of a receptor for a small number of lead compounds of known biological activities, and identifies possible pharmacophoric points of ligand-receptor interaction. These are commonly heteroatoms, lone pair electrons, electron-rich aromatic areas and planar substituent groups. Computational modelling and graphical facilities are integral parts of the rational drug design process allowing the calculation and visualisation of certain properties of novel compounds. The results of conformational searches of different lead compounds can be displayed and compared, and the spatial arrangements of points or areas of the conformers likely to interact with a receptor protein examined. Graphical manipulations such as superimpositions and volume analyses of conformers of different ligands can assist in the development of pharmacophore models of the active ligand binding site on the receptor.

Compounds that have not yet been synthesised or biologically evaluated can be modelled and various properties compared with known compounds. These data can then influence and direct future synthetic strategies. New compounds containing the defined pharmacophoric properties can be designed and synthesised by the organic chemist and evaluated for affinity at the relevant receptor sites. In this way, the number of drug
candidates is greatly reduced in comparison to traditional drug design methods and the chances of synthesising high affinity ligands at the receptor are increased.

In this chapter molecular modelling techniques have been used to attempt to rationalise some of the structure-activity relationships that have been observed for imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines at the BZR and PBR in Chapters I-VI of this thesis. The work was carried out using the SYBYL software package installed on a Silicon Graphics workstation. Conformational searches of the compounds have been performed and low energy conformers identified. The low energy conformers of different molecules have been superimposed on known BZR and PBR ligands and pharmacophoric points of interaction identified. Volume analyses of the superimposed groups of BZR and PBR ligands have identified the areas of common volume and excluded volume at the receptor sites. Pharmacophoric models of interaction between imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines and their binding sites at the BZR and PBR have been developed, compared to existing pharmacophore models at these receptor sites, and used in the syntheses of other novel compounds with improved BZR or PBR selectivity.

VII-2 Procedure

All molecular modelling was carried out on a Silicon Graphics Personal Iris workstation. Imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines with high affinity for either the BZR or the PBR (as determined by nanomolar IC$_{50}$ values for the displacement of [${}^{3}$H]diazepam from these receptor sites) were selected for the molecular modelling study. The molecular models of the compounds were constructed with standard bond lengths and angles as defined using the SYBYL 6.1 molecular modelling software. The structures were energy minimised using the TRIPOS force field with Gasteiger-Hückel charges. These minimisations were carried out using the MINIMIZE command within SYBYL, using the Powell Method with Simplex initial optimisation and a Termination Gradient of 0.05 kcal/mol. The structures of diazepam

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A This work was carried out under the supervision of Dr Margaret G. Wong at the School of Chemical Sciences, Swinburne University of Technology, Melbourne.
and Ro5-4864 were constructed in the boat conformation found in crystalline diazepam and thought to represent the most probable active conformation of the molecule.47

VII-2.1 Conformational searches

Systematic conformational searches were undertaken on the energy minimised structures of the selected imidazo[1,2-b]pyridazines. The SEARCH command within SYBYL was used for this purpose. Rotatable bonds were selected and varied from 0-359° with 30° increments. In the case of phenyl or symmetrically substituted phenyl substituent groups, the bonds were varied from 0-179° in 30° increments. The conformational searches were then filtered using the FILTER command to within 5 kcal/mol of the global energy minimum for each molecule. From these filtered systematic searches, the twenty lowest energy conformers were taken and energy minimised. The energy minimised conformers within 2 kcal/mol of the lowest energy minimised conformer were then selected for the superimpositions.

VII-2.2 Superimpositions

The molecular superimpositions were performed using the FIT ATOMS command within SYBYL. Four points of possible hydrogen bonding or lipophilic interaction on the imidazo[1,2-b]pyridazines or imidazo[1,2-a]pyridines were selected, and superimposed on similar areas of known BZR or PBR ligands.

All the minimised conformers within 2 kcal/mol of the global energy minimum, as described above in Chapter VII-2.1, were selected for superimposition with the known BZR or PBR ligands to allow for the possibility that the biologically active conformations of the molecules were not necessarily the lowest energy conformations, due to the electronic and steric requirements of the receptor binding sites. The conformer of each molecule that gave the best superimposition with the known BZR or PBR (as appropriate) ligand, as determined by root mean square (rms) calculations for the four fitted atoms, was then used for the volume comparisons.
VII-2.3 Volume analyses

The MULTIPLE VOLUME CONTOUR command within SYBYL was used for the volume analyses. Separate volume analyses were carried out for the BZR and the PBR ligand binding sites. In the case of the BZR, a series of imidazo[1,2-b]pyridazines with high BZR affinities were superimposed on diazepam (as described above in Chapter VII-2.2). The total volume occupied by the superimposed ligands was calculated. The common volume (the volume shared by the superimposed compounds), otherwise known as the receptor essential volume,\(^{41}\) was also defined for the BZR. Similar total and common volume calculations were obtained for a series of imidazo[1,2-b]pyridazines with selective high PBR affinities, superimposed on Ro5-4864 and PK11195 (see Figure I-8, p22 for the chemical structures of these compounds). A superimposition of the total volumes of the BZR ligands with the total volumes of the PBR ligands enabled differences in the structural requirements of certain areas of the two receptor sites to be defined.

VII.3 Results and discussion

VII-3.1 3-Methoxy- and 3-benzamidomethyl-imidazo[1,2-b]pyridazine BZR ligands

In Chapter II, the syntheses of a number of high affinity BZR ligands were described. Some of these ligands were 2-aryl-3-methoxy-6-(substituted benzylthio)-imidazo[1,2-b]pyridazines (II.9) and others were 6-alkylthio-2-aryl-3-benzamidomethyl-imidazo[1,2-b]pyridazines (II.13). In the following Chapters III-VI, emphasis was placed on the syntheses of variously substituted compounds containing a 3-benzamidomethyl group.

It was therefore of interest initially to determine whether the interactions of the structurally diverse 3-methoxy and 3-benzamidomethyl compounds could theoretically be accounted for at the same ligand binding site of the BZR. For the purposes of this preliminary modelling study, 3-methoxy-6-(3,4-methylenedioxybenzylthio)-2-(3,4-methylendioxyphenyl)imidazo[1,2-b]pyridazine (II.9e) (IC\(_{50}\) 1 nM) and 3-benzamidomethyl-2-(3,4-methylendioxyphenyl)-6-methylthioimidazo[1,2-b]pyridazine (II.131)
(IC$_{50}$ 2 nM)$^{263}$ were selected as high affinity prototype ligands with high BZR affinity from each class of compounds.

Systematic conformational searches were undertaken for both compounds. The rotatable bonds were labelled and varied from 0-360° (or 0-179° for symmetrically substituted phenyl groups) in 30° increments. As described previously in Chapter VII-2.2, the conformational searches were filtered to within 5 kcal/mol of the global energy minimum for the molecules and the 20 lowest energy conformers from this filtered systematic search were examined.

The lowest energy conformers of (II.9e) identified in this way were characterised by a similar orientation of the 6-(3,4-methylenedioxybenzylthio) group. The phenyl ring of the substituted benzylthio group was orientated towards the imidazo[1,2-b]pyridazine nucleus, rather than pointing away from it. Similar studies with 2-aryl-3-methoxy-6-(substituted benzylamino)imidazo[1,2-b]pyridazines of known nanomolar BZR affinities$^{271}$ showed that the low energy conformers of these compounds also had the phenyl ring of the benzylamino group orientated towards the imidazo[1,2-b]pyridazine nucleus (unpublished results). Conformers with the 6-(substituted benzylthio) groups in this position therefore appear to be the most likely of the possible conformers to interact with the active binding site of the BZR.

The lowest energy conformers of (II.13l) were all found to have the 3-benzamidomethyl group orientated towards, rather than away from, the 2-(3,4-methylenedioxyphenyl) group. The 2- and 3-position groups were approximately parallel to each other.

