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Molecular modelling, design and synthesis of α₁-adrenergic receptor antagonists

Burak Coban

University of Wollongong

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MOLECULAR MODELLING, DESIGN AND SYNTHESIS OF $\alpha_1$-ADRENERGIC RECEPTOR ANTAGONISTS

By

BURAK COBAN
M.Sc.

Submitted fulfilment of the requirements for the degree of Doctor of Philosophy.

The University Of Wollongong, Wollongong June, 1999.
Author’s Statement.

This thesis contains no material which has been accepted for the award of any other higher degree or graduate diploma in any tertiary institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

Burak ÇOBAN

22.6.1999
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Abstract

The $\alpha_1$-adrenergic receptors ($\alpha_1$-ARs) mediate many effects of the sympathetic nervous system. Like other adrenergic receptors, $\alpha_1$-ARs are activated by the catecholamines, adrenaline and noradrenaline. The $\alpha_1$-ARs are membrane proteins and members of the G-protein coupled receptor superfamily. Three distinct subtypes have been confirmed by cloning techniques.

The $\alpha_1$-ARs have a divergent affinity for many synthetic drugs. Drugs interacting as agonists and antagonists with the subtypes selectively have been used in the treatment of a variety of diseases including hypertension, asthma and prostatic hypertrophy. However, there is still no antagonist available to discriminate between the subtypes by 1000 or more fold.

In order to design such selective antagonists, firstly, ligand based methods have been used by means of Apex-3D and Catalyst software of Molecular Simulations, Inc (MSI). Pharmacophores of the $\alpha_{1A}$- and $\alpha_{1B}$-ARs were generated together with a pharmacophore for the $\alpha_{1D}$-AR. These are the first $\alpha_1$-subtype specific pharmacophores for antagonists reported. Tetrahydroisoquinoline ring-containing compounds were designed, synthesised and tested as ligands on the cloned and expressed human $\alpha_1$-ARs. These results have also been evaluated via other computer-aided techniques, such as molecular electrostatic potential mapping and docking. Secondly, structure-based drug design methods have been developed using Insight software of MSI. New models of $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptors have been built and by the use of docking procedures, more detailed information about the binding pocket in the receptor for the ligands obtained and some
modifications on the target compounds have been proposed to design novel ligands with potentially higher activity and selectivity. Finally, these two techniques, ligand-based and structure-based design methods have been brought together to produce a novel approach called docking-derived pharmacophore modelling to create more detailed pharmacophore models for the $\alpha_{1A}$- and $\alpha_{1B}$-AR subtypes. The overall results are discussed and future directions proposed.
CHAPTER 1
GENERAL INTRODUCTION

1.1. INTRODUCTION

Membrane bound receptors are very important in maintaining a range of physiological processes in living systems. A receptor is a protein or a protein complex in or on a cell. It specifically recognizes and binds to a compound acting as a molecular messenger (neurotransmitter, hormone, lymphokine, lectin, drug, etc). In a broader sense, the term receptor is often used as a synonym for any specific drug binding site. Among the many different types of receptors, $\alpha_1$-adrenergic receptors ($\alpha_1$-ARs) are members of the G-protein-coupled-receptor super family and are activated by the catecholamines, adrenaline (1) and noradrenaline (2) (Figure 1.1). $\alpha_1$-ARs are expressed in a wide variety of tissues including brain, liver, myocardium, and vascular smooth muscle, and these receptors have a divergent affinity for many synthetic drugs. As new pharmacological tools have been identified, they have been subdivided into an increasing number of distinctive receptor subtypes. Drugs interacting with these subtypes have proven useful in a variety of diseases involving all of the major organ systems. Some of these diseases include hypertension, angina pectoris, congestive heart failure, cardiac arrhythmia, asthma, depression, prostatic hypertrophy and glaucoma. However, there is no useful subtype-selective drug readily available that can discriminate subtypes without causing any side effects. Hypotension is a common side effect of non-selective $\alpha_1$-AR blocking agents. Thus, development of highly selective drugs for the subtypes may help in the treatment of subtype-related disorders.
1.2. G-PROTEIN COUPLED RECEPTORS

G-protein coupled receptors are all activated by their own associated ligand leading to a conformational change resulting in increased affinity of the receptor for the guanine nucleotide proteins (G-proteins), which then in turn activate adenylate cyclases, phosphatidyl biphosphate hydrolases or the activity of certain ion cannels. General characteristics of a G-protein coupled receptors are shown in Figure 1.2. There are a great variety of receptors belonging to this family together with their subtypes including α₁, α₂ and β adrenergic receptors, serotonin receptors, muscarinic acetylcholine receptors, dopamine receptors, neurokinin (NK) peptide receptors and light receptors (rhodopsin). Each member of this family consists of seven hydrophobic regions (Figure 1.2) that are believed to form a bundle of α-helical transmembrane (TM) domains of 20-28 amino acids each. These TM domains are connected by alternating intra- and extra-cellular hydrophilic loops (Figure 1.2). The α₁-ARs are single polypeptide chains ranging from 429 to 561 amino acids in length. The amino-terminal region is exposed on the extra-cellular face of the membrane and the carboxyl-terminal region is on the cytoplasmic face. The regions of sequence similarity with other G-protein coupled receptors lie within the TM helices of the receptor, whereas the hydrophilic loop regions
are more divergent. A conserved intra-molecular disulfide bond between the first and second extra-cellular loops maintains the tertiary structure of the receptor.

Despite the wide variety of functions mediated by GPCRs, considerable structural homology exists among receptors of this class. For example, a large number of this type of receptor have a series of conserved residues located within the TM segments. Several of the GPCRs, including α₁-ARs, have been cloned and their sequences determined. Natural agonists of these receptors bind to sites located in the upper part of the TM helices interacting with specific amino acid residues. Antagonists that are chemically very similar to the agonists have been shown to occupy much of the same space. However, additional sites of interaction have been identified by site directed mutagenesis.
Because of the similarities in the transmembrane sequences of the family and their common interactions with G-proteins, it has been assumed that the basic structure of GPCRs is the same. It was also widely assumed that receptors have a similar structure to bacteriorhodopsin, the X-ray crystal structure of which is known\textsuperscript{13-15}, because the sequences of rhodopsin, which is a GPCR, and bacteriorhodopsin (the light activated proton pump of \textit{Halobacterium halobium}; non-GPCR) can be aligned and both are activated by the same function of the retina. The projection map of the GPCR bovine rhodopsin was recently determined by electron crystallography of two dimensional crystals.\textsuperscript{16} The projection structure of rhodopsin is less elongated, slightly wider and the helices are tilted differently in comparison with bacteriorhodopsin. A probable arrangement of the seven helices has been modelled by Baldwin\textsuperscript{9} based on structural information which was extracted from a detailed analysis of 204 GPCR sequences. In this study, each helix was fitted to the peaks in the projection map of rhodopsin and a tentative 3D arrangement of the helices was proposed by means of detailed helical wheel projection models. In addition, the analysis of occurrence of polar residues at certain positions in the helices of the GPCRs suggested that they might share a common packing arrangement that differs from that of the helices in bacteriorhodopsin. Recently, a model for the $\alpha$-carbon positions in the seven TM helices in the rhodopsin family of GPCRs was presented by Baldwin \textit{et al.}\textsuperscript{17} The model incorporates structural information derived from the analysis of about 500 sequences of this family. Many GPCR transmembrane domains have been modelled on the basis of the known structure of bacteriorhodopsin\textsuperscript{18-24} and rhodopsin. Some of the newer models of these receptors have been built using both of the templates, copying helix main axes from the bacteriorhodopsin template, but building the topography of the amino acid positions almost the same as in the bovine-rhodopsin input structure.\textsuperscript{24} In other studies,\textsuperscript{11}
bacteriorhodopsin-like models have been refined with the information from the bovine-rhodopsin structure. Bacteriorhodopsin based models of the adrenergic receptors\(^1\) have been modified with the information from the \(\alpha\)-carbon template\(^2\) of the helices of the rhodopsin family of GPCRs and used in this project to study their interactions with our designed compounds.

### 1.3. THE \(\alpha_1\)-ADRENERGIC RECEPTOR SUBTYPES

The subdivision of \(\alpha_1\)-ARs was derived principally from ligand binding studies. So far three \(\alpha_1\)-ARs have been cloned and pharmacologically characterised, namely \(\alpha_{1A}\), \(\alpha_{1B}\) and \(\alpha_{1D}\). The antagonist, prazosin (3) (Figure 1.3) binds to all these subtypes very strongly (pKi= 9-10). The agonist oxymetazoline (4) and antagonists WB 4101 (5) and phentolamine (6) were used to discriminate higher affinity sites as \(\alpha_{1A}\) and lower affinity sites as \(\alpha_{1B}\). Subsequently, the \(\alpha_{1B}\) subtype but not the \(\alpha_{1A}\) subtype, was shown to be completely inactivated by the alkylating agent chloroethylclonidine (7). In addition, cloning of these receptors has shown the existence of the \(\alpha_{1D}\) subtype.\(^2\) The differences between the subtypes are shown in Table 1.1 and Figure 1.4. The functional evidence of further distinct subtypes, showing low affinity for prazosin (eg. the \(\alpha_{1L}\) group), is still awaiting confirmation from molecular biology.\(^2\) The relatively high expression of the \(\alpha_{1A}\) subtype in the human prostate has led to interest in selective \(\alpha_{1A}\) antagonists for the treatment of benign prostatic hyperplasia.\(^2\) Similarly, the relatively high expression of the \(\alpha_{1B}\) receptor in the human aorta is indicative of the importance of this subtype in mediating vascular hypertension.
<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>$\alpha_{1A}$</th>
<th>$\alpha_{1B}$</th>
<th>$\alpha_{1D}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other names</td>
<td>$\alpha_{1c}$</td>
<td>$\alpha_{1b}$</td>
<td>$\alpha_{1\alpha, \alpha_{1\beta, \alpha_{1D}}}$</td>
</tr>
<tr>
<td>Potency order</td>
<td>NA$\geq$adrenaline</td>
<td>adrenaline=NA</td>
<td>adrenaline=NA</td>
</tr>
<tr>
<td>Selective agonists</td>
<td>A61603</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Selective antagonists</td>
<td>KMD3213 (10.4)</td>
<td>AH1111OA (7.1)</td>
<td>BMY7378 (8.4)</td>
</tr>
<tr>
<td></td>
<td>(+)niguldipine (10.0)</td>
<td>chloroethylclonidine</td>
<td>(+)noradrenaline</td>
</tr>
<tr>
<td></td>
<td>SNAP5089 (9.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-methyluropidil (9.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RS17053 (9.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SNAP5272 (8.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-selective agents</td>
<td>prazosin, WB 4101, phentolamine, benzoxathiain, abanoquil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of amino acids</td>
<td>h466</td>
<td>h519</td>
<td>h572</td>
</tr>
<tr>
<td></td>
<td>r466</td>
<td>r515</td>
<td>r561</td>
</tr>
<tr>
<td>Prototype tissues</td>
<td>rat kidney &amp; submaxillary gland, rabbit liver, human prostate.</td>
<td>rat spleen, liver &amp; hearth.</td>
<td>rat aorta &amp; cerebral cortex.</td>
</tr>
</tbody>
</table>

Table 1.1. The differences between $\alpha_{1}$-AR subtypes,\(^5,26\) pKi values are shown in parentheses.

Currently there are few ligands that are recognised by one $\alpha_{1}$-subtype with at least 100- to 1000-fold higher affinity than by the other two subtypes. 5-Methyluropidil (8) and SNAP (9) 5089 have 100-fold selectivity for the $\alpha_{1A}$-AR subtype and KMD-3213 (10) has a 500-fold lower affinity at the $\alpha_{1B}$-AR, and a 56-fold lower affinity at the $\alpha_{1D}$-AR with Ki=0.036 nM at the $\alpha_{1A}$-AR. On the other hand, cyclazosin\(^27\) (11) has a 40-fold and AH-11101A (12) has 30-fold selectivity for the $\alpha_{1B}$-AR over the other two subtypes. Finally BMY 7378 (13) and SK&F 105854 (14) have been reported to be selective for the $\alpha_{1D}$-AR.\(^5\)
Figure 1.3. Known antagonists for α₁-ARs.
The boxed sequences refer to the extracellular half of the TM domains that contain the putative ligand binding site. The amino acids that differ only in one subtype are typed in bold, whereas the amino acids that differ within all subtypes are shadowed.
1.4. COMPUTER-ASSISTED DRUG DESIGN

Computer-assisted drug design (CADD) represents applications of molecular modeling programs as tools in pharmaceutical research. Molecular modeling is the science of representing molecular structures numerically and simulating their behaviour with the equations of quantum and classical physics. Molecular data can be generated and presented including geometries (bond lengths, bond angles, torsion angles), energies (heat of formation, activation energy, etc.), electronic properties (moments, charges, ionisation potentials, electron affinity) and bulk properties (volumes, surface areas). In considering this topic, it is important to emphasise that computer analysis cannot substitute for a clear understanding of the system, but may only act as an additional tool to gain better insight into the chemistry of the problem.¹⁰

The starting point for many CADD studies is generally a two-dimensional drawing of a compound of interest. These diagrams are translated into a connection table in which definitions of the atom types in the molecule, their hybridisation and the ways they are bonded to each other, are stored. However, tables must be transformed into three-dimensional representations of chemical structure to study chemical properties. To accomplish this task, mathematical descriptions of physical chemistry provided by quantum and molecular mechanics are employed.
1.4.1. CALCULATION METHODS

1.4.1.1. Quantum mechanics

Quantum mechanical theory uses well known physical constants such as the velocity of light, values for the masses and charges of nuclear particles and differential equations to calculate molecular properties and geometries. This is also called *ab initio* quantum mechanics. The equation from which molecular properties can be derived is the Schrödinger equation:

\[ H\Psi = E\Psi \]

where E is energy of the system, \( \Psi \) is the wave function which defines the Cartesian and spin coordinates of the atomic particles and H is the Hamiltonian operator that includes terms for both potential and kinetic energies. Unfortunately, the Schrödinger equation can only be solved for very small molecules such as hydrogen and helium. Approximations must be introduced to use the method for polyatomic systems.\(^{28}\)

While recent publications have reported calculations on large molecules, but they require a longer computing time required for calculations and a large amount of disk space to store intermediate data files. Thus, alternative approaches have been developed to computing structures and properties by simplifying portions of the calculation. These methods are referred to collectively as semiempirical quantum methods.

1.4.1.2. Semi-empirical methods

Semiempirical methods use approaches which are similar to *ab initio* methods, but several approximations are introduced to simplify the calculations.\(^{29}\) While semiempirical methods require less computer resource, they still compute extensively.
In general, calculations are performed on compounds which contain up to 100 atoms. However, compounds which range in size from several hundred atoms (drug candidates, monomers, etc.) to several thousand atoms (proteins, nucleic acids, carbohydrates, polymers, etc.) are under investigation in medicinal chemistry projects. Due to the computational requirements for quantum mechanical approaches, these types of compounds are impossible to work with. Thus, a further simplification is required. This approach is the molecular mechanics or force field method.

1.4.1.3. Molecular mechanics (force field) method

Molecular mechanics is a mathematical formalism which attempts to reproduce molecular geometries, energies and other features by adjusting bond lengths, bond angles and torsion angles to equilibrium values that are dependent on the hybridisation of an atom and its bonding scheme. Rather than using quantum physics, the method relies on the laws of classical Newtonian physics and experimentally derived parameters to calculate geometry as a function of steric energy. The general form of the force field equation\(^3^0\) is:

\[
E = \sum E_{\text{stretching}} + \sum E_{\text{bending}} + \sum E_{\text{dihedral}} + \sum E_{\text{van-der-Waals}} + \sum E_{\text{electrostatic}} + \sum E_{\text{hydrogen-bonding}}
\]

\(1\) (2) (3) (4) (5) (6)

\(E\) is total steric energy, which is the difference between the energy of a real molecule and an ideal molecule, the first three energy terms describe the bond energy and the last three energies belong to non-bonded interactions. Each of the energy terms is either experimentally known or theoretically estimated. Therefore, a force field consists of a set of analytical energy functions and their associated sets of numerical parameters.\(^3^0\)
In contrast to \textit{ab initio} methods, molecular mechanics is used to compute molecular properties which do not depend on electronic effects including geometry and rotational barriers. Since the calculations are fast and efficient, molecular mechanics can be used to examine systems containing thousands of atoms.\textsuperscript{30} However, unlike \textit{ab initio} methods, molecular mechanics relies on experimentally derived parameters so that calculations on new molecular structures may be misleading.

Each of the methods described above are used to calculate the energy of a compound in a specific 3D orientation and to optimise the geometry as a function of energy (i.e., to adjust the coordinates of each of the atoms and recompute energy of the molecule until a minimum energy is obtained). They also can be used to simulate the time-dependent behaviour of molecules (molecular dynamics) and explore their conformational flexibility (conformational search).\textsuperscript{30}

\textbf{1.4.1.4. Molecular dynamics}

Molecular dynamics is another commonly used method which combines energy calculations from force field methodology with the laws of Newtonian mechanics to investigate the conformational space of a molecule.\textsuperscript{30} The simulation is performed by numerically integrating Newton's equations of motion over small time steps, usually 1 fs. The simulation is initialised by providing the location and assigning a force vector for each atom in the molecule. Once the acceleration and velocities are computed, new atom locations and the temperature of the assembly can be calculated. These values are used to calculate trajectories, or time dependent locations, for each atom. Over a period of time, these values can be stored on disk and played back after the simulation has completed.\textsuperscript{30}
Molecular dynamics simulations have been used in a variety of biomolecular applications. Structural dynamic and thermodynamic data from molecular dynamics has provided insight into structure-function relationships, binding affinities, mobility and stability of proteins, nucleic acids and other macromolecules.

1.4.1.5. Conformational search

While molecular dynamics provides an excellent approach to searching regions of conformational space, it does not cover all the possible conformations. The active conformation of a molecule can be missed as the dynamics simulation skips over the hills and valleys of the potential energy surface. Since the active conformation at a receptor may not always be the minimum energy structure it is important to examine all potentially accessible conformations.\(^{30}\)

For small molecules with a limited number of freely rotating bonds, this can be accomplished by driving each torsion angle stepwise over a 360 degree range. The theoretical number of conformations for a molecule can be calculated by the formula:\(^{30}\)

\[
\text{Number of conformers} = \left(\frac{360}{\text{angle increment}}\right)^{\text{# rotatable bonds}}
\]

Thus a molecule with four rotatable bonds searched in a 60 degree increment will generate \((360/60)^4\) or 1,296 distinct conformations. If the energy for each structure is evaluated in one second, the search and evaluation will require approximately 22 minutes. This is acceptable for small and medium sized molecules but not reasonable for proteins and peptides.
1.4.1.6. Molecular surfaces, molecular fields and electrostatic potential

The computational methods discussed above are used to optimise molecular geometry and calculate physical and electronic properties. An equally important aspect of CADD is the ability to display these properties with the structural features. Molecular surfaces play an important role in these studies. Electrostatic potential, lipophilicity potential and steric field are examples of fields where each lattice point contains a value on the surface of the molecule.\textsuperscript{31}

Molecules can interact well beyond their van der Waals surfaces. Positively charged molecules are attracted to negatively charged ones, \( \pi \) systems tend to stack in particular orientations and hydrogen bonds can form between suitable sites. The strength of these interactions will be moderated by the dielectric constant of the intervening regions but dielectric constants are measured for the bulk properties of materials and so are hard to define on an atomic level. Generally, non-bonded interactions are treated as a combination of van der Waals repulsions and electrostatic interactions and are calculated from Coulomb's law through a partial charge given to each atom.\textsuperscript{31}

Molecular electrostatic potential has been the most widely used molecular field. The electrostatic interaction has the longest range of the forces responsible for binding of drugs to enzymes and receptors, and is thus likely to play an important role in the early stages of the interaction. Once a complex has formed, high affinity ligands should have complementary potential to those of the receptor. Lower electrostatic complementarity is one reason for lower ligand affinities.
When the macro-molecular structure is unknown, a comparison of a series of analogues for the receptor may enable a model to be generated to rationalise the spread of biological activity and to predict the activity of untested structures. Such an analysis on dopamine antagonists\textsuperscript{32} is one of many examples where electrostatic potential, either alone or in combination with other factors, has been used to gain insight into the drug-receptor interaction.

1.4.2. DRUG DESIGN METHODS

CADD applications are used to find a ligand that will interact specifically with the binding site of the receptor. Binding of a ligand to a receptor may include hydrophobic, electrostatic and hydrogen-bonding interactions. This approach to CADD optimises the fit of a ligand in a receptor site. However, optimum fit in a target site does not guarantee that the desired activity of the drug will be enhanced or that undesired side effects will be diminished. Moreover, this approach does not consider the pharmacokinetics of the drug.

The approach used in CADD is dependent on the amount of information that is available about the ligand and receptor.

1.4.2.1. Ligand based design (pharmacophore mapping)

The ligand-based approach is applicable when the 3D structure of the receptor is unknown, and a series of compounds has been identified that show the activity of interest.\textsuperscript{30} To use this approach most effectively, one should have structurally similar compounds with high activity, no activity and a range of intermediate activities. In recognition site mapping, the aim is to identify a pharmacophore, which is a template
derived from the structures of these compounds, representing the geometry of the receptor site as a collection of functional groups in three-dimensional space.\textsuperscript{33}

It should be pointed out that the lowest energy conformation is not necessarily the binding conformation but the energy of the binding conformation is not expected to be much greater than that of the global minimum. Because the bioactive conformation of the compounds is not generally known, a set of lower energy conformations of each compound must be used. In applying this approach, conformational analysis will be required; the extent of that will be dependant on the flexibility of the compounds under investigation. Ultimately, common functional groups (descriptor centres) such as hydrophobic groups and aromatic rings will be identified, considering all conformers of each compound, then common three-dimensional arrangements of the descriptor centres will be identified as a pharmacophore. The pharmacophore model can give a good negative image of the binding site. This template may then be used to develop new compounds with functional groups in the desired positions.\textsuperscript{34}

Pharmacophore development approaches sometimes rely on incorrect assumptions, for example, that all compounds in the training set bind to the receptor in the same mode (same binding site, same receptor conformation). There can be multiple binding modes, which will make pharmacophore mapping much more difficult. A problem can arise if two compounds are very similar but bind in different modes, because of minor differences in shape.

A pharmacophore for agonists and antagonists at the \(\alpha_1\)-AR for both agonists and antagonists has been reported\textsuperscript{35} and there have also been many QSAR studies for \(\alpha_1\)
adrenergic receptors.\textsuperscript{36-38} An earlier study\textsuperscript{35} was focused on an agonist pharmacophore and concluded that all phenethylamines bind to the $\alpha_1$-adrenergic receptor similar to that in the Easson-Stedman hypothesis which involves a phenyl ring, $\beta$-hydroxyl group and a basic nitrogen atom (Figure 1.5)\textsuperscript{35}. The distance between the nitrogen atom and the phenyl ring was also defined. Other types of agonists do not fit on this pharmacophore but there was no subtype specific information. In a QSAR study,\textsuperscript{36} antagonists at the $5HT_{1A}$ receptor and the $\alpha_1$-AR were chosen to superimpose on each other to find out the common features, i.e. the protonated amine function, two aromatic nuclei, and a bulky aliphatic substituent. However, there was no assessment of subtype selectivity with respect to the $\alpha_1$ receptors in this study. There are other studies reported\textsuperscript{37,38} where more detailed information is available, but again without the subtype selectivity, without clear distances or angles being defined.

![Figure 1.5. Easson-Stedman hypothesis.\textsuperscript{35}](image-url)
1.4.2.2. Structure based design (docking)

"Structure based design is a design strategy for new chemical entities based on the three dimensional structure of the target obtained by X-ray or nuclear magnetic resonance (nmr) studies, or from protein homology models."³⁹ "The main assumption of structure based design is that good inhibitors must possess significant structural and chemical complementarity to their target receptor."⁴⁰ The computational techniques for the exploration of the possible binding modes of a ligand to a given receptor is also called docking which depends on the availability of the three dimensional structure of the receptor. A docking study can be used to determine the binding conformation of a ligand and calculate the binding energy, which then can be used to compare with other ligands.

Docking algorithms vary but the basic method involves placing the ligand at the active site of the receptor and simulating the complex with various minimisation techniques such as molecular dynamics. After that, the binding conformation of the ligand, interacting amino acids of the receptor and other possible interactions with neighbouring amino acids and interaction energies can be determined.⁴⁰ This data can then be used to make modifications on the ligand or to design novel drugs.

1.5. CONFORMATIONALLY CONSTRAINED MOLECULES

In the design process, conformationally constrained molecules can be used to overcome conformational flexibility problems. It has been assumed that most of the antagonists have only slight selectivity between the subtypes because of their flexibility. Different conformations of a drug can be active on a different subtype or even a different receptor.
Thus, higher selectivity could not be created with any substitution on those flexible compounds, but conformationally rigid templates may be used to increase selectivity instead. It is also useful to have an active compound which is conformationally rigid so that the binding conformation is known and can be used as a template. The isoquinoline template has been chosen in this work because of the high activity of known antagonists of this group on the α₁-ARs.

![Diagram](image)

Figure 1.6. Isoquinoline ring containing known compounds. The name isoquinoline is used to include the ring skeleton itself and partially reduced derivatives.

