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Pharmacophore development for antagonists at α_1 adrenergic receptor subtypes

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Summary

Many receptors, including α_1 adrenergic receptors, have a range of subtypes. This offers possibilities for the development of highly selective antagonists with potentially fewer detrimental effects. Antagonists developed for α_{1A} receptors, for example, would have potential in the treatment of benign prostatic hyperplasia. As part of the molecular design process, structural features necessary for the selective affinity for α_{1A} and α_{1B} adrenergic receptors have been investigated. The molecular modelling software (particularly the Apex module) of Molecular Simulations, Inc. was used to develop pharmacophore models for these two subtypes. Low-energy conformations of a set of known antagonists were used as input, together with a classification of the receptor affinity data. The biophores proposed by the program were evaluated and pharmacophores were proposed. The pharmacophore models were validated by testing the fit of known antagonists, not included in the training set. The critical structural feature for selectivity between the α_{1A} and α_{1B} adrenergic receptor sites is the distance between the basic nitrogen atom and the centre of an aromatic ring system. This will be exploited in the design and synthesis of structurally new selective antagonists for these sites.

Introduction

Receptors of the α_1 adrenergic subtype are members of the G-protein-coupled receptor (GPCR) superfamily. At least three distinct α_1 receptor subtypes have been reported on the basis of both pharmacology and molecular biology [1–6]. The GPCRs are integral membrane proteins and contain seven hydrophobic domains which are believed to be arranged in seven membrane spanning helices [7–9]. These helices are responsible for passing signals across the cell membrane after interaction with their ligand on the extracellular side. The binding of the ligand elicits a response felt by the G-protein on the intracellular side. The activated G-protein then sets a second messenger system in train. Different GPCRs recognize different ligands. For adrenergic receptors, the activating ligands are norepinephrine and epinephrine. There are, however, only a few different types of G-proteins, each of which is used by many different receptors and to initiate a specific type of intracellular signalling. In addition, each receptor type may use more than one G-protein for signalling, and recent studies have indicated that stimulation of different α_1 adrenergic receptor subtypes, for example, can lead to different physiological effects, e.g. a decrease in the propensity for abnormal heart rhythms (α_{1B} subtype [10]) versus promotion of arrhythmias and automaticity during myocardial ischaemia and the development of benign prostatic hyperplasia (α_{1A} subtype [11]). Therefore, the development of α_1 subtype specific drugs to either block or enhance receptor function would be important for disorders thought to be related to one subtype only, such as benign prostatic hyperplasia.

In order to design such ligands, structural information on the receptor subtypes, especially their binding sites, would be invaluable. There is, however, no X-ray structure available for any GPCRs. Many GPCR transmembrane domains have been modelled on the basis of the known structure of bacteriorhodopsin, but these models are only qualitative in nature and are still under development [7–9]. From these models, which also take into

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TABLE 1
AFFINITY ESTIMATE (pK_i) VALUES OF α_1 ADRENERGIC ANTAGONISTS

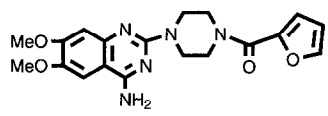
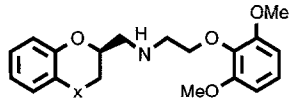
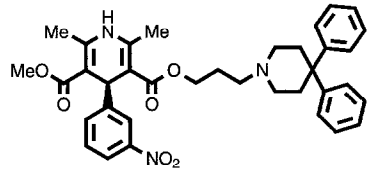
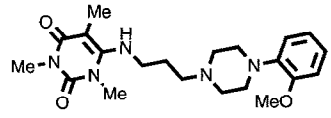
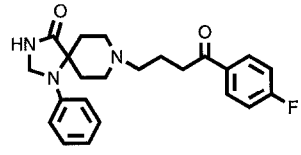
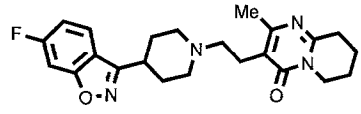
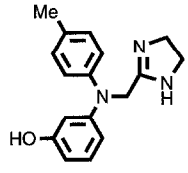
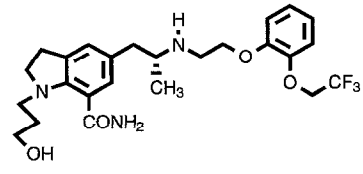
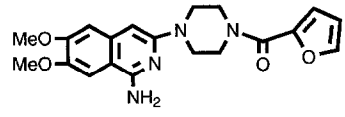
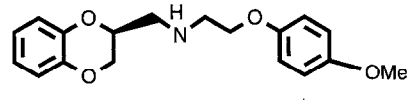
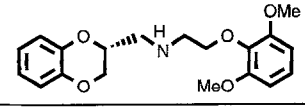
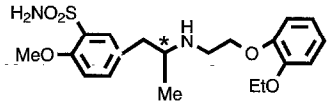
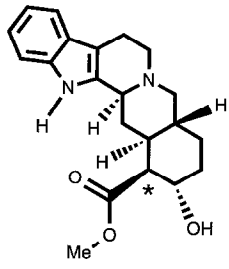
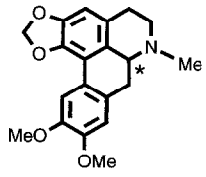
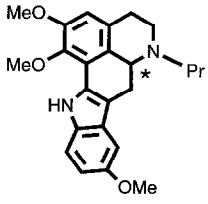
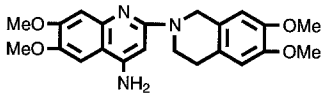
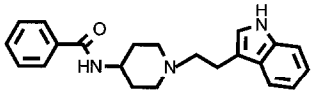
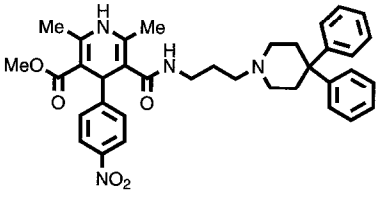
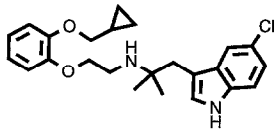
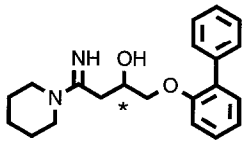
Antagonists			α_{1A}	pK_i	α_{1B}	Reference
1		Prazosin	10.1		10.2	2
2		X = O, (*S*)-WB4101	10.1		8.8	2
3		X = S, (*S*)-Benoxathian	9.7		8.4	2
4		(+)-Niguldipine	9.6		7.6	2
5		5-Methyluropidil	9.2		7.6	2
6		Spiperone	8.1		9.3	2
7		Risperidone	6.6 8.6		8.6 8.1	26 23
8		Phentolamine	8.8		7.5	2
9		KMD-3213	10.4		7.7	27
10		Inactive prazosin analogue	No adrenergic activity			22
11		Inactive WB4101 analogue		3.9 for α_1		17
12		(*R*)-WB4101 analogue		7.4 for α_1		28

TABLE 1
(continued)