Areas of likely ligand-receptor interaction that would help to define the BZR pharmacophore for (II.9e) were the phenyl rings of the 6-(substituted benzylthio) and 2-aryl groups, the oxygen atoms of the methylenedioxy and methoxy groups, and the electron rich aromatic areas and ring nitrogen atoms of the imidazo[1,2-b]pyridazine nucleus. Similar possible pharmacophoric points for (II.13l) were the phenyl rings of the 2-aryl and 3-benzamidomethyl groups, the aromatic areas and ring nitrogens of the imidazo[1,2-b]pyridazine nucleus, the oxygen atoms of the methylenedioxy and carbonyl groups and the sulphur atom of the methylthio group. It is unlikely that all these areas
described interact directly with the BZR. It is probable, however, that areas with similar
electronic or hydrophobic properties located in similar regions in space for both (II.9e)
and (II.13i) are involved in stabilising the binding of these ligands to the BZR.

It is clear that if the low energy conformers of (II.9e) are superimposed on the
low energy conformers of (II.13i) with overlap of the imidazo[1,2-b]pyridazine nucleus,
there will be poor overlap of the respective 6- and 3-position groups of the two
molecules. If, however, (II.9e) is superimposed on to (II.13i) with the phenyl ring of the
6-(3,4-methylenedioxybenzylthio) group of (II.9e) on the phenyl ring of the
3-benzamidomethyl group of (II.13i), the sulphur of the 6-(substituted benzylthio) group
of (II.9e) on to a methylenedioxy oxygen of the 2-aryl group of (II.13i), a
methylenedioxy oxygen of the 2-aryl group of (II.9e) on to the sulphur of the
6-methylthio group of (II.13i) and a methylenedioxy oxygen of the 6-(substituted
benzylthio) group of (II.9e) on to the carbonyl oxygen of the 3-benzamidomethyl group
of (II.13i), then a good overall superimposition of the two molecules is obtained as
shown in Figure VII-1.

Despite the different substituent groups present in the 6- and 3-positions of
(II.9e) and (II.13i), superimposition of the low energy conformers of the two molecules
as outlined above leads to the close proximity of certain features likely to be involved
with interaction with the BZR. The overall geometric properties of these conformers of
the two compounds are similar as there are no regions in space occupied by one ligand
alone when the two are superimposed in this way. Both compounds could therefore
theoretically be accommodated at the same site on the BZR protein.

Points of ligand-receptor interaction for (II.9e) could be postulated as the
sulphur atom, methylenedioxy oxygens and phenyl ring of the 6-substituted benzylthio
group and the methylenedioxy oxygens of the 2-aryl group. Similar pharmacophoric
points for (II.13i) would be the carbonyl oxygen and phenyl ring of the 3-benzamido-
methyl group, the methylenedioxy oxygens of the 2-(3,4-methylenedioxy-phenyl) group
and the sulphur of the 6-methylthio group. It is difficult to identify areas of the
imidazo[1,2-b]pyridazine nucleus that contribute to receptor-ligand interactions on the
basis of the superimposition of these two ligands alone, however this will be explored
Figure VII-1 Superimposition of (III.9e) and (III.13i)
in more detail with other imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines in the following sections of this chapter.

It is of interest that the possible pharmacophoric points of (II.9e) include the phenyl ring, methylenedioxy oxygens and sulphur atom of the 6-(substituted benzylthio) group. This is consistent with the observed biological activities of the series of 3-methoxyimidazo[1,2-b]pyridazines reported in Chapter II where 2-aryl-6-(chloro or methylthio)-3-methoxyimidazo[1,2-b]pyridazines had low BZR affinities and a 6-(substituted benzylthio) group was required for high affinity binding to the BZR. Similarly, the methylenedioxy oxygens of the 2-(3,4-methylenedioxyphenyl) group are potential pharmacophoric points and this is also consistent with the observed beneficial effects on BZR affinity of a 2-(3,4-methylenedioxyphenyl) substituent group in the 2-aryl-3-methoxy-6-(substituted benzylthio) compounds.

This introductory study has demonstrated that it is theoretically possible for the 2-aryl-3-methoxy-6-(substituted benzylthio)imidazo[1,2-b]pyridazines to occupy the same ligand binding site on the BZR protein as the 6-alkylthio-2-aryl-3-benzamidomethyl analogues. The structures of low energy conformers of (II.9e) and (II.13l), representative of both classes of compounds, indicate that it is probable that they bind to a common site on the BZR, but adopt different orientations at this receptor site.

In the following sections of this chapter, other 3-benzamidomethyl compounds will be examined and superimposed on diazepam, Ro5-4864 and PK11195 (as appropriate) to determine the structural and electronic requirements of the binding site of these compounds at the BZR and PBR.

VII-3.2 6-Alkoxy-2-aryl-3-benzamidomethylimidazo[1,2-b]pyridazine BZR ligands

The work reported above in Chapter VII-3.1 has demonstrated that it is theoretically possible for 3-methoxy and 3-benzamidomethylimidazo[1,2-b]pyridazines to interact at a common BZR binding site. The properties of the ligand binding site will now be examined in more detail by modelling the structures of selected 6-alkoxy-2-aryl-3-benzamidomethylimidazo[1,2-b]pyridazines (III.6) reported in Chapter III. These compounds showed a decrease in BZR affinity as the size of the 6-alkoxy group was
increased, and it was therefore implied that the bulkier 6-position groups were interacting with a sterically hindered area of the BZR protein.

The 3-benzamidomethyl-6-(methoxy, ethoxy and propoxy)-2-(3,4-methylenedioxyphenyl)imidazo[1,2-b]pyridazines (III.6a,h,o) (IC\textsubscript{50} values of 7, 25 and 116 nM respectively) were selected for modelling purposes. The structure of 3-benzamidomethyl-6-(2-methoxyethoxy)-2-(3,4-methylenedioxyphenyl)imidazo[1,2-b]pyridazine (III.6v) was also generated. This compound was not actually synthesised in Chapter III, as the 2-phenyl and 2-(p-methylphenyl) analogues (III.6s) and (III.6t) showed low BZR affinities, though it was used for the modelling study to allow the comparison of a series of imidazo[1,2-b]pyridazines with a consistent 2-position substituent group. It was estimated, using the BZR affinities of 2-(p-methylphenyl) analogues as a guide, that (III.6v) would have a likely IC\textsubscript{50} value of approximately 200-300 nM.

The rotatable bonds of the molecules were selected and conformational searches carried out as outlined previously. The 20 lowest energy conformers were energy minimised and the structures of the resultant minimised conformers for each of the compounds (III.6a,h,o,v) examined as candidates for superimposition on diazepam. It was desired, if possible, to identify minimised conformers of (III.6a,h,o,v) within 2 kcal/mol of the global energy minimum for each compound, that had common structural features. These could then be hypothesised to be the biologically active conformations that bind to the same receptor site.

The energy minimised conformers (a)-(d) of (III.6a) and (III.6h) showed relatively small variations in structure (Figures VII-2 and VII-3). The 3-benzamidomethyl and 2-(3,4-methylenedioxyphenyl) groups were positioned in close proximity to each other and were approximately parallel. The 6-ethoxy group in (III.6h) adopted a linear conformation in all the minimised conformers of this molecule, directed away from the imidazo[1,2-b]pyridazine nucleus. Conformer (a) of (III.6a) and conformer (d) of (III.6h) were selected for superimposition on diazepam.
Figure VII-2 Energy minimised conformers of (III.6a)

Anticlockwise from top right: conformer (a), 42.61 kcal/mol; conformer (b), 43.25 kcal/mol; conformer (c), 43.63 kcal/mol; conformer (d), 42.71 kcal/mol.
Figure VII-3 Energy minimised conformers of (III.6h)

Anticlockwise from top right: conformer (a), 43.14 kcal/mol; conformer (b), 42.77 kcal/mol; conformer (c), 43.74 kcal/mol; conformer (d), 42.23 kcal/mol.