Although the isoquinoline motif is very common among natural products, a relatively small number of those show useful biological activity. For example, an alkaloid papaverine (15) (Figure 1.6), extracted from *Papaver somniferum*, has been used as a
muscle relaxant. The other isoquinoline based drugs include the antiviral compound famotine (16), the antipsychotic agent tetrabenazine (17) and a β-adrenergic agonist trimethoquinol (18).41

The starting set of isoquinolines used in this study which has high affinities for the α1-ARs consists of synthetic compounds that were developed in our laboratories including IQC (19) (a reduced isoquinolino[8,1-ab]carbazole derivative)42 and alkaloids such as dicentrine (20) and derivatives.43 IQC (19) has a 16-fold selectivity of binding for the α1A subtype over the α1B subtype and dicentrine (20) (an alkaloid extracted from Lindera megaphylla) has moderate affinity for α1-adrenergic receptors, although not tested specifically on the subtypes. Other derivatives related to dicentrine including glaucine (21), predicentrine (22) and boldine (23), which are selective for the α1A subtype.44 Discretamine (24) is also a natural product, isolated from the plant Fissistigma glaucescens, and it has a high affinity (Ki= 25 nM) for the α1D subtype45; it also has a 25-fold lower affinity at the α1A, and a 14-fold lower affinity at the α1B AR’s.

1.6. PROJECT AIM

There are still no useful antagonists that can bind to the α1 adrenergic subtypes with great selectivity. Such subtype selective antagonists could be used as a cure for the particular diseases related to the α1-AR subtype targets including benign prostatic hyperplasia and hypertension.
The central aim of the project was to design, synthesise and evaluate new subtype selective \(\alpha_1\)-adrenergic ligands. In order to do this, computer-aided ligand design techniques were to be used. Firstly, the lack of structural information about the actual receptors led us to start with a ligand-based design process. It was planned to obtain three dimensional pharmacophores for the three subtypes by exploiting the common structural information from a set of known \(\alpha_1\)-adrenergic antagonists. Selective antagonists can be used with their associated activities as input data and the use of two sophisticated computer programs, Apex-3D and Catalyst was planned to generate selective pharmacophores.

A structure based design technique, docking, was also planned by using computer models of the \(\alpha_{1A}\)-and \(\alpha_{1B}\)-adrenergic receptor subtypes. This approach can give us an opportunity to visualise the proposed binding pockets of the receptor subtypes, major hydrophobic interactions and possible hydrogen bonding interactions. Comparisons between the subtypes were also to be investigated.

According to the information obtained from the pharmacophore and docking studies, isoquinoline derivatives will be designed which are proposed to bind selectively to each of the subtypes of the \(\alpha_1\)-AR. Synthetic routes can then be studied and finally the synthesis of these compounds and biological tests on cloned receptors can also be undertaken.
There is NO p. 22 in original document
CHAPTER 2

DEVELOPMENT OF $\alpha_{1A}$ AND $\alpha_{1B}$ PHARMACOPHORES AND TARGET LIGANDS

2.1. PHARMACOPHORE DEVELOPMENT

This is the most commonly used approach to drug design where the three dimensional structure of the receptor is not known. In this case, structures of known ligands are characterised with their pharmacological data on the same receptor and used to find structure-activity relationships to design novel drug candidates. The relative orientation of the functional groups necessary for biological activity of the ligands defines a pharmacophore which, ideally, accounts for the binding of a structurally diverse set of ligands to a common receptor. The basic assumption in the development of a pharmacophore model for a set of ligands is that they bind to the same site of the receptor. Thus, functional groups common to all ligands interact with the complementary components of the receptor and would be expected to orient similarly in the active site.

If the structure-activity relationship data of a set of ligands is examined, it is sometimes possible to identify the parts of the ligands that are important for binding. These important functional groups are then superimposed in the 3D space of the energetically accessible conformations of the ligands. These functional groups are often electron lone pairs, hetero atoms, centroids of a collection of atoms (an aromatic ring), surfaces, and
volumes. In addition, directions of the hydrogen bonds representing the features in the receptor can also be mapped.

Ideally, an active rigid molecule can be used as a starting template. If this is not possible, semi-rigid or flexible molecules are used with all possible conformations taken into account to overlay and draw good alignment of important pharmacophoric elements. Because of the conformational flexibility problem and the large numbers of alignment possibilities, these searches must be automated by means of sophisticated computer programs. Two of these programs, Apex-3D and Catalyst of Molecular Simulations, Incorporated, were used in this study.

In Catalyst and Apex-3D, inactive ligands can be included into training sets. This allows one to filter only important common features i.e. the chemical features on the inactive compound are excluded when common features are searched, but the reason for inactivity of a drug can also be the result of a number of properties of the ligand rather than their structures, such as chemical or metabolic instability, or insolubility. Thus this feature must be used cautiously. The conformational search is restricted to low energy conformations of the ligands in these programs because they only allow a certain number of conformers (100) in the training set. The energies of the matching conformations are not used to rank the pharmacophore model candidates. Both programs also use alternative mappings of the large number of conformations to the pharmacophore models.
Considering a wide variety of chemical structures, a previous pharmacophore study pointed out some features linked with $\alpha_1$-adrenergic receptor antagonism\textsuperscript{35}. This study was based on a process which involved choosing an active constrained molecule followed by a subsequent comparison with more flexible compounds in order to obtain a pharmacophore. Basically, corynanthine (25), the most constrained compound was chosen as a template to be compared with the conformationally free compounds. The resulting pharmacophore (Figure 2.1) has three features including an aromatic region, a basic nitrogen which has to be protonated at physiological pH and a semipolar region or a bulky lipophilic area. However, this early study did not consider $\alpha_1$ receptor subtypes.

A number of linear QSAR models were developed from $\alpha_1$ adrenergic antagonists.\textsuperscript{36} Complete geometry optimisation was performed for the protonated forms of the compounds considered taking the extented conformations as starting geometries. From these studies, it was strongly suggested that the protonated amine function is very important for binding. In addition, two aromatic ring systems on either side of the protonated amine function are also necessary for the $\alpha_1$-adrenergic activity. No clear trend was observed for the angle value but the distances between aromatic ring and the nitrogen are 4 and 7 Å. In the earlier QSAR models developed from a noncongeneric series of $\alpha_1$ adrenergic antagonists also showed that the protonated nitrogen is the key element for the binding. Other important functions found in this model are an aromatic nucleus (quinazoline ring for prazosin, phenyl piperazine for niguldipine analogues,
phenyl ring of benzodioxane of WB4101 etc.), polar residue, and an additional aromatic ring system which is present in the antagonists with high affinities.37,38

![Figure 2.1. The pharmacophore for the α₁-adrenergic receptor.](image)

2.1.1. APEX-3D PHARMACOPHORES

In the Apex-3D algorithm, chemical structures are represented by descriptor centres which interact hypothetically with the receptor. Descriptor centres can be either atoms or pseudo atoms which can participate in the interaction on the basis of the following properties:47-49

(i) electrostatic interaction  
(ii) hydrogen bonds  
(iii) charge transfer complexes  
(iv) hydrophobic interaction  
(v) van der Waals dispersion forces

These properties correlate with quantum chemical indices such as:

(i) atomic point charges  
(ii) π populations  
(iii) electron donor and acceptor indices  
(iv) HOMO and LUMO coefficients

Apex-3D starts with the separation of training set compounds into activity classes according to their actual activities and submission of a number of conformers for each
flexible compound in the set by employing user-defined conformational models (imported from other software.). These models can be derived from various modelling methods such as molecular mechanics or quantum mechanics methods. In this case, Insight II version 95.0 molecular modelling system with discover and search compare modules with the CVFF force field (molecular mechanics method), was used to do the conformational search by employing molecular dynamics, energy minimisation and systematic conformational search methods (see experimental section). It should be pointed out, however, that the number of conformers for each molecule is restricted to a hundred in this program. Then, Apex-3D identifies the common structural patterns for all compounds belonging to a given activity class or classes and calculates the number of occurrences of all identical structural patterns. These occurrence numbers are then used to calculate statistical estimates such as the probability, $p$ (explained in the filters section), that novel compounds having a given feature will belong to a certain activity class. Finally, pharmacophores are selected if they have a probability higher than a certain threshold. Prediction of the biological activity of novel compounds suggested for synthesis, based on the selected pharmacophore, is also possible in this software.
Table 2.1a. Apex-3D training set for the $\alpha_{1A}$ and $\alpha_{1B}$ ligands.

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>$\alpha_{1A}$</th>
<th>p$K_i$</th>
<th>$\alpha_{1B}$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin (3)</td>
<td>10.1</td>
<td>10.2</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>X=O, WB-4101 (5)</td>
<td>10.1</td>
<td>8.8</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>X=S, Benoxathian (26)</td>
<td>9.7</td>
<td>8.4</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>(+)-Niguldipine (27)</td>
<td>9.6</td>
<td>7.6</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>5-Methyluropidil (8)</td>
<td>9.2</td>
<td>7.6</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Spiperone (28)</td>
<td>8.1</td>
<td>9.3</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2.1b. Apex-3D training set for the $\alpha_{1A}$ and $\alpha_{1B}$ ligands.

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>$\alpha_{1A}$</th>
<th>p$K_i$</th>
<th>$\alpha_{1B}$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risperidone (29)</td>
<td>6.6</td>
<td>8.6</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.6</td>
<td>8.1</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Phentolamine (6)</td>
<td>8.8</td>
<td>7.5</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>KMD-3213 (10)</td>
<td>10.4</td>
<td>7.7</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Inactive prazosin analogue (30)</td>
<td>No adrenergic activity</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactive WB-4101 analogue (31)</td>
<td>3.9 for $\alpha_1$</td>
<td>38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the first experiment, the training set consisted of nine antagonists (Table 2.1) and two inactive molecules including their ten minimum energy conformations which were
obtained (see experimental section) within a 10 kcal per mol energy window with respect to the minimum energy. The compounds were classified into three activity classes (Table 2.2). This input data produced hundreds of pharmacophore hypotheses, called “biophores” for each subtype. Biophores were filtered automatically and manually to reduce the number.

<table>
<thead>
<tr>
<th>Class name</th>
<th>$\alpha_{1A}$ condition $(pK_a)$</th>
<th>$\alpha_{1B}$ condition $(pK_a)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very active</td>
<td>$&gt;9$</td>
<td>$&gt;8$</td>
</tr>
<tr>
<td>Active</td>
<td>$&gt;8$ and $&lt;9$</td>
<td>$&gt;6$ and $&lt;8$</td>
</tr>
<tr>
<td>Inactive</td>
<td>$&lt;8$</td>
<td>$&lt;6$</td>
</tr>
</tbody>
</table>

Table 2.2. Activity classes used in Apex-3D

The automatic filters within the software that has been used are shown in the Table 2.3.

<table>
<thead>
<tr>
<th>Probability</th>
<th>$\alpha_{1A}$ subtype</th>
<th>$\alpha_{1B}$ subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td># of Centres</td>
<td></td>
<td></td>
</tr>
<tr>
<td>size</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>active</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2.3. Filter criteria for the $\alpha_{1A}$ and $\alpha_{1B}$ ligands in Apex-3D.

The first filter $p$ stands for a probability which is defined as $p = (m_{rk} + 1) / (m_r + 2)$ where $m_{rk}$ is the number of very active compounds possessing the given biophore, and $m_r$ is the total number of compounds possessing the given biophore. The second filter is size which controls the number of the descriptor centres. For example, in this experiment every biophore has to have at least 3 descriptor centres. The last filter is active which specifies the least number of the very active compounds that the given biophore has to contain. In this experiment biophores have to contain at least five very active compounds for $\alpha_{1A}$ and four very active compounds for the $\alpha_{1B}$ subtype.
After the automatic filters, the biophores were further filtered manually. All the biophores containing any inactive molecules except risperidone for the \( \alpha_{1A} \) subtype and also any biophores with less than three independent descriptor centres (see Figure 2.2) were removed. The next criterium which was considered for the filtration was that the most basic nitrogen atom of prazosin and WB-4101 must be one of descriptor centres and must be located at the same position. The most basic nitrogen atom of prazosin was determined according to experimental data\(^{56} \) and is shown in Figure 2.3 as N1. The most basic nitrogen atom theoretically and experimentally protonates and interacts\(^{11} \) with the highly conserved aspartate residue in the third transmembrane helix of each of the \( \alpha_{1A} \) and \( \alpha_{1B} \) receptor subtypes.
After the filtration of the biophores, there were two biophores left for each subtype including one with an aromatic ring as proposed previously by De Marinis et al. However, neither of our biophores contained the most basic nitrogen atoms of all compounds as a descriptor centre aligning in the same spot. This problem was encountered by De Marinis and solved there by fitting another nitrogen atom (N2), not the most basic nitrogen atom (N1) of prazosin, onto the pharmacophore. Figure 2.3 also shows the alternative nitrogen atom (N2) which fits the previously generated pharmacophore model by De Marinis et al. However, the protonation of this nitrogen atom is unlikely because the calculated energy difference for protonation of this nitrogen atom rather than the most basic one is about 20 kcal/mol. Therefore the high affinity of prazosin cannot be explained by these pharmacophores, and it is possible it may bind in a different region.

The protonated forms of the antagonists were also investigated, but conformational analysis experiments in the gas phase on these compounds gave unrealistic folded conformers forming intra-molecular hydrogen bonds and/or aromatic-aromatic interactions. When the protonated nitrogen atoms form intra-molecular hydrogen bonds they are sterically unavailable for interaction with the receptor (see section 2.1.2). Solvated molecules were not allowed as input to the Apex 3-D software, so this option was not available.

In the second experiment, only selective compounds were put into the training set and a larger number of conformations were utilised for each compound. The selective
compounds were selected and 100 conformers were generated for each molecule using a systematic conformational search as discussed in the experimental section.

2.1.1.1. Results for the α1A subtype

Eighty-nine conformations of KMD3213 and ninety-nine conformations each for 5-methyluropidil and (+)-niguldipine were used for this task. The probability was chosen to be ≥ 0.8, size ≥ 3, active = 5 and the biophores which did not contain the most basic nitrogen atom as a descriptor centre at the same spot were removed. This left nine biophores, all except one having an aromatic ring. The distances between the nitrogen atom and the centre of the aromatic ring are very similar to the pharmacophore proposed by De Marinis et al.\(^\text{35}\) There is also a third descriptor centre in seven of the biophores which is a hetero atom associated with the second unsaturated six membered ring system which also agrees with the same pharmacophore.

![Figure 2.4. The α1A pharmacophore model.](image)

The new pharmacophore model for the α1A subtype according to this trial is sketched in Figure 2.4. It contains an aromatic ring 5.2-5.8 Å away from the basic nitrogen atom
and on the other side of the molecule 6-8 Å away from the basic nitrogen atom, an aromatic or six membered unsaturated ring with polar substituents.57

### 2.1.1.2. Results for the α₁B subtype

Forty conformations of risperidone and forty-two conformations of spiperone were used for this task. The probability was chosen to be ≥ 0.75, size ≥ 3, active = 4 and the biophores which did not contain the most basic nitrogen atom as a descriptor centre at the same spot were removed. This left nine biophores, all except one having an aromatic ring. The distance between the basic nitrogen and the centre of the aromatic ring is larger for this subtype but less well defined as 6.2-7.8 Å. In most of the biophores there is a third descriptor centre for a hetero atom associated with an aromatic ring or unsaturated six-membered ring system. The proposed pharmacophore model for the α₁B subtype is shown in the Figure 2.5.57

![Figure 2.5. The α₁B pharmacophore model.](image)

### 2.1.1.3. Validation of the pharmacophore models:

As a test for the models, the relevant distances were measured in a series of known molecules (Table 2.4), and the new compounds are shown in Table 2.5. The distances
are shown as ranges encountered in a number of conformations calculated within 10 kcal per mol energy range of global minimum for each molecule using molecular dynamics. Two conclusions can be drawn from this table.

(1) Prazosin and phentolamine can not fit either of the pharmacophore models and may bind to a different binding site of the receptor.

(2) All antagonists apart from 5-methyluropidil, corynanthine and dicentrine fit both proposed pharmacophores.\(^{57}\)

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>N-aromatic centre (Å)</th>
<th>N-ring-polar groups (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha_{1A}) pharmacophore</td>
<td>5.2 - 5.8</td>
<td>5.2 - 6.7</td>
</tr>
<tr>
<td>(\alpha_{1B}) pharmacophore</td>
<td>6.2 - 7.8</td>
<td>5.2 - 7.5</td>
</tr>
<tr>
<td>(3) Prazosin</td>
<td>2.7 or 7.6 - 8.8</td>
<td>2.7 or 7.6 - 8.8</td>
</tr>
<tr>
<td>(6) Phentolamine</td>
<td>3.0</td>
<td>3.7</td>
</tr>
<tr>
<td>(5) WB-4101</td>
<td>5.4 - 6.3 or 4.1 - 6.2</td>
<td>5.4 - 6.3 or 4.1 - 6.2</td>
</tr>
<tr>
<td>(27) (+)-Niguldipine</td>
<td>4.9 - 5.8 or 4.2 - 10.3</td>
<td>4.3 - 8.6 or 5.8 or 4.2 - 10.3</td>
</tr>
<tr>
<td>(8) 5-Methyluropidil</td>
<td>5.7</td>
<td>5.2 - 7.8 or 5.7</td>
</tr>
<tr>
<td>(10) KMD-3213</td>
<td>4.6 - 5.2 or 4.0 - 6.4</td>
<td>4.6 - 5.2 or 4.0 - 6.4</td>
</tr>
<tr>
<td>(29) Risperidone</td>
<td>6.3 - 7.0 or 5.6</td>
<td>6.3 - 7.0 or 5.6 or 4.1 - 5.2</td>
</tr>
<tr>
<td>(28) Spiperone</td>
<td>5.8 - 6.1 or 4.9 - 7.8</td>
<td>5.8 - 6.1 or 4.9 - 7.8 or 4.3</td>
</tr>
<tr>
<td>(26) Benoxathian</td>
<td>5.5 - 6.2 or 5.3 - 6.3</td>
<td>5.5 - 6.2 or 5.3 - 6.3</td>
</tr>
<tr>
<td>(25) Corynanthine</td>
<td>5.7</td>
<td>3.9</td>
</tr>
<tr>
<td>(32) YM-617</td>
<td>4.5 - 6.4 or 4.1 - 5.2</td>
<td>4.5 - 6.4 or 4.1 - 5.2</td>
</tr>
<tr>
<td>(33) Indoramin</td>
<td>4.7 - 6.1 or 7.5</td>
<td>4.7 - 5.1</td>
</tr>
<tr>
<td>(34) Abanoquil</td>
<td>6.3 or 2.7</td>
<td>6.3 or 2.7</td>
</tr>
<tr>
<td>(35) RS-17053</td>
<td>4.5 - 6.5 or 5.3 - 6.3</td>
<td>4.5 - 6.5 or 5.3 - 6.3</td>
</tr>
<tr>
<td>(12) AH-1110A</td>
<td>3.7 - 8.8 or 5.0 - 7.9</td>
<td>-</td>
</tr>
<tr>
<td>(19) IQC</td>
<td>3.9 or 5.0 or 6.6</td>
<td>3.9 or 5.0 or 6.6</td>
</tr>
<tr>
<td>(20) Dicentrine</td>
<td>3.9 or 5.3</td>
<td>3.9 or 5.3</td>
</tr>
</tbody>
</table>

Table 2.4. Distances between the basic nitrogen and two ring centres for selected \(\alpha_{1}\)-AR antagonists.\(^{57}\)
### Table 2.5. Test compounds for pharmacophore models.

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>(\alpha_{1A})</th>
<th>pK_i</th>
<th>(\alpha_{1B})</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-YM-12617 (32a)</td>
<td>10.5</td>
<td>9.2</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>(S)-YM-12617 (32b)</td>
<td>8.4</td>
<td>7.0</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Indoramin (33)</td>
<td>8.4</td>
<td>7.4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Abanoquil (34)</td>
<td>10.4</td>
<td>10.1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>RS-17053 (35)</td>
<td>9.3</td>
<td>7.8</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>AH-11110A (12)</td>
<td>5.6</td>
<td>7.1</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

From these results it can be seen that, non-selective antagonists like WB-4101, Indoramin or YM-617 can fit both models by changing their orientation, since the polar group of one subtype and the aromatic group of the other can overlap with each other very well (see **Figure 2.6**). For example, the benzodioxane side of WB-4101 can serve as an aromatic ring for the \(\alpha_{1A}\) model, while the aromatic ring with three oxygens can
serve as a polar group. The same group may be an aromatic centre while the benzodioxane ring may be the polar group for the $\alpha_{1B}$ model. The lower polarity of the benzodioxane ring may then explain the 20-fold less affinity of WB-4101 for the $\alpha_{1B}$ receptor (see Figure 2.6). The selective antagonists AH-11110A and spiperone have a long distance between the nitrogen atom and the aromatic ring centre and therefore fit the $\alpha_{1B}$ model better. The missing polar group on AH-11110A may explain why it has such a low affinity for the $\alpha_{1A}$ receptor. Similarly, in the case of (+)-niguldipine, RS-17053, KMD-3213 and 5-methyluropidil ($\alpha_{1A}$ selective antagonists) the distance between nitrogen and the aromatic ring centre can only fit the $\alpha_{1A}$ model. However, different affinities for the stereoisomers of YM-617 and WB-4101 can not be explained by these models.$^{57}$

### 2.1.1.4. Design of more selective antagonists

According to the pharmacophore models, an ideal $\alpha_{1A}$ selective ligand must possess:

(i) a basic nitrogen atom which is accessible and should be protonated at physiological pH; (ii) an aromatic ring at a distance of 5.2 - 5.8 Å from the nitrogen atom; (iii) a preferably non-aromatic ring with polar substituents at a distance of 6 - 8 Å from the nitrogen atom.

On the other hand, an ideal $\alpha_{1B}$ selective ligand should possess:

(i) a basic nitrogen atom which is accessible and should be protonated at physiological pH; (ii) an aromatic ring at a distance of 6.2 - 7.8 Å from the nitrogen atom;
(iii) a preferably non-aromatic ring with polar substituents at a distance of 5 - 6 Å from the nitrogen atom.

Figure 2.6. Overlap of the $\alpha_{1A}$ and $\alpha_{1B}$ pharmacophores.

To design such ligands, conformationally rigid lead compounds were selected (Table 2.6), such as IQC, a ring D-indole analogue of the aporphine alkaloid system. If a selective ligand can be designed from a rigid molecule, then more detailed data about the binding site can be obtained, for example the angles between the essential functional groups.

The most important feature of our pharmacophores is the distance difference between the basic nitrogen atom and the aromatic ring. IQC fits on both pharmacophore models because the aromatic region over the indole moiety of this compound is very large and the distance between the basic nitrogen atom and the aromatic ring varies from 5.0 to 7.0 Å. To produce selectivity for one subtype over the other, the positioning of an aromatic ring in the region of the indole ring moiety would need to be modulated.
Table 2.6. Rigid compounds used for design purposes.

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>$\alpha_{1A}$</th>
<th>pK$_i$</th>
<th>$\alpha_{1B}$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racemic IQC (19)</td>
<td>8.6 for $\alpha_1$</td>
<td></td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>(S)-(+-)-Dicentrine (20)</td>
<td>8.3 for $\alpha_1$</td>
<td></td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>Corynanthine (25)</td>
<td>6.8</td>
<td>6.3</td>
<td></td>
<td>58</td>
</tr>
</tbody>
</table>

For the $\alpha_{1A}$ subtype, the indole system could be replaced with a five membered ring system to prevent $\alpha_{1B}$ binding. This makes the molecule very compact and it would fit only the $\alpha_{1A}$ binding site with a distance of 5.0 Å between the basic nitrogen atom and the aromatic ring. The heteroaromatic thiadiazole$^{62}$ ring was chosen to replace the indole unit in this way because of its ease of synthesis.

Timolol, with an isomeric 1,2,5-thiadiazole moiety, has shown (Figure 2.7) blocking activity at $\beta$-adrenergic receptors.$^{63}$ The $\beta$-blocking activity of phenethylamine derivatives is retained when an oxymethylene group is interposed between the aromatic ring and the ethanolamine side chain as in prenalterol.$^{64}$
The analogue of IQC, thiadiazole (36), was designed and synthesised for the $\alpha_{1A}$ site with a nitrogen-heteroaromatic ring distance of 5.0 Å (too short for the $\alpha_{1B}$ site) (Figure 2.8).

For the $\alpha_{1B}$ subtype, aromaticity of the five membered ring portion of the indole system would need to be abolished to prevent $\alpha_{1A}$ binding and at the same time the rigidity of the molecule must be preserved to prevent buckling which produces shortening of the nitrogen-aromatic ring E distance. Therefore instead of reducing the ‘2,3 double bond’ of the indole moiety, alkylation of the ‘3’ position was chosen as a synthetic target. This forces the double bond to migrate to the ‘1,2’ position and removes the aromatic component at 5 Å from the isoquinoline nitrogen, while retaining the rigidity of the molecule (Figure 2.8).
Figure 2.8. Design of IQC analogues for possible selective binding for the α_{1A} and α_{1B} receptors.

Subsequent pharmacophore work was then undertaken taking into account the problematical nature of risperidone (29). Even though risperidone was listed as an α_{1B}-selective antagonist in a recent review^{27}, this selectivity had been questioned in an earlier study^{54} which found risperidone only slightly selective for the α_{1A} subtype over α_{1B} (see Table 2.1a). This led us to repeat our pharmacophore development study. Risperidone was taken out of the training set and a number of new compounds (Tables 2.7, 2.8 and 2.9), reported after the Apex-3D results, were also included. These experiments were performed using the programme Catalyst, an MSI product which had subsumed the former Biosym product Apex-3D.
2.1.2. CATALYST PHARMACOPHORES

Catalyst treats molecular structures as templates consisting of strategically positioned chemical functions that will bind to the receptor. Molecular flexibility is taken into account by considering each compound as a collection of conformers representing different areas of conformational space available to the molecule within a given energy range.\textsuperscript{52}

The geometry of compounds is built with the catalyst builder and optimised by using the generalised CHARM-like force field implemented in the program.\textsuperscript{50} Catalyst automatically generates the conformational models for each compound using the poling algorithm.\textsuperscript{51} A visual inspection is necessary to register the conformers in the model.