Antagonists			α_{1A}	pK_i	α_{1B}	Reference
13		(*R*)-YM-12617	10.5		9.2	29
14		(*S*)-YM-12617	8.4		7.0	19
		YM-617 = Tamsulosin				
15		Corynanthine	6.8		6.3	30
16		(*S*)-(+)-Dicentrine		8.3 for α_1		25
17		Racemic IQC		8.6 for α_1		31
18		Abanoquil	10.4		10.1	11
19		Indoramin	8.4		7.4	11
20		SNAP 5089	9.7		6.7	32
21		RS 17053	9.3		7.8	33
22		AH 11110A	5.6		7.1	24

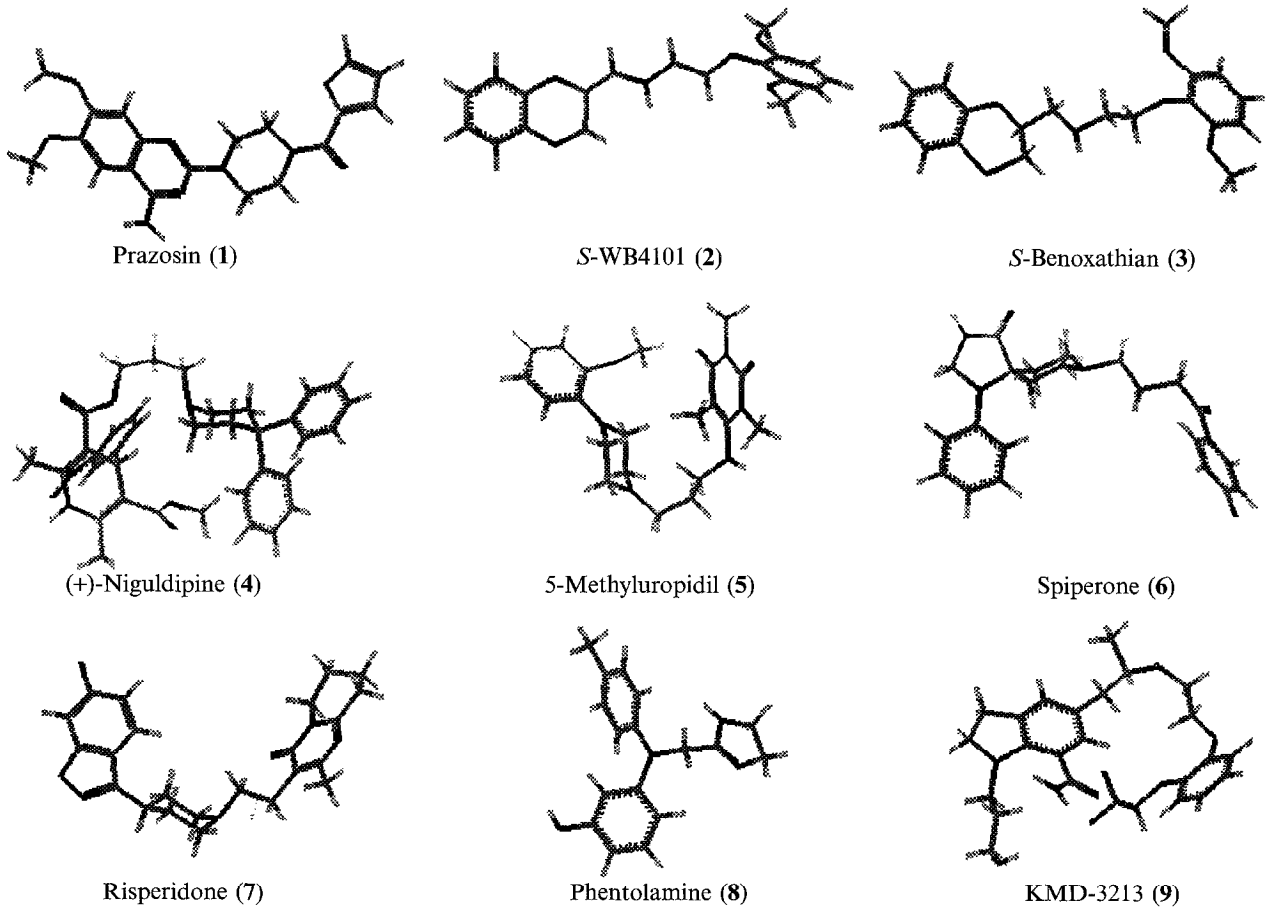


Fig. 1. Global minimum structures for antagonists **1–9**, calculated as described in the text. Light grey: hydrogens; grey: carbons; black: heteroatoms.

account mutagenesis and other experimental data, it is believed that the agonist binding pocket is located in the transmembrane domain near the extracellular face and that an aspartate side chain in the third transmembrane helix binds the hydrogen of the protonated amine of both agonists and antagonists. Close to this residue is also a large hydrophobic pocket containing conserved aromatic and serine/cysteine residues believed to bind the catechol ring of the agonists.

Another commonly adopted approach to drug design [12,13] relies on establishing the structure–activity relationships of a number of known ligands of a receptor in order to develop a pharmacophore, i.e. the essential molecular fragment which selectively recognizes the binding site. This is the approach adopted here using the Apex-3D software package by Molecular Simulations, Inc. (formerly Biosym Technologies, San Diego, CA, U.S.A.). This program 'employs advanced statistical techniques and 3D pattern-matching algorithms to draw its own generalizations from existing chemical data' [14].

There have been attempts in the literature to develop a pharmacophore for the α_1 adrenergic receptors for both agonists and antagonists [15–19], but no mention has been made of the specific structural requirements for affinity to the various α_1 subtypes. Table 1 shows a variety of antagonists and their affinities for the two subtypes considered here.

This paper reports studies directed towards defining antagonist pharmacophores for the α_{1A} and α_{1B} adrenergic receptors.

Methods

The molecular modelling software of Molecular Simulations, Inc. was used, specifically the following modules: INSIGHT II, BUILDER, DISCOVER, Search-Compare and Apex-3D. The CVFF force field was used throughout.

Antagonists **1–9** from Table 1 were built and their geometry was optimized. The ligand molecules were then further investigated by using dynamics simulations (900

TABLE 2
ACTIVITY CLASSES USED IN APEX-3D

Class name	α_{1A} condition (pK_i)	α_{1B} condition (pK_i)
Very active	> 9	> 8
Active	> 8 and < 9	> 6 and < 8
Inactive	< 8	< 6

K, time = 20 ps, time step = 1.0 fs, sample conformation taken every 2 ps). The 10 conformations sampled were minimized again (until the rms derivative was smaller than 0.001 kcal/Å). The final 10 conformations chosen were all within a 10 kcal/mol energy window with respect to the global minimum.

The global minimum conformations so determined are depicted in Fig. 1.