The energy minimised conformers of (III.6h) were characterised by the substituent groups pointing away from the imidazol(1,5-a)pyrimidine nucleus. Figure VII-4 shows the possible low energy conformers (a) - (d) of (III.6v). The energy of conformer (d) (44.7 kcal/mol) is slightly lower than that of conformer (d) (45.2 kcal/mol), the structural similarity of conformer (d) with the proposed biologically active conformer of (III.6v) suggests that it is the biologically active conformer of (III.6v).
For (III.60), the low energy conformers had similarly orientated 2- and 3-positon substituent groups to (III.6a) and (III.6h), but three different conformations of the 6-propoxy group. Conformers containing these three orientations of the 6-propoxy group, and their conformational energies, are shown in Figure VII-4. It can be seen from this that conformers (c) and (d), containing a linear 6-propoxy group orientated in a similar direction to the 6-ethoxy group in (III.6h), are approximately 3 kcal/mol lower in energy than the alternative conformers (a) and (b). Conformers (c) and (d) therefore represented the most likely conformation of (III.60) at the ligand binding site of the BZR and as the two conformers were very similar (differing only slightly in torsion angles), both would give almost identical results in the superimpositions. In the studies presented here, conformer (c) was selected for further superimposition.

All the low energy conformers of (III.6v) were characterised by linear 6-(2-methoxyethoxy) substituent groups pointing away from the imidazo[1,2-b]pyridazine nucleus. Figure VII-5 shows the possible low energy conformers (a)-(d) of (III.6v). The difference between them is the orientation of the 2- and 3-position groups. Although the energy of conformer (c) (44.7 kcal/mol) is slightly lower than that of conformer (d) (45.2 kcal/mol), the structural similarity of conformer (d) with the proposed biologically active conformers of (III.6a,h,o) suggests that it is the biologically active conformer of (III.6v).

The torsion angles of the conformers of (III.6a,h,o,v) selected for superimposition with diazepam are shown in Table VII-1. The similar overall geometries of these conformers are characterised by the 2- and 3-position groups close to and parallel to each other, and linear 6-position alkoxy groups orientated in a similar direction.
Figure VII-4 Energy minimised conformers of (III.60)

Anticlockwise from top right: conformer (a), 46.90 kcal/mol; conformer (b), 46.98 kcal/mol; conformer (c), 43.30 kcal/mol; conformer (d), 43.40 kcal/mol
Figure VII-5 Energy minimised conformers of (III.6v)

Anticlockwise from top right: conformer (a), 46.57 kcal/mol; conformer (b), 45.17 kcal/mol; conformer (c), 44.75 kcal/mol; conformer (d), 45.16 kcal/mol

The diazepin molecule was then examined for possible likely points of ligand-receptor interaction. These were found to be the fused phenyl and 5-phenyl rings, the carbon, oxygen, the 7-clinor group and the N-4 atom. The best superimposition of diazepin (III.6v) to 1,2-bipyridazines on to diazepin was obtained using the interatomic distances defined in Table VII. Conformers (C1 to C3, O, N4 to N-4 (diazepin)) and N-4(1,10-phenylenediamine) to O4 gave a very close fitting of the selected points of superimposition. The selected atoms in this case, however, had different electronic properties and were therefore unlikely to interact with the same area of the receptor protein.
Table VII-1 Torsion angles $\tau(\circ)^{\circ}\text{Å}$ of conformers selected for superimposition

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\tau_1$</th>
<th>$\tau_2$</th>
<th>$\tau_3$</th>
<th>$\tau_4$</th>
<th>$\tau_5$</th>
<th>$\tau_6$</th>
<th>$\tau_7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>III.6a</td>
<td>0.0</td>
<td>53.0</td>
<td>-164.7</td>
<td>61.6</td>
<td>-20.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III.6b</td>
<td>-0.5</td>
<td>131.2</td>
<td>168.5</td>
<td>-62.0</td>
<td>21.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III.6o</td>
<td>0.5</td>
<td>140.7</td>
<td>148.6</td>
<td>136.6</td>
<td>17.6</td>
<td>-179.8</td>
<td>-</td>
</tr>
<tr>
<td>III.6v</td>
<td>0.0</td>
<td>-38.6</td>
<td>145.2</td>
<td>137.6</td>
<td>-156.9</td>
<td>179.9</td>
<td>179.8</td>
</tr>
</tbody>
</table>

A: The torsion angles ($\circ$) were defined as follows: $\tau_1 = \tau (N5, C6, O1c, C2c)$; $\tau_2 = \tau (C2a, C1a, C2, N1)$; $\tau_3 = \tau (N2b, C1b, C3, N4)$; $\tau_4 = \tau (C3b, N2b, C1b, C3)$; $\tau_5 = \tau (C5b, C4b, C3b, N2b)$; $\tau_6 = \tau (O1c, C2c, C3c, X4c)$; $\tau_7 = \tau (C2c, C3c, X4c, C5c)$

The diazepam molecule was then examined for possible likely points of ligand-receptor interaction. These were found to be the fused phenyl and 5-phenyl rings, the carbonyl oxygen, the 7-chloro group and the N-4 atom. The best overall superimposition of the imidazo[1,2-b]pyridazines on to diazepam was achieved by superimposing the selected atoms or centroids defined in Table VII-2 as follows: C1 to C1, C2 to C2, O* to N-4(diazepam), and N-1(imidazo[1,2-b]pyridazine) to O#. The root mean square (r.m.s) distances (Å) between the four fitted atoms or ring centroids are given. It was possible to select other atoms as the points of superimposition and hence obtain lower r.m.s values, without altering the overall geometry of the superimpositions. For example, the superimposition C1 to C1, C2 to C2, N* to N-4 (diazepam), and N-4(imidazo[1,2-b]-pyridazine) to O# gave a very close fitting of the selected points of superimposition. The selected atoms in this case, however, had different electronic properties and were therefore unlikely to interact with the same area of the receptor protein.
Table VII-2 Superimpositions of selected conformers with diazepam

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_{\text{min}}$</th>
<th>$E_{\text{cnf}}$</th>
<th>Fitted Atoms</th>
<th>rms</th>
</tr>
</thead>
<tbody>
<tr>
<td>III.6a</td>
<td>42.61</td>
<td>42.61</td>
<td>C1-C1, C2-C2, O*-N4, N1-O#</td>
<td>0.96</td>
</tr>
<tr>
<td>III.6a</td>
<td>42.61</td>
<td>42.61</td>
<td>C1-C1, C2-C2, N*-N4, N4-O#</td>
<td>0.57</td>
</tr>
<tr>
<td>III.6h</td>
<td>42.23</td>
<td>42.23</td>
<td>C1-C1, C2-C2, O*-N4, N1-O#</td>
<td>1.01</td>
</tr>
<tr>
<td>III.6h</td>
<td>42.23</td>
<td>42.23</td>
<td>C1-C1, C2-C2, N*-N4, N4-O#</td>
<td>1.09</td>
</tr>
<tr>
<td>III.6o</td>
<td>43.30</td>
<td>43.30</td>
<td>C1-C1, C2-C2, O*-N4, N1-O#</td>
<td>1.65</td>
</tr>
<tr>
<td>III.6o</td>
<td>43.30</td>
<td>43.30</td>
<td>C1-C1, C2-C2, N*-N4, N4-O#</td>
<td>0.61</td>
</tr>
<tr>
<td>III.6v</td>
<td>44.70</td>
<td>45.16</td>
<td>C1-C1, C2-C2, O*-N4, N1-O#</td>
<td>1.70</td>
</tr>
<tr>
<td>III.6v</td>
<td>44.70</td>
<td>45.16</td>
<td>C1-C1, C2-C2, N*-N4, N4-O#</td>
<td>0.64</td>
</tr>
</tbody>
</table>

A: $E_{\text{min}}$ is the global energy minimum for the molecule (kcal/mol). $E_{\text{cnf}}$ is the energy of the proposed biologically active conformation of the molecule (kcal/mol). The rms value is the root mean square distance (Å) between the fitted atom points on the imidazo[1,2-b]pyridazines and diazepam selected above for the superimpositions.