Catalyst provides two numbers to assess the validity of a hypothesis:

(i) the cost of an ideal hypothesis which is a lower bound on the cost of the simplest possible hypothesis that still fits the data perfectly.

(ii) the cost of the null hypothesis which assumes that there is no statistically significant structure in the data and that the experimental activities are normally distributed about their mean.

According to the assessment criteria a generated hypothesis with a score that is substantially below the cost of the null hypothesis is likely to be statistically significant. The program gives ten of these hypotheses as a result of an experiment by default.
Table 2.7. New antagonists used in the Catalyst derived pharmacophores.
Table 2.8. Niguldipine analogues.

Table 2.9. Prazosin analogues.
A training set of forty antagonists (Tables 2.7, 2.8 and 2.9) was selected and the protonated forms of the antagonists were built to predefine at least one common feature as a positive ion between all molecules. Conformational searching was done using a ten kcal/mol energy threshold with respect to the energy minimum. The catalyst software uses molecular mechanics (force field) calculations and a particular way to handle the conformational space sampling. Unreasonable conformations of the molecules were removed from the collection because these interactions would interfere with the surface accessibility of the functional groups. This in turn may block any possible interaction with the receptor. These conformers were created because of the intra-molecular π-π interactions between the two aromatic rings or hydrogen bonding of the basic nitrogen atom to the other hetero-atoms within the molecules (Figure 2.9). These interactions are very common when the experiments are run in the gas phase.

![Figure 2.9. A folded conformation of KMD3213 (10). The structure is in Table 1.4.](image)

A training sets of molecules, with their associated conformational models was submitted to generate hypotheses for a general α₁ model. The chemical functions used in this
generation step included positive ion, hydrogen bond acceptor and donor, aromatic ring and hydrophobic group. The aromatic ring function allows consideration of directionality of π–π interactions.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound Name</th>
<th>Activity $K_i = \text{nM}$</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Prazosin</td>
<td>$\alpha_{1A}$: 0.2; $\alpha_{1B}$: 0.25; $\alpha_{1D}$: 0.32</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>WB-4101</td>
<td>$\alpha_{1A}$: 0.16; $\alpha_{1B}$: 2.5; $\alpha_{1D}$: 0.25</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>Phentolamine</td>
<td>$\alpha_{1A}$: 1.6; $\alpha_{1B}$: 7.9; $\alpha_{1D}$: 7.9</td>
<td>58</td>
</tr>
<tr>
<td>8</td>
<td>5-Methyluropidil</td>
<td>$\alpha_{1A}$: 0.63; $\alpha_{1B}$: 40; $\alpha_{1D}$: 10</td>
<td>67</td>
</tr>
<tr>
<td>9</td>
<td>SNAP-5089</td>
<td>$\alpha_{1A}$: 0.23; $\alpha_{1B}$: 13; $\alpha_{1D}$: 66</td>
<td>67</td>
</tr>
<tr>
<td>10</td>
<td>KMD-3213</td>
<td>$\alpha_{1A}$: 0.04; $\alpha_{1B}$: 20; $\alpha_{1D}$: 2.0</td>
<td>67</td>
</tr>
<tr>
<td>11</td>
<td>Cycloazosin</td>
<td>$\alpha_{1A}$: 12; $\alpha_{1B}$: 0.13; $\alpha_{1D}$: 3.2</td>
<td>54</td>
</tr>
<tr>
<td>12</td>
<td>AH-111101A</td>
<td>$\alpha_{1A}$: 2500; $\alpha_{1B}$: 76; $\alpha_{1D}$: 2750</td>
<td>60</td>
</tr>
<tr>
<td>13</td>
<td>BMY-7378</td>
<td>$\alpha_{1A}$: 250; $\alpha_{1B}$: 630; $\alpha_{1D}$: 6.3</td>
<td>67</td>
</tr>
<tr>
<td>14</td>
<td>SKF-104856</td>
<td>$\alpha_{1A}$: 44; $\alpha_{1B}$: 63; $\alpha_{1D}$: 5.2</td>
<td>24</td>
</tr>
<tr>
<td>19</td>
<td>IQC</td>
<td>$\alpha_{1A}$: 4; $\alpha_{1B}$: 250; $\alpha_{1D}$: 100</td>
<td>new</td>
</tr>
<tr>
<td>21</td>
<td>Glaucine</td>
<td>$\alpha_{1A}$: 75; $\alpha_{1B}$: 1200; $\alpha_{1D}$: -</td>
<td>44</td>
</tr>
<tr>
<td>22</td>
<td>Predicentrine</td>
<td>$\alpha_{1A}$: 7.4; $\alpha_{1B}$: 400; $\alpha_{1D}$: -</td>
<td>44</td>
</tr>
<tr>
<td>23</td>
<td>Boldine</td>
<td>$\alpha_{1A}$: 4.9; $\alpha_{1B}$: 316; $\alpha_{1D}$: -</td>
<td>44</td>
</tr>
<tr>
<td>24</td>
<td>Discretamine</td>
<td>$\alpha_{1A}$: 616; $\alpha_{1B}$: 360; $\alpha_{1D}$: 25</td>
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</tr>
<tr>
<td>25</td>
<td>Corynanthine</td>
<td>$\alpha_{1A}$: 142; $\alpha_{1B}$: 517; $\alpha_{1D}$: 253</td>
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<tr>
<td>26</td>
<td>Benzoxathian</td>
<td>$\alpha_{1A}$: 0.2; $\alpha_{1B}$: 4.0; $\alpha_{1D}$: 0.4</td>
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</tr>
<tr>
<td>27</td>
<td>(+)-Niguldipine</td>
<td>$\alpha_{1A}$: 0.15; $\alpha_{1B}$: 55; $\alpha_{1D}$: 100</td>
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</tr>
<tr>
<td>28</td>
<td>Spiperone</td>
<td>$\alpha_{1A}$: 7.9; $\alpha_{1B}$: 0.5; $\alpha_{1D}$: 13</td>
<td>25</td>
</tr>
<tr>
<td>32a</td>
<td>(-)-YM-617</td>
<td>$\alpha_{1A}$: 0.035; $\alpha_{1B}$: 0.7; $\alpha_{1D}$: 0.11</td>
<td>58</td>
</tr>
<tr>
<td>32b</td>
<td>(+)-YM-617</td>
<td>$\alpha_{1A}$: 4.3; $\alpha_{1B}$: 96; $\alpha_{1D}$: 22</td>
<td>58</td>
</tr>
<tr>
<td>33</td>
<td>Indoramin</td>
<td>$\alpha_{1A}$: 4.0; $\alpha_{1B}$: 40; $\alpha_{1D}$: 160</td>
<td>67</td>
</tr>
<tr>
<td>34</td>
<td>Abanoquil</td>
<td>$\alpha_{1A}$: 0.04; $\alpha_{1B}$: 0.08; $\alpha_{1D}$: 0.04</td>
<td>7</td>
</tr>
<tr>
<td>35</td>
<td>RS-17053</td>
<td>$\alpha_{1A}$: 0.6; $\alpha_{1B}$: 16; $\alpha_{1D}$: 16</td>
<td>67</td>
</tr>
<tr>
<td>37</td>
<td>A-131701</td>
<td>$\alpha_{1A}$: 0.22; $\alpha_{1B}$: 6.95; $\alpha_{1D}$: 0.97</td>
<td>67</td>
</tr>
<tr>
<td>38</td>
<td>NAN-190</td>
<td>$\alpha_{1A}$: 2.0; $\alpha_{1B}$: 15; $\alpha_{1D}$: 0.8</td>
<td>24</td>
</tr>
<tr>
<td>39</td>
<td>WAY-100635</td>
<td>$\alpha_{1A}$: 144; $\alpha_{1B}$: 186; $\alpha_{1D}$: 63</td>
<td>24</td>
</tr>
<tr>
<td>40</td>
<td>RS-100,975</td>
<td>$\alpha_{1A}$: 1.0; $\alpha_{1B}$: 79; $\alpha_{1D}$: 100</td>
<td>67</td>
</tr>
<tr>
<td>41</td>
<td>REC-15/2739</td>
<td>$\alpha_{1A}$: 1.0; $\alpha_{1B}$: 32; $\alpha_{1D}$: 2.5</td>
<td>67</td>
</tr>
<tr>
<td>42</td>
<td>SNAP-1069</td>
<td>$\alpha_{1A}$: 16; $\alpha_{1B}$: 200; $\alpha_{1D}$: 790</td>
<td>67</td>
</tr>
<tr>
<td>43</td>
<td>SL-89.0591</td>
<td>$\alpha_{1A}$: 2.5; $\alpha_{1B}$: 13; $\alpha_{1D}$: 2.5</td>
<td>67</td>
</tr>
<tr>
<td>44</td>
<td>JHT-601</td>
<td>$\alpha_{1A}$: 0.4; $\alpha_{1B}$: 1.2; $\alpha_{1D}$: 1.2</td>
<td>67</td>
</tr>
<tr>
<td>45</td>
<td>GG-818</td>
<td>$\alpha_{1A}$: 0.2; $\alpha_{1B}$: 16; $\alpha_{1D}$: 25</td>
<td>67</td>
</tr>
<tr>
<td>46</td>
<td>Uropidil</td>
<td>$\alpha_{1A}$: 288; $\alpha_{1B}$: 1320; $\alpha_{1D}$: 1660</td>
<td>24</td>
</tr>
<tr>
<td>47</td>
<td>Bromotopsentin</td>
<td>$\alpha_{1A}$: 12000; $\alpha_{1B}$: 740; $\alpha_{1D}$: -</td>
<td>68</td>
</tr>
<tr>
<td>48</td>
<td>REC-15/2615</td>
<td>$\alpha_{1A}$: 1.9; $\alpha_{1B}$: 0.3; $\alpha_{1D}$: 2.6</td>
<td>70</td>
</tr>
</tbody>
</table>
Table 2.10. \( \alpha_{1A}, \alpha_{1B}, \alpha_{1D} \) antagonists and their activities.

<table>
<thead>
<tr>
<th></th>
<th>Alfuzosin</th>
<th>10</th>
<th>10</th>
<th>3.16</th>
<th>67</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Doxazosin</td>
<td>3.16</td>
<td>1.0</td>
<td>4.0</td>
<td>67</td>
</tr>
<tr>
<td>51</td>
<td>Terazosin</td>
<td>6.3</td>
<td>2.0</td>
<td>2.5</td>
<td>67</td>
</tr>
<tr>
<td>52</td>
<td>Bunazosin</td>
<td>0.5</td>
<td>1.0</td>
<td>1.0</td>
<td>69</td>
</tr>
<tr>
<td>53</td>
<td>REC-15/2627</td>
<td>0.63</td>
<td>0.5</td>
<td>0.5</td>
<td>70</td>
</tr>
<tr>
<td>54</td>
<td>A prazosin deriv.</td>
<td>950</td>
<td>206</td>
<td>1550</td>
<td>67</td>
</tr>
<tr>
<td>55</td>
<td>SNAP-5399</td>
<td>0.65</td>
<td>324</td>
<td>631</td>
<td>67</td>
</tr>
<tr>
<td>56</td>
<td>SNAP-5150</td>
<td>1.9</td>
<td>331</td>
<td>400</td>
<td>67</td>
</tr>
</tbody>
</table>

Activity data were also included in the input of the Catalyst experiment (shown in Table 2.10). The absence of the positive ion in the hypotheses shows that the training set compounds do not share the same binding site at the receptor. Moreover, the problem with the basic nitrogen atom of prazosin-like compounds has already been discussed. In addition, site directed mutagenesis studies have suggested that niguldipine and its analogues bind to a different site of the receptor\(^{12,102}\). These findings led to the investigation of smaller groups of training sets including only selective antagonists for each subtype.

### 2.1.2.1. Hypothesis for the \( \alpha_{1A} \) subtype

Only 5-methyluropidil (8), KMD-3213 (10), RS-17053 (35), RS-1009756 (40), REC-15/2739 (41), GG-818 (45) and uropidil (46) were used for the \( \alpha_{1A} \) training set. The chemical functions used in the generation step were the same as above.
Nine lowest cost hypotheses were obtained. Even though hypothesis 3 had a slightly higher cost than hypothesis 2, this hypothesis was chosen as our best model due to the existence of a positive ion feature. Hypotheses 5, 6 and 7, which also have a positive ion, were discarded because they were very similar to hypothesis 3 with a higher cost. Hypothesis 3 consists of four features including a positive ion in the middle of the molecule, two hydrogen bond acceptor groups on either side of the positive ion and a hydrophobic group. There is no aromatic ring system in this hypothesis but the hydrophobic group can also be an aromatic ring (Figure 2.10) and this hypothesis had a very good correlation between the experimental activities and the estimate values as shown in the Figure 2.11.

The hypothesis for the $\alpha_{1A}$ subtype overestimates ($pK_a$=9-10) compounds with very low activity like (13) and (47). This could be due to the great differences in the structures of these compounds. This hypothesis does, however, estimate activities for the isoquinoline derivatives, including dicentrine derivatives and IQC (19), very well (see Table 2.11a).
Table 2.11a). The correlation between the experimental activity and the estimated activities of selected compounds for the $\alpha_{1A}$ hypothesis are shown in Table 2.11b.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound Name</th>
<th>Activity $K_i$ nM</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Prazosin</td>
<td>0.2</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>WB-4101</td>
<td>0.16</td>
<td>5.7</td>
</tr>
<tr>
<td>8</td>
<td>5-Methyleneurodil</td>
<td>0.63</td>
<td>2.8</td>
</tr>
<tr>
<td>9</td>
<td>SNAP-5089</td>
<td>0.23</td>
<td>9.4</td>
</tr>
<tr>
<td>10</td>
<td>KMD-3213</td>
<td>0.04</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>Cyclazosin</td>
<td>12</td>
<td>-4.3</td>
</tr>
<tr>
<td>12</td>
<td>AH-111101A</td>
<td>2500</td>
<td>-33</td>
</tr>
<tr>
<td>13</td>
<td>BMY-7378</td>
<td>250</td>
<td>-270</td>
</tr>
<tr>
<td>14</td>
<td>SKF-104856</td>
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</tr>
<tr>
<td>19</td>
<td>IQC</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>21</td>
<td>Glaucine</td>
<td>75</td>
<td>-10</td>
</tr>
<tr>
<td>23</td>
<td>Boldine</td>
<td>4.9</td>
<td>6.1</td>
</tr>
<tr>
<td>27</td>
<td>(+)-Niguldipine</td>
<td>0.15</td>
<td>3.8</td>
</tr>
<tr>
<td>28</td>
<td>Spiperone</td>
<td>7.9</td>
<td>-7.6</td>
</tr>
<tr>
<td>32b</td>
<td>(+)-YM-617</td>
<td>4.3</td>
<td>-2</td>
</tr>
<tr>
<td>33</td>
<td>Indoramin</td>
<td>4.0</td>
<td>7</td>
</tr>
<tr>
<td>35</td>
<td>RS-17053</td>
<td>0.6</td>
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</tr>
<tr>
<td>38</td>
<td>NAN-190</td>
<td>2.0</td>
<td>-3.2</td>
</tr>
<tr>
<td>39</td>
<td>WAY-100635</td>
<td>144</td>
<td>-47</td>
</tr>
<tr>
<td>40</td>
<td>RS-100,975</td>
<td>1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>43</td>
<td>SL-89.0591</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>44</td>
<td>JHT-601</td>
<td>0.4</td>
<td>-1.1</td>
</tr>
<tr>
<td>45</td>
<td>GG-818</td>
<td>0.2</td>
<td>16</td>
</tr>
<tr>
<td>46</td>
<td>Uropidil</td>
<td>288</td>
<td>-3.9</td>
</tr>
<tr>
<td>47</td>
<td>Bromotopsentin</td>
<td>12000</td>
<td>-170</td>
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</table>

Table 2.11a. Experimental activity and estimated activities of selected compounds for the $\alpha_{1A}$ hypothesis.
Table 2.11b. Correlation between experimental activity 
\((K_I=M)\) and estimated activities of selected compounds 
for the \(\alpha_{1A}\) hypothesis. The green line represents the ideal 
situation where true activity is equivalent to estimated activity. 
It is simply a guide and the line of best fit is not actually shown, 
only the correlation is given.

2.1.2.2. Hypothesis for the \(\alpha_{1B}\) subtype

Only AH-111101A (12), spiperone (28) and bromotopsentin (47) were used for the \(\alpha_{1B}\) 
training set. These compounds have structural variety and they bind to the \(\alpha_{1B}\) subtype 
with some selectivity. The same chemical functions were used in the generation step.
Nine lowest cost hypotheses were obtained. Even though hypothesis 3 had slightly higher cost than hypothesis 2, this hypothesis was chosen as the best model due to the existence of the positive ion. Hypothesis 3 consisted of three features including a positive ion in the middle of the molecule, a hydrogen bond donor group on one side of the positive ion and a hydrophobic group on the other. There was no aromatic ring system in this hypothesis, but the hydrophobic group can also be an aromatic ring (Figure 2.12) and this hypothesis had very good correlation between the experimental activities and the estimate values as shown in Figure 2.13a.

The hypothesis for the $\alpha_{1B}$ subtype underestimates nonselective compounds with very high binding ($pK_i = 9-10$) including (5), prazosin and its analogues. The problem with prazosin analogues was mentioned earlier in the literature. This hypothesis also estimated activities for the isoquinoline derivatives ((21), (22) and (23)), including dicentrine derivatives, and IQC (19), very well (see Table 2.12a). The correlation
between the experimental binding activity and the estimated activities of selected compounds for the $\alpha_{1B}$ hypothesis are shown in Table 2.12b.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound Name</th>
<th>Activity $K_i$ = nM</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Prazosin</td>
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<tr>
<td>5</td>
<td>WB-4101</td>
<td>2.5</td>
<td>2300</td>
</tr>
<tr>
<td>8</td>
<td>5-Methylurodil</td>
<td>40</td>
<td>2300</td>
</tr>
<tr>
<td>9</td>
<td>SNAP-5089</td>
<td>13</td>
<td>2300</td>
</tr>
<tr>
<td>10</td>
<td>KMD-3213</td>
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<td>230</td>
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<tr>
<td>11</td>
<td>Cyclazosin</td>
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<tr>
<td>23</td>
<td>Boldine</td>
<td>320</td>
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<td>(+)-Niguldipine</td>
<td>55</td>
<td>2300</td>
</tr>
<tr>
<td>28</td>
<td>Spiperone</td>
<td>0.5</td>
<td>59</td>
</tr>
<tr>
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<td>(+)-YM-617</td>
<td>96</td>
<td>70</td>
</tr>
<tr>
<td>33</td>
<td>Indoramin</td>
<td>40</td>
<td>410</td>
</tr>
<tr>
<td>35</td>
<td>RS-17053</td>
<td>16</td>
<td>52</td>
</tr>
<tr>
<td>38</td>
<td>NAN-190</td>
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<td>2300</td>
</tr>
<tr>
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<td>Uropidil</td>
<td>1300</td>
<td>2300</td>
</tr>
<tr>
<td>47</td>
<td>Bromotopsentin</td>
<td>740</td>
<td>1300</td>
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</table>

Table 2.12a. Experimental activity and estimated activities of selected compounds for the $\alpha_{1B}$ hypothesis.
Table 2.12b. Correlation between experimental activity ($K_i$) and estimated activities of selected compounds for the $\alpha_{1B}$ hypothesis. The green line was explained under Table 2.11b.

**Figure 2.11.** Experimental activity ($K_i$=M) and estimate correlations of the training set of compounds for the $\alpha_{1A}$ hypothesis.

**Figure 2.13.** Experimental activity ($K_i$=M) and estimate correlations of the training set of compounds for the $\alpha_{1B}$ hypothesis.

### 2.1.2.3. Comparison of the Catalyst pharmacophores

The pharmacophore models for the subtypes are very similar when they are overlapped especially the positive ions and the hydrophobic functional groups located in the same
position. However, only possible target for the design is the hydrogen bond acceptor functional groups for the $\alpha_{1A}$ subtype and the hydrogen bond donor functional group for the $\alpha_{1B}$ subtype (see Figure 2.14)

![Overlap of the $\alpha_{1A}$ and $\alpha_{1B}$ pharmacophores.](image)

Overall prediction of the test compounds gave some good results where niguldipine analogues have the right ($\alpha_{1A}$) selectivity with a lower affinities whereas pharmacophore models could not predict affinities nor selectivities of prazosin analogues. They have very low affinities with $\alpha_{1A}$ selectivity. It is well known that, cyclazosin (11) has $\alpha_{1B}$ over $\alpha_{1A}$ and the other prazosin analogues used here have no selectivity.

It was also shown that, the $\alpha_{1A}$ subtype gave overestimated affinities for some compounds with low affinities whereas, the $\alpha_{1B}$ subtype gave underestimated affinities for some compounds with high affinities. This could be due to the training sets of the pharmacophore models where the $\alpha_{1A}$ subtype has selective compounds with very high
affinities and there are only a few the $\alpha_{1B}$ selective compounds available with low affinities.

### 2.1.2.4. Designing new targets from the pharmacophore models

In this part of the design process, IQC (19), which was the main lead compound for the earlier design strategy, was discarded as a template for new selective ligands because one part of the molecule (the indole ring) did not map onto the hypotheses (Figure 2.15). Another known isoquinoline derivative, tetrabenazine (17), was used as a structurally advanced template instead, because this compound was available in the laboratory and it had the same structural features considered necessary for $\alpha_1$ binding (Table 2.13).

![Figure 2.15. IQC (19) on the $\alpha_{1A}$ and $\alpha_{1B}$ hypotheses.](image)

The benzoquinolizidine derivative tetrabenazine (17) has clinical use of an antipsychotic and antidyskinetic agent because of its action in depleting neuronal noradrenaline or dopamine stores. It exerts a sedative action and has a beneficial effect on various
dopamine stores. It exerts a sedative action and has a beneficial effect on various symptoms of schizophrenia. It also has dopaminergic activity which indicates that it binds to a dopaminergic receptor which is a G-protein coupled receptor.

Tetrabenazine (17) showed a high affinity for the $\alpha_{1A}$ pharmacophore model whereas, it had a low predicted affinity for the $\alpha_{1B}$ subtype. After mapping tetrabenazine (17) on the $\alpha_{1A}$ and $\alpha_{1B}$ hypotheses (Figure 2.16), a number of possible improved target compounds were created (Table 2.13) and tested on the hypotheses. To improve $\alpha_{1B}$ binding a hydrogen bond donor group was attached at the carbonyl group position. This modification increased affinity on the $\alpha_{1B}$ subtype (57, 60, 61). To lower the $\alpha_{1A}$ affinity, methoxy groups which were mapping onto the hydrophobic group on the pharmacophore were modified (58, 59, 60, 61, 63). In addition, a thiol group (62, 63) was interchanged with the hydroxyl group to lower the hydrogen bond acceptor capability at this site, but still retain a hydrogen bond donor group necessary for the $\alpha_{1B}$ activity. The best result was obtained when the thiol group was attached at the 2 position (see (62) in Table 2.13). However, using tetrabenazine derivatives did not give any selectivity between the subtypes. The reduced tetrabenazine derivative (57) was synthesised to test binding affinity to cloned and prepared human $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$ receptors of this compound and tetrabenazine (17).
Figure 2.16. Tetrabenazine on the $\alpha_{1A}$ and $\alpha_{1B}$ hypotheses.
Table 2.13. Designed tetrabenazine analogues and estimated activities (K, M).
Grey spheres are common features for both subtypes, whereas red spheres are for $\alpha_{1A}$ and blue spheres are for $\alpha_{1B}$ subtypes only. HBA and HBD are indicating H-bond acceptor and donor groups, PI is positive ion and H is hydrophobic group.
A further design route was also followed by developing a smaller compound from IQC (19), which was already known to be an α1A antagonist with relatively high binding to the subtypes. When we compared the Catalyst pharmacophore models, hydrophobic sites and the positive ion sites of both subtypes can be superimposed with an overlapping hydrogen bond donor functional group for the α1B subtype and a hydrogen bond acceptor functional group for the α1A subtype. This suggested that a group with only hydrogen bond donor functionality, but not acceptor functionality, could be useful to design a selective α1B antagonist or vice versa. In order to do this a number of possibilities were considered (Tables 2.14 and 2.15) including:

i) An amine group which could be protonated at physiological pH, thus retaining hydrogen bond donor but not acceptor capacity. (70, 72, 73, 77 and 78)

ii) A hydroxyl group adjacent to the amine group which is hydrogen bonded to the hydroxyl group as an acceptor, hence the amine would not be able to serve as a hydrogen bond acceptor to the receptor. (79)

iii) Incorporation of the nitrogen in a secondary amine or lactam moiety, with electron delocalisation which reduces nitrogen lone pair electron availability for hydrogen bond acceptance. (75 and 76)
The compounds in Tables 2.14 and 2.15 were designed considering the availability of intermediates and the ease of synthesis. Target ligands shown in Table 2.15 have very high estimated affinities for the $\alpha_{1A}$ pharmacophore. Because of the very high affinity of the training set compounds ($10^{-9} - 10^{-10}$ M), only two fitting features (the positive ion and hydrophobic group) gave $10^{-8}$ M affinity for this subtype. To locate a poor compound for the $\alpha_{1A}$ subtype two isoquinoline derivatives were modelled. As can be seen in Table 2.16, the compound with methoxy groups (65) still gave at least 100nM binding affinity whereas without methoxy groups (64) the affinity was lower.
Table 2.15. Estimated activities (KJ, M) of some fused hexahydroisoquinoline derivatives at the α1B subtype. Estimated activities of these target compounds at the α1A subtype are all the same KJ=7.4x10^8 M.