The protonated forms of the nine antagonists (**1**–**9** in Table 1) were also investigated in the same way and two inactive derivatives of (*S*)-WB4101 and prazosin were also considered (**10** and **11** in Table 1). The inactive ligands were chosen from previous modelling studies on α_1 adrenoceptor antagonists [17,18] and were included to assist the program to induce features which are probably not related to biological activity [14]. The global minimum conformations for compounds **10** and **11** and for the test compounds were determined as above. For selected antagonists, structure basis sets containing more than 10 conformations per molecule were also prepared by systematic conformational searching in the following way.

An optimized conformation was used and the bonds around which systematic rotations were to be performed were identified (bonds in rings cannot be treated in this way). The increment for the rotations was selected (usually 60° or 90°) in such a way as to obtain an estimate of about a few hundred sterically allowed conformers. These were then minimized (until the rms derivative was smaller than 0.001 kcal/Å) and duplicates removed. This resulted in about 50–100 conformers for the molecules chosen, again all conformations were within 10 kcal/mol from the lowest conformation, which in all cases coincided with the one previously determined (by dynamics).

The Apex-3D software [14,20,21] takes as input a number of molecules which bind to the same receptor site (or are presumed to do so) and a classification of each molecule according to its activity. The thresholds used for this classification are based on the pK_i values in Table 1 and are listed in Table 2. The program then identifies common functional groups (descriptor centres) for these molecules. The descriptor centres can be quantitative, such as electron donor and acceptor reactivity indices (these are calculated using the semiempirical MOPAC method), or they can qualitatively describe an atom (heteroatom, hydrogen-bond donor or acceptor, etc.). Descriptor centres can also refer to pseudoatoms such as ring centroids. Because the bioactive conformation of the ligands is not generally known, a set of representative conformations is generated for each molecule as described above, and the property and distance matrices are calculated for each conformer. The next step is then a search for common 3D arrangements of the descriptor centres identified. The inductive inference procedures used are based on similarity (common structural patterns in different compounds with similar affinity), dissimilarity (different structural patterns in compounds with different affinity) and concomitant variations (variations in structural patterns in compounds with varying affinity). This approach 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 common 3D arrangements of varying numbers of common descriptor centres. These have been called 'biophores' [14,21]. The program provides statistical filtering options for these biophores, but further evaluation proved necessary to be able to propose pharmacophore models.

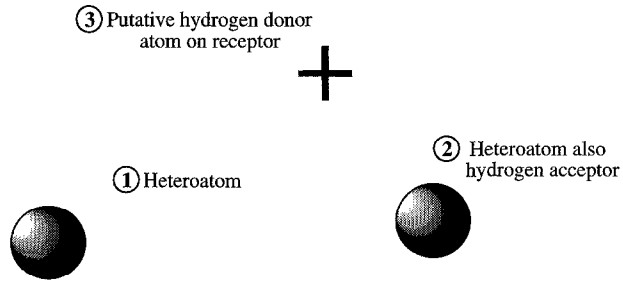


Fig. 2. Independent descriptor centres. A hypothetical biophore is shown which would be listed as containing three descriptor centres: a heteroatom (1), a heteroatom (2) and a hydrogen acceptor (2). Together with a hydrogen acceptor atom, the program always shows the approximate position of the hydrogen donor atom on the receptor (3). Since two of the three descriptor centres are connected to the same atom, they are not independent and this biophore is viewed as containing only two descriptor centres.

Results and Discussion

Inclusive pharmacophores

As a first attempt, 10 conformations each of all nine antagonists and the two inactive molecules (compounds **1**–**11** in Table 1) were used together with the classification data from Table 2. Because the binding conformation of the antagonists at the receptors is not known and cannot be assumed to coincide with the global minimum conformation, a number of minimum-energy conformations of different geometries need to be taken into consideration for each molecule. This was done by using 10 conformations within an energy window of 10 kcal/mol (see also the Restricted pharmacophores section).

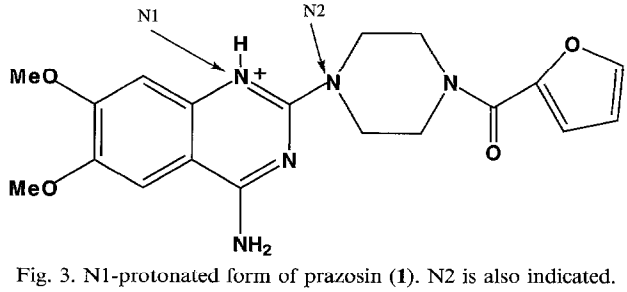


Fig. 3. N1-protonated form of prazosin (**1**). N2 is also indicated.

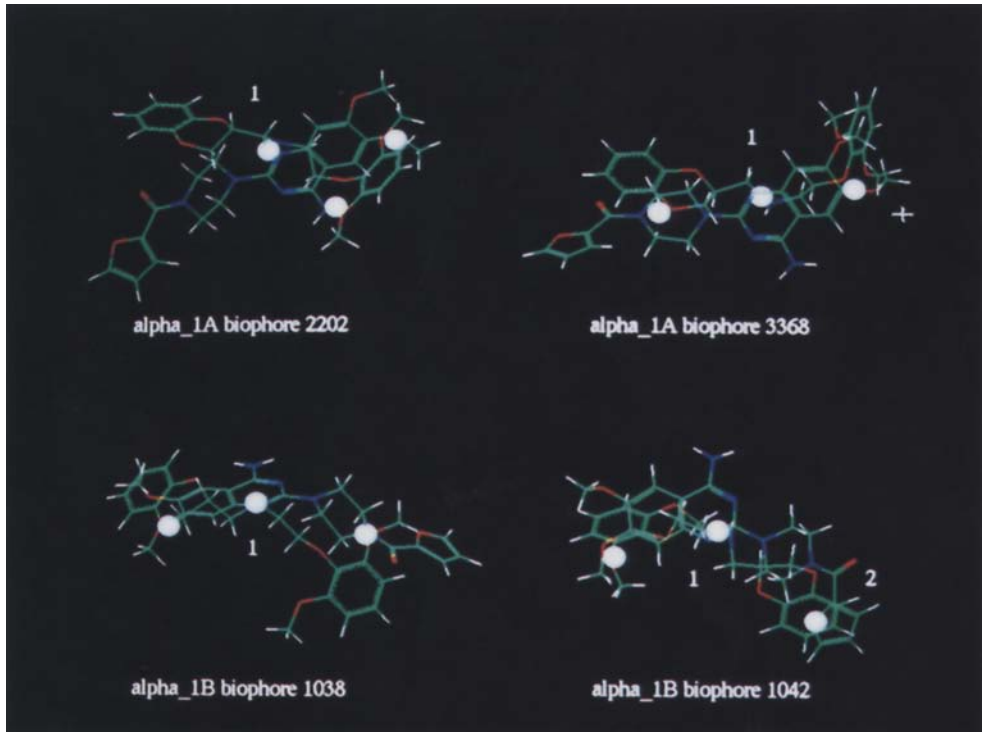


Fig. 4. Inclusive biophores for the α_{1A} and α_{1B} adrenergic receptor subtypes. For clarity, only two molecules are shown for each biophore: prazosin (**1**) and WB4101 (**2**). Green: carbon; white: hydrogen; red: oxygen; blue: nitrogen. The white spheres depict the descriptor centres for each biophore, the number 1 next to a descriptor centre denotes a basic nitrogen common to prazosin and WB4101, and the number 2 similarly denotes the centre of an aromatic ring. White crosses are connected with heteroatom descriptor centres that can function as hydrogen-bond donors; the cross denotes the estimated position of the hydrogen donor on the receptor.