A: The $N^*$ atom is part of a hydrogen bond donating NH group, whereas N-4 (diazepam) is a hydrogen bond acceptor. The N-4 atom of the imidazo[1,2-b]pyridazine nucleus is also a less likely candidate for hydrogen bonding interaction than N-1, as theoretical calculations of atomic charges in substituted imidazo[1,2-b]pyridazines have identified N-4 as an area of low electronic charge in comparison to N-1 (Dr P. Matyus, personal communication). It was therefore considered that the superimpositions described initially gave the most convincing overlap of common areas of pharmacophoric interaction.
The superimposition of (III.6h) with diazepam is shown in Figure VII-6. Only one imidazo[1,2-b]pyridazine is shown superimposed in this figure for the sake of clarity, however (III.6a,o,v) gave similar superimpositions. With the imidazo[1,2-b]-pyridazines superimposed on diazepam in this way it can be noted that the oxygens of the 2-(3,4-methylenedioxyphenyl) substituents are in close proximity to the 7-chloro group of diazepam. The electronegative oxygen atoms of the 2-(3,4-methylenedioxy-phenyl) group could stabilise the binding of the imidazo[1,2-b]pyridazines to the BZR by interaction with the same area of the receptor as the 7-chloro group of diazepam. This would provide a possible explanation for the higher BZR affinities observed in Chapters II and III for 6-(alkylthio or alkoxy)-3-benzamidomethyl-2-(3,4-methylenedioxyphenyl)imidazo[1,2-b]pyridazines compared to the 2-phenyl analogues.

The 6-alkoxy groups of the imidazo[1,2-b]pyridazines are orientated away from the 7-membered ring of diazepam, in the opposite direction to its fused phenyl ring. The 6-alkoxy groups appear to interact with an area of the BZR inaccessible to diazepam. Alkoxy groups larger than ethoxy in size seem to exceed the steric limits of this area of the BZR and cause a reduction in the affinity for the receptor of the imidazo[1,2-b]-pyridazines containing these substituent groups.

The molecular modelling study of this series of 6-alkoxy-3-benzamidomethyl-2-(3,4-methylenedioxyphenyl)imidazo[1,2-b]pyridazines has defined a number of likely pharmacophoric points for these molecules. These points can be divided into areas of lipophilic interaction (the phenyl rings of the 2-aryl and 3-benzamidomethyl groups) and hydrogen bond acceptor sites (the carbonyl oxygen of the 3-benzamidomethyl group and possibly the methylenedioxy oxygens from the 2-aryl group, and N-1 of the imidazo[1,2-b]pyridazine nucleus). Additionally, an area of steric hindrance has been defined, occupied by 6-alkoxy-2-aryl-3-benzamidomethylimidazo[1,2-b]pyridazines containing linear 6-alkoxy groups greater in size than ethoxy.

Figures VII-7 and VII-8 show the volume calculations for the superimpositions of (III.6a,h,o,v) on diazepam. Figure VII-7 shows the total volume (green) and the common volume (purple) for the BZR ligands. Figure VII-8 shows the total volume (green) and the volume only occupied by (III.6o,v) (yellow). The yellow volume defines
Figure VII-6 Superimposition of (III.6h) with diazepam

Figure VII-7 Total volume (green) and common volume (purple) for the superimposition of (III.6k,e) with diazepam
Figure VII-7 Total volume (green) and common volume (purple) for the superimposition of (III.6a,h,o,v) with diazepam
Figure VII-8  Total volume (green) and volume of (III.6o,v) only (yellow) for the superimposition of (III.6a,h,o,v) with diazepam
the area of receptor excluded volume which when occupied by a ligand results in a reduction in its BZR affinity.

The N-5 atom of the imidazo[1,2-b]pyridazine nucleus does not appear from these studies to be an important pharmacophoric point. This is consistent with the previously observed general similarity between the BZR affinities of the substituted imidazo[1,2-b]pyridazines and analogous imidazo[1,2-a]pyridines. In the following section, the 6(7 and 8)-chloroimidazo[1,2-a]pyridines will be modelled to further examine the BZR binding site.

VII-3.3 6(7 and 8)-Chloroimidazo[1,2-a]pyridine BZR ligands

The 2-aryl-3-benzamidomethyl-6(7 and 8)-chloroimidazo[1,2-a]pyridines reported in Chapter IV had BZR affinities of varying magnitude. In general, the order of magnitude of the BZR affinities of isomers in this series were 6-Cl > 7-Cl > 8-Cl.

The structures of 3-benzamidomethyl-6(7 and 8)-chloro-2-phenylimidazo[1,2-a]pyridines (IV.6a,c,f), with IC₅₀ values (or percentage displacement of [³H]diazepam binding at 1000 nM) at the BZR of 47 nM, (58%) and (12%) respectively, were constructed using SYBYL and examined as examples from this series of compounds. It was of interest to determine whether substitution at the 6-, 7- or 8-position of the imidazo[1,2-a]pyridines led to marked structural alterations of their respective low energy conformers. If the geometries of the low energy conformers of the 6(7 and 8)-chloroimidazo[1,2-a]pyridines were similar, it could be inferred that the reduced BZR affinities of (IV.6c,f), compared with (IV.6a), were a result of unfavourable ligand-receptor interactions between the 7- or 8-chloro groups rather than differently orientated 2- or 3-position groups.

The conformational searches for the compounds were performed as described previously and the energy minimised conformers for each of the compounds were studied. Figure VII-9 shows the energy minimised conformers for (IV.6a). The conformer (d), with 2- and 3-position groups aligned close to each other and parallel (in a similar orientation to the 2- and 3-position groups of the proposed biologically active
Figure VII-9  Energy minimised conformers of (IV.6a)

Anticlockwise from top right: conformer (a), 34.93 kcal/mol; conformer (b), 35.24 kcal/mol; conformer (c), 34.88 kcal/mol; conformer (d), 32.37 kcal/mol.

The geometrical similarities between the low-energy conformations of (IV.6a-6f) of (3H-3,4a,5,6-tetrahydro-3H-1,2-benzisimidazole) suggest that the 2- and 3-positioned imidazo[1,2-a]pyridazines can act as BZR by activating the benzene rings at the active ligand binding site, and do not interfere in BZR by activating the 6-chloro and 5-chloro in (IV.6g) and (IV.6h) molecule. The overlap between the phenyl rings of the 2- and 3-positioned groups of the imidazo[1,2-a]pyridazines and the fused phenyl and 5-phenyl rings of diphenyl the carbonyl oxygen from the 3-heteroatom-imidazo group and N-4 of diphenyl and N-1 of the imidazo[1,2-a]pyrididine and the carbonyl oxygen of diphenyl were noted as in Chapter VII-3.2.
conformers of the 6-alkoxyimidazo[1,2-b]pyridazines described above in Chapter VII-3.2) was over 2 kcal/mol lower in energy than the other conformers (a)-(c). The results of the conformational analyses of (IV.6c) and (IV.6f) were similar, and showed that the conformers with the 2- and 3-position groups in this orientation were at least 2 kcal/mol lower in energy than the other possibilities.

The geometrical similarities between the low energy conformers of (IV.6a,c,f) and of (III.6a,h,o,v) (described above in Chapter VII-3.2) suggest (i) that the 2-aryl-3-benzamidomethyl-6(7 and 8)-substituted imidazo[1,2-a]pyridines and 2-aryl-3-benzamidomethyl-6-substituted imidazo[1,2-b]pyridazines interact with the BZR by adopting similar orientations at the same ligand binding site, and (ii) that the differences in BZR affinities amongst the 6(7 and 8)-substituted imidazo[1,2-a]-pyridines could most probably be accounted for by unfavourable ligand-receptor interactions at an area of the receptor inaccessible to the 6-chloro group.