Target ligands in Table 2.14 were derived from an intermediate structure (66) in the design of aporphine analogues. The best result was achieved with the compound (67) which could be synthesised by reducing (66). This compound did not show selectivity
but it did indicate very high estimated affinity for the $\alpha_{1B}$ pharmacophore which would be useful for further design.

Table 2.16. Estimated activities ($K_i$, M) for the tetrahydroisoquinoline derivatives.

<table>
<thead>
<tr>
<th></th>
<th>$\alpha_{1A}$</th>
<th>$K_i$</th>
<th>$\alpha_{1B}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(64)</td>
<td>$1.4 \times 10^{-5}$</td>
<td></td>
<td>$2.3 \times 10^{-4}$</td>
</tr>
<tr>
<td>(65)</td>
<td>$7.4 \times 10^{-8}$</td>
<td></td>
<td>$2.3 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

Finally it was decided to prepare target compounds (17), (57), (66), (67) for the binding assays and to test the pharmacophore design concepts.
CHAPTER 3
SYNTHESIS OF RECEPTOR LIGANDS

3.1. SYNTHESIS OF RING-D APORPHINE ANALOGUES

The aporphines are part of the isoquinoline group of alkaloids and consist of four six-membered rings labelled A-D and this is represented by the dicentrine (20) structure in Figure 3.1. The nitrogen of ring-B is usually tertiary and often with a methyl substituent. The positions 1 and 2 are always substituted with oxygen containing functionalities such as hydroxy, methoxy or methylenedioxy groups. Many naturally occurring aporphines and their synthetic derivatives have biological activities mediated by the central nervous system. These include sedative, narcotic, analgesic, hypotensive and dopaminergic activities. The most extensively studied aporphine analogue is apomorphine (83), a morphine derivative, which is a partial agonist at dopamine D2 receptors.

Fused D-ring analogue studies first began with Schneider and co-workers (1973), in the development of ligands with hypotensive activity. Following this, Bremner and Browne (1975) synthesised a series of aporphine analogues with the D-ring replaced with an indole moiety (84). These compounds exhibited significant α1-adrenergic activity. In recent years, many other heteroatomic ring-D aporphine analogues have been synthesised with rings such as pyrrole, pyrazole, isoxazole, thiazole and 1,2,3-thiadiazole (Berney 1982), but their biological activities here not reported.
Thiadiazole (36) and MET-IQC (37) were discussed in Chapter 2 as potential ligand targets for synthesis. They are ring-D aporphine analogues in which the D ring is replaced with 1,2,3-thiadiazole and 3-methyl substituted 3H-indole rings respectively. The synthesis of these compounds was performed via the intermediate tricyclic ketone (66) (a reduced 5,6-dimethoxybenzo[de]quinolin-7-one) which incorporates three rings of the aporphine structure. The carbonyl group in the 7 position was then used to build the D ring.

3.1.1. SYNTHESIS OF 1-PROPYL-5,6-DIMETHOXY-2,3,8,9-TETRAHYDROBENZO[de]QUINOLIN-7-ONE (66)

The multi-step sequence for synthesis of the ketone (66) involved an isoquinoline precursor. Two different routes were examined in an earlier study (Groenewoud 1991) although variations on these are feasible. The first route (Scheme 3.1), via the ester (86), involved Bischler-Napieralski cyclisation of a succinic anhydride derivative, but did not result in as good a yield of (66) as the second route (Scheme 3.2) involving
chain extension of the 1-methyl substituted derivative (87) via the cyclisation of the enamine (89). Therefore the second route of Groenewoud\textsuperscript{42} was chosen to synthesise the ketone in this study, with some further investigation of reaction conditions.

[Diagram]

Scheme 3.1. Synthesis of the ketone (66) via succinic anhydride cyclisation.

In the second route the 1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline (87) was prepared by the method of Brossi \textit{et al.} (1977) using phosphoryl chloride as the condensing agent.\textsuperscript{77} The \textit{N}-propyl derivative (88) was prepared in a sealed tube at 90°C with a 90% yield. The bromide salt of the ester derivative (90) should be prepared (75% yield) using fresh enamine derivative (89) after the basification (89%) of the iodide salt (88), because of its lack of stability. The reduction of (90) and the preparation of the amino acid hydrochloride salt derivative (92) was achieved in over 90% yield. Cyclisation of the amino acid hydrochloride salt derivative (92) was investigated by using triflic anhydride, phosphorus pentoxide or finally, oleum (20% free SO\textsubscript{3}) as the cyclising agents. Yields of the first two reactions (10% and 20% respectively) were not as good as that from the oleum cyclisation (45%). However, careful temperature control was essential to maximise the yield of (66) and to minimise further demethylation of the 6-methoxy group to the carbonyl group, as noted by Groenewoud.\textsuperscript{42}
3.1.1.1. Preparation of the isoquinoline precursor (87)

The acetylation of homoveratrylamine (85) and subsequent Bischler-Napieralski cyclisation to the isoquinoline was undertaken according to the method of Brossi et al. (1977). The acetylation was achieved in 80% yield. However, only 76% yield was obtained after recrystallisation of the product. The structure of the acetylated homoveratrylamine was confirmed by the $^1$H n.m.r. and mass spectra. The lack of an aromatic proton (doublet) at 6.75 ppm in the $^1$H n.m.r. spectrum of the dihydroisoquinoline (87) indicated that cyclisation had occurred. Cyclisation with phosphorus oxychloride in this step gave (87) in 76% yield.

3.1.1.2. N-alkylation of the isoquinoline (87)

Propylation of (87) was achieved by heating a mixture of the dihydroisoquinoline (87) and an excess of 1-iodopropane in a sealed tube at 90°C. The yield (84%) was
maximised by keeping the temperature between 86-90°C. The peaks at 1.950 and 1.092 ppm in the \(^1\)H n.m.r. spectrum of (88) showed that the alkylation occurred.

**3.1.1.3. Preparation of the ester (91) via enamine formation**

The enamine derivative (89) was produced in high yield (89%) by basification of the propyl iodide salt (88) with concentrated sodium hydroxide. Because of its reported low stability, this compound was immediately reacted with ethyl bromoacetate under a nitrogen atmosphere to form the ester bromide salt (90). Pure and very fresh starting material led to a very good yield in this reaction (83%). The ester carbonyl peak appeared at 176ppm in the \(^13\)C n.m.r. spectrum as reported. Sodium borohydride reduction of the ester bromide salt (90) resulted in formation of the ester (91) as a colourless syrup. The \(^1\)H n.m.r. spectrum and mass spectrum confirmed reduction had proceeded as expected.

**3.1.1.4. Formation of the amino acid hydrochloride salt (92) and oleum cyclisation**

Hydrolysis of the ester (91) was achieved by stirring in 2 M hydrochloric acid solution for three days, and the very hygroscopic amino acid salt (92) was isolated as a white powder in 90% yield.

The ketone was then prepared by an oleum Friedel-Crafts cyclisation of the salt (92). This reaction was dependent on the temperature. Even though the temperature was kept constant at 35°C, the product formed in a low yield (40%, lower than the reported\(^42\) yield of 70%). This may have been due to hot spots in the reaction flask and larger increments of (92) being added to the oleum. The yield was maximised to 75% by
lowering the temperature to 25°C while the hydrochloride salt (92) was being added and no further temperature control was needed while the reaction proceeded for five minutes.

One major product was apparent by thin layer chromatography and medium pressure flash column chromatography was used to purify the ketone (66) from other products. The $^1$H n.m.r. and mass spectra supported the ketone formation while there was only one aromatic proton apparent at 6.843 ppm in the $^1$H n.m.r. spectra.

The yield loss of this reaction is probably mainly due to sulfonation side reactions. To prevent these reactions, Friedel-Crafts cyclisation was attempted with phosphorus pentoxide. This reaction resulted in a low yield (20%).

Cyclisation of the acid (92) was also attempted using a solution of trifluoromethanesulfonic anhydride in dichloromethane with N,N-dimethylaminopyridine (DMAP) as a catalyst. However, the yield of the ketone (66) from this reaction was very low (16%) and starting material was partly recovered. Thus, oleum cyclisation was used to form the ketone (66) in this project.

3.1.2. SYNTHESIS OF THE THIADIAZOLE (36)

Synthesis of 1,2,3-thiadiazoles has been reviewed recently. The most convenient method is the one involving the reaction of thionyl chloride with $\alpha$-methylene hydrazones, which can be obtained in turn from the corresponding ketones (Scheme 3.3).
The synthesis of the 1,2,3-thiadiazolo[4,5-g]quinoline (93) from the corresponding semicarbazone hydrochloride has been described by Berney et al., (1982).\(^79\)

The thiaodiazole (36) was prepared from the amino ketone (66) (Scheme 3.4). Conversion of the amino ketone to the semicarbazone (94) was achieved in good yield. However, reaction of (94) with thionyl chloride gave the required thiaodiazole (36) in only 14% yield. The yield is very low in comparison to the derivative (93) which was synthesised previously.\(^79\) The lower yield may be due to side reactions involving the methoxy groups. In the \(^1\)H n.m.r. spectrum of the thiaodiazole (Figure 3.2), one proton singlet appeared at \(\delta 6.73\) for the only aromatic hydrogen, and a one proton doublet at \(\delta\)
3.8 was assigned to the hydrogen atom at the stereogenic carbon. The mass spectrum of thiadiazole (36) also confirmed the molecular weight for this compound.

Figure 3.2. $^1$H n.m.r. data for (36).

3.1.3. SYNTHESIS OF IQC (19)

The amino ketone derivative is a convenient starting material for heteroaromatic ring-D aporphine analogues. Preparation of the indole ring-D aporphine skeleton was established by Bremner et al. (Scheme 3.5) via Fischer indolisation from the ketone derivatives.\textsuperscript{75,80} In this procedure $p$-methoxyphenylhydrazine (95) reacted with the amino ketone (66). This was achieved with the use of glacial acetic acid as a catalyst resulting in the immediate precipitation of the aryl hydrazone derivative (96). The
hydrazone (96) was then cyclised to form the indole (19) using boron trifluoride etherate as the acid catalyst (64% yield).

The $^1$H n.m.r. spectroscopy of (19) provided strong evidence for the indolic NH, with a peak at 9.25 ppm integrating as one proton. The $^{13}$C n.m.r. spectrum also correlated with the structure. The molecular formula was confirmed via mass spectrometry, the spectrum is exhibiting a very strong molecular ion peak at m/z 392.

3.1.4. METHYLATION OF THE INDOLE (19)

As discussed in Chapter 2, a further synthetic target was the methylated indole derivative (37). In non-fused indoles, it has been reported that C-3 alkylation can be achieved by reaction of the corresponding 2,3-dialkylindole magnesium salt with alkyl halides.
Alternatively the N-lithiated analogues may be used (Scheme 3.6a). This is a simple and short procedure for the direct formation of 3-alkyl-2,3-dimethyl-indolines from the lithium salt of 2,3-dimethylindole (Scheme 3.6b). This reaction can be carried out by addition of n-butyllithium in tetrahydrofuran to a solution of 2,3-dimethylindole in tetrahydrofuran (THF) at -78°C. The electrophile is then added and the solution allowed to warm to room temperature. The results of a number of methylation experiments of non-fused indoles, with yields varying between 36-98%, have been published. It was thus decided to use this procedure with IQC (19) to try and prepare the target (37) (Scheme 3.6c). However, the reaction did not produce the expected compound (78) probably due to the steric hindrance from the bulky fused ring system. The indole (19) was essentially recovered unreacted from this procedure.

![Scheme 3.6. The C-3 alkylation of indole derivatives](image-url)
3.2. SYNTHESIS OF THE TETRABENAZINE DERIVATIVES

Tetrabenazine (17) was first synthesised by Brossi et al. in 1958 by Bischler-Napieralski cyclisation followed by constructing of ring C (Figure 3.8). The reaction of homoveratrylamine (85) with the half-acid chloride from methyl malonate gives the expected amide (97). This compound is then subjected to the cyclodehydration reaction and the product is reduced to the tetrahydroisoquinoline (98) by catalytic hydrogenation. Treatment of (98) with dimethyl isobutyl malonate and formaldehyde leads to a Mannich reaction that results in formation of the alkylation product (99). Hydrolysis of the ester followed by decarboxylation of the diacid and then reesterification gives the diester (100). Base-catalysed Dieckman cyclisation leads to formation of the carbomethoxycyclohexanone ring. The remaining carbomethoxy group in the product (101) is then removed by repeating the hydrolysis and carboxylation sequence. Thus, tetrabenazine (17) is finally obtained. In this current study, tetrabenazine was obtained pre-made from the former Hoffmann La-Roche Pty. Ltd., Dee Why, NSW.
Scheme 3.7. Synthesis of tetrabenazine (17) by Brossi et al.\textsuperscript{82}

Figure 3.3. X-ray structure of tetrabenazine (17).
As it was important to have unequivocal stereochemical information on tetrabenazine for the structure-activity studies, an X-ray structural analysis was undertaken by Assoc. Prof. A. H. White, University of Western Australia. The relative configuration of (17) obtained by X-ray crystallography is shown in Figure 3.3. There are two very similar configurations preferred on the equatorial R group. A number of derivatives of tetrabenazine have been in the literature. These include compounds with different 3-alkyl substituents, derivatives of (57) with alkyl substituents at the 2-position and benzoisoquinoline derivatives with amino, amido, and ester functional groups at the 2-position. The reduction of (17) was prepared by using borohydride and isomers were purified by high performance liquid chromatography which resulted (see Scheme 3.8) in separation of the more abundant isomer (17a).

### 3.2.1. REDUCTION OF TETRABENAZINE (17)

In this work, sodium borohydride reduction of (17) gave a very high yield of (2S*,3R*,11bR*)-2-hydroxy-3-isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizine (57a). However, a new stereogenic site was generated by this reaction at the 2-position and this was clearly seen in thin layer chromatography as two very close spots for the diastereomers. The separation of the diastereomers was not possible by column chromatography. Thus, the stereoselective reduction of this ketone (17) (and (66) see section 3.3) was performed by using L-selectride (Lithium tri-sec-butylborohydride, see Scheme 3.8).
Stereoelectronic control in the reduction of cyclic ketones by lithium aluminium hydride favors axial attack on the rigid molecule, whereas L-selectride, being very bulky, delivers the hydride by the least-hindered face of the molecule and gives selectively the axial alcohol with over 90% yield (Scheme 3.10a & b). Other borohydrides which can be used for this type of reaction and their stereo-selectivities are given below (Scheme 3.9).\(^8\)

### Scheme 3.9. Stereoisomer ratios of some reducing agents for the reduction of a cycloketone derivative.

<table>
<thead>
<tr>
<th>Reducing Agent</th>
<th>%1</th>
<th>%2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAH/THF</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>NaBH₄/MeOH</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>NBH₄/CeCl₃/MeOH</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>LiBH₄/THF</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>LTBA/THF</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>Li(sec-Bu)₂BH/THF</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>K(sec-Bu)₃BH/THF</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>9-BBN/THF</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Thexyl BHC!·Me₂S</td>
<td>44</td>
<td>56</td>
</tr>
</tbody>
</table>

The n.m.r. data in a deuterated chloroform solution is for (57) shown in Figure 3.5. The broad singlet at 4.045 ppm integrating as one hydrogen provides strong evidence for the reduction. The alcohol peak is also present at 1.660 ppm. The relative configuration of (57) in was confirmed by X-ray crystallographic analysis (Figure 3.4).
Scheme 3.10 Reduction of ketones using L-selectride.

Figure 3.4. X-ray structure of (57).
3.2.2. SYNTHESIS OF \((2S^*, 3R^*, 11bR^*)\)-2-HYDROXY-3-ISOBUTYL-1,3,4,6,7,12b-HEXAHYDRO-2\(H\)-[1,3]BENZODIOXOLO[5,6-\(a\)]QUINOLIZINE (60)

The methylenedioxy group is of interest in chemistry because it occurs in many natural products and has been suggested as a protecting group for catechols. However, attempts to carry out the direct methylation of catechol have often afforded low yields of products. Synthesis of methylenedioxy derivative (58) of tetrabenazine (17) was attempted as set out in Scheme 3.11. First, boron tribromide has been widely used to cleave ethers especially demethylation of methyl aryl ethers. Various methylation methods have been used for the protection of catechol groups. This was first done by
Fitting and Remsen in 1873 by heating 3,4-dihydrobenzoic acid with methylene iodide and potassium hydroxide. Since then, a number of procedures have been developed to methylenate catechols. One such method involves reaction of a catechol with a dihalomethane in dimethylformamide in the presence of an excess of potassium or caesium fluoride resulting in a very high yield of the corresponding methylenedioxy compound in a short time. A convenient method using a phase transfer catalysis (Adogen 464) was also reported.

![Chemical structures](image)

Scheme 3.11. Synthesis of (25*,3R*,11bR*)-2-hydroxy-3-isobutyl-1,3,4,6,7,12b-hexahydro-2H-[1,3]benzodioxolo[5,6-a]quinolizine (60)

In the last step, the methylenedioxy compound (58) was planned to be reduced by using L-selectride to afford (60). This reaction was explained in section 3.2.1.

### 3.2.2.1. Demethylation of tetrabenazine (17)

Boron tribromide was used to remove the ether groups by treating (17) with excess boron tribromide at -78°C. This resulted in formation of the very polar catechol (59) with a 57% yield. An ES mass spectrum of (59) gave an MH⁺ peak at m/z 290 consistent with the formula C₁₇H₂₄NO₃. In the ¹H n.m.r. spectrum (Figure 3.6), the
absence of the singlets at 3.8 ppm integrating for six protons (2x-OMe) confirmed the
product (59). The $^{13}$C n.m.r. spectrum also confirmed the absence of two methoxy
carbons and the existence of three methine, five methylene and two methyl carbons.

![Chemical structure diagram](image)

Figure 3.6. $^1$H n.m.r. data for (59).

### 3.2.2.2. Methylene dioxy derivative of (17)

The dihydroxy derivative (59) was treated with sodium hydroxide solution to create the
dianion to react with dibromomethane in water in the presence of, Adogen 464, a phase
transfer catalyst. This resulted in only 18% yield of the expected product (58). The ES
mass spectrum of (58) gave an MH$^+$ ion at m/z 302 consistent with the formula. The $^1$H
n.m.r. spectrum also showed a singlet at $\delta$ 5.9 which integrated for two protons and
assigned to the methylenedioxy protons (Figure 3.7)
3.2.2.3. Future syntheses

In order to prepare compound (60) it will be necessary to access (58) in higher yields. This could possibly be done by reducing (59) first to the alcohol, followed by the methylation reaction. This would obviate one potential synthetic problem, namely enolate anion formation from (59) in the presence of strong base which could result in alternative reaction path options.
3.3. FORMATION OF THE ALCOHOL (67)

The ketone (66) was treated with L-selectride in THF at -78°C. This resulted, after workup and recrystallisation, in the preparation of (67) as colourless crystals in 53% yield. The relative configuration of \((7S^*,9aR^*)\)-1-propyl-5,6-dimethoxy-7-hydroxy-2,3,7,8,9,9a-hexahydro-1H-benzo[de]quinoline (67) was confirmed by X-ray crystallography (Figure 3.8). In the \(^1\)H n.m.r. spectrum, the singlet at 5.0 ppm integrating for one hydrogen (H-7) and the singlet at 1.7 ppm integrating for one hydrogen (OH) strongly suggested that (67) was formed (Figure 3.9).

![Figure 3.8. X-ray structure of (67).](image)
Figure 3.9. $^1$H n.m.r. data for (67).
CHAPTER 4

RECEPTOR MODELLING AND DOCKING STUDIES

4.1. RECEPTOR MODELLING

The most common drug action targets are proteins such as enzymes, ion channels, antibodies and membrane bound receptors. Tissue cells receive information from their environment by integral membrane receptors. The information is transmitted to an effector located inside the cell membrane. These effectors may include enzymatically active sites, ion channels or binding sites for intra-cellular proteins. A common mechanism for receptor signal transmission is through binding of the receptor to a G-protein which in turn activates other enzymes. Drugs can act either as an agonist which mimics the effect of an endogenous ligand or as an antagonist which blocks the signal.

Modern drug discovery has been focused on the three dimensional structural information of the macro-molecular target. The atomic coordinates of the site of drug action are derived from X-ray crystallographic or nuclear magnetic resonance structural determinations. Electron diffraction may provide useful lower resolution information especially for membrane bound macromolecules for which the required crystallisation for X-ray diffraction is difficult.\textsuperscript{30}

In the absence of the three dimensional experimental structure, models of the macro-molecular target can be used. For example, if the primary structure of a protein of interest is known and it shares a certain degree of sequence homology with one or more
proteins for which detailed structural information is available, it is possible to construct a homology model of the target protein.

The first step in homology modelling is to determine the registration or alignment of the target sequence onto the experimental structure allowing for insertions and deletions. Following this, mutations of the amino acids of the experimental structure to match those of the target protein need to be done. The final step is the examination of the structure to make sure it is reasonable.

In the G-protein coupled receptor field, molecular cloning has revealed substantial structural similarity among the receptor proteins, despite the great diversity of their ligands. They all contain seven hydrophobic regions where most of the sequence homology is located. The N-terminus of the receptors is located extracellularly and the C-terminus intracellularly. Adjacent helices are antiparallel to each other and the third intracellular loop has been shown to be responsible for coupling to the G-proteins. In the absence of a high resolution crystal structure of any member of the receptor family, models of the G-protein coupled receptors as membrane spanning helical bundles have been built on the basis of a low resolution X-ray structure of bacteriorhodopsin and an electron diffraction structure of bovine rhodopsin. Bacteriorhodopsin is a membrane protein that functions as a light driven proton pump in Halobacterium halobium. Bacteriorhodopsin is not a member of the GPCR family. However, on the basis of experimental evidence it has a 3D folding identical to the GPCR family and has a functional resemblance to mammalian opsins such as rhodopsin. They both are involved in photo isomerisation of a covalently linked retinal chromophore for proton pumping. Bovine rhodopsin is a GPCR and its comparison with bacteriorhodopsin is
very important to assess the 3D structure of the GPCRs. Although the projection map of bovine rhodopsin indicates the presence of seven transmembrane α-helices, their arrangement appears to be different from those in bacteriorhodopsin. The structural information derived from the analysis of ~200 sequences of the GPCRs was used in conjunction with the projection map to derive a model for the 3D arrangements of the seven TM helices. Recently, a model for the α-carbon positions in the TM helices in the rhodopsin family of GPCRs was published. This model incorporates structural information derived from the analysis of ~500 sequences in this family.

Three dimensional models of the GPCRs can be prepared with the following method. Firstly the amino acids involved in the TM helices are identified on the basis of hydrophobicity and primary sequence homology analyses of the members of the GPCR family. Then, helices are built as ideal α-helices and they are packed and positioned as in the bacteriorhodopsin or bovine rhodopsin experimental structure. The rotational orientations of the helices are fine-tuned according to the positions of the polar residues, conserved residues and amino acids known to be involved in ligand binding. They all should point towards inside towards the core of the receptor. To identify specific molecular interactions between ligands and receptors, site directed mutagenesis (substitution of the amino acid side chains) of the receptor has been combined with modification of the ligand. The information from such experiments can then be applied to the receptor model to dock the ligand into the binding pocket and the orientation of the receptor helices adjusted to reflect the experimental data. The refined model can suggest other ligand-receptor interactions. This in turn can serve as a target for medicinal chemistry in an iterative process to optimise binding interactions.
4.2. STRUCTURE BASED QSAR AND BINDING ENERGY

Recently, a QSAR study investigated the antagonist selectivity towards three subtypes of the α1 adrenergic receptor using docking techniques on the computer models of these subtypes. Molecular dynamics simulations were used to analyse the ligand-receptor interactions and binding energies of ligands were calculated and compared with the experimental binding affinities. The results showed that the TM domains of these subtypes have different dynamic behaviours and different topographies of the binding sites. In particular, the α1A-AR binding site is more flexible and topographically different from the other two subtypes. Non-selective antagonists gave similar binding energies for different subtypes, whereas antagonists showing selectivity towards one subtype gave higher stabilisation for this subtype over the other two subtypes. This shows that binding as well as selectivity is guided by the dynamic complementarity between ligand and receptor. Higher flexibility of the α1A subtype over the other two subtypes was also suggested in this study because docking the antagonists into the α1A subtype model was much easier than for the other two subtypes. However, no details on interacting residues of the receptor nor binding sites of the antagonists were reported in this study.