Apex produced hundreds of biophores for each subtype from this input, even when only the biophores connected to the class of very active ligands were considered, and it was clearly necessary to use the automatic filtering the software provides in order to reduce this number. The filters selected were:

for the α_{1A} subtype – $p \geq 0.6$, size ≥ 3 and active ≥ 5;

for the α_{1B} subtype – $p \geq 0.6$, size ≥ 3 and active ≥ 4.

This means that the probability (p) that a novel compound possessing the biophore under consideration will be a 'very active' compound is only moderate. p is defined as follows:

$$p = (m_{rk} + 1)/(m_r + 2)$$

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. Only biophores with at least three descriptor centres were considered (size ≥ 3) and the biophores were also required to contain at least five (α_{1A}) or four (α_{1B}) very active molecules (active ≥ 5 or 4).

This still left a large number of biophores (87 in the case of the α_{1A} subtype). These were now further edited by manually removing all biophores containing one of the two inactive molecules (risperidone (**7**) was classified as inactive for the α_{1A} subtype, but biophores containing this ligand were not discarded at this stage, because in comparison with the truly inactive analogues **10** and **11**, **7** shows some affinity for the α_{1A} receptor site) and also any biophores with less than three independent descriptor centres (see Fig. 2). The next step was to check if the biophores conformed to the idea that the most basic nitrogen atom in each molecule will be the one to be protonated most easily and therefore also the one to interact with the highly conserved aspartate residue in the third transmembrane helix of the receptor. For prazosin (**1**), this nitrogen has been determined both experimentally and theoretically [22]; we also confirmed that the most stable protonated form of prazosin is the one shown in Fig. 3, where the hydrogen ion is attached to N1.

Therefore, a further evaluation criterion for the biophores was the requirement that the N1 of prazosin be one of the descriptor centres and that the nitrogen in WB4101 (**2**) be located at the same spot.

This left only two biophores for each subtype, which are depicted in Fig. 4. Only one of these four biophores (1038) contains an aromatic ring centre as a descriptor. This has been proposed as a pharmacophore element by De Marinis et al. [15]. None of the biophores has the most basic nitrogens of all molecules aligned in the same spot as would have to be the case for a credible pharma-

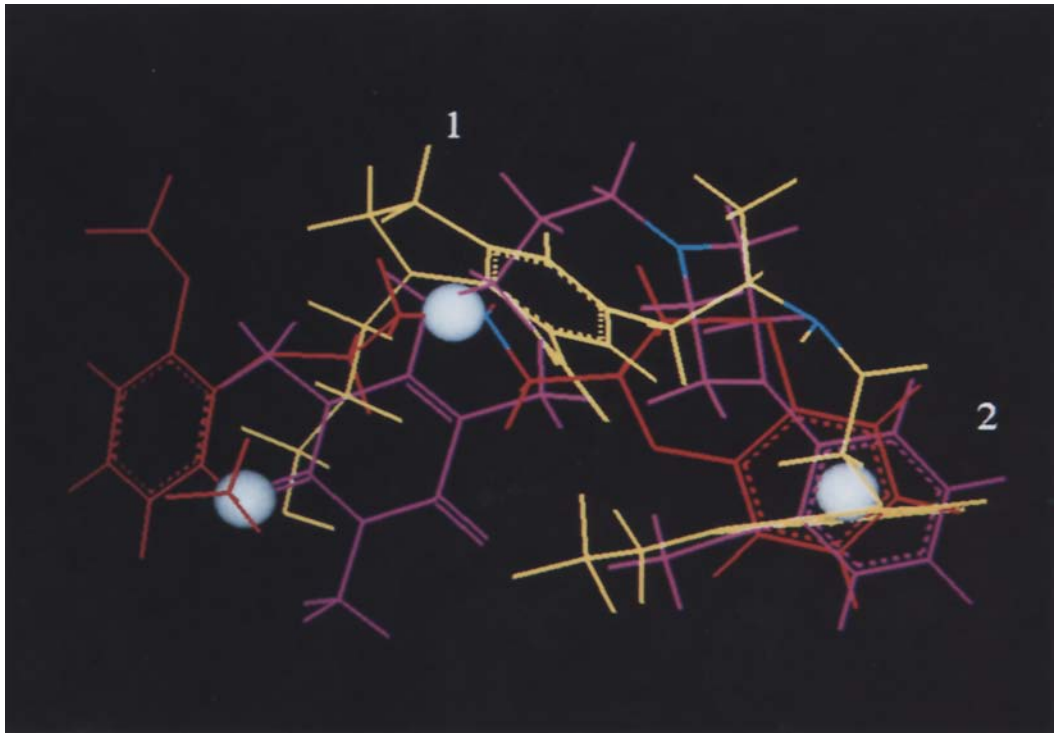


Fig. 5. α_{1A} inclusive biophore 1042 (α_{1B}) with 5-methyluropidil (in purple), benoxathian (in red) and KMD-3213 (in yellow). The most basic nitrogen atom of each compound is shown in light blue, illustrating the fact that these atoms do not overlap in the biophore.

cophore for a bioamine receptor (see Fig. 5 for an example). This problem is further illustrated by a discussion of the pharmacophore for α_1 adrenergic receptor antagonists (no subtype specified) as proposed by De Marinis et al. [15], which suffers from the same problem. To make prazosin fit their proposed pharmacophore, the authors align it so that the nitrogen in the piperazine ring (N2 in Fig. 3) is the basic nitrogen used for interaction with the