The compounds (IV.6a,c,f) were superimposed on diazepam in a similar manner to the imidazo[1,2-b]pyridazines (III.6a,h,o,v) and these superimpositions are shown in Figure VII-10. The overlap between the phenyl rings of the 2- and 3-position groups of the imidazo[1,2-a]pyridines and the fused phenyl and 5-phenyl rings of diazepam, the carbonyl oxygen from the 3-benzamidomethyl group and N-4 of diazepam and N-1 of the imidazo[1,2-a]pyridine and the carbonyl oxygen of diazepam were noted as in Chapter VII-3.2.

The 6(7 and 8)-chloro groups of these compounds are not sterically bulky. One possible explanation for the reduced BZR affinities of the compounds containing the 7- and 8-chloro groups therefore appears to be electronic repulsion between ligand and receptor at these points. It is possible that the BZR protein in this area contains electronegative atoms or groups that repel the 7- and 8-chloro groups and destabilise the binding of imidazo[1,2-a]pyridines containing these substituents to the BZR.

Alternatively, this area of the active ligand binding site of the receptor could be subject to particularly severe steric constraints. Therefore, in addition to the area of steric hindrance of the BZR ligand binding site defined in Chapter VII-3.2, there also appears to be an area of unfavourable electronic and/or steric interaction that can be accessed.
Figure VII-10  Superimposition of (IV.6a,c,f) with diazepam
by the 7- and 8-position substituent groups of 2-aryl-3-benzamidomethyl-7(and 8)-substituted imidazo[1,2-a]pyridines.

VII-3.4 2-(Styryl, p-cyclohexylphenyl and p-t-butylphenyl)imidazo[1,2-b]-pyridazine PBR ligands

Chapters V and VI of this thesis described the syntheses of imidazo[1,2-b]-pyridazines with bulkier groups in the 2-position than the substituted phenyl substituents present in the 2-positions of the BZR ligands of Chapters II and III. In general, these compounds were found to have low affinities for the BZR, but in some cases compounds with nanomolar PBR affinities were identified. A number of these compounds with high, specific PBR affinity were selected for further molecular modelling studies.

The compounds selected were (V.3e) (PBR IC$_{50}$ 10 nM), (VI.3h) (IC$_{50}$ 37 nM), (VI.3s) (IC$_{50}$ 52 nM) and (VI.3r) (IC$_{50}$ 155 nM), to provide a variety of substituents at the 2- and 6-positions of the imidazo[1,2-b]pyridazines. Conformational searches of these molecules were performed and the low energy conformers for each of the compounds compared.

The low energy conformers of (VI.3h,s,r) were all characterised by the 2- and 3-position groups orientated parallel to each other, as observed for the proposed biologically active conformers of the BZR ligands described in Chapter VII-3.1-3.3. Figure VII-11 shows the structures of the four lowest energy conformers (a)-(d) of (V.3e). These all show the 6-(m-nitrobenzylthio) group orientated around and towards the imidazo[1,2-b]pyridazine nucleus in a manner similar to that observed for the 2-aryl-3-methoxy-6-(substituted benzylthio)imidazo[1,2-b]pyridazines discussed in Chapter VII-3.1. Conformer (c) was selected as the most likely candidate for the biologically active conformer as it had the closest commonality with the low energy conformers of (VI.3h,s,r).
Figure VII-11 Energy minimised conformers of (V.3e)

Anticlockwise from top right: conformer (a), 31.75 kcal/mol; conformer (b), 29.00 kcal/mol; conformer (c), 30.00 kcal/mol; conformer (d), 30.59 kcal/mol
Due to the observed geometrical similarities between the low energy conformers of the imidazo[1,2-b]pyridazines with high PBR affinities and the low energy conformers of other imidazo[1,2-b]pyridazines with high BZR affinities, the principle pharmacophoric points of the imidazo[1,2-b]pyridazines at both the BZR and PBR appear to be similar. These points can be described as the phenyl rings of the 2-position groups and the 3-benzamidomethyl substituent, the carbonyl oxygen of the 3-benzamidomethyl group and the N-1 atom of the imidazo[1,2-b]pyridazine (or imidazo[1,2-a]pyridine) nucleus. This is consistent with the PBR / BZR affinities observed in the 1,4-BZs. Diazepam has nanomolar affinity for both the peripheral and central receptor sites whereas Ro5-4864, differing structurally only slightly from diazepam by the presence of a 5-(p-chlorophenyl) group instead of a 5-phenyl group, is PBR specific.\textsuperscript{12} The major pharmacophoric points of both diazepam and Ro5-4864 are identical.

Superimpositions of (\textit{V.3e}) and (\textit{VI.3h,s,r}) on Ro5-4864 were performed using C1, C2, O* and N-1 of the imidazo[1,2-b]pyridazines on to C1, C2, N-4 and O# of Ro5-4864 (points defined as for the BZR superimpositions shown in Table VII-2). The superimposition of (\textit{V.3e}) on to Ro5-4864 is shown separately in Figure VII-12. It is interesting to note in this case that the NO\textsubscript{2} oxygen atoms from the 6-(m-nitrobenzylthio) group are orientated close to both the carbonyl oxygen and the N-4 atom of Ro5-4864 and may therefore further stabilise the binding of this molecule (thus contributing to its particularly high PBR affinity) by additional hydrogen bonding interactions at these areas of the receptor protein.

Figures VII-13, VII-14 and VII-15 show the superimpositions of (\textit{V.3e}) and (\textit{VI.3h,s,r}) on both Ro5-4864 and PK11195. The PBR ligand PK11195 was included in these superimpositions, though it is uncertain whether it binds to the same site on the PBR protein as Ro5-4864 (discussed in Chapter I-3). Though the exact details of the PK11195 binding site are not known, it does have both a pendant and a fused phenyl ring and a carbonyl oxygen (on the carboxamide substituent) located at similar positions in space to the pendant and fused phenyl rings and carbonyl oxygen of Ro5-4864. The possibility that the two ligands do share a common PBR binding site therefore cannot be excluded.
Figure VII-12 Superimposition of (V.3e) with Ro5-4864
Figure VII-13  Superimposition of (V.3e) and (VI.3h,s,r) with Ro5-4864 and PK11195
Figure VII-14 Total volume (green) and common volume (purple) for the superimposition of (V.3e) and (VI.3h,s,r) with Ro5-4864 and PK11195
Figure VII-15 Total volume (green) and volume of Ro5-4864 and PK11195 only (orange) for the superimposition of (V.3e) and (VI.3h,s,r) with Ro5-4864 and PK11195
Figure VII-13 shows the structures of the proposed biologically active conformations of the various PBR ligands and areas of overlap. Volume analyses of the superimposed structures are shown in Figures VII-14 and VII-15. In Figure VII-14, the total volume of the superimposed ligands is represented in green, and the common volume in purple. It can be seen that the volume occupied by the imidazo[1,2-b]-pyridazines differs most from the common volume in the region of the 6-position substituent groups of the imidazo[1,2-b]pyridazines. Figure VII-15 shows the total volume of all the ligands (green) and the total volume of Ro5-4864 and PK11195 only (orange). In this case it is clear that the volume occupied by the imidazo[1,2-b]-pyridazines does not greatly exceed the volume occupied by Ro5-4864 and PK11195. It is therefore feasible that these compounds can be accommodated at the PBR ligand binding site.

3.5 BZR and PBR pharmacophores

A number of pharmacophore models have been developed for the BZR and these have been reviewed by Villar et al. The results of research reported since the publication of this review have contributed further to this area. In this section a BZR pharmacophore model will be developed for the imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines described in this thesis, which will be compared with the pharmacophore models already published.