Interaction and binding energies are important terms that measure the strength of the interaction between the receptor and ligand. The interaction energy (IE) which is calculated by the software automatically between the ligand and receptor is given by:

\[ IE = E_{RL} - (E_R + E_L) \]
where \( E_{RL} \) is the energy of the ligand-receptor complex, \( E_R \) is the energy of the isolated receptor and \( E_L \) is the energy of the isolated ligand. These values are normally calculated with molecular mechanics force fields for molecules in vacuo but there are some examples of \textit{ab initio} calculations as well.\textsuperscript{92} A number of minimisation procedures should be used to optimise the structure of the complex. The simulation of the complex allows large movements of the side chains of the receptor and the ligand atoms.\textsuperscript{93} The binding energy (BE) is calculated manually to get a better quantitative measurement for comparison with the biological activity using the following formula:

\[
BE = IE + ED_R + ED_L
\]

where \( IE \) is the ligand-receptor interaction energy and \( ED_R \) and \( ED_L \) are the distortion energies of the receptor and of the ligand respectively.\textsuperscript{24}

\textbf{4.3. RECEPTOR-LIGAND INTERACTIONS}

Before analysing the ligand-receptor docking experiments in the study described in this thesis, the nature of the interactions should be explained in depth by considering first what forces are involved in the ligand-receptor interaction. The interaction of a ligand with a receptor can be explained with a number of forces

- electrostatic interactions
- hydrogen bonds
- hydrophobic effects
- van der Waals interactions

The electrostatic interactions are the strongest and long range interactions between the ligand and the receptor. A familiar example for this type of interaction is that the amine function of the adrenaline molecule, which has a \( pK_a > 7 \) so that the ligand will be partly
or fully positively charged at neutral pH, interacts with the conserved aspartate residue in the $\alpha_1$ adrenoceptor, which is negatively charged.

Hydrogen bonding is the most common interaction occurring in the receptor ligand interactions. A hydrogen bond occurs when two electronegative atoms compete for the same hydrogen atom. The hydrogen atom is bonded covalently to one of the atoms, the donor, but also interacts with the other, the acceptor. The strengths of the hydrogen bonds are usually between 2 and 10 kcal/mol. The reason for the wide range is the variety of hydrogen bonds. The chemical groups that most commonly serve as hydrogen bond donors are N-H, O-H and sometimes S-H and C-H groups. The most common acceptor groups are $\text{O=}$, $\text{-O-}$, $\text{-N=}$ and sometimes $\text{-S-}$ and the $\pi$ electrons of aromatic groups. Charge assisted hydrogen bonds are much stronger than neutral hydrogen bonds. The serine residues in the TM5 of ARs interacting with the catechole ring can be given as an example for H-bonding interactions.

Hydrophobic interaction is another important factor which occurs between non-polar groups which cannot participate in hydrogen bonding interactions. These are additional factors related to molecular shape rather than distribution of electrons. Hydrophobic interactions occur usually between carbon chains. Hydrophobic groups disturb the structure of water as a solvent and this will lead to entropically unfavourable ordering of water molecules around the hydrophobic side groups. These unfavourable forces are reduced by the existence of other hydrophobic groups nearby so these hydrophobic groups are pushed together for the reduction of unfavourable forces.
The van der Waals interaction is energetically and structurally very important and takes the form of repulsion when atoms approach each other.\textsuperscript{95} As they come near enough for their electron orbitals to begin overlap, the repulsion increases. The optimal distance for the interaction of two atoms is usually 0.3 - 0.5 Å greater than the sum of their van der Waals radii.\textsuperscript{95}

The non-covalent interactions involved in the formation of molecular complexes, that are of major importance as contributors to the binding energy, are electrostatic interactions including hydrogen bonds, van der Waals attraction and hydrophobic interactions. All three of these interactions are involved in complex formation in biological systems. There are also other attractive forces such as aromatic stacking interactions. It is well known that there are arrangements of aromatic rings which involve either offset-face-to-face or edge-to-face interactions (\textbf{Figure 4.1}) which are favourable energetically. A large number of aromatic ring interactions in the X-ray structures of proteins was analysed and it was found that the edge-to-face orientation predominates. The stabilisation of the perpendicular stacking is thought to derive from the electrostatic interaction between partially positively charged hydrogens on one ring with the partially negatively charged centre of the second.\textsuperscript{96} Phenyl ring centroids are separated by a preferential distance of between 4.5 and 7.0 Å and dihedral angles approaching 90° are the most common (50°-90°). The free energies of the formation of the interaction are between -0.6 and -1.3 Kcal/mol. This is relatively small compared to other types of interactions, but if the number of the aromatic rings are considered in protein structures, this type of interaction is very important.\textsuperscript{96}
In addition to the $\pi-\pi$ interactions, aromatic rings are involved in CH–$\pi$ interactions. These are weak attractive forces between CH groups and $\pi$-systems. Recently, involvement of CH–$\pi$ interactions in the crystal structures of proteins was investigated. According to theory and experimental evidence, the study suggests that the CH–$\pi$ interaction is originated by a charge transfer process from the $\pi$-system to the antibonding orbital of the C-H bond ($\pi \rightarrow \sigma^*$). Apart from its role in molecular association, the CH–$\pi$ interaction can also play an important role in stabilisation for the specific binding of interacting molecules.

The principal forces driving drug binding are the hydrophobic effect and electrostatic interactions (hydrogen bonds and salt bridges). The hydrophobic component (non-directional) contributes primarily to affinity whereas the hydrogen bond, because of its directional nature, contributes principally to specificity.

4.4. BINDING POCKET OF $\alpha_1$-ADRENERGIC RECEPTORS AND OTHER CONSERVED RESIDUES

The adrenergic receptors, like other G-protein coupled biogenic amine receptors have a conserved aspartic acid residue in the third TM helix, which is essential for activity and
is assumed to bind a protonated amine counter ion present in the ligands of these receptors. For example, the direct binding of adrenaline to D8 in TM3 of β2-ARs was identified by site directed mutagenesis. The catechol ring of adrenaline interacts with serine residues at the top of TM5 of the same receptor and also performs a π-π interaction with phenylalanine in TM7. The α1-ARs share a similar structure but it is clear that their ligand binding sites may differ in each subtype and that the binding sites of different classes of ligands may involve different helices. Therefore, to get a better understanding of ligand interactions, it is important to focus on each subtype individually to investigate the binding modes of a variety of structurally diverse ligands.

The binding site for α1-AR subtypes is expected to be in the same location as in β2-ARs. However, contradictory site directed mutagenesis studies have been published. Mutation of D125 to A by one group suggested that this substitution totally impaired the ability of the α1B-AR to bind both agonists and antagonists whereas, the same mutation showed no significant difference in ligand binding for antagonists and agonist binding decreased only about 3 fold according to another study.

The serine residues of TM5 are another target for the ligands, especially for agonists. Another controversy exists in mutation studies for two serine residues in this helix for the α1A subtype. One group found that S192 (but not S188) is important for both agonists and antagonists except niguldipine (27) and analogues. Another group showed that only the combined mutation of both serine residues decreases adrenaline binding which indicates that both serines (S188 and S192) interact with the catechol ring of adrenaline. The α1B and α1D subtypes have one extra serine residue (Figure
4.2), which for the $\alpha_{1B}$ subtype was demonstrated as having no effect on agonist binding affinity and functional responsiveness.\textsuperscript{10} The first serine residue (S207) was also studied for the $\alpha_{1B}$ and established as the one interacting with the catechol ring of adrenaline.\textsuperscript{11}

The aromatic residues involved in a $\pi$-$\pi$ interaction with the catechol ring of adrenaline differ between the subtypes. For the $\alpha_{1A}$ subtype F193 in TM5 (F310 in TM6 for the $\alpha_{1B}$ subtype)\textsuperscript{101} was found to be important for binding due to a decrease in the adrenaline binding to the F193L mutant of this subtype.\textsuperscript{12} Visualisation of this interaction using a model based on the structure of bacteriorhodopsin failed because F193 was pointing outside the binding pocket. The model was modified by rotating TM5 20\textdegree clockwise to bring the phenylalanine residue inside the binding pocket.\textsuperscript{12}

<table>
<thead>
<tr>
<th>human $\alpha_{1A}$</th>
<th>human $\alpha_{1B}$</th>
<th>human $\alpha_{1D}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>...F187</td>
<td>S A L G S F Y...</td>
<td></td>
</tr>
<tr>
<td>...F206</td>
<td>S S L G S F Y...</td>
<td></td>
</tr>
<tr>
<td>...F257</td>
<td>S S V C S F Y...</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.2. Proposed alignment of TM5 amino acid residues for $\alpha_{1}$-ARs. The serine residues which may be involved in binding and activation by catecholamines are highlighted.\textsuperscript{7}

There are also other important amino acids, for example the Y203A (TM5) and Y338A (TM7) mutants of the $\alpha_{1B}$ subtype showed that these residues play important roles in preserving the receptor structure rather than directly interacting with the agonists.\textsuperscript{11}

Investigation of the role of the amino acids which are different between the $\alpha_{1A}$ and $\alpha_{1B}$ subtypes indicates that mutations of the A204 and L314 of the $\alpha_{1B}$ subtype to the $\alpha_{1A}$ subtype counterparts (the A204V and L314M mutants of the $\alpha_{1B}$ subtype) changed the
binding profile of agonists from the $\alpha_{1B}$ to the $\alpha_{1A}$ phenotype and reversal of these residues in the $\alpha_{1A}$ to those in the $\alpha_{1B}$ (V185A and M293L) reversed the binding profile to that of the $\alpha_{1B}$. These results were modelled and docking studies showed that agonists with ortho substituents in the phenyl ring are interacting with these residues.

Niguldipine (27) and its analogues, which were not affected by S188A, S192A and F193L mutations, were found to interact with F86 in TM2 of the $\alpha_{1A}$ receptor, because they were adversely affected by a F86M mutation. In addition, the selectivity of these antagonists may be explained with the same mutation because at this position only the $\alpha_{1A}$ subtype has the phenylalanine residue.

4.5. SOME INTER- AND INTRA-HELICAL INTERACTIONS

The proline residues in the TM helices were the focus of a recent study which included interactions of the prolines with surrounding residues and investigates their involvement in the stabilisation of the overall structure of the receptor. A number of proline containing helices were examined and it was found that the existence of a proline residue in a helix causes a kink or a change in the helical direction. The extent of the kink in the proline containing helix is dependent on the surrounding environment and the type and number of hydrogen bonds that are formed. There are three conformations of the helix present:

1. Regular kink: created by a loss of one or two H-bonds in the backbone.

3. Tight turn: no loss in H-bonding. This is only possible if the neighbouring amino acid is β-branched including valine, isoleucine and tyrosine.

In addition to the interruption of α-helical structure within the TM helices this study also concentrated on the CH-O interactions involving gamma and delta hydrogens of the pyrrolidine ring of the prolines. As an overall conclusion to this study, it was suggested that the conservation and frequent occurrence of TM prolines may be due to important structural and functional roles.

4.6. α1A AND α1B ADRENERGIC RECEPTOR MODELS

There are a number of models of the α1-ARs that have been built for various reasons, for example, visualisation of the experimental results of site directed mutagenesis, structure based QSAR studies using docking techniques, investigation of receptor dynamics to find out the active and inactive conformations of the receptor, and determination of inter- and intra-helical interactions including proline residues. Our α1B model in this work was initially built to investigate the unique interactions of the proline residues. In the building process, the size of the TM domains was determined by using an algorithm based on the weighted pairwise comparisons of aligned residues of α1 sequences. The TM regions were constructed as α-helices by overlaying of putative α1-AR transmembrane residues with the transmembrane coordinates of bacterio-rhodopsin, with data files generated using the Insight II molecular modelling software from Molecular Simulations Inc. These helices were subsequently packed with respect to the adjacent helices according to where possible the
Site directed mutagenesis studies were used to identify the binding pocket of the α_{1A} model.\textsuperscript{10-12} The aspartate residue (D106) in TM3 and serine (S192) and the phenylalanine (F193) residues in the TM5 were found to be important. A visual examination of our initial α_{1A} model showed that the F193 was not in the suggested binding pocket. In addition, docking experiments did not give the expected ligand-receptor interactions. This problem was reported in the literature previously and solved there by rotating the helix 5 until the residue F193 is in the binding pocket.\textsuperscript{12}

After publication of the α-carbon template of the rhodopsin-like GPCRs,\textsuperscript{17} we had an opportunity for a new approach to this problem by revising the models according to this template and this was done as described in Section 4.7.

4.7. REVISION OF THE RECEPTOR MODELS

The packing of the trans-membrane helices was revised on the basis of Baldwin’s α-carbon template\textsuperscript{17} without changing the helices themselves from the previous model.\textsuperscript{9} Firstly, the initial model was positioned onto the template and the helices moved individually to match with the template. Homology data was used to move each helix to
anchor, matching a few conserved residues. In the case of helix 2, it was found that 96% of the GPCRs have a leucine residue in position 10 of this helix. Using this data, helix 2 was rotationally moved to align the residues at the position 10 of both models. The rest of the helices were aligned according to this homology data to match the highly conserved residues shown in Table 4.1. Figure 4.3 shows the superimposition of the $\alpha_{1A}$ model onto the template before revisions. The major difference is due to the fact that the helices in the template are ideal helices. However, in our model, helices were built individually according to biophysical considerations allowing them to kink at the point of the proline residues in helices 2, 4, 5, 6 and 7. Helices 6 and 7 are far away from the ideal helix structure compared to the others because of the different conformational isomers of the proline residues and the number of proline residues in these helices.

<table>
<thead>
<tr>
<th>conserved residues</th>
<th>TM2</th>
<th>TM3</th>
<th>TM4</th>
<th>TM5</th>
<th>TM6</th>
<th>TM7</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_{1A}$</td>
<td>N9</td>
<td>L10</td>
<td>A11</td>
<td>E/D24</td>
<td>R25</td>
<td>Y26</td>
</tr>
<tr>
<td></td>
<td>91%</td>
<td>97%</td>
<td>96%</td>
<td>99%</td>
<td>100%</td>
<td>74%</td>
</tr>
<tr>
<td>$\alpha_{1B}$</td>
<td>N86</td>
<td>L87</td>
<td>A88</td>
<td>D142</td>
<td>R143</td>
<td>Y144</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W170</td>
<td>Y223</td>
<td>P309</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1. The most conserved residues within the GPCRs used to revise our models.

After revision of the models, an intensive minimisation process was used to optimise the structure allowing the side chains to relax. All the hydrogen bonds in the backbone of the helices were restrained during minimisation. The amino acid pairs which did not have the expected backbone hydrogen bonds are listed in Table 4.2. Usually, in the backbone of the $\alpha$-helix the carbonyl oxygen of an amino acid is hydrogen bonded to the NH of the fourth following amino acid. However, in the presence of proline which does not have an NH group in the backbone, this is not possible and the helix tilts
according to the conformational isomer of the proline. This may prevent other neighbouring amino acids forming hydrogen bonds.

<table>
<thead>
<tr>
<th></th>
<th>$\alpha_1$</th>
<th></th>
<th></th>
<th>$\alpha_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S77</td>
<td>-</td>
<td>P81</td>
<td>Y82</td>
<td>-</td>
</tr>
<tr>
<td>T78</td>
<td>-</td>
<td>F82</td>
<td>F96</td>
<td>-</td>
</tr>
<tr>
<td>I157</td>
<td>-</td>
<td>P161</td>
<td>I176</td>
<td>-</td>
</tr>
<tr>
<td>S192</td>
<td>-</td>
<td>P196</td>
<td>S177</td>
<td>-</td>
</tr>
<tr>
<td>F193</td>
<td>-</td>
<td>L197</td>
<td>S211</td>
<td>-</td>
</tr>
<tr>
<td>Y194</td>
<td>-</td>
<td>A198</td>
<td>F212</td>
<td>-</td>
</tr>
<tr>
<td>L283</td>
<td>-</td>
<td>P287</td>
<td>Y213</td>
<td>-</td>
</tr>
<tr>
<td>F289</td>
<td>-</td>
<td>P293</td>
<td>L296</td>
<td>-</td>
</tr>
<tr>
<td>S323</td>
<td>-</td>
<td>P327</td>
<td>F311</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S341</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.2. The lost hydrogen bonds in the backbones. (C=O of AA n to NH of AA n+4)

Figure 4.3. Overlap of Baldwin's $\alpha$-carbon template\textsuperscript{17} and our original $\alpha_{1A}$ model.
In the next stage of the modelling, adrenaline-receptor complexes were generated and binding energies were compared with the experimental data to test the revised models. As was expected, adrenaline binds to the $\alpha_{1A}$ subtype slightly better than it does to the $\alpha_{1B}$ subtype.

Apart from the salt bridge between the protonated nitrogen atom and the deprotonated aspartate residue (D106 in the $\alpha_{1A}$ and D125 in the $\alpha_{1B}$ subtype) in TM3, and various hydrophobic interactions, there are two important interactions which have been established by experimental studies. These are a $\pi-\pi$ interaction between the catechol ring of adrenaline and the F193 residue in TM5 of the $\alpha_{1A}$ subtype (F310 in TM6 of the $\alpha_{1B}$ subtype) and hydrogen bonding between S192 of TM5 in the $\alpha_{1A}$ subtype (S211 in TM5 of the $\alpha_{1B}$ subtype) and the para-hydroxyl group of the catechol ring of adrenaline (Figure 4.4 & 4.5). Both these interactions were observed in the results of our docking experiment in agreement with the experimental data.\textsuperscript{10-12,100,106} There is an additional interaction found between the $\beta$-hydroxyl group of adrenaline and the carbonyl oxygen of D125 in the $\alpha_{1B}$ subtype. This interaction is important because it is possible only for the (R) conformation of adrenaline and has not been reported previously. In the binding of adrenaline to the $\beta$-adrenergic receptors, the $\beta$-hydroxyl group interacts with of TM4, but this residue is not present in $\alpha_1$-ARs. In addition, binding energies were calculated and shown in Table 4.3.
Table 4.3. Activity and energy data for adrenaline on both subtypes.

<table>
<thead>
<tr>
<th>pK_10</th>
<th>BE</th>
<th>IE</th>
<th>DL</th>
<th>DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>α_1A</td>
<td>6.1</td>
<td>-141</td>
<td>-152</td>
<td>4</td>
</tr>
<tr>
<td>α_1B</td>
<td>5.8</td>
<td>-134</td>
<td>-189</td>
<td>10</td>
</tr>
</tbody>
</table>

BE = UE + DL + DR
IE = interaction energy, DL = distortion energy of the ligand, DR = distortion energy of the receptor and BE = binding energy.

As can be seen from the table, binding energies from our docking experiments correlate with the experimental binding affinities of the ligand. According to these docking results of the natural ligand, our receptor models were considered sufficiently sound to continue with the docking experiments with rigid molecules.
4.9. DOCKING OF IQC (19)

After the examination of the adrenaline-receptor complexes, the receptor models could be used to visualise interactions with other ligands. It can be assumed that the other ligands bind to the same binding pocket as adrenaline.\textsuperscript{11,12,99} Because our design study focused on rigid compounds, the docking study commenced with IQC (19).
IQC was placed into the binding pocket with the indole ring pointing towards the extracellular side, the protonated nitrogen atom close to the aspartate (D106 for the $\alpha_{1A}$ and D125 for the $\alpha_{1B}$ subtype) and the dimethoxy part close to the serine residues in TM5. The routine minimisation process was run to optimise the complex structure and the interaction energy was calculated for both subtypes (Table 4.4). The binding data correlates with the binding energies very well. This suggests that the binding pocket for IQC in the $\alpha_{1A}$ and $\alpha_{1B}$ AR subtypes may be the same site where adrenaline binds. This was expected due to the geometric similarities between IQC and adrenaline (see Figure 4.6).

<table>
<thead>
<tr>
<th></th>
<th>$pK_i$</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_{1A}$</td>
<td>8.4</td>
<td>-164</td>
</tr>
<tr>
<td>$\alpha_{1B}$</td>
<td>6.6</td>
<td>-134</td>
</tr>
</tbody>
</table>

Table 4.4. Activity and energy data for IQC on both subtypes.

The complexes were analysed and we found that the main interaction between IQC and the receptor models is the salt bridge between the conserved aspartate and the basic nitrogen of the ligand. The propyl group of IQC was surrounded by amino acid side chains from TM3 and TM4 in both subtypes. The highest activity for IQC derivatives was reported with the propyl chain rather than methyl, ethyl or butyl chains on the nitrogen in the experimental binding studies. Other important interactions are the same as in the adrenaline-receptor complexes. The serines (S208 and S192 for $\alpha_{1A}$ and $\alpha_{1B}$ respectively) are H-bonded to one of the oxygens on the methoxy groups and phenylalanine groups (F193 and F310 for $\alpha_{1A}$ and $\alpha_{1B}$ respectively) interact with the aromatic side of the isoquinoline system of IQC. In addition, another aromatic residue
TRP285 also interacts with the same aromatic system in the $\alpha_{1A}$ subtype (Figure 4.7 and 4.8).

The differences in the docking between the two subtypes is the weak interaction of the indole ring of IQC with the $\alpha_{1B}$ model, where there is a $\pi$-$\pi$ stacking interaction between F330 and the indole ring of IQC and a hydrogen bond between the carbonyl oxygen atom of L314 and the NH group of the indole ring (Figure 4.8).

Figure 4.7a. IQC-$\alpha_{1A}$ adrenergic receptor complex, H-bond, salt bridge and van der Waals interactions.

Figure 4.7b. IQC-$\alpha_{1A}$ adrenergic receptor complex, aromatic interactions.
In a further examination of the complexes, it was found that in the bottom end of the binding pocket, where the top of the isoquinoline system rests, there are two sulfur containing residues (C110 and M292 for $\alpha_{1A}$ and C128 and C129 for $\alpha_{1B}$) which can be utilised to modify the interactions between IQC and the receptors for better selectivity. An addition of an amine, methoxy or alcohol group to the “4” position of IQC (Figure 4.9) could interact with these cysteines in this region.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>BE for $\alpha_{1A}$</th>
<th>BE for $\alpha_{1B}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQC (19)</td>
<td>-164</td>
<td>-134</td>
</tr>
<tr>
<td>(R)-4-amino-IQC (102)</td>
<td>-177</td>
<td>-147</td>
</tr>
<tr>
<td>(R)-4-hydroxyl-IQC (103)</td>
<td>-171</td>
<td>-142</td>
</tr>
<tr>
<td>(R)-4-methoxy-IQC (104)</td>
<td>-167</td>
<td>-139</td>
</tr>
</tbody>
</table>

Table 4.5. Binding energies for IQC and its derivatives.
Both enantiomers of these two derivatives of IQC were modelled and docked into the subtypes to measure the comparative binding energies. It can be seen from Table 4.5 that the addition of these groups improved binding of IQC to the models however, the selectivity of the ligand did not change. This may be due to the high flexibility of the $\alpha_{1A}$ receptor which can adopt a conformation to get maximum interaction with the ligand. These H-bond acceptor and donor groups could interact with the aspartate carbonyl group in this subtype.

![Figure 4.9. 4-substituted derivatives of IQC. R=NH$_2$ (102), OH (103), OMe (104)](image)

4.10. PHARMACOPHORES DERIVED FROM DOCKING STUDIES

The binding information extracted from the IQC-receptor complexes of the docking experiments suggests that the receptor subtype imposes steric requirements as well as some electrostatic features on ligand binding. Therefore the docking derived pharmacophores for the subtypes according to IQC binding were built using the Catalyst software and the information about the regions sterically forbidden to the ligand by the receptor were also included in our docking derived pharmacophore as excluded volumes.
Excluded volumes can be created by using the structure based information. The information about the complex is usually extracted from the X-ray structure of the complex. The atoms of the surrounding amino acids in the complex structure can be specified as excluded volumes and this pharmacophore can be used to assess the important chemical features as well as the size and shape necessary for recognition by the receptor. In this way steric factors involved in the ligand-receptor interactions can be taken into account.

In our study, coordinates of atoms essential for binding were determined from the complex model. In addition to pharmacophoric points derived from the ligand, the atomic coordinates of the surrounding residues of the receptor were used to define a large number (80-100) of excluded volumes which the ligand is not allowed to penetrate. This information was put into a command script and pharmacophores were built by using the hypoedit command.

4.10.1. THE $\alpha_{1A}$ PHARMACOPHORE

The pharmacophore was created by using the binding information in the complex model. Firstly, the interactions were identified and the coordinates were obtained from the modelling data and the features were created by using Catalyst software.
The docking derived $\alpha_{1A}$ pharmacophore can be seen in Figure 4.10. The red sphere represents the positive ion interacting with the D106. The blue spheres are the hydrophobic groups for the propyl chain on the protonated nitrogen atom and the methyl moiety of the upper methoxy group. The aromatic part of the isoquinoline ring of IQC is involved in an aromatic interaction with F193 and this is represented by a brown sphere and a blue plane. The last feature is the H-bond acceptor group on the oxygen atom of the upper methoxy group interacting with S192 which is represented by a green sphere.

The excluded volumes were also included and the binding conformation of IQC was docked into this pharmacophore (Figure 4.11). As can be seen the ligand is surrounded by the receptor atoms of the binding pocket. The picture was taken from the opening of
the extra-cellular receptor binding site. The black spheres are representing the excluded volumes.

Figure 4.11. The docking derived $\alpha_{1A}$ pharmacophore with excluded volumes.

4.10.2. THE $\alpha_{1B}$ PHARMACOPHORE

The docking derived pharmacophore for the $\alpha_{1B}$ subtype is more complicated (Figure 4.12). The red sphere again for the positive ion is again located on the protonated nitrogen atom interacting with the D125. Hydrophobic groups on the propyl chain, two of the methoxy groups of the isoquinoline ring and the methoxy group on the indole ring are blue. Aromatic rings are represented with brown spheres and the blue planes located on two phenyl rings of the molecule. There is one H-bond acceptor group located on the oxygen atom of the lower methoxy group (green spheres) and a H-bond donor group on the NH group of the indole ring (purple spheres).
The excluded volumes were also included in this subtype (Figure 4.13).
4.11. COMPARING THE DOCKING DERIVED PHARMACOPHORES

The comparison between the pharmacophores can give an indication of the selectivity features between these subtypes.