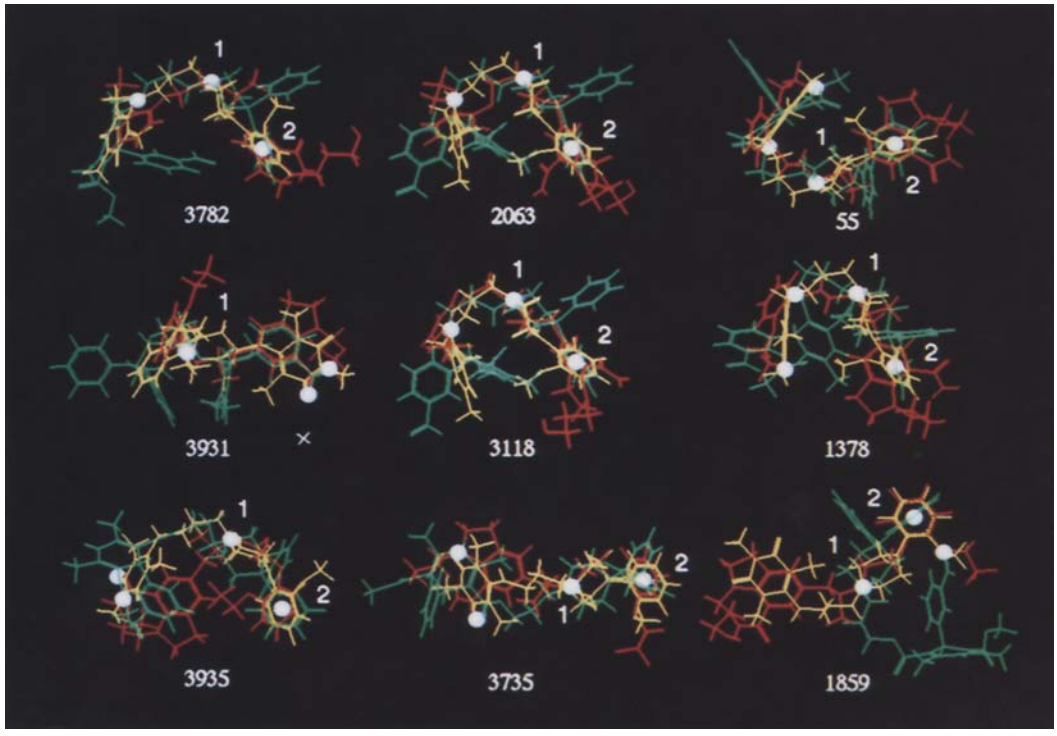


Fig. 6. Restricted biophores for the α_{1A} subtype. KMD-3213 (**9**): red; 5-methyluropidil (**5**): yellow; niguldipine (**4**): green. Symbols as in Fig. 4.

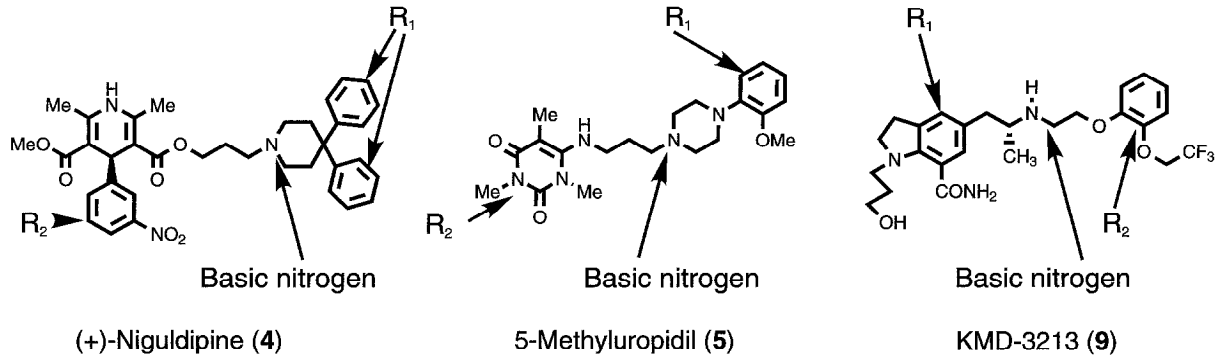


Fig. 7. Chemical structures of **4**, **5** and **9**, with various important structural features highlighted.

receptor. They point out that 'only protonation at the 2-amino group gives a proton-aromatic distance and an N-H to aromatic plane angle that resembles those found in the structural classes previously discussed'. From previous studies it is quite unlikely that this nitrogen is protonated. De Benedetti et al. [22] estimated that the protonation energy difference for protonation at N1 and N2 is about 20 kcal/mol. The very high (but subtype-unselective) affinity of prazosin for α_1 adrenergic receptors cannot be explained by such a pharmacophore which necessitates the involvement of a very much less favourable protonated form. The protonated forms of the antagonists were also investigated, but the results are not reported here because of the problem of intramolecular hydrogen bonding when looking at isolated molecules (gas phase). These interactions heavily biased the conformations towards unrealistically folded ones where the hydrogen on the nitrogen would not be available for interaction with the receptor. Apex does not allow one to use solvated molecules in its structure base, but the study of solvated protonated bases will still be undertaken with the aim of finding reasonable conformations for treatment with the Apex module.

Restricted pharmacophores

Because of the problems outlined above when trying to include all nine antagonists and the two inactive molecules in the training set, a different approach was taken whereby only the most selective and very active antagonists for each subtype were used to try and develop a pharmacophore, but a larger number of conformations were utilized for each molecule chosen.

Results for the α_{1A} subtype

Eighty-nine conformations of KMD-3213 (**9**) and 99 conformations each of 5-methyluropidil (**5**) and niguldipine (**4**) were used for this task. The probability was chosen to be ≥ 0.8 and all the most basic nitrogens were required to be an element in the biophores and to coincide as well. This left nine biophores, all except one (3931) having an aromatic ring as a descriptor centre as well (Fig. 6). The distances between these two critical descriptors are very similar in all eight biophores (5.2–5.8 Å) and also agree well with the pharmacophore proposed by De Marinis. 5-Methyluropidil and niguldipine are always aligned in the same way in the proposed biophores (Fig. 7): aromatic centres R1 overlapping. In KMD-3213, both possible aromatic rings (R1 and R2 in Fig. 7) have been used in the alignments. For seven of the biophores in Fig. 6, there is a third (and sometimes fourth) descriptor centre at the other end of the molecules for a heteroatom associated with the second aromatic (or unsaturated six-membered ring) centre. This is also in agreement with the pharmacophore of De Marinis.

The pharmacophore this suggests for antagonists binding to this receptor is sketched in Fig. 8. It consists of an aromatic ring centre 5.2–5.8 Å away from the basic nitrogen and, on the other side of the molecule, 6–8 Å away from the basic nitrogen, of an aromatic or six-membered unsaturated ring with polar substituents, and it is essentially the same as the pharmacophore proposed by De Marinis et al.

Results for the α_{1B} subtype

Forty conformations of risperidone (**7**) and 42 conformations of spiperone (**6**) were used for this task. The probability was chosen to be ≥ 0.75, the number of descriptor centres was ≥ 3 and all the most basic nitrogens were required to be an element in the biophores and to coincide as well. This left 12 biophores, all but one of which (138) contain an aromatic ring centre. Figure 9 shows nine of these biophores (the remaining three bio-

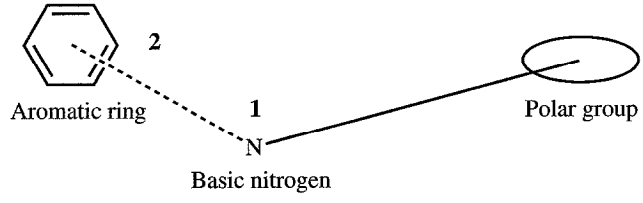


Fig. 8. Pharmacophore model for α_{1A} adrenergic receptor antagonists. Numbers as in Fig. 4.