A fundamental assumption in the development of these BZR pharmacophore models is that the different chemical classes of BZR ligands (1,4-BZs, β-carbolines, imidazo[1,2-a]pyridines, imidazo[1,2-b]pyridazines etc) bind to a single functional class of receptor. Though there is a considerable theoretical heterogeneity in the structure of the GABA_A/Cl^- channel complex and associated BZ binding site (due to the combinations of various forms of α, β and γ subunits of the GABA_A receptor discussed in Chapter I-2.4) BZR pharmacophoric models have not yet developed to account for this.

The BZR pharmacophore models that have been developed have used the superimposition of BZR ligands (with geometries determined using X-ray
crystallographic data or computer simulations and conformational analyses) to determine possible common points of ligand-receptor interaction. Both the affinities and the efficacies of the BZR ligands have been evaluated in an attempt to identify structural or physiochemical effects that influence these properties.

Figure VII-16 shows six of the principal BZR pharmacophore models that have been developed for BZR agonists. These models have been developed by considering the properties of several different classes of BZR ligands, however diazepam is shown in Figure VII-16 as the prototypical BZR ligand. The models vary in complexity from that of Fryer,9 which requires only an aromatic group (the fused phenyl ring in the case of diazepam) and a proton accepting group located 4-5.5 Å away (the carbonyl oxygen of diazepam) as the minimum number of points of pharmacophoric interaction, to that of Codding and Muir,46 which requires six pharmacophoric points for 1,4-BZ agonists. These are two areas of lipophilic interaction L1 and L2 (the fused phenyl and 5-phenyl rings of diazepam), two proton accepting groups (the carbonyl oxygen and N-4 atoms of diazepam), one proton donor group (N-1 of diazepam) and an electron withdrawing group (the 7-Cl group of diazepam).

Two of the BZR pharmacophore models define areas of negative ligand-receptor interaction. The model presented by Villar et al.48 defines an area of steric hindrance S1 in the vicinity of the 7-Cl group of diazepam, and also relates the value of the angle θ between the two proton accepting groups H1 and H2 and the most lipophilic group L1 of the molecule (the carbonyl oxygen, N-4 atom and 5-phenyl ring respectively of diazepam) to the efficacy (agonist / antagonist / inverse agonist) of the ligands. Similarly, the model defined by Cook et al.52 also contains an area of steric hindrance S1 close to the 7-Cl group of diazepam and a second area S2 around the N-1 atom of diazepam.

Prior to the definition of pharmacophoric points for the imidazo[1,2-b]-pyridazines and imidazo[1,2-a]pyridines it was necessary to determine the efficacies of the compounds at the BZR. The IC50 values of (III.6a,h,o) were determined for the in vitro displacement of [3H]flumazenil from rat brain membranes in the presence and absence of GABA. (This work was carried out by Dr L.P.Davies) The ratio of the IC50 values for the compounds in the presence and absence of GABA (known as GABA shift
ratios) gives an indication of the efficacies of the ligands. In general, inverse agonists have a GABA shift ratio of less than 1, antagonists approximately 1, partial agonists 1 to 1.5, and full agonists greater than 1.5. The radioligand used in the GABA shift assay is [3H]flumazenil rather than [3H]diazepam because flumazenil is a BZR antagonist and therefore binds to the BZR with the same affinity in the presence or absence of GABA. The results of the GABA shift experiment are shown in Table VII-3. All compounds gave GABA shift ratios of over 1.5, consistent with full agonist properties at the BZR. Further GABA-shift experiments with a range of 3-methoxy and 3-benzamidomethylimidazo[1,2-b]pyridazines with nanomolar affinities for the BZR also demonstrated full agonist properties for these compounds (Dr L.P. Davies, personal communication). It was therefore assumed that the BZR pharmacophore developed in this thesis applied to agonists.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (GABA)&lt;sup&gt;A&lt;/sup&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (no GABA)&lt;sup&gt;B&lt;/sup&gt;</th>
<th>GABA Shift Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>III.6a</td>
<td>9.8</td>
<td>18.9</td>
<td>1.93</td>
</tr>
<tr>
<td>III.6b</td>
<td>28.9</td>
<td>68.1</td>
<td>2.36</td>
</tr>
<tr>
<td>III.6o</td>
<td>95.5</td>
<td>243.1</td>
<td>2.55</td>
</tr>
</tbody>
</table>

A: IC<sub>50</sub> values determined for the displacement of [3H]flumazenil binding from rat brain membranes in the presence of 100 µM γ-aminobutyric acid (GABA)

B: IC<sub>50</sub> values determined for the displacement of [3H]flumazenil binding from rat brain membranes in the absence of GABA
Figure VII-16 Literature BZR pharmacophore models showing areas of interaction with diazepam

![Chemical structures](image)

- **Fryer**
- **Wong**
- **Borea et al**
- **Cook et al**
- **Villar et al**
- **Coding-Muir**

Figure VII-17 shows the pharmacophore developed for the imidazo[1,2-b]pyridazine and imidazo[1,2-a]pyridine BZR ligands. Diazepam is also shown fitted to this model for comparative purposes. There are many similarities between this pharmacophore model and the models shown in Figure VII-16, in particular the model of Cook et al.52 Two areas of lipophilic interaction (the phenyl rings of the 2-aryl and 3-benzamidomethyl groups) and two proton accepting groups (the carbonyl oxygen of the 3-benzamidomethyl group and the N-1 atom) are defined. Additionally, three areas of steric hindrance are described.

The first of these areas, S1, is below the para position of the 2-phenyl ring. Occupation of this area, for example by the bulky 2-((p-cyclohexylphenyl, p-t-butylphenyl
or styryl) groups (as reported in Chapters V and VI) results in a considerable decrease in BZR affinity. Fitting the diazepam molecule in this pharmacophore model leads to this region S1 being in the region of the 7-chloro group of diazepam. This is consistent with the areas of steric hindrance S1 in the models defined by Cook et al.\textsuperscript{52} and Villar et al.\textsuperscript{48} shown in Figure VII-16.

**Figure VII-17 Imidazo[1,2-b]pyridazine and imidazo[1,2-a]pyridine BZR pharmacophore**

The second area S2 is around the 7- and 8-position of the imidazo[1,2-b]-pyridazine or imidazo[1,2-a]pyridine nucleus. Substitution at these positions of the molecules leads to a significant reduction in their affinities for the BZR. When diazepam is fitted into this pharmacophore, the region S2 appears in the vicinity of the N-1 atom. This is similar to the area S2 defined by Cook et al.\textsuperscript{52} and shown in Figure VII-16.

The third area of steric hindrance S3 is occupied by bulky linear substituent groups in the 6-position of imidazo[1,2-b]pyridazines (and by analogy, imidazo[1,2-a]-pyridines also). This area of negative ligand-receptor interaction has not previously been defined for BZR agonists. For diazepam this area S3 would be positioned beyond the carbonyl oxygen atom. The series of 6-alkoxy-2-aryl-3-benzamidomethylimidazo[1,2-b]-pyridazines reported in Chapter III of this thesis clearly show that occupation of the S3

\[
\begin{align*}
\text{S2} & \quad \text{S3} \\
\text{R} & = \text{halogeno, alkylthio, alkoxy} \\
X & = \text{CH} / N
\end{align*}
\]
region results in a reduction of BZR affinity. 6-Alkylthio analogues with bulkier alkylthio groups (reported in Chapter II) also show a reduction in BZR affinities due to this interaction with S3. A new area of negative ligand-receptor interaction for the BZR pharmacophore has therefore been defined.

The distances between the proposed points of pharmacophoric interaction H1, H2, L1 and L2 (as defined in Figure VII-17) for the imidazo[1,2-b]pyridazine (III.6a) and for the 1,4-BZ diazepam (taken as prototype BZR ligands from both classes of compounds) were measured using the MEASURE command within SYBYL. The results are recorded in Table VII-4 and are similar for both (III.6a) and diazepam.