4.11.1. THE $\alpha_{1A}$-SUBTYPE VERSUS $\alpha_{1B}$-SUBTYPE

The docking derived pharmacophores are very similar over the isoquinoline ring system including positive ion, H-bond acceptor group, and the hydrophobic groups (Figures 4.10, 112 and 14). However, the main difference between the subtypes is that there is no feature on the indole ring involved in the interaction with the $\alpha_{1A}$ subtype. On the other hand, interaction between the indole ring and the $\alpha_{1B}$ subtype is very strong. A H-bond donor group, an aromatic ring and a hydrophobic group are involved in the interaction (Figures 4.10 and 4.12). This difference can be seen in Figure 4.14 which shows the two pharmacophores superimposed on each other.
Figure 4.14. Superimposition of the two docking derived pharmacophores. Features unique to the $\alpha_{1B}$ pharmacophore are highlighted. The other features are on the isoquinoline system of IQC which are very similar in both pharmacophores.

The other major difference is that the binding pocket of the $\alpha_{1A}$ subtype is very tight leading to a better affinity for the ligand whereas the $\alpha_{1B}$ subtype has a very large space on top of the ligand. This space could be used as a target to design new selective ligands for the $\alpha_{1B}$ subtype (Figure 4.13). The potential problem here is that the $\alpha_{1A}$ pocket seems to be more flexible, as noted also by De Benedetti et al. This is probably the reason why just about every ligand known, including adrenaline (1) and IQC (19), has a higher affinity for the $\alpha_{1A}$ receptor, since the pocket can mould itself around the ligand.
4.11.2. LIGAND BASED VERSUS DOCKING DERIVED PHARMACOPHORES

The docking derived pharmacophores (DDP) were also compared with the ligand based pharmacophores (LBP) which are explained in Chapter 2. In the case of the \( \alpha_{1A} \) pharmacophores, the three of the features (hydrogen bond acceptor, positive ion and hydrophobic group) overlapped well. This is remarkable because the LBP was obtained using only subtype selective antagonists which did not include IQC (19) whereas the DDP was obtained only from IQC. This information also strongly suggests that IQC may interact with the same binding site at the receptor as the selective ligands as well as adrenaline. However, the second H-bond acceptor group in the LBP, which is shown in Figure 4.15 as a green sphere on the right hand side, is not indicated in the DDP. In addition, the aromatic ring and the second hydrophobic group of the DDP, are not present in the LBP.

![Figure 4.15. Superimposition of LBP and DDP for the \( \alpha_{1A} \) subtype. Common features are not highlighted in the figure.](image)
The ligand based $\alpha_{1B}$ pharmacophore has only three features, namely positive ion, hydrophobic and H-bond acceptor groups. These features can be superimposed onto the same features of the DDP of the $\alpha_{1B}$ subtype with a very reasonable degree of agreement as depicted in Figure 4.16. However, the DDP derived from IQC (19) also has three other hydrophobic groups, two aromatic rings and a hydrogen bond acceptor groups which well may be related only to the IQC binding.

Figure 4.16. Superimposition of LBP and DDP for the $\alpha_{1B}$ subtype. Features common to both pharmacophores are highlighted, the other additional features are of the DDP.
4.12. DESIGN OF SELECTIVE LIGANDS

As was mentioned in the comparison of the docking derived pharmacophores, the large space on top of IQC (19) in the binding pocket could be used to design an $\alpha_{1B}$ selective ligand. The target compound, MET_IQC (37), designed from the Apex-3D pharmacophores could serve for this purpose if the methyl group is added stereoselectively (R enantiomer). When this compound was tested on the docking derived pharmacophore with excluded volumes, the fit value was very high (5.48) compared to the other targets (Table 4.6). As can be seen in Figure 4.17, the space can be used as a potential target by filling it with a bulky hydrophobic group. In addition, this space can be filled with a H-bond donor group to interact with the carbonyl group of A123. As a result, the bis-propyl derivative (105) of IQC (19) is suggested as a target for synthesis and evaluation as a selective $\alpha_{1B}$ ligand.

Another way of designing $\alpha_{1B}$ selective ligands is by targeting the H-bond donor group and/or the second phenyl ring (the indole ring in IQC(19)). The target (67) designed from the ligand based pharmacophore is also can be mapped well onto the docking derived $\alpha_{1B}$ pharmacophore with excluded volumes. It has a fit value of 4.5 for this pharmacophore (Table 4.6).
<table>
<thead>
<tr>
<th>Ligands</th>
<th>$\alpha_{1A}$</th>
<th>$\alpha_{1B}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1QC (19)</td>
<td>3.17</td>
<td>4.00</td>
</tr>
<tr>
<td>Thiadiazole (36)</td>
<td>1.17</td>
<td>2.95</td>
</tr>
<tr>
<td>Alcohol (67)</td>
<td>2.93</td>
<td>4.48</td>
</tr>
<tr>
<td>2H-TBA (57)</td>
<td>1.88</td>
<td>2.49</td>
</tr>
<tr>
<td>MET-IQC (37)</td>
<td>1.88</td>
<td>5.48</td>
</tr>
<tr>
<td>Propyl-IQC (105)</td>
<td>not possible*</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Table 4.6. The target ligands and their fit values on the DDPs with excluded volumes. The last row has the binding energies derived from docking information for (99).
* Compound could not be mapped.

Docking of (105) showed that the binding energy of this ligand to the $\alpha_{1A}$-subtype is still lower than it is for $\alpha_{1B}$-subtype. This may be due to the flexibility of side chains in the binding pocket of $\alpha_{1A}$-subtype as mentioned by the other group.$^{24}$
Figure 4.17. Illustration of the space and the designed target (105).
Right to left: 1. From the top of isoquinoline system of IQC.
2. From the propyl chain side of IQC.
Bottom right: normal to the plane of IQC.
5.1. INTRODUCTION

The \(\alpha_{1D}\)-adrenergic receptor is one of the three subtypes of the \(\alpha_1\)-ARs that have been cloned and characterised.\(^3\) Determination of the anatomical distribution and physiological function of this receptor subtype is hindered currently because of the lack of subtype-selective ligands.

![Selecting antagonists for the \(\alpha_{1D}\) subtype.](image)

There are a few antagonists known which show some \(\alpha_{1D}\) selectivity (Figure 5.1). For example, SKF104856 (14) shows 8 and 12 fold selectivity against the \(\alpha_{1A}\) and \(\alpha_{1B}\) subtypes respectively.\(^24\) BMY7378 (13) is another selective antagonist having over
hundred fold selectivity for the $\alpha_{1D}$ subtype over the other two subtypes but with similar selectivity for the $D_3$ dopaminergic receptor.\textsuperscript{112} Recently, the compound SNAP8719 (106), a new derivative of BMY7378 (13) has been reported to show greater selectivity over the other subtypes of $\alpha_1$-ARs and any other bioamine receptors. However, the selectivity of SNAP8719 (106) is still only 125-fold over the $\alpha_{1B}$ subtype, and higher selectivity would be desirable for pharmacological and possible therapeutic studies.\textsuperscript{112}

5.2. THE $\alpha_{1D}$ PHARMACOPHORE

No pharmacophores have been published for the $\alpha_{1D}$ ligand binding. The ligand based pharmacophore development approach was used to design novel ligands for the $\alpha_{1D}$ subtype with potentially high selectivity. A number of selective ligands in their protonated forms were modelled as a training set. These were BMY7378 (13), SKF104856 (14), discretamine (24), NAN190 (38) and WAY100635 (39). The same chemical features were used in the generation step as for the $\alpha_{1A}$ and $\alpha_{1B}$ subtypes. Ten pharmacophore models (hypotheses) were derived by the Catalyst software. The best hypothesis (Figure 5.2) consisted of four features including a positive ion, an aromatic ring and two hydrophobic groups. The hypothesis has very good correlation (Figure 5.3a, Figure 5.3b) within the training set of compounds and also with the test set of aporphine derivatives (Table 5.1), such as IQC (19) (experimental affinity=estimated affinity= 100 nM, Figure 5.4). However, compound (3) has very low estimated activity due to the prazosin problem.
Figure 5.2. The $\alpha_{1D}$ pharmacophore and BMY7378 (13).

Figure 5.3. Correlations between experimental activity ($\alpha_{1D}$) and the estimated activity for
a. Training set molecules. b. Test compounds.

The green line represents the ideal situation where true activity is equivalent to estimated activity. It is simply a guide and the line of best fit is not actually shown, only the correlation is given.
<table>
<thead>
<tr>
<th>No</th>
<th>Compound Name</th>
<th>Activity</th>
<th>$K_i = \text{nM}$</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Prazosin</td>
<td>found 0.3</td>
<td>estimated 96</td>
<td>320</td>
</tr>
<tr>
<td>5</td>
<td>WB-4101</td>
<td>found 0.3</td>
<td>estimated 80</td>
<td>270</td>
</tr>
<tr>
<td>8</td>
<td>5-Methyluropidil</td>
<td>10</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>SNAP-5089</td>
<td>66</td>
<td>110</td>
<td>1.7</td>
</tr>
<tr>
<td>10</td>
<td>KMD-3213</td>
<td>2</td>
<td>76</td>
<td>38</td>
</tr>
<tr>
<td>11</td>
<td>Cyclazosin</td>
<td>3.2</td>
<td>120</td>
<td>37</td>
</tr>
<tr>
<td>12</td>
<td>AH-111101A</td>
<td>2750</td>
<td>9300</td>
<td>3.4</td>
</tr>
<tr>
<td>13</td>
<td>BMY-7378</td>
<td>6.3</td>
<td>4.2</td>
<td>-1.5</td>
</tr>
<tr>
<td>14</td>
<td>SKF-104856</td>
<td>5.2</td>
<td>3.7</td>
<td>-1.4</td>
</tr>
<tr>
<td>19</td>
<td>IQC</td>
<td>100</td>
<td>130</td>
<td>1.3</td>
</tr>
<tr>
<td>24</td>
<td>Discretamine</td>
<td>25</td>
<td>250</td>
<td>10</td>
</tr>
<tr>
<td>27</td>
<td>(+)-Niguldipine</td>
<td>100</td>
<td>77</td>
<td>-1.3</td>
</tr>
<tr>
<td>28</td>
<td>Spiperone</td>
<td>13</td>
<td>84</td>
<td>6.5</td>
</tr>
<tr>
<td>32b</td>
<td>(+)-YM-617</td>
<td>22</td>
<td>100</td>
<td>4.7</td>
</tr>
<tr>
<td>33</td>
<td>Indoramin</td>
<td>160</td>
<td>190</td>
<td>1.2</td>
</tr>
<tr>
<td>35</td>
<td>RS-17053</td>
<td>16</td>
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<td>38</td>
<td>NAN-190</td>
<td>0.8</td>
<td>0.9</td>
<td>1.1</td>
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<td>39</td>
<td>WAY-100635</td>
<td>63</td>
<td>75</td>
<td>1.2</td>
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<tr>
<td>40</td>
<td>RS-100,975</td>
<td>100</td>
<td>87</td>
<td>-1.1</td>
</tr>
<tr>
<td>43</td>
<td>SL-89.0591</td>
<td>2.5</td>
<td>100</td>
<td>42</td>
</tr>
<tr>
<td>44</td>
<td>JHT-601</td>
<td>1.2</td>
<td>7.4</td>
<td>6.1</td>
</tr>
<tr>
<td>45</td>
<td>GG-818</td>
<td>25</td>
<td>2500</td>
<td>100</td>
</tr>
<tr>
<td>46</td>
<td>Uropidil</td>
<td>1660</td>
<td>100</td>
<td>-16</td>
</tr>
</tbody>
</table>

Table 5.1. Experimental activity and estimated activities of selected compounds for the $\alpha_{1D}$ hypothesis.
5.3. DESIGNING NEW TARGETS

To design a new ligand for the $\alpha_{1D}$ hypothesis an aporphine derivative was chosen with an alkane chain attached to interact with the lower hydrophobic group. The compounds (see Table 5.2) proposed for future synthesis and pharmacological evaluation are given in Table 2.1.
Table 5.2. Target ligands for the $\alpha_{1D}$ subtype and their estimated activities.

<table>
<thead>
<tr>
<th></th>
<th>$\alpha_{1D}$</th>
<th>$\alpha_{1A}$</th>
<th>$\alpha_{1B}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$4.7 \times 10^{-8}$</td>
<td>$7.7 \times 10^{-8}$</td>
<td>$2.3 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>$2.0 \times 10^{-7}$</td>
<td>$7.5 \times 10^{-8}$</td>
<td>$2.3 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

Figure 5.5. The $\alpha_{1D}$ pharmacophore and (107).
CHAPTER 6

PHARMACOLOGICAL RESULTS AND EVALUATION

6.1. INTRODUCTION

Development of compounds as potential drugs involves the necessity for an appropriate initial *in vitro* screen on the cloned receptors. Once a compound has been positively assessed, more sophisticated procedures are employed involving tissue preparations and animal models. Thus many tests must be undertaken before a new drug may be used in human clinical trials.

These *in vitro* tests measure binding affinity in terms of the quantity $pK_i$, which is the negative logarithm of $K_i$, the inhibition constant. $K_i$ is calculated via the equation:

$$K_i = \frac{IC_{50}}{1 + C / K_d}$$

where $K_d$ is the dissociation constant of the radiolabelled ligand of concentration $C$. The $IC_{50}$ value represents the concentration of unlabelled antagonist which inhibits 50% of the radioligand binding. Thus, $K_i$ is a measure of strength of binding to a specific receptor and a first indication of pharmacological activity. A $pK_i$ value above 6 is regarded as significant for a lead compound; most clinically useful drugs would have $pK_i$ values of approximately 9, showing activity at nanomolar concentrations.
6.2. RESULTS AND EVALUATION

The activity of IQC (19) on $\alpha_1$-ARs was already known, but subtype selectivity was tested in order design more selective derivatives. The thiadiazole (36), which was designed from the Apex-3D pharmacophore models (see Chapter 2), was an example of this type of compound which was tested on the cloned human receptors. The Catalyst derived pharmacophores were used to design tetrabenazine derivatives. Tetrabenazine (17), the reduced tetrabenazine derivative (57) and two benzo[de]quinoline derivatives (66) and (67) were also tested. The results of these tests are shown in Table 6.1. For the synthesis of these compounds see Chapter 3. Testing was done by Songhai Chen at The Victor Chang Cardiac Research Institute, Sydney.

<table>
<thead>
<tr>
<th></th>
<th>$\alpha_{1\text{A}}$</th>
<th>$\alpha_{1\text{B}}$</th>
<th>$\alpha_{1\text{D}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQC (19)</td>
<td>8.2</td>
<td>6.9</td>
<td>7.0</td>
</tr>
<tr>
<td>Thiadiazole (36)</td>
<td>4.3</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Tetrabenazine (17)</td>
<td>3.6</td>
<td>1.3</td>
<td>3.4</td>
</tr>
<tr>
<td>2H-tetrabenazine (57)</td>
<td>5.0</td>
<td>4.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Ketone (66)</td>
<td>4.0</td>
<td>3.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Alcohol (67)</td>
<td>3.6</td>
<td>3.2</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Table 6.1. Target compounds and their binding affinities at cloned and expressed human $\alpha_1$-ARs.

As can be seen in Table 6.1, the thiadiazole (36), has very low affinity for the $\alpha_1$-ARs. This may be explained by the electrostatic potential distribution of this compound to be discussed in Section 6.3.
The low affinity of tetrabenazine (17) and the reduced tetrabenazine derivative (57) can be explained by our docking studies. Tetrabenazine was placed in the same binding pocket as IQC (19) in both receptor subtypes. However, the final complex after minimisations suggested that it bound outside of the pocket in both subtypes, probably because of its bulky alkyl group. A long range salt bridge keeps the compound docked in the receptor, but the rest of the molecule is distorted and pushed away from the pocket.

Finally ketone (66) and alcohol (67) have also very low affinity for the \( \alpha_1 \) adrenergic receptor subtypes. It can be suggested that the 3-methoxyindole moiety of IQC (19) must be important for the binding of this compound to the \( \alpha_1 \) adrenergic receptor subtypes. This group could interact with the residues in the extracellular loop region of the receptor as hydrophobic group.

6.3. MOLECULAR ELECTROSTATIC POTENTIAL (MEP) MAPPING

Electrostatic forces play an important role in determining the essential stereoelectronic features of biomolecules and drugs in order to study QSAR. They also play an important role in determining the orientations of the molecules when approaching their receptors because of their long range action compared to other intermolecular interactions. Qualitative arguments can be based on electrostatic properties of certain functional groups. For example, carboxyl oxygen atoms are negatively charged at physiological pH and interact with protons forming salt bridges or/and hydrogen bonds.
However, the nature of directly bonded functional groups and even a more distant functionality can have significant effect by polarising the charge distribution. Both these effects are represented in charge distribution calculated by *ab initio* methods which is the best source of information about the electrostatic properties of a molecule.

Firstly, the molecular wave function for a specific compound is calculated and a representation of the *ab initio* charge (electron) density must be chosen so that the MEP and its associated field can be evaluated rapidly at many points. Secondly, this information needs to be displayed in a form which can be easily interpreted. In this area of study most work has concentrated on colour coding or contouring the potential onto predefined molecular surfaces such as the van der Waals surface. These representations are informative and readily observed from a photograph. The biological activity and electrostatic properties of 1,2,4-thiadiazolyl-tryptamine and its oxadiazole analogue are described by Castro *et al.*\(^{113}\) and despite the fact that the geometries of the two molecules are very similar, their biological activities are remarkably different. This was explained by calculating MEP models of the two compounds. Another MEP approach to QSAR was used to draw comparisons between apomorphine, a dopaminergic drug, and ergoline and some of its analogues, creating a dopaminergic pharmacophore.\(^{32}\) The MEP patterns were calculated and superimposition of the molecules suggests that they might bind to the same receptor site. In these studies, it has been shown that the MEP is generally a reliable reactivity index for noncovalent interactions of a series of structurally related polar ligands with a common receptor.
6.3.1. THE MOLECULAR ELECTROSTATIC POTENTIAL (MEP) MAPPING OF APORPHINE DERIVATIVES

Models of the thiadiazole (36), IQC (19) and boldine (23) (an aporphine derivative with high activity AT $\alpha_1$-ARs) were built, optimised using $ab$ initio calculations at the RHF/STO 3-21G level and the electrostatic potentials were mapped on to the total electron density surfaces. The calculated electrostatic potential surfaces for the compounds are displayed side by side and on the same scale and colour coded. The value of the potential was measured at important points and presented in Figure 6.1.

![Figure 6.1. MEP for (23), (19) and (36) respectively.](image)

In Figure 6.1, calculations of the MEPs from the wave functions suggested that the majority of the differences between (36) and (19) or (23) lay in the electron density pattern located on the lower ring system which is the thiadiazole ring on (36), the dimethoxybenzene ring for (23) and the 3-methoxy-indole ring for (19). Compound (36) has a negative potential section situated on the nitrogen atom shown as a red region, whereas the other two aporphine analogues, (23) and (19), have an area of positive potential situated at the corresponding part of the lower aromatic ring.
The positively charged edge of the aporphine skeleton can be an element of the $\alpha_1$-AR pharmacophore. In contrast to this, the same edge is occupied by the nitrogen atom in the thiadiazole ring of (36), which is negatively charged (red colour). This could cause an unfavorable interaction with the receptor, and hence possibly explain the poor affinity of (36) for $\alpha_1$-ARs. It should be noted that, pharmacophores take no direct account of electrostatic potentials and only features necessary for binding can be taken into account not detrimental features.

One way to reduce the negative electrostatic potential observed for (36), but still remain an aromatic ring, would be to synthesise and evaluate a fused 1,3-oxazole analogue. The basicity of the nitrogen in such derivatives would be very low, although the ring would still be aromatic.
CHAPTER 7

CONCLUSION

7.1. DISCUSSION

The $\alpha_1$-adrenergic receptor subtypes, although structurally similar in their TM domains, have significant differences in their ligand binding affinities and their physiological effects. Most subtype selective antagonists do not discriminate between the subtypes to a great extent (>1000 fold) to prevent side effects. In particular, there is a shortage of ligands with selective affinity for the $\alpha_{1B}$ and $\alpha_{1D}$ subtypes. Such compounds could be useful therapeutically, for example, for the treatment of cardiovascular conditions.

A number of computer-aided drug design techniques were used to develop subtype selective ligands in this study.

7.1.1. PHARMACOPHORE DEVELOPMENT, SYNTHESIS AND TESTING (CHAPTERS 2, 3 AND 6)

The lack of structural information about the receptor led us to start with a ligand based approach to the problem. Preliminary models of the pharmacophores for the $\alpha_{1A}$ and $\alpha_{1B}$ subtypes were generated\textsuperscript{57} by using Apex-3D\textsuperscript{47-49} by Molecular Simulations Inc. The pharmacophores contain a protonated amine centre which is necessary for binding to the aspartate residue in the TM3 of the receptor. This feature was reported to be important by many authors.$^{12,35-38,56}$ The pharmacophore models also have other features such as an aromatic region and a polar group. These features were also reported\textsuperscript{35} previously for the $\alpha_1$-adrenergic receptor but without analysing the specific
subtypes. The main differences between subtypes according to these models are the distances between:

1. The protonated amine function and the aromatic region,
2. The protonated amine function and the polar region.

The first distance for the $\alpha_{1A}$ subtype is in the range of 5.2-5.8 Å, whereas this distance is between 6.2 and 7.8Å for the $\alpha_{1B}$ subtype. The second distance was less well defined (5.2-6.7 and 5.2-7.5Å for the $\alpha_{1A}$ and $\alpha_{1B}$ subtypes respectively). The minimal ligand approach was used and the distance between the nitrogen and the aromatic ring system was the target of the design process. A thiadiazole aporphine analogue (36), for which this distance is 5.2 Å, was synthesised as a good target for the $\alpha_{1A}$ subtype. Although, many aporphine derivatives, including (19), (20), (21), (22), (23) and (24), have slight selectivity for the $\alpha_{1A}$ subtype, and bind quite strongly, our compound (36) had very low affinity for $\alpha_1$-ARs. This was possibly due to an unfavourable electron rich region around the thiadiazole ring system, as noted by application of the electrostatic potential mapping technique.

New pharmacophore models were developed by using the Catalyst software of MSI (which superseded Apex-3D). These pharmacophore models also include the protonated amine function as a positive ion. The $\alpha_{1A}$ model also has two hydrogen bond acceptor groups and a hydrophobic group, whereas the $\alpha_{1B}$ model has a hydrogen bond donor group and a hydrophobic group. The pharmacophore models predicted the affinities of the aporphine derivatives very well, but the models were too similar to design a selective compound from isoquinoline ring-containing compounds. The H-
bond acceptor group of the \( \alpha_{1A} \) model and the donor group of the \( \alpha_{1B} \) model could be overlapped. This suggested that an \( \alpha_{1B} \) selective compound should contain a group with only H-bond donor functionality, but not a H-bond acceptor functionality. A number of isoquinoline containing compounds have been designed with this constraint in mind and two of them ((57) and (67)) were synthesised.

Syntheses of (57) and (67) with control of relative stereochemistry were achieved by L-Selectride reduction of (17) and (66) (Section 3.2.1). The relative configurations of compounds (57) and (67) were proven by X-ray crystallographic analysis and supported by n.m.r. data.

The compounds (57) and (67) had very low affinity for the \( \alpha_1 \)-ARs. The low affinity of (57) may be explained on the basis of an unfavourable steric interaction due to the bulky alkyl chain of this compound. This was found by using docking techniques (see Chapter 4). The low activity of (67) indicated the importance of the aromatic ring proposed by the earlier pharmacophore models.\(^{35,57}\)

### 7.1.2. STRUCTURE BASED DESIGN (CHAPTER 4)

Models of the \( \alpha_{1A} \) and \( \alpha_{1B} \)-ARs were built and binding pockets of the subtypes were determined by using docking techniques by docking adrenaline, the natural ligand of these receptors, into the receptor models. Experimental data was confirmed\(^{10-12,100,106}\) from the docking experiments and the calculated binding energies also correlated with the experimental affinities (Table 4.3). This information was used to dock IQC (19) and further analysis of the interacting residues of the receptor and the groups of the
ligand suggested new design opportunities. A new set of potential interacting residues of the $\alpha_{1B}$-model was determined (see Section 4.9) and targets were proposed and docked into receptor models to interact better with only the $\alpha_{1B}$-model. However, the results of these experiments showed that the target ligands still have higher binding energies for the $\alpha_{1A}$ subtype. This was explained by the flexibility of this receptor subtype which was also reported in an earlier study.24

In the last part of the design process a novel technique was developed by creating pharmacophores using the model of the IQC-receptor complexes from the docking studies. These pharmacophores also include a number of excluded volumes which allow the user to consider the regions sterically forbidden for the ligand (Section 4.10). These pharmacophores had common features with the ligand based pharmacophores developed by the Catalyst software. However, additional features specific to IQC-binding were found and could be utilised for designing new derivatives of this compound. Analysis of the pharmacophores indicated that the $\alpha_{1B}$-pharmacophore had an unfilled space which could be useful to design an $\alpha_{1B}$ selective target. A bis-propyl derivative (99) of IQC (19) had a good fit on the $\alpha_{1B}$-pharmacophore and, this compound could not be mapped onto the $\alpha_{1A}$ pharmacophore. Unfortunately, this compound gave higher binding energy for the $\alpha_{1A}$ receptor model when it was docked suggesting, as reported24 before, the flexibility of this subtype. However, it may be possible to design other substituents which will increase $\alpha_{1B}$ binding but preclude $\alpha_{1A}$ binding on steric and/or electronic grounds.
7.2. FUTURE DIRECTIONS

The ligand based approach can be modified by using a different training set of structurally related antagonists. This will result in different sets of common features according to the training sets of compounds. These different pharmacophores could be tested using aporphine analogues to find more useful templates to design more selective ligands.