TABLE 3
DISTANCES BETWEEN THE BASIC NITROGEN AND TWO RING CENTRES FOR SELECTED α_1 ADRENERGIC ANTAGONISTS

Antagonist	N-aromatic centre (Å)	N-ring with polar groups (Å)
α_{1A} pharmacophore	5.2–5.8	5.2–6.7
α_{1B} pharmacophore	6.2–7.8	5.2–7.5
Prazosin	2.7 or 7.6–8.8	2.7 or 7.6–8.8
Phentolamine	3.0	3.7
WB4101	5.4–6.3 or 4.1–6.2	5.4–6.3 or 4.1–6.2
(+)-Niguldipine	4.9–5.8 or 4.2–10.3	4.3–8.6 or 5.8 or 4.2–10.3
5-Methyluropidil	5.7	5.2–7.8 or 5.7
KMD-3213	4.6–5.2 or 4.0–6.4	4.0–6.4 or 4.6–5.2
Risperidone	6.3–7.0 or 5.6	6.3–7 or 5.6 or 4.1–5.2
Spiperone	5.8–6.1 or 4.9–7.8	5.8–6.1 or 4.9–7.8 or 4.3
Benoxathian	5.5–6.2 or 5.3–6.3	5.5–6.2 or 5.3–6.3
Corynanthine	5.7	3.9
YM-617	4.5–6.4 or 4.1–5.2	4.5–6.4 or 4.1–5.2
Indoramin	4.7–6.1 or 7.5	4.2–5.1
Abanoquil	6.3 or 2.7	6.3 or 2.7
RS 17053	4.5–6.5 or 5.3–6.3	4.5–6.5 or 5.3–6.3
AH 11110A	3.7–8.8 or 5.0–7.9	3.7–3.8
IQC	3.9 or 5.0 or 6.6	3.9 or 5.0 or 6.6
Dicentrine	3.9 or 5.3	3.9 or 5.3

phores are repeats of one of those nine). The distances between the basic nitrogen and the aromatic ring centre are less well defined than in the α_{1A} subtype, but are definitely longer (6.2–7.8 Å). The greater variability arises from the fact that different alignments are possible for the two molecules, depending on which aromatic centre is chosen (Fig. 10). Most of the time, R1(risperidone) overlaps R1(spiperone) and mostly the two F atoms on these rings overlap too. For most of the biophores in Fig. 9, there is a third (and sometimes fourth) descriptor centre at the other end of the molecules for a heteroatom associated with the second aromatic (or unsaturated six-membered ring) centre. The pharmacophore this suggests for antagonists binding to this receptor is sketched in Fig. 11. It is quite different to the α_1 pharmacophore proposed by De Marinis et al. This can be rationalized by the fact that the α_1 antagonists used in their study are (where such data are available, see Table 1) more effective ligands at the α_{1A} receptor subtype and therefore, when they consider an α_1 receptor, they really are looking at an α_{1A} receptor.

Validation of pharmacophores

To test the validity of the proposed pharmacophores depicted in Figs. 8 and 11, the relevant distances were measured in a series of other molecules known to bind to the two receptor subtypes. The results are shown in Table 3; the chemical structures and references for the compounds are in Table 1. Two distances are shown in Table 3 for each compound. In the second column, the distances between the most basic nitrogen atom and the centre of each aromatic ring are listed (some compounds such as prazosin have two or more aromatic rings and, therefore, two distances appear here, separated by 'or'). In the last column of Table 3 are the distances between the nitrogen and the centre of any ring which could be considered to be polar. The distances are shown as the ranges encountered in a number of low-energy conformations calculated for each molecule using molecular dynamics as described in the Methods section. The relevant distances for the two pharmacophores proposed here are also included in the table. Two conclusions can be drawn from these results:

(1) Prazosin and phentolamine do not fit either of the proposed pharmacophores and may bind to a different receptor site.

(2) All antagonists apart from 5-methyluropidil, corynanthine and dicentrine fit both the proposed pharmacophores, as is required by the fact that all of them show activity at both receptor subtypes.

Comparing Figs. 8 and 11, one can conclude that nonselective antagonists like (*S*)-WB4101, indoramin and YM-617 can fit both receptor types by simply reversing their orientation. At the α_{1A} receptor site, the benzodioxane side of WB4101, for example, would function as the required aromatic ring and the phenyl ring substituted with three oxygens would be the second unsaturated ring that is required to be substituted by polar groups. At the α_{1B} receptor site, the phenyl ring would function as the aromatic ring and the benzodioxane system as the polar ring (this arrangement is not as favoured, probably because of the lower polarity of the benzodioxane system, which would explain the almost 20-fold preference of WB4101 for the α_{1A} site). It is difficult, however, to explain why the WB4101 analogue **11** is inactive.

The preferential binding of spiperone, risperidone and AH 11110A to the α_{1B} subtype can be rationalized as fol-

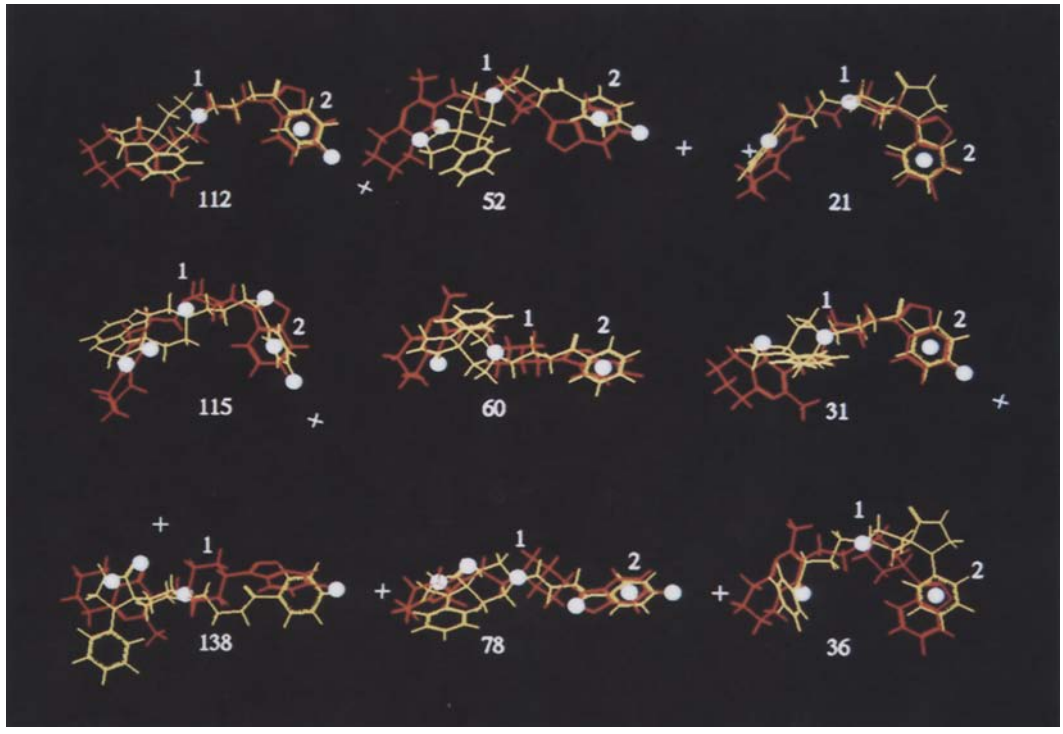
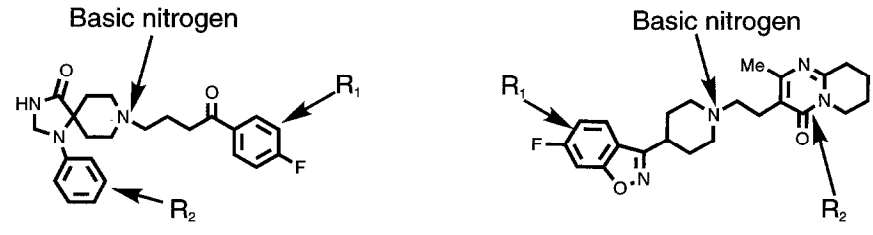