Table VII-4 Measurement of distances\textsuperscript{A} between the proposed points of pharmacophoric ligand-receptor interaction\textsuperscript{B} for diazepam and (III.6a)

<table>
<thead>
<tr>
<th>Pharmacophoric points</th>
<th>Distance / Å</th>
<th>Pharmacophoric points</th>
<th>Distance / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 - H1 (O)</td>
<td>4.95</td>
<td>L1 - H1 (N-1)</td>
<td>3.95</td>
</tr>
<tr>
<td>L1 - H2 (N-4)</td>
<td>3.80</td>
<td>L1 - H2 (O)</td>
<td>3.89</td>
</tr>
<tr>
<td>L2 - H1 (O)</td>
<td>6.76</td>
<td>L2 - H1 (N-1)</td>
<td>6.49</td>
</tr>
<tr>
<td>L2 - H2 (N-4)</td>
<td>3.69</td>
<td>L2 - H2 (O)</td>
<td>3.65</td>
</tr>
<tr>
<td>H1 - H2</td>
<td>3.46</td>
<td>H1 - H2</td>
<td>4.13</td>
</tr>
<tr>
<td>L1 - L2</td>
<td>4.86</td>
<td>L1 - L2</td>
<td>3.46</td>
</tr>
</tbody>
</table>

A: Distances measured using the MEASURE command within SYBYL.
B: Distances including L1 and / or L2 were taken from the centre of the appropriate phenyl ring.
The greatest differences in the distances between the pharmacophoric points for (III.6a) and for diazepam are between the points L1 and H1, and L1 and L2. In the case of the distances between L1 and H1, however, \( [4.95 \, \text{Å} \text{ for diazepam and } 3.95 \, \text{Å} \text{ for (III.6a)}] \) both values fall into the range stated by Fryer of approximately 4-5.5 Å for BZR agonist ligands.\(^9\) The areas of lipophilic interaction L1 and L2 are slightly closer for (III.6a) than for diazepam, but this does not appear to have any significant effect on its BZR affinity. The similar geometric and spatial positions of the pharmacophoric points for both (III.6a) and diazepam, and the structural similarities between the proposed biologically active conformer of (III.6a) and the other imidazo[1,2-\(b\)]pyridazines and imidazo[1,2-\(a\)]pyridines as determined by the conformational analyses reported in this chapter, suggests that the imidazo[1,2-\(b\)]pyridazines, imidazo[1,2-\(a\)]pyridines and 1,4-BZs are able to bind to the BZR by interaction with a common ligand binding site on the receptor protein.

To summarise, the pharmacophoric features derived from the BZR binding studies of imidazo[1,2-\(b\)]pyridazines and imidazo[1,2-\(a\)]pyridines reported in this thesis consist of two areas of lipophilic interaction and two proton accepting sites. These areas are similar to those of pharmacophore models previously described in the literature and presented in Figure VII-16. Three areas of steric hindrance have also been defined, one of which has not been previously reported. Occupation by a ligand of any one of these three areas results in a considerable reduction in its BZR affinity.

**ii PBR pharmacophore** In contrast to the BZR pharmacophore, little work has been done to develop a model for the PBR pharmacophore. As mentioned previously in Chapter VII-3.4, it is known that amongst the 1,4-BZs, diazepam has high affinity for both the BZR and the PBR, whereas 4'-chlorodiazepam (Ro5-4864) has specific affinity for the PBR. It has therefore been assumed that the principal pharmacophoric features of the ligand binding site for 1,4-BZs at the BZR are similar to those at the PBR, and that there is a sterically hindered area of the BZR in the vicinity of the \( \text{para} \) position of the 5-phenyl ring of diazepam that is not present at the PBR binding site.

Recently, Fiorini *et al.*\(^{162}\) have published the syntheses and pharmacological evaluation of a series of 6-arylpyrrolo[2,1-\(d\)]benzothiazepine derivatives as specific PBR
ligands. Using the structure-activity relationships of these compounds they have presented a PBR pharmacophore model. This model (shown in Figure VII-18 for these compounds and Ro5-4864) identifies two areas of lipophilic interaction (the fused phenyl and 5-phenyl rings of Ro5-4864), one proton accepting group (the N-4 atom of Ro5-4864), an electronegative moiety π1 (the carbonyl oxygen of Ro5-4864) able to undergo an ionic dipole interaction with a positively charged site on the receptor, and an area of steric hindrance S1 (directed away from the C-3 atom of Ro5-4864).

Figure VII-18 The PBR pharmacophore model developed by Fiorini et al.162

Kozikowski et al.161 have also reported high specific PBR affinities for a series of 2-arylindole-3-acetamides (Figure VII-19). The structural features of this series leading to high PBR affinities were an indole nitrogen at position-1, the carbonyl group (and the presence of two n-hexyl groups on the amide nitrogen) of the 3-position amide group, and a halogeno substituent at the 5-position of the indole ring and / or the para-position of the 2-aryl ring.

A compound with high specific PBR affinity containing these substituents is shown in Figure VII-19. It is possible to identify some common pharmacophoric points of this molecule that are consistent with the PBR pharmacophore model proposed by Fiorini et al.162 Two areas of lipophilic interaction L1 and L2 (the fused phenyl and 2-phenyl rings) and a proton accepting group H1 (the carbonyl oxygen of the 3-position amide group) are present. The introduction of a second methylene group at the 3-
position between the amide residue and the indole ring resulted in a reduction of PBR affinity.\textsuperscript{161} It is therefore possible that the extended 3-position group is interacting with the same area of receptor steric hindrance defined as S1 by Fiorini \textit{et al.}\textsuperscript{162} The 2-arylindole-3-acetamides, however, do not contain a group corresponding to the \pi 1 moiety previously described, and the presence of the proton donating indole NH group at position-1 was also found to be beneficial for high PBR affinity.

**Figure VII-19 PBR pharmacophore interaction of 2-arylindole-3-acetamides**

The imidazo[1,2-b]pyridazines reported in this thesis with high specific PBR affinities contained the same principal points of pharmacophoric interaction as the imidazo[1,2-b]pyridazine BZR ligands. These were L1 and L2 (the 2-aryl and 3-benzamidomethyl phenyl rings) and H1 and H2 (the 3-benzamidomethyl carbonyl oxygen and N-1 atoms). Figure VII-20 shows the superimposition of Ro5-4864, (\textbf{III.6a}) (BZR IC\textsubscript{50} 7 nM), (\textbf{III.6h}) (BZR IC\textsubscript{50} 25 nM), (\textbf{III.5}) (PBR IC\textsubscript{50} 8 nM), (\textbf{V.3e}) (PBR IC\textsubscript{50} 10 nM), (\textbf{VI.3p}) (PBR IC\textsubscript{50} 32 nM) and (\textbf{VI.3r}) (PBR IC\textsubscript{50} 155 nM). Volume analyses of this superimposition are shown in Figures VII-21, VII-22 and VII-23. Figure VII-21 identifies the total volume (green) and common volume (purple) for all seven of the PBR and/or BZR ligands. Figure VII-22 shows the total volume of all the ligands (green) and the total volume of the BZR ligands (\textbf{III.6a,h}) only (orange). It can be seen from this diagram that the PBR ligands occupy greater volumes in the regions of the 6- and 3-position substituent groups of the imidazo[1,2-b]pyridazines than the BZR ligands. This is also illustrated in Figure VII-23, which shows the total volume of all the ligands (green) and the total volume of the PBR ligands only (yellow).
Figure VII-20  Superimposition of (III.5), (III.6a), (III.6h), (V.3e), (VI.3p), (VI.3r)
and Ro5-4864
Figure VII-21  Total volume (green) and common volume (purple) for the superimposition of (III.5), (III.6a), (III.6h), (V.3e), (VI.3p), (VI.3r) and Ro5-4864
Figure VII-22 Total volume (green) and volume of (III.6a) and (III.6h) only (orange) for the superimposition of (III.5), (III.6a), (III.6h), (V.3e), (VI.3p), (VI.3r) and Ro5-4864
Figure VII-23  Total volume (green) and volume of (III.5), (V.3e), (VI.3p), (VI.3r) and Ro5-4864 only (yellow) for the superimposition of (III.5), (III.6a), (III.6h), (V.3e), (VI.3p), (VI.3r) and Ro5-4864
The (yellow) additional volumes occupied by the larger 6- and 3-position substituent groups of the PBR ligands can be seen.