The receptor models should also be modified according to new findings of the site directed mutagenesis studies and receptor dynamics could be investigated to obtain more information about the structure of these macromolecules. There are 48 amino acid differences in the TM domains between $\alpha_{1A}$ and $\alpha_{1B}$ subtypes, and a subset of these residues must be critical for the subtype selectivity.

Other possible modifications (Table 7.1) could be undertaken on the IQC structure to test on the new pharmacophores as well as on the receptor models to design selective ligands to bind to the $\alpha_{1A}$ and $\alpha_{1B}$ adrenergic receptors selectively. Compound (111)
incorporates features which should enhance $\alpha_{1B}$ binding over $\alpha_{1A}$ binding, while (110) should bind more strongly to the $\alpha_{1A}$ receptor subtype.
CHAPTER 8

EXPERIMENTAL

8.1. PHARMACOPHORE GENERATION (CHAPTERS 2 and 5)

8.1.1. APEX-3D EXPERIMENTS
Throughout this study all molecular modeling work was performed on a Silicon Graphics Indy workstation using Biosym software of Molecular Simulations, Inc., specifically, the modules INSIGHT II, BUILDER, DISCOVER, Search-Compare and Apex-3D, under an IRIX 5.2 operating system. The CVFF force field was used throughout.

Antagonists in the Apex-3D training set from Table 2.1 were built and their geometry was optimised by using the BUILDER module of the software package. The next series of steps were performed using the DISCOVER module, starting with an initial minimisation to relieve any strain on the molecules before the dynamics was started. This minimisation invoked 100 steps of steepest descents minimisation followed by 100 steps of conjugate gradients until the maximum derivative was less than 0.0001 kcal/mol. The dynamics was initialised at 900 K for 1 ps (1000 steps of 1 fs). During the initialisation process, the system was raised to and partially equilibrated at the target temperature. The major conformational changes were seen in this process. The first step in the loop was to resume the dynamics at 900 K for 2 ps, then a sample conformation was saved to an archive file, finally the dynamics was resumed for another step and so on for a total of 10 cycles. The ten conformations sampled were
minimized again until the maximum derivative was less than 0.0001 kcal/mol by using the same method as the initial minimisation process. Finally, the global minimum energy conformations were determined.

The next step involved in systematic conformational searching. Global minimum energy conformations were used for this part and the bonds around which systematic rotations were to be performed were identified. The increment (usually 60° or 90° increments) for the rotations was selected in such a way as to obtain an estimate of about a few hundred sterically allowed conformers. These were then minimized until the maximum derivative was less than 0.0001 kcal/mol by using the same method as the initial minimisation process and with duplicates removed. This resulted in about 50 to 100 conformers for the molecules chosen by means of the Search-Compare module.

The Apex-3D module takes as input a number of molecules which bind to the receptor site and the classification of each molecule according to its activity. All the structures obtained from the conformational analysis were used as data for the Apex-3D module after they were classified into three activity classes according to their pKi values at the receptor site.

The program then identifies common functional groups (descriptor centres) for these molecules, including heteroatom, and hydrogen bond donor and acceptor groups. These functional groups can refer to pseudo atoms such as ring centroids and hydrogen bond direction. After the descriptor centres are identified, the program searches for common 3D arrangements (biophores) of these centres. This program works best for a set of molecules which are structurally different and include subsets of structurally related
compounds with very different activity. The software usually finds a large number of biophores and provides statistical filtering options to evaluate them, but further evaluation is necessary to be able to propose pharmacophore models.

8.1.2. CATALYST EXPERIMENTS
Throughout this aspect of the study all molecular modeling work was performed on a Silicon Graphics O₂ workstation using the Catalyst package of Molecular Simulations, Inc., under an IRIX 5.2 operating system. A generalised CHARMM-like force field was used throughout.

A training set of subtype selective α₁-antagonists was selected according to their binding affinities for the subtypes. The selectivities of these molecules range from 5 to 1000 fold with at least 1000 nM affinity.

Molecules were edited within the Catalyst package using the CHARMM-like force field and conformational models for each compound were generated automatically using the poling algorithm. This emphasised representative coverage over a 10 kcal/mol energy range above the estimated global minimum and the "best searching procedure" was chosen.

The training subset of molecules with their associated conformational models and activities was submitted to Catalyst by using the generate hypothesis command. The chemical functional groups used in this generation step included hydrogen bond donor and acceptor, hydrophobic, positive ion and ring aromatic groups.
The statistical relevance of the various hypotheses so obtained is assessed on the basis of their cost relative to the null hypothesis and their correlation coefficients $R^{51.65}$. The null hypothesis is an ideal hypothesis which is the simplest possible hypothesis that fits the data and presumably there is no statistically significant structure including that the experimental activities are normally distributed about their mean. The cost function consists of two terms. The first penalises the deviation between the estimated activity and the experimental activity and the second penalises the complexity of the hypothesis. The hypotheses were then used to estimate the activities of the target compounds. These activities are derived from those conformers displaying the smallest root-mean square (RMS) deviations when projected onto the hypothesis.
8.2. SYNTHESIS (CHAPTER 3)

8.2.1. GENERAL PROCEDURES

All melting points were determined using a Reichert hot stage melting point apparatus and are uncorrected. The $^1$H nuclear magnetic resonance spectra (nmr) were determined at 300 or 400 MHz with a Varian Unity-300 or 400 spectrometer. The $^{13}$C nmr spectra were recorded using the same instruments at 75 or 100 MHz. Unless otherwise stated, the spectra were obtained on solutions in CDCl$_3$ and referenced to TMS. The electron impact (EI) mass spectra were obtained on a Shimadzu QP-5000 mass spectrometer using and the direct insertion technique, with an electron beam energy of 70 eV and a source temperature of 260°C. The peak intensities, in parentheses, are expressed as a percentage abundance. For the chemical ionisation (CI) mass spectra, isobutane was used as the ionising gas over a Shimadzu QP-5000 mass spectrometer. The low resolution electro spray mass spectra were obtained on a Vacuum General - Quattro mass spectrometer using 50% aqueous acetonitrile as solvent and a skimmer cone voltage of 25V. Where electro spray MS data is reported, it refers to the positive ion mode. The high resolution mass spectra were obtained using a Vacuum General Austospec-OA-TOF mass spectrometer using the same condition above and a resolution of 5000.

Analytical thin layer chromatography (TLC) was performed on Merck Kieselgel 60PF$_{254}$ silica on plastic backed sheets. $R_f$ values were recorded from the centre of the spots. All chromatographic solvent proportions are volume by volume. Column chromatography was performed using Merck silica gel (0.032-0.064) under medium
pressure. X-ray crystallography was done by Assoc. Prof. Allan White, Department of Chemistry, University of Western Australia.

All reagents and solvents were purified and dried by standard techniques. The drying of chloroform, diethyl ether or dichloromethane extracts was done with anhydrous magnesium sulfate. Solvents were removed under reduced pressure in a rotary evaporator. Light petroleum used had a boiling point range of 40-60°C. Tetrabenazine was obtained from the former Hoffmann La-Roche Pty. Ltd., Dee Why, NSW.

8.2.1. SYNTHESIS OF RING-D APORPHINE ANALOGUES

8.2.1.1. Preparation of N-Acetylhomoveratrylamine (112)

To a stirred mixture of homoveratrylamine (85) (30.09g, 145mmol) and dry pyridine (15mL; distilled over potassium hydroxide) under nitrogen was added acetic anhydride (19mL) dropwise to maintain a reaction temperature of 60-70°C. The temperature was then increased to 90°C for 1h. The solution was left at room temperature overnight. Pyridine and acetic acid were distilled and the crude N-acetylhomoveratrylamine (112) recrystallised from ethyl acetate to afford colourless crystals (23.81g, 107mmol, 79%); m.p. 98-100°C.42

M.s. (EI): m/z 223 (M+*, 100%).

1H n.m.r. δ: 6.822 (d, J 8.3 Hz, 1H, ArH); 6.753 (s, 1H, ArH); 6.732 (d, J 7.5 Hz, 1H, ArH); 3.876 (s, 6H, 2xAr-OCH3); 5.560 (s, NH).

13C n.m.r. δ: 169.9 (C=O), 148.8 (C-3), 147.5 (C-4), 131.2 (C-1), 120.4, 111.7, 111.2 (C-2, C-5, C-6), 55.7 (2xAr-OCH3), 40.6 (C-2’), 35.0 (C-1’), 23.1 (CH3).
8.2.1.2. Preparation of 6,7-Dimethoxy-1-methyl-3,4-dihydroisoquinoline (87)

To a mixture of dry toluene (90mL) and N-acetylhomoveratrylamine (112) (15.4g, 69mmol), at 60°C, freshly distilled phosphoryl chloride (18mL) was added dropwise over 30 min. The mixture was refluxed until hydrogen chloride evolution ceased (2.5h), and then was cooled on ice. The toluene was decanted and the crystals washed with light petroleum containing a few drops of toluene. The crystals were dissolved in water (50mL) and the solution cooled on ice. Basification to pH 11 of the mixture with 32% sodium hydroxide (50mL) was undertaken with ice added to maintain a reaction temperature below 30°C. The product was extracted with portions of dichloromethane (3x20mL) which were combined, washed with water (5mL) and dried. The product was collected after evaporation as a yellow powder (10.86g, 53mmol, 76%), which yielded 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline (87) as colourless crystals after recrystallisation from diethyl ether; m.p. 98-100°C.42

M.s. (EI): m/z 205 (M⁺, 100%), 190 (55), 174 (15), 160 (12), 147 (10), 131 (8).

¹H n.m.r. δ: 6.991 (s, 1H, ArH); 6.694 (s, 1H, ArH); 3.923 (s, 6H, 2xAr-OCH₃); 3.662 (t, J 7.6 Hz, 2H, H3); 2.636 (t, J 7.6 Hz, 2H, H4); 2.372 (s, 3H, C-1').

¹³C n.m.r. δ: 163.4 (C-1), 150.6, 147.2 (C-6, C-7), 130.9 (C-8a), 122.3 (C-4a), 110.0, 108.8 (C5, C8), 56.0, 55.8 (2xAr-OCH₃), 46.8 (C-3), 25.6 (C-4), 23.2 (C-1').
8.2.1.3. Preparation of 6,7-Dimethoxy-1-methyl-2-propyl-3,4-dihydroiso-quinolinium Iodide (88)

Excess 1-iodopropane (1.6mL; freshly distilled over anhydrous potassium carbonate) and the finely powdered 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline (87) (1.86g, 9mmol) were placed in a sealed tube. This mixture was heated at 90°C for 24h. The solid was removed from the tube with a small amount of dichloromethane and diethyl ether was added to recrystallise the crude product. This was filtered and washed with diethyl ether to give the iodide salt (88) (2.85g, 7.6mmol, 84%) as a pale yellow powder; m.p. 179-180°C.42

M.s. (ES): m/z 247 (375-HI, 45%), 232 (30), 216 (9), 205 (97), 190 (49), 174 (18), 160 (12), 142 (100).

$^1$H n.m.r. δ: 7.282 (s, 1H, ArH); 6.917 (s, 1H, ArH); 4.112 (t, J 7.6 Hz, 4H, 2CH$_2$); 4.013, 3.916 (s, 6H, 2xAr-OCH$_3$); 3.253 (t, J 7.7 Hz, 2H, H$_4$); 3.032 (s, 3H, CH$_3$); 1.987-1.903 (m, 2H, H-2'); 1.092 (t, J 7.4 Hz, 3H, CH$_3$).

$^{13}$C n.m.r. δ: 173.7 (C-1); 156.3, 148.6 (C-6, C-7); 137.8 (C-8a); 119.7 (C-4a); 112.4, 110.6 (C-5, C-8); 59.2 (C-3); 56.9, 56.8 (2xAr-OCH$_3$); 51.1 (C-1'); 26.1 (C-4), 21.2 (C-2'); 20.3 (CH$_3$); 11.1 (C-3').
8.2.1.4. Preparation of 6,7-dimethoxy-1-methylidene-2-propyl-1,2,3,4-tetrahydro-isoquinoline (89)

A solution of the iodide salt (88) (16.75g, 45mmol) in water (50mL) was basified with 32% sodium hydroxide. This mixture was then extracted rapidly with portions of diethyl ether (3x20mL), the extracts combined, dried and the solvent evaporated to give crude enamine (89) (9.79g, 40mmol, 89%) as a yellow-orange syrup.\(^{42}\)

\textbf{M.s. (EI):} m/z 247 (M\(^+\), 45%), 232 (30), 219 (8), 205 (100), 190 (45), 174 (15), 160 (10), 142 (8).

\textbf{\(^1\)H n.m.r.} \(\delta\): 7.203 (s, 1H, ArH); 6.573 (s, 1H, ArH); 4.30 (bs, integrated less than 2H, C-1’); 3.90 (s, 3H, Ar-OCH\(_3\)); 3.84 (s, 3H, Ar-OCH\(_3\)); 3.23-3.18 (m, 4H, 2CH\(_2\)); 2.80 (t, \(J\) 5.9 Hz, 2H, H4); 1.73-1.60 (m, 2H, C-2’); 0.94 (t, \(J\) 7.4 Hz, 3H, C-3’).

\textbf{\(^{13}\)C n.m.r.} \(\delta\): 148.5 (C-1), 147.2, 146.2 (C-6, C-7), 127.5 (C-8a), 124.8 (C-4a), 110.2, 107.9 (C-5, C-8), 75.3 (C-1’), 55.7, 55.5 (2xAr-OCH\(_3\)), 54.0 (C-3), 47.7 (C1’), 29.8 (C-4), 18.6 (C-2’), 11.5 (C-3’).

8.2.1.5. Preparation of 1-[2-(ethoxycarbonyl)ethyl]-6,7-dimethoxy-2-propyl-3,4-dihydroisoquinolinium bromide (90)

To the crude enamine (89) (9.79g, 40mmol), at 0°C and under nitrogen gas, ethyl bromoacetate (40mL) was added dropwise with stirring. The solution was then heated to 60°C for 30 min. Dry toluene was added and the product
was collected as cream crystals (13.64g, 33mmol, 83%). Recrystallisation from dry ethanol/diethyl ether afforded pure 1-[2-(ethoxycarbonyl)ethyl]-6,7-dimethoxy-2-propyl-3,4-dihydroisoquinolinium bromide (90) as a yellow powder; m.p. 176-178°C. \textsuperscript{42}

\textbf{M.s. (ES):} m/z 334 (M-Br, 100%).

\textsuperscript{1}H n.m.r. \(\delta\): 7.502 (s, ArH); 6.963 (s, ArH); 4.262-4.059 (m, 3CH\(_2\)); 4.011 (s, OCH\(_3\)); 4.098 (s, OCH\(_3\)); 3.752 (t, \(J 7.7\) Hz, H4); 3.235 (t, \(J 7.5\) Hz, H-3); 2.942 (t, \(J 7.1\) Hz, CH\(_2\)); 2.097-1.924 (m, 2H, CH\(_2\)); 1.223 (t, \(J 7.1\) Hz, CH\(_3\)); 1.097 (t, \(J 7.4\) Hz, CH\(_3\)).

\textsuperscript{13}C n.m.r. \(\delta\): 176.0 (C=O), 171.2 (C-1), 156.3, 148.4 (C-6, C-7), 133.7 (C-8a), 118.1 (C-4a), 112.6, 110.8 (C-5, C-8), 61.4 (C-3), 59.2 (C-4''), 57.1, 56.7 (2xAr-OCH\(_3\)), 51.5 (C-1'), 32.7 (C-2''), 26.4 (C-4), 26.0 (C-1''), 21.6 (C2'), 14.0 (C-5'') 11.1 (C-3').

8.2.1.6. Preparation of ethyl 3-[6,7-dimethoxy-2-propyl-1,2,3,4-tetrahydroisoquinolin-1-yl] propanoate (91)

To a stirred solution of the bromoester salt (90) (2.00g, 4.8mmol) in ethanol (15mL) at 0°C, was added sodium borohydride (0.42g) over 1h. This was left overnight at room temperature. The ethanol was then evaporated and the residue dissolved in water (4mL). The solution was extracted with portions of diethyl ether (3x10mL) which were combined, dried with sodium sulphate and the solvent evaporated leaving a clear, pale green syrup (1.54g, 4.6mmol, 95%). This was purified by medium pressure column chromatography to yield pure ethyl 3-[6,7-dimethoxy-2-propyl-1,2,3,4-tetrahydroisoquinolin-1-yl] propanoate (91) as a colourless syrup.

\textbf{M.s. (ES):} m/z 336 (MH\(^+\), 100%).
1H n.m.r. δ: 6.583 (s, 1H, ArH); 6.552 (s, 1H, ArH); 4.127 (q, J 7.1 Hz, 2H, CH2); 3.856 (s, 6H, 2OCH3); 3.544 (t, J 6.5 Hz, 1H, H-1); 3.156-3.108 (m, 1H); 2.801-2.744 (m, 2H); 2.543-2.402 (m, 5H); 2.033-1.949 (m, 2H); 1.535-1.462 (m, 2H, CH2); 1.264 (t, J 7.1 Hz, 3H, CH3); 0.891 (t, J 7.4 Hz, 3H, CH3).

13C n.m.r. δ: 174.3 (C=O), 147.2 (C-6, C-7), 129.9 (C-8a), 126.7 (C-4a), 111.2, 110.5 (C-5, C-8), 63.1 (C-3), 62.5 (C-1), 60.0 (C-4'''), 59.8 (C-1''), 55.9, 55.8 (2xAr-OCH3), 55.4 (C-11), 43.7 (C-4); 31.0 (C-2'''), 23.7 (C-1'''), 21.1 (C-2''), 14.2 (C-5'''), 11.8 (C-3').

### 8.2.1.7. Preparation of 1-[2-(carboxyethyl)]-6,7-dimethoxy-2-propyl-1,2,3,4-tetrahydroisoquinolinium chloride (92)

The ester (91) (1.04g, 3.10mmol) was dissolved in 2M hydrochloric acid (3mL). This solution was stirred at room temperature under nitrogen gas and shielded from light for four days. The solvents were removed in vacuo over 24 h, the crude product collected as a white foam (0.96g, 2.79mmol, 90%). This was recrystallised from dry methanol/diethyl ether to yield pure 1-[2-(carboxyethyl)]-6,7-dimethoxy-2-propyl-1,2,3,4-tetrahydroisoquinolinium chloride (92) as a colourless powder; m.p. 168-170°C.42

M.s. (CI): m/z 308 (M-Cl, 1%).

M.s. (ES): m/z 308 (M-Cl, 100%), 292 (2), 249 (8).

1H n.m.r. δ: 6.804 (s, 1H, ArH); 6.644 (s, 1H, ArH); 4.502 (t, 1H, H1); 3.891, 3.871 (s, 6H, OCH3); 3.752 (m, 1H); 3.51 (m, 1H); 3.031 (m, 4H); 2.854 (m, 1H); 2.697 (m, 2H); 2.123-2.052 (m, 4H); 0.943 (t, J 7.4 Hz, CH3).
$^{13}$C n.m.r. $\delta$: 174.4 (C=O), 149.3, 149.1 (C-6, C-7), 122.0, 121.0 (C-8a, C-4a), 110.1, 110.8 (C-5, C-8), 67.6 (C-3), 64.3 (C-1), 61.7 (C-1'), 56.2, 55.9 (2xAr-OCH$_3$), 42.2 (C-4), 30.8 (C-2''); 21.4 (C-1'') 17.9 (C-2'), 11.1 (C-3').

8.2.1.8. Preparation of 5,6-dimethoxy-1-propyl-2,3,7,8,9,9a-hexahydro-1H-benzo-nicinolin-7-one (66) using oleum

Dry, finely powdered, amino acid salt (92) (0.96g, 2.79mmol) was sprinkled over the surface of stirred oleum (6mL; 20% free sulfur trioxide) at room temperature. The reaction mixture was stirred for a further 5 min. It was then quenched with ice and the solution basified to pH 11 with 32% sodium hydroxide solution. The basic solution was extracted with dichloromethane (3x50mL), the extracts combined, dried and the solvent evaporated to leave 5,6-dimethoxy-1-propyl-2,3,7,8,9,9a-hexahydro-1H-benzo[nicinolin-7-one (66) as a dark green gum (0.61g, 2.12mmol, 74%).

M.s. (CI): m/z 290 (MH$^+$, 100%).

$^1$H n.m.r. $\delta$: 6.842 (s, 1H, ArH); 3.864, 3.852 (s, 6H, 2xAr-OCH$_3$); 3.33 ('apparent doublet', $J$ 11.6 Hz, 1H, H-9a); 3.203-3.157 (m, 1H); 3.111-2.948 (m, 1H); 2.832-2.379 (m, 7H); 1.712-1.562 (m, 3H); 0.936 (t, $J$ 6.8 Hz, CH$_3$).

$^{13}$C n.m.r. $\delta$: 196.9 (C-7); 151.8 (C-6); 147.5 (C-5); 133.4 (C-6a); 129.4 (C-9b); 125.6 (C-3b); 116.7 (C-4); 61.3 (C-9a); 59.7, 55.9 (2xAr-OCH$_3$); 55.1 (C-2); 49.1 (C-1'); 36.4 (C-8); 28.7, 27.3 (C-3, C-9); 19.4 (C-2'); 11.8 (C-3').
8.2.1.9. Attempted cyclisation of (91) with phosphorus pentoxide

A solution of the amino acid (91) (200mg, 0.6mmol) in 1,2-dichloroethane (7mL) was added to a solution of phosphorus pentoxide (426mg, 3mmol) in 1,2-dichloroethane (5mL) at room temperature under nitrogen gas, and the mixture was then stirred for 24 h at room temperature. The reaction mixture was diluted with 3% sodium hydroxide and dichloromethane (80mL). The organic phase was extracted, dried and the solvent evaporated. The residue was chromatographed on silica gel (methanol:dichloromethane, 2:98) to give the ketone (3mg, 0.1mmol, 20%).

8.2.1.10. Attempted cyclisation of (92) with triflic anhydride

A 1.10M solution of triflic anhydride (1.05mL, 1.16mmol) in anhydrous dichloromethane was added over a period of 15 min. to a cooled (ice-water bath) solution of the amino acid salt (92) (100mg, 0.23mmol) and 4-N,N-dimethylaminopyridine (DMAP) (85mg, 0.69mmol) in dichloromethane (6mL). The reaction mixture was left to stir for 16 h while the ice bath was kept in place but no further additions of ice were made. The reaction mixture was then diluted with dichloromethane (10mL), washed with saturated aqueous sodium carbonate (5mL) and then the organic solvent solution dried. The evaporation of the solvent gave products include small amount of the ketone (66), starting material (92) and other derivatives.
8.2.1.11. Preparation of 1,2,9-trimethoxy-6-propyl-4,5,6,6a,7,12-hexahydroisoquinolino[8,1-ab]carbazole (19)

To a solution of the ketone (66) (1.2g, 4.2mmol) in dry ethanol (2.5mL) and glacial acetic acid (3 drops) was added fresh p-methoxyphenylhydrazine (0.36g, 7.5mmol) in dry methanol (2mL). The reaction mixture was refluxed for 2 h under nitrogen and then the solvents evaporated. To the residue was added glacial acetic acid (4mL) and redistilled boron trifluoride etherate (0.5mL). The mixture under nitrogen was brought to reflux as rapidly as possible and heated for 10 min. Ice was added (50g), the solution was basified to pH 11 with 32% sodium hydroxide solution, and the mixture was then extracted with chloroform (3x20mL). The extracts were combined, washed with water and dried. Evaporation of the solvent afforded the crude product as a cream foam (1.05g, 2.6mmol, 64%). Recrystallisation from dichloromethane/light petroleum (40-60°C) afforded 1,2,9-trimethoxy-6-propyl-4,5,6,6a,7,12-hexahydroisoquinolino [8,1-ab]carbazole (19) as tan crystals; m.p. 120-122°C.\textsuperscript{42}

M.s. (Cl): m/z 393 (MH\textsuperscript{+}, 100%).

\textsuperscript{1}H n.m.r. \(\delta\): 9.243, (s, 1H, NH); 7.311, (d, \(J\) 8.8 Hz, 1H, ArH); 7.032, (s, 1H, ArH); 6.858 (d, \(J\) 8.7 Hz, 1H, ArH); 6.519, (s, 1H, H-3); 3.408-3.337, (m, 1H); 3.232-2.942, (m, 3H); 2.671-2.514, (m, 4H); 1.663-1.593, (m, CH\textsubscript{2}); 0.997, (t, \(J\) 7.2 Hz, CH\textsubscript{3}).