Fig. 9. Restricted biophores for the α_{1B} subtype. Risperidone (**7**): red; spiperone (**6**): yellow. Symbols as in Fig. 4.

lows. At the α_{1A} site, risperidone would have to use the aromatic five-membered heterocycle as the aromatic centre, which is apparently not as favourable as using a larger six-membered aromatic ring. Therefore, the binding of risperidone to the α_{1A} site is much reduced. Recently, the selectivity of risperidone has been questioned [23] on the basis of results with recombinant receptors. The compound is, however, still listed in a very recent review [5] as an α_{1B}-selective agent.

Compound AH 11110A has only very recently been reported as an α_{1B}-selective antagonist [24]. As would be expected from its moderate selectivity, it fits both pharmacophores, with the imine nitrogen being the most basic site. In most of its low-energy conformations, the aromatic rings are too far from the imine nitrogen to fit comfortably on the α_{1A} site. It has fairly low affinity ($pK_i = 7.1$ for α_{1B}), which may be due to the fact that it does not really have a polar group attached to the end of the molecule away from the aromatic ring. The fluoro-substituted aromatic ring of spiperone is too far away in most low-energy conformations to comfortably fit on the α_{1A} site.

Similarly, the preferential binding of KMD-3213, 5-methyluropidil, RS 17053, niguldipine and the very closely related SNAP 5089 to the α_{1A} subtype can be rationalized as follows. The reduced α_{1B} activity of niguldipine can be explained by the fact that this molecule has only one polar end, and the phenyl rings at the other end are not substituted by the required polar groups. The pharmacophore is still not refined enough to account for the much reduced affinity of SNAP 5089 to the α_{1B} site, making this compound much more α_{1A} selective than niguldipine. 5-Methyluropidil, on the other hand, has only one aromatic ring, which does not fit very well on the α_{1B} site. This compound may utilize the pyrimidine dione ring as an 'aromatic' ring when binding to an α_{1B}



Spiperone (**6**) Risperidone (**7**)

Fig. 10. Chemical structures of **6** and **7**, with various important structural features highlighted.

site. The two aromatic rings of KMD-3213 are not far enough from the nitrogen to fit comfortably on the α_{1B} site and, in most of the conformations encountered for RS 17053, both aromatic rings are too close to the nitrogen to fit the α_{1B} site either.

The differences in selectivity for the (*R*) and (*S*) forms of WB4101 and YM-617 have not yet been scrutinized. Abanoquil appears to be more closely related to prazosin than to the other types of antagonists. Corynanthine was used by De Marinis et al. [15] for the development of their pharmacophore and will be discussed in detail later, together with IQC and dicentrine.

Results for a prazosin–WB4101 study

It was decided to study the prazosin problem more closely by building a task consisting of 99 conformations of prazosin and 99 conformations of WB4101. Filtering the resulting biophores using $p \geq 0.75$ and size ≥ 3 resulted in 58 biophores. Requiring the N1 of prazosin to be an element of the biophores reduced that number to nine biophores. Only three of those nine biophores also contained the nitrogen of WB4101 and only one of them had both nitrogen atoms coinciding (11 in Fig. 12). This biophore did not include an aromatic ring as a descriptor centre and covered only a part of each molecule. This finding confirms the suggestion put forward above that prazosin binds to a different site at the receptors under consideration here than do the other antagonists such as WB4101.

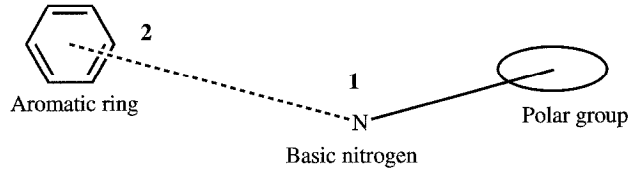


Fig. 11. Pharmacophore model for α_{1B} adrenergic receptor antagonists. Numbers as in Fig. 4.

Comparison of the proposed pharmacophores with the pharmacophore proposed for agonists at bioamine receptors

De Marinis et al. [15] reviewed the structure–activity relationships of α_1 adrenergic receptor agonists of the phenethylamine type and concluded that 'The dominant factors in the correlation of structure and activity for all phenethylamines have been embodied in the Easson–Stedman hypothesis...'. They also point out, however, that this hypothesis 'does not correctly predict the effects observed with the imidazolines or the 2-aminotetralins'.

A common feature of both types of agonists is, however, a 'two-point mode of attachment involving only the phenyl ring and one nitrogen'. Figure 13 shows this common pharmacophore for α_1 receptor agonists and it can be seen that the distance between the phenyl ring and the nitrogen is the same as that required by the proposed pharmacophore for α_{1A} antagonists, but quite different to the proposed pharmacophore for α_{1B} antagonists.

It therefore appears that the α_{1A} antagonists make use of the same two points of attachment as used by all the