The areas of steric hindrance at the PBR ligand binding site appear to be less restrictive than the corresponding areas of ligand-receptor interaction at the active ligand binding site of the BZR. The area S1 of the imidazo[1,2-b]pyridazine BZR pharmacophore defined previously in this section (Figure VII-17) does not exist at the PBR, as bulky 2-position groups can be accommodated, eliminating BZR affinity while maintaining nanomolar PBR affinity. Compounds such as (VI.4c), however, containing a 2-(p-cyclohexylphenyl) group may be reaching the steric limits of this area of the PBR, as the affinity of (VI.4c) (IC₅₀ 155 nM) is reduced compared to the 2-(p-t-butylphenyl) analogue (VI.4d) (IC₅₀ 32 nM).

It is probable that there is an area of steric hindrance of the PBR pharmacophore close to the 7- and 8-positions of the imidazo[1,2-b]pyridazine nucleus (corresponding to S2 of the BZR pharmacophore). The 2-aryl-3-benzamidomethyl-7(and 8)-(chloro or methoxy) compounds reported in Chapter IV show a reduction in PBR affinities compared with 6-substituted analogues, with the exception of (III.6g) discussed in Chapter IV. The relative differences in the PBR affinities of these compounds, however, are not as great as their relative differences in BZR affinities. This suggests that this area of negative steric interaction is not as restrictive at the PBR binding site as it is at the BZR binding site. Alternatively, it may be that the binding of the 6-substituted compounds is stronger due to a favourable ligand-receptor interaction between the 6-position group and the receptor protein, and that the absence of this favourable interaction (as in the 7- and 8-substituted analogues) results in decreased affinity for the receptor.

The PBR pharmacophore model developed in this thesis is shown in Figure VII-24. The areas of lipophilic interaction L1 and L2, and proton accepting groups H1 and H2 are identical to the BZR pharmacophore. Only one area of steric hindrance S1, however, has been defined on the basis of the imidazo[1,2-b]pyridazine PBR ligands reported here. In particular, the area of steric hindrance in the BZR pharmacophore between the 7-Cl group and the 5-phenyl ring of diazepam does not appear to be present.
in the PBR pharmacophore. The area of steric hindrance defined by Fiorini et al.\textsuperscript{162} (Figure VII-18) for the substituted arylpyrrolo[2,1-d][1,5]benzothiazepines is not occupied by the imidazo[1,2-b]pyridazines synthesised here (assuming that the two classes of molecules adopt the suggested orientations at the PBR binding site) so no further details of this area have been obtained.

**Figure VII-24 PBR pharmacophore for imidazo[1,2-b]pyridazines**

It was observed that, if the imidazo[1,2-b]pyridazines did interact with the PBR (and BZR) in the way that the pharmacophore models imply, the phenyl ring of the 3-benzamidomethyl group would be superimposed over the 5-phenyl ring of diazepam. Substitution of the 5-phenyl ring of diazepam by a para-chloro group forms Ro5-4864, a PBR specific ligand. Therefore chloro-substitution of the phenyl ring of the 3-benzamidomethyl group should have a similar effect in increasing selectivity for the PBR.

3-Benzamidomethyl-6-chloro-2-(p-chlorophenyl)imidazo[1,2-b]pyridazine (III.5) was found in Chapters III and VI to have a BZR IC\textsubscript{50} of 26 nM and a PBR IC\textsubscript{50} of 8 nM. It therefore bound with very high affinity to both central and peripheral receptor types, in an analogous manner to diazepam. Analogues of (III.5), containing 3-(o, m and p)-chlorobenzamidomethyl groups (VI.3k,l,m respectively) had BZR IC\textsubscript{50}'s of 488, 67 and 401 nM and PBR IC\textsubscript{50}'s of 13, 20 and 10 nM. PBR selectivity was therefore
improved ca 10-fold in the 3-(o and p)-chlorobenzamidomethyl compounds. This result is consistent with the BZR and PBR pharmacophore models and orientation of imidazo[1,2-b]pyridazines within them as proposed in this chapter.

VII-4 Conclusion

Variously substituted imidazo[1,2-b]pyridazines and imidazo[1,2-a]pyridines have proven to be useful ligands to develop pharmacophoric models at the BZR and PBR. Further work is required to develop and refine the BZR and PBR pharmacophore models presented in this thesis. It would be of particular interest for the BZR model to synthesise compounds containing 3-CH₂NHCH₂Ph and 3-CH₂CH₂COPh substituents to compare with the 3-benzamidomethyl analogues, and hence determine the importance of the carbonyl oxygen (suggested as the proton acceptor group H2) and of the proton donating NH group. Similarly, the phenyl rings of the 2- and 3-position substituent groups could be replaced by alkyl groups of varying chain length to further examine the properties of the proposed sites of lipophilic interaction L1 and L2. For the PBR pharmacophore it would be informative to examine the limitations imposed by other possible areas of steric hindrance at the active ligand binding site of the receptor by synthesising ligands with substituent groups that interact with these areas. It would also be of interest to synthesise compounds containing proton donor groups in various locations and attempt to relate the PBR affinities of these compounds to the PBR pharmacophore models presented in this thesis and by others.

The compounds could also be examined for in vitro affinities for recombinant GABA_{A} receptors expressing different combinations of α, β and γ subunits, and the pharmacological profiles compared with those of other known BZR ligands. This may enable certain structural or electronic features conferring selectivity for either receptor subtype to be identified. In vivo studies with some of the high affinity BZR and / or PBR ligands would assist in the identification of potential behavioural or therapeutic effects of these compounds. A series of PBR specific imidazo[1,2-b]pyridazines are currently
undergoing evaluation as possible agents to assist in the recovery from brain damage resulting from stroke and other types of CNS trauma.\textsuperscript{A}

\textsuperscript{A} This work is being carried out at the Institute for Drug Research, Budapest, Hungary.
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PUBLICATIONS


PUBLICATIONS
(Based on the work described in this thesis)

1. Barlin, G.B.; Davies, L.P.; Harrison, P.W.; Jacobsen, N.W. and Willis, A.C.
   "Imidazo[1,2-b]pyridazines. XVI. Synthesis and Central Nervous System
   Activities of Some 6-(Chloro, Alkylthio, Phenylthio, Benzylthio or Pyridinylmethylthio)-
   3-(unsubstituted, benzamidomethyl or methoxy)-2-(styryl or benzoyl)imidazo[1,2-b]-
   pyridazines."

2. Barlin, G.B.; Davies, L.P.; Davis, R.A. and Harrison, P.W.
   "Imidazo[1,2-b]pyridazines. XVII. Synthesis and Central Nervous
   System Activity of Some 6-(Alkylthio and chloro)-3-(methoxy, unsubstituted and
   benzamidomethyl)-2-arylimidazo[1,2-b]pyridazines Containing Methoxy,
   Methyleneedioxy and Methyl Substituents."

3. Barlin, G.B.; Davies, L.P. and Harrison, P.W.
   "Imidazo[1,2-b]pyridazines. XVIII. Syntheses and Central Nervous
   System Activities of Some 6-, 7- and 8-(Chloro and methoxy)imidazo[1,2-a]pyridine
   Analogues."