\textsuperscript{13}C n.m.r. \(\delta\): 154.2 (C-1), 151.0 (C-2), 142.3 (C-9), 131.5, 131.2 (C11a, C12a), 126.5 (C-7b), 126.2 (C-12c), 121.4 (C-12b), 112.6 (ArH), 112.0 (ArH), 110.6 (ArH), 99.9 (ArH), 60.8 (C-6a), 60.1 (Ar-OCH\textsubscript{3}), 56.0 (CH\textsubscript{2}), 55.9, 55.7 (2xAr-OCH\textsubscript{3}), 47.3 (C-1'), 41.5 (C-5), 29.4 (C-7), 25.2 (C-4), 18.9 (C-2'), 12.1 (C-3').
8.2.1.12. Attempted preparation of the fused indole derivative 1,2,9-trimethoxy-7a-methyl-6-propyl-4,5,6,6a,7,7a-hexahydroisoquinolino[8,1-ab]carbazole (37)

To a solution of the indole (19) (270mg, 0.69mmol) in tetrahydrofuran at -78°C was added a solution of n-butyllithium (430μL, 25% in hexane). The solution was stirred at -78°C for 30 min before iodomethane (43mL) was added. The mixture was allowed to warm to room temperature and then stirred for a further 2.5 h before pouring into water (4mL). The organic phase was extracted with diethyl ether (2x20mL). The extracts were combined, dried and the solvents evaporated. Most of the starting material (19) was recovered (85%), and compound (37) could not be detected (1H-n.m.r.; M.s.).

8.2.1.13. Preparation of 15,6-dimethoxy-1-propyl-7-semicarbazono-2,3,7,8,9,9a-hexahydro-1H-benzo[de]quinoline (94)

Anhydrous sodium ethanoate (180mg, 3.0mmol) was added to a hot solution of semicarbazide hydrogen chloride (180mg, 1.6mmol) in ethanol (20mL). The sodium chloride precipitate which formed was removed by filtration. To the filtrate was added the amino ketone (66) (360mg, 1.2mmol) and this mixture was heated under reflux for 1 h. After cooling, the solid was filtered, washed with cold ethanol and dried to afford, after recrystallisation from methanol, the semicarbazone (94) (180mg, 0.5mmol, 42%) m.p. 188.7-199°C.

M.s. (CI): m/z 347 (MH+, 100%).
M.s. (ES): m/z 347 (MH⁺, 100%), accurate mass 347.2081, C₁₈H₂₇N₄O₃ requires 347.2083.

¹H n.m.r. δ: 9.392 (s, 1H, NH), 6.624 (s, 1H, H-4), 3.849, 3.773 (s, 6H, 2xArO-CH₃), 3.237-2.985 (m, 3H), 2.910-2.335 (m, 7H), 2.041 (s, 2H, NH₂), 1.750-1.280 (m, 3H), 0.995-0.890 (t, 3H, CH₃).

¹³C n.m.r. δ: 159.0 (C=O), 151.9 (C-5), 145.4 (C-6), 143.9 (C-7), 130.3 (C-3a), 128.46 (C-6b), 124.9 (C-6a), 112.5 (C-4), 60.7, 59.6 (2xAr-OCH₃), 55.8 (C-9a), 55.5 (C-8), 48.7 (C-2), 28.5 (C-3), 26.0 (C-1’), 25.0 (C-9), 19.2 (C-2’), 11.9 (C-3’).

8.2.1.14. Preparation of 1,2-dimethoxy-6-propyl-5,6,6a,7-tetrahydro-4'H-benzo[de]thiadiazolo[4,5-g]quinoline (36)

To thionyl chloride (2mL) was added the semicarbazone (94) (40mg, 0.12mmol) portionwise at 0°C with stirring. The mixture was stirred for a further 3 h at 0°C and then kept overnight at room temperature. Dichloromethane (25mL) was added and the mixture was poured with stirring into a cooled solution of sodium carbonate (6g in 25mL of water). The organic phase was separated, dried and the dichloromethane evaporated to give crude product. The crude product was purified via flash column chromatography (silica gel, methanol:dichloromethane 5:95). Recrystallisation from methanol gave 1,2-dimethoxy-6-propyl-5,6,6a,7-tetrahydro-4'H-benzo[de][1,2,3]-thiadiazolo[4,5-g]quinoline (36) (8mg, 0.02mmol, 16%); m.p. 185-186°C.

M.s. (CI): m/z 332 (MH⁺, 100%).

M.s. (ES): m/z 332 (MH⁺, 79%, accurate mass 332.1432, C₁₇H₂₂N₃O₂S requires 332.1433).
**1H n.m.r.** δ: 6.711 (s, 1H, H-4); 3.987, 3.900 (s, 6H, Ar-OCH₃); 3.727 (dd, J₁ 6.0, J₂ 15.0 Hz, 1H, H-6a); 3.56-3.50 (dd, J₁ 3.0, J₂ 15.0 Hz, 1H, one of C-7); 3.24-3.16 (m, 1H, one of C-5); 3.122-3.014 (m, 1H, one of C-5); 2.90-2.647 (m, 2H, CH₂); 2.587-2.430 (m, 2H, CH₂); 1.639-1.543 (m, 2H, C-2’); 0.955 (t, J 6.0 Hz, 3H, C-3’).

**13C n.m.r.** δ: 152.6 (C-10a), 147.7 (C-7a, Ar-OCH₃), 144.2 (Ar-OCH₃), 130.8 (C-10b), 125.2 (C-10c), 121.6 (C-3a), 112.8 (C-3), 61.5 (C-6a), 59.2 (C-7), 56.3, 56.0 (2xAr-OCH₃), 49.4, 29.1 (C-1’, C-3), 26.3 (C-4), 19.4 (C-2’), 12.0 (C-3’).

### 8.2.1.15 Preparation of (7S*,9aR*)-5,6-dimethoxy-7-hydroxy-1-propyl-2,3,7,8,9,9a-hexahydro-1H-benzo[de]quinoline (67)

A 1 M L-selectride solution in tetrahydrofuran (0.7mL, 0.7mmol) was cooled to -78°C under nitrogen gas. A solution of the ketone (66) (150mg, 0.52mmol) in tetrahydrofuran (5mL) was added portionwise maintaining the temperature below 0°C. The mixture was stirred vigorously for 2 h at -78°C and then allowed to warm to room temperature (1 h). The reaction mixture was hydrolysed with water (1mL) and ethanol (4mL) was added. The organoborane was oxidised with 6M sodium hydroxide (3mL) and 30% hydrogen peroxide (4mL). The aqueous phase was saturated with potassium carbonate. The organic phase separated and the aqueous phase was extracted with diethyl ether (3x20mL). The combined organic extracts and organic phase was dried and the solvents evaporated to yield (7S*,9aR*)-5,6-dimethoxy-7-hydroxy-1-propyl-2,3,7,8,9,9a-hexahydro-1H-benzo[de]quinoline (67) (80mg, 0.27mmol, 53%) as colourless crystals; m.p. 52-54°C.

**M.s. (CI):** m/z 292 (MH⁺,100), 174 (MH⁺-H₂O, 95).

**M.s. (ES):** m/z 292 (MH⁺ accurate mass 292.1917, C₁₁H₂₆NO₃ requires 292.1913).
$^1$H n.m.r. $\delta$: 6.61 (s, 1H, H-4); 4.99 (s, 1H, H-7); 3.90, 3.84 (s, 6H, 2xAr-OCH$_3$); 3.29-3.20 (m, 2H, two of C-3); 3.06-3.02 (m, 1H, H-9a); 2.80-2.62 (m, 3H, one of C-1’ and two of C-2); 2.49-2.39 (m, 1H, one of C-1’); 2.24-2.18 (m, 1H, one of C-9); 2.06-2.00 (m, 1H, one of C-9); 1.90-1.70 (m, 2H, two of C-8); 1.73 (s, 1H, OH); 1.60-1.53 (m, 2H, two of C2’); 0.91 (t, 3H, C-3’).

$^{13}$C n.m.r. $\delta$: 150.9, 145.4 (C-5, C-6), 131.8 (C-6a), 130.0, 127.5 (C-3a, C-9b), 112.2 (C-4), 62.7 (C-7), 61.5 (C-3), 61.0 (C-9a), 55.73, 53.61 (2xAr-OCH$_3$), 49.92 (C-2), 29.87 (C-1’), 27.76 (C-8), 22.36 (C-9), 19.43 (C-2’), 12.01 (C-3’).

8.2.2. SYNTHESIS OF TETRABENAZINE (17) DERIVATIVES

8.2.2.1. Reduction of tetrabenazine (17) by sodium borohydride

To a stirred solution of tetrabenazine (17) (500mg, 1.6mmol, in ethanol (20mL) at 0°C, was added sodium borohydride (160mg, 4.0mmol) over 1 h. The reaction was continued for 5 h at room temperature. The ethanol was then evaporated and the residue was dissolved in water (3mL) and extracted with dichloromethane (3x10mL), dried and the solvent evaporated leaving a white solid. 2-hydroxy-3-isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[α]quinolizine was obtained as colourless crystals (350mg, 1.1mmol, 70%) after recrystallisation from diethyl ether; m.p. 150-155°C.

M.s. (CI): m/z 320 (MH$^+$, 100%).

$^1$H n.m.r. $\delta$: 6.660 (s, 1H, H-11); 6.575 (s, 1H, H-8); 4.091 (d, $J$ 6.9 Hz, 1H, H-2); 3.805 (s, 6H, 2xAr-OCH$_3$); 3.686 (s, 1H), 3.561 (d, $J$ 11.7 Hz, 1H, H-11b); 3.212-2.966 (m, 2H); 2.775-2.380 (m, 3H); 2.058-1.605 (m, 4H); 1.338-1.096 (m, 3H, include. OH); 0.926 (t, $J$ 5.4 Hz, 6H, 2xC-3’).
$^{13}$C n.m.r. δ: 147.4, 147.2 (C-10, C-9), 129.3, 126.85 (C-11a, C-7a), 111.44, 107.98 (C-8, C-11), 74.6 (C-2), 60.9, 60.0 (C-4, C-11b), 55.9, 55.8 (2xAr-OCH$_3$), 51.9 (C-6), 41.6 (C-1), 40.5 (C-1'), 39.7 (C-3), 29.1 (C-7), 25.3 (C-2'), 24.1, 21.7 (2xC-3').

8.2.2.2. Preparation of (2$S^*,3R^*,11b R^*$)-2-hydroxy-3-isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizine

An L-selectride solution in tetrahydrofuran (1M, 7ml) was cooled to -78°C under nitrogen gas. A cold (0°C) solution of tetrabenazine (17) (1.8g, 5.6mmol) in tetrahydrofuran (6mL) was then added (maintained temperature below 0°C). The mixture was stirred vigorously for 2 h at -78°C and allowed to warm to room temperature (over 1 h). The reaction mixture was hydrolysed with water (1mL) and ethanol (4mL) was added. The organoborane was oxidised with 6M sodium hydroxide (3mL) and 30% hydrogen peroxide (4mL). The aqueous phase was saturated with potassium carbonate. Organic phase separated and aqueous phase was extracted with diethyl ether (3x20mL), the ether extract dried and the solvent evaporated to give the crude product (57) (1.2g, 3.8mmol, 68%) which yielded (2$S^*,3R^*,11b R^*$)-2-hydroxy-3-isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizine as colourless crystals after recrystallisation from diethyl ether; m.p. 156-158°C.

M.s. (CI): m/z 320 (MH$^+$, 100%), 302 (MH$^+$-H$_2$O, 25)

M.s. (ES): m/z 320 (MH$^+$, 100%, accurate mass 320.2221, C$_{19}$H$_{30}$NO$_3$ requires 320.2226).

$^1$H n.m.r. δ: 6.636 (s, 1H, H-11); 6.545 (s, 1H, H-8); 4.045 (s, 1H, C-2); 3.805 (s, 6H, 2xOCH$_3$); 3.462 (d, J 11.5 Hz, 1H, H-11b); 3.079 (m, 1H, one of C-7); 2.942-2.901 (m, 1H, one of C6); 2.661-2.511 (m, 3H, one of C-4, one of C-6 and one of C-7); 2.408-
2.352 (m, 2H, one of C-4 and one of C-1); 1.950 (d, \( J = 6.0 \) Hz, 1H, H-3); 1.694-1660 (m, 3H, H-2', OH and one of C1); 1.261-1.112 (ddd, \( J = 9.0 \), \( J_2 = J_3 = 6.0 \) Hz, 2H, two of C1'); 0.895 (t, \( J = 6.0 \) Hz, 6H, 2xCH3).

\[ ^{13}C \text{n.m.r.} \delta: 147.27, 147.02 \text{ (C-9, C-10)}, 130.00, 126.85 \text{ (C-7a, C-11a)}, 111.44, 107.98 \text{ (C-8, C-11)}, 67.89 \text{ (C-2)}, 56.32, 56.26 \text{ (C-4, C-11b)}, 55.90, 55.74 \text{ (2xAr-OCH3)}, 52.40 \text{ (C-6)}, 39.14 \text{ (C-1)}, 38.79 \text{ (C-1')}, 37.71 \text{ (C-3)}, 29.13 \text{ (C-7)}, 24.77 \text{ (C-2')}, 22.88, 22.86 \text{ (2xC-3')}.

### 8.2.2.3. Preparation of the methiodide salt of (57)

A solution of the alcohol (57) (100mg, 0.3mmol) in dry acetone (5mL) was treated with iodometahane (150µmL) and allowed to stand at room temperature for 1 h. The precipitate which had formed was collected and recrystallised from methanol-diethyl ether to afford colourless needles of the methiodide salt derivative of (57) (130mg, 0.28mmol, 93%); m.p. 255-255.5°C. The \(^1H\)- and \(^{13}C\)-n.m.r. spectra were done in dimethyl-d6 sulfoxide solution.

\[ ^1H \text{n.m.r.} \delta: 6.999 \text{ (s, 1H, ArH)}; 6.914 \text{ (s, 1H, ArH)}; 4.850 \text{ (dd, \( J = 12.6 \) Hz, 1H); 4.590-4.454 \text{ (m, 1H)}; 4.082 \text{ (s, 1H)}; 3.865, 3.848 \text{ (s, 6H, 2xAr-OCH3)}; 3.775-3.637 \text{ (m, 2H)}; 3.390 \text{ (s, 3H, N-CH3)}; 3.312-3.160 \text{ (m, 2H)}; 2.654-2.234 \text{ (m, 2H)}; 1.892-1.744 \text{ (m, 3H)}; 1.568-1.428 \text{ (m, 2H)}; 1.242-1.116 \text{ (m, 1H)}; 0.964 \text{ (dd, \( J = 2.4 \), 6.3 Hz, 6H, 2xC-3')}.

\[ ^{13}C \text{n.m.r.} \delta: 148.6, 147.8 \text{ (C-9, C-10)}, 123.9, 120.3 \text{ (C-7a, C-11a)}, 111.7, 110.0 \text{ (2xAH)}, 62.7, 62.3, 55.5, 49.7, 49.0, 48.6, 37.1, 23.8, 22.8, 22.4, 10.3 \text{ (2xC-3')}.
8.2.2.4. Preparation of (3R*,11bR*)-2-oxo-3-isobutyl-9,10-dihydroxy-1,3,4,6,7,11b-hexahydro-2H-benzo[α]quinolizine

Boron tribromide (6.0mL, 1M, 6.0mmol) was added to a solution of tetrabenazine (17) (220mg, 0.7mmol) in dry dichloromethane (3 ml) under nitrogen, at -78°C. The solution was allowed to stir for 6 h at this temperature. It was then cooled in an ice bath and a saturated aqueous solution of sodium bicarbonate was added (pH=7.5). The resulting mixture was extracted with dichloromethane (40mL) and ethyl acetate (2x30mL). The combined organic extracts were dried, evaporated and flash chromatographed (silica gel; dichloromethane:methanol, 98:2) to give (3R*,11bR*)-2-oxo-3-isobutyl-9,10-dihydroxy-1,3,4,6,7,11b-hexahydro-2H-benzo[α]quinolizine (59) as a colourless powder (120mg, 0.4mmol, 57%); m.p. 186-188°C.

**M.s. (ES):** m/z. 290 (MH⁺, 100%, accurate mass 290.1770, C₁₇H₂₄N⁰₃ requires 290.1756).

**¹H n.m.r. δ:** 6.806 (s, 1H, H-8); 6.604 (s, 1H, H-11); 4.460 (d, J 11.7 Hz, 1H, C-11a); 3.900 (s, 1H); 3.602-3.052 (m, 3H); 2.744 (d, J 12 Hz, 1H); 2.459-2.223 (m, 2H); 2.072-2.029 (m, 1H); 1.748-1.440 (m, 2H); 1.358-1.250 (m, 2H); 1.148-1.036 (m, 2H); 0.910 (t, J 6.6 Hz, 6H, 2xC-3’).

**¹³C n.m.r. δ:** 164.9 (C-2), 147.1, 146.7 (C-9, C-10), 130.7, 127.8 (C-7a, C-11a), 117.9 (C-11), 114.6 (C-8), 65.0 (C-11b), 64.0 (C-7), 52.8 (C-6), 49.9 (C-4), 49.6 (C-1), 37.8 (C-3), 31.7 (C-2’), 28.0 (C-1’), 25.3, 24.4 (2xC-3’).
8.2.2.5. Preparation of \((3R^*,11bR^*)\)-2-oxo-3-isobutyl-1,3,4,6,7,12b-hexahydro-2H-
[1,3]benzo-dioxolo[5,6,\(\alpha\)]quinolizine (58)

A mixture of water (5mL), dibromomethane (0.35mL, 3mmol) and adogen 464 (8.9mg, 20\(\mu\)mol) was vigorously
stirred and heated to reflux under nitrogen gas. A solution of (59) (580mg, 2mmol) and sodium hydroxide (2.0g, 50mmol) in water was added at such a rate that the addition was completed after two hours. After that, the reaction mixture was stirred and refluxed for a further hour. Dibromomethane was distilled and the product was extracted with chloroform (20mL), diethyl ether (40mL) and dichloromethane (20mL). The product was separated by flash column chromatography (silica gel; methanol:dichloromethane, 2:98). The methylenedioxy compound (58) was collected as a solid after evaporation of the solvent (110mg, 0.36mmol, 18%).

M.s. (ES): \(m/z\) 302 (MH\(^+\), 100%, accurate mass 302.1766, \(\text{C}_{18}\text{H}_{24}\text{N}_{2}\text{O}_{3}\) requires 302.1756).

\(^1\text{H} \text{n.m.r.} \delta: 6.690 (s, 1H, Ar-H); 6.665 (s, 1H, Ar-H); 5.910 (s, 2H, metdioxy); 3.454 (d, \(J\ 12\ Hz,\ 1H\)); 3.254 (dd, \(J\ 6.0,\ 11.0\ Hz,\ 3.160-2.880\) (m, 3H); 2.820 (dd, \(J\ 3.0,\ 13.2\ Hz,\ 1H\)); 2.782-2.426 (m, H); 2.344 (t, \(J\ 11.7\ Hz,\ 1H\)); 1.854-1.452 (m, 3H); 1.052-0.950 (m, 1H); 0.886 (q, \(J\ 3.9,\ 6.6,\ 6H,\ 2x\text{C}-3'\)).

\(^{13}\text{C} \text{n.m.r.} \delta: 146.3, 146.1 (C-9, C-10), 129.5, 127.1 (C-7a, C11a), 108.4, 104.9, 100.8, 62.7, 61.3, 50.4, 47.4, 47.3, 35.0, 29.7, 25.3, 23.1, 22.0.
8.3. DOCKING (CHAPTER 4)

Throughout this part of the study all molecular modeling work was performed on a Silicon Graphics Indy workstation using a Biosym software of Molecular Simulations, Inc., specifically the following modules: INSIGHT II, BUILDER, DISCOVER and docking, under IRIX 5.2 system. The CVFF force field was used throughout.

8.3.1 INITIAL RECEPTOR MODELS

The initial receptor models $\alpha_{1A}$ and $\alpha_{1B}$-AR were obtained from Dr. Peter Riek, Victor Chang Cardiac Research Institute, Sydney (see Chapter 4).

The adrenaline and $\alpha_{1B}$-AR complex was obtained by manually docking the protonated agonist into the binding site of the minimized structure of the $\alpha_{1B}$-AR carrying the aspartate D125 in its protonated form. The proposed binding pocket for the $\alpha_{1B}$-AR is the space between helices 3, 4, 5 and 6 including the side chains, aspartate D125, serine S207 and phenylalanine F310$^{11,12,99}$. The binding site of the $\alpha_{1A}$-AR model was also discussed in the earlier chapters 2 and 4. The other ligand-receptor complexes were obtained by manually positioning the protonated ligand into the binding pockets and keeping the positively charged nitrogen atom within 2 to 4 Å of the negatively charged aspartate side chain. The complexes then were energy minimized. Minimisations were repeated several times, with different initial orientations of both the ligand and the receptor side chains to optimise the interactions known to be important by the site-directed mutagenesis experiments.$^{11,12,99}$ Before minimisation the backbone of the receptor was constrained using the DISCOVER module, starting with an initial
minimisation iteration based on 100 steps of steepest descents, followed by 2000 steps of conjugate gradients iteration, until the maximum derivative was less than 0.001 kcal mol\(^{-1}\).

Intercation energies (IE) were calculated using the “DOCKING” module and distortion energies of the receptors (ER) and the ligands (EL) were also calculated in order to estimate the binding energies (BE) by using the following equation:\(^24\)

\[
BE = IE + ER + EL
\]

Correlation between the binding energies and the experimental binding affinities was drawn and graphs were presented for each subtype.

8.3.2. REVISION OF THE MODELS

The helical packing of the receptors was regenerated starting from a common topography for the amino acids in the most conserved positions superimposing onto the Baldwin’s \(\alpha\)-carbon template of rhodopsin.\(^17\) The initial models were energy minimized by the same method as noted in Section 8.3.1 but with constrains only the hydrogen bonds in the backbones by using the generic distance command. In this way, the helices were allowed to move together with the side chains of the amino acids.

The previous docking procedures were used to obtain the ligand-receptor complex structures and the calculated binding energies presented in the tables.

8.3.3. DOCKING DERIVED PHARMACOPHORES

The IQC-receptor complexes were prepared by creating a subset including a zone within 9 Å distances from the C7 of IQC (19). This subset was further analysed to identify
atoms of IQC involved in interactions with the receptor. The cartesian coordinates of
these atoms and pseudo atoms for the ring centres were noted. Atoms of the receptor
close to IQC (19) were also identified to produce the excluded volumes.

These coordinates were used to edit a template command file (see Table 8.1) to
translate into catalyst pharmacophores (hypotheses). In the command file, all features
(chemical groups) were described by giving 3D coordinates, size and feature.

```
$CATALYST_GOODIES/hypoedit \
-dict $CATALYST_CONF/Dictionary.chm \
-feat HYDROPHOBIC \ 
-feat HYDROPHOBIC \ 
-feat HYDROPHOBIC \ 
-feat "HB DONOR" \ 
-feat "HB ACCEPTOR" \ 
-blob 100.0 100.0 100.0 150.0 1 \ 
-blob 200.0 200.0 200.0 150.0 2 \ 
-blob 300.0 300.0 300.0 150.0 3 \ 
-blob 400.0 400.0 400.0 150.0 4 \ 
-blob 500.0 500.0 500.0 200.0 5 \ 
-blob 600.0 600.0 600.0 150.0 6 \ 
-blob 700.0 700.0 700.0 200.0 7 \ 
-xvol 800.0 800.0 800.0 250.0 \ 
-xvol 900.0 900.0 900.0 250.0 \ 
-xvol 1000.0 1000.0 1000.0 250.0 \ 
-xvol 1100.0 1100.0 1100.0 250.0 \ 
crystalQuery.chm
```

Table 8.1. The template of the hypothesis command file.

<table>
<thead>
<tr>
<th>feat= feature, blob= sphere, xvol= excluded volume</th>
</tr>
</thead>
</table>

| for blobs and xvols the first three numbers are for the 3D coordinates, the fourth number is the size |
| the last number is the feature number. H-bond donor, acceptor and aromatic rings have two |
| blobs. The last line is the hypothesis name. The file is case and space sensitive. All the |
| measurements are in Å. |

The template of the hypothesis command file will produce a simple hypothesis
including 5 features (3 hydrophobic, 1 H-bond donor and 1 H-bond acceptor) and 4
excluded volumes.
8.4. PHARMACOLOGICAL TESTS (CHAPTER 6)

COS-1 membranes were prepared as described in the literature.\textsuperscript{115} The cDNAs encoding the human $\alpha_1$-adrenergic receptors were subcloned into the mammalian expression vector pMT2\textsuperscript{+}.\textsuperscript{115} Plasmid DNA was used to transfect cells. Transient expression in COS-1 cells was accomplished by the diethylaminoethane-dextran method.\textsuperscript{116} The ligand binding characteristics of the expressed receptors were determined in a series of radioligand binding studies using the $\alpha_1$-antagonist radioligand $[^{125}\text{I}]$HEAT as described previously.\textsuperscript{115} Nonspecific binding was determined in the presence of $10^4$M phentolamine. Binding data were analysed by the iterative curve-fitting program LIGAND.

8.5. MOLECULAR ELECTROSTATIC POTENTIAL MAPPING (CHAPTER 6)

This particular molecular modeling work was performed on a Silicon Graphics Indy workstation using Spartan SGI version 4.0.3. GL software, under IRIX 5.2 system. Quantum mechanics ($ab$ \textit{initio}) theory was used throughout this study.

The ligands were built and optimised by using an $ab$ \textit{initio} calculation at the Hartree-Fock level with the minimal STO-3G basis set. The electron density of the molecules have also been calculated in three dimensions generating the surface (0.002 electron/au\textsuperscript{3}) and electrostatic potential (-10 kcal/mol) of each point was then colour coded onto this surface from the most negative (red) to the most positive (blue).
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