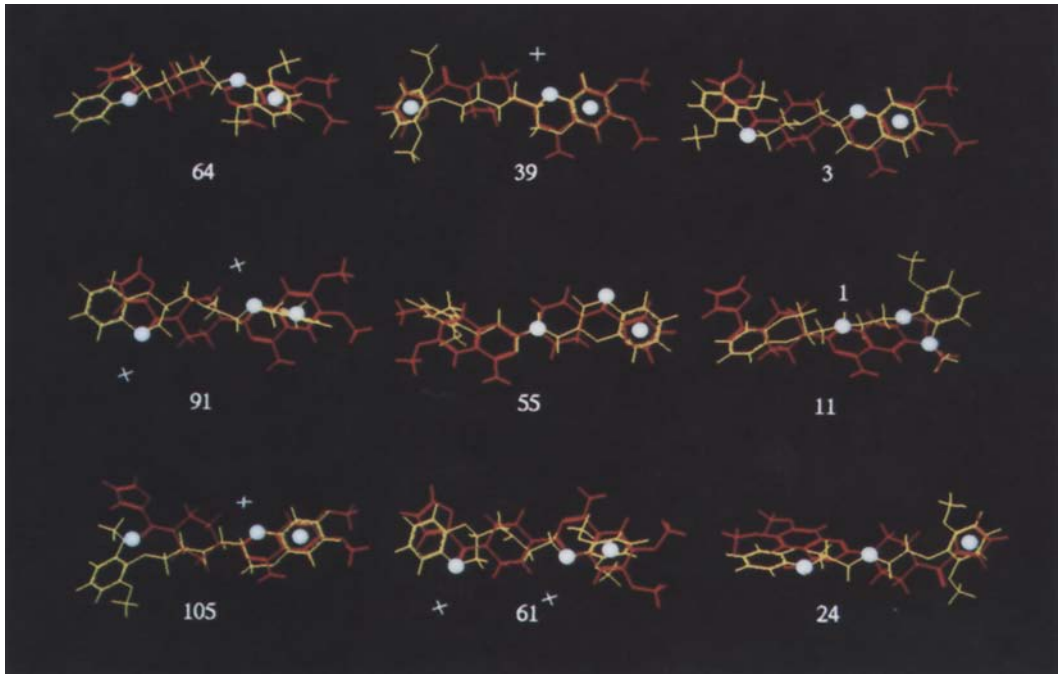


Fig. 12. Nine biophores from the prazosin–WB4101 study. Prazosin (**1**): red; WB4101 (**2**): yellow. Symbols as in Fig. 4.

agonists and then extend further to a pocket accommodating their polar rings. The α_{1B} antagonists and prazosin, on the other hand, use only the common aspartate point of attachment and extend to other as yet unidentified regions of the receptor.

Both the proposed antagonist pharmacophores are also in qualitative agreement with the modelling studies undertaken by Trumpp-Kallmeyer et al. and others [8,9] on the α_2 adrenergic receptor agonist binding pocket as described in the Introduction section. These studies do not provide any distances between the hydrophobic pocket for aromatic rings and the aspartate residue to bind the hydrogen on the basic nitrogen, but they do suggest the importance of the basic nitrogen close to an aromatic ring system.

Design of more selective antagonists

From the above considerations, it can be summarized that the ideal α_{1A} antagonist should have (i) a basic nitrogen that is accessible and can easily be protonated at physiological pH; (ii) a six-membered aromatic ring at a distance of 5.2–5.8 Å from the nitrogen atom; and (iii) a (preferably nonaromatic) ring substituted with polar groups at a distance of 6–8 Å from the nitrogen atom.

On the other hand, an ideal α_{1B} antagonist should have (i) a basic nitrogen that is accessible and can easily be protonated at physiological pH; (ii) a six-membered aromatic ring at a distance of 6.2–7.8 Å from the nitrogen atom; and (iii) a (preferably nonaromatic) ring substituted with polar groups at a distance of 5–6 Å from the nitrogen atom.

Conformationally restricted α_1 antagonists

Conformationally constrained molecules can be used as probe molecules and to simplify the problem of finding a pharmacophore. De Marinis et al. used this approach in the development of their pharmacophore and it will be used here to design and synthesize potentially more subtype-selective α_1 antagonists based on the IQC (reduced isoquinolino[8,1-*ab*]carbazole derivative, **17**) and dicentrine (**16**) molecules. One analogue, where the distance between the nitrogen and the aromatic ring is too short for the α_{1B} site, is currently being synthesized, and analogues where this critical distance is too long to fit the α_{1A} site are being designed.

Dicentrine and IQC show very similar affinity for α_1 sites, whereas corynanthine is considerably less active. In all the three cases, the distance between the polar ring centre and the basic nitrogen is 3.9 Å, which is much shorter than the ones proposed in Figs. 8 (6–8 Å) and 11 (5–6 Å). This is, however, in agreement with the findings of De Marinis that the polar group(s) at the other end of the molecule relative to the aromatic ring is (are) much less sterically sensitive.

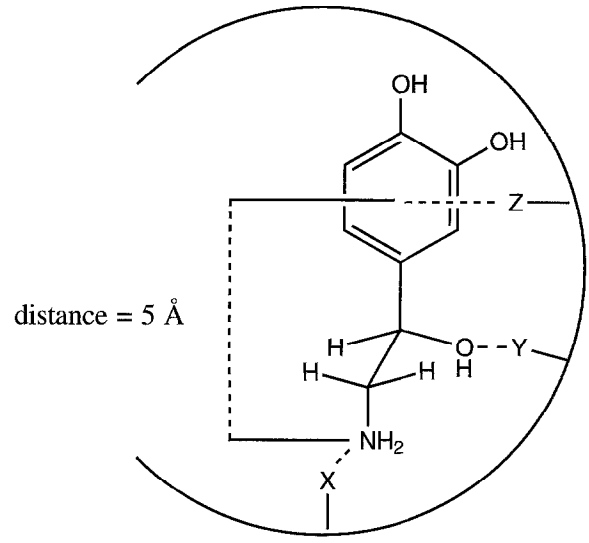


Fig. 13. Schematic representation of the Easson–Stedman hypothesis.

So far, the 'pharmacophore angle' between the two distance vectors in each proposed pharmacophore has not been studied in detail. This angle can vary in the flexible ligands such as WB4101, whereas it is fixed for the three constrained molecules. In this context, it is interesting to note that a much more acute angle, such as in IQC and dicentrine, is associated with higher affinity compared to corynanthine.

Dicentrine itself should be selective for the α_{1A} subtype, because it has no aromatic ring far enough from the nitrogen to be able to fit into the α_{1B} site comfortably. This has not been tested yet. Yu et al. [25] tested dicentrine in human prostate tissue, which contains predominantly the α_{1A} receptor subtype [11]. Similarly, corynanthine should be selective for the α_{1A} subtype, but this is not so. It has, however, much lower affinities for both subtypes than any other compound classified as active in this study. IQC would need to use the five-membered indolic ring to fit on the α_{1A} site and it is therefore predicted that this molecule should show some selectivity for the α_{1B} subtype.

Conclusions

Pharmacophores for antagonists were developed for the α_{1A} and α_{1B} adrenergic receptor subtypes. The distance between the basic nitrogen atom and the aromatic ring centre was found to be the critical feature for distinguishing selective antagonists for the two subtypes, and this will be exploited in the design and synthesis of structurally new selective antagonists. These pharmacophores describe the structural features necessary for the binding of selective antagonists, such as KMD-3213, niguldipine, 5-methyluropidil, risperidone and spiperone. They also explain the binding of nonselective antagonists, such as WB4101, benoxathian, YM-617 and others. Further re-

finement of these pharmacophores is required, however, to account for the differences in binding of the optical isomers of WB4101, YM-617 and others and to explore the requirements of the polar groups in greater depth.

The nonselective, but strongly binding, antagonist prazosin does not conform to either of the proposed pharmacophores and it is suggested that this ligand may utilize a different binding site which is common to both subtypes. The pharmacophore for prazosin and related compounds will be investigated in the future.

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