J Comput Aided Mol Des (2013) 27:305–319
DOI 10.1007/s10822-013-9647-5

5-HT_{1A} receptor pharmacophores to screen for off-target activity of α_1-adrenoceptor antagonists

Tony Ngo · Timothy J. Nicholas · Junli Chen · Angela M. Finch · Renate Griffith

Received: 23 January 2013 / Accepted: 13 April 2013 / Published online: 27 April 2013

Abstract The α_1-adrenoceptors (α_1-ARs), in particular the α_{1A}-AR subtype, are current therapeutic targets of choice for the treatment of urogenital conditions, such as benign prostatic hyperplasia (BPH). Due to the similarity between the transmembrane domains of the α_1-AR subtypes, and the serotonin receptor subtype 1A (5-HT_{1A}-R), currently used α_1-AR subtype-selective drugs to treat BPH display considerable off-target affinity for the 5-HT_{1A}-R, leading to side effects. We describe the construction and validation of pharmacophores for 5-HT_{1A}-R agonists and antagonists. Through the structural diversity of the training sets used in their development, these pharmacophores define the properties of a compound needed to bind to 5-HT_{1A} receptors. Using these and previously published pharmacophores in virtual screening and profiling, we have identified unique chemical compounds (hits) that fit the requirements to bind to our target, the α_{1A}-AR, selectively over the off-target, the 5-HT_{1A}-R. Selected hits have been obtained and their affinities for α_{1A}-AR, α_{1B}-AR and 5-HT_{1A}-R determined in radioligand binding assays, using membrane preparations which contain human receptors expressed individually. Three of the tested hits demonstrate statistically significant selectivity for α_{1A}-AR over 5-HT_{1A}-R. All seven tested hits bind to α_{1A}-AR, with two compounds displaying K_i values below 1 μM, and a further two K_i values of around 10 μM. The insights and knowledge gained through the development of the new 5-HT_{1A}-R pharmacophores will greatly aid in the design and synthesis of derivatives of our lead compound, and allow the generation of more efficacious and selective ligands.

Keywords Pharmacophore · 5-HT_{1A} receptor · α_1-Adrenergic receptors · Drug selectivity · Radioligand binding · GPCR

Introduction

The monoamine receptors belong to the G protein-coupled receptor (GPCR) superfamily. Members of this class of receptors were originally classified based on their affinity for endogenous monoamine ligands, such as norepinephrine, serotonin and dopamine, in addition to their systemic distribution [1]. These receptors function via the transmission of an extracellular stimulus through their seven transmembrane helices to intracellular loops that then facilitate the activation of signalling proteins [2]. Collectively, they play a crucial role in the physiology of the cardio-pulmonary, renal, urogenital, gastrointestinal and central nervous systems [3–6]. Pharmacologically, this makes these receptors targets for the treatment of a number of pathological conditions including hypertension, benign prostatic hyperplasia (BPH), prostate cancer, schizophrenia and depression [7–12]. However, owing to the similarity in structure of their endogenous ligands, the binding pockets of these receptors are highly similar, with members across the three families sharing up to 50 % amino acid sequence identity [13–15]. Thus many clinically used drugs unintentionally exhibit high affinity for other monoamine receptors outside of their intended therapeutic target [16]. Due to the wide systemic distribution of these receptors,

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this can result in a number of side effects, dependent on the drug's off-target receptor interactions.

The α_1-adrenoceptors (α_1-ARs) respond to the biogenic amines norepinephrine and epinephrine [17]. Three α_1-AR subtypes have been identified with the α_{1A} subtype expressed predominately in prostate and urethral smooth muscle [18], whilst the α_{1B} subtype is predominately located in the smooth muscle of skeletal and mesenteric arteries of older men [19]. Knowledge of the localization of the α_{1D} subtype is currently limited due to its low level of cell surface expression and a limited number of selective small molecule ligands capable of activating or antagonizing this receptor [20]. The α_{1A} subtype is the current therapeutic target of choice for the treatment of BPH. Selective antagonism of this receptor has been demonstrated to improve urine flow rate, and relieve lower urinary tract symptoms associated with this disease [9, 21]. In addition, antagonism of the α_{1B}-ARs reduces arterial and venous vasoconstriction and consequently decreases peripheral resistance, lowering arterial blood pressure in hypertensive patients [11].

The difficulty in developing a ligand with α_1-AR subtype selective properties is compounded by the similarity between the transmembrane domains of the α_1-ARs, and the serotonin receptor subtype 1A (5-HT_{1A}-R) [14]. It has been demonstrated that the two most prevalently used α_1-AR selective drugs for the treatment of BPH (tamsulosin and naftopidil) show no selectivity between their intended target (the α_{1A}-AR) and the 5-HT_{1A}-R [22, 23]. Furthermore, several other α_1-AR subtype selective compounds such as BMY7378, which is selective for the α_{1D}-AR over the α_{1A} and α_{1B}-AR, have been demonstrated to have no selectivity for the α_{1D}-AR over the 5-HT_{1A}-R [14].

The 5-HT_{1A}-R acts primarily as a presynaptic autoreceptor, regulating neurotransmitter release across the synaptic junction [24]. Interference with its actions is known to deregulate sleep patterns, cause anxiety like behaviour and interrupt neural tone to the iris sphincter muscle [25–27]. Systemic interference with 5-HT_{1A}-R signalling may also lead to sexual dysfunction and inhibited bladder control [28]. Thus it represents an important off-target to avoid in the design of next generation α_1-AR ligands.

There is at present no crystal structure for any of the α_1-ARs or the 5-HT_{1A}-R, thus identifying differences between these receptors that may be manipulated in order to design a more α_1-AR selective drug is limited to ligand-based structure–activity studies and in silico methodologies such as pharmacophore modelling. We have previously developed pharmacophores for selective antagonists at the α_1-AR subtypes [29], these will be used in the current work and will be referred to as "α_{1X}-AR pharmacophore" (X can be A, B, or D) for short. We have also demonstrated their usefulness for retrieving novel compounds with micromolar α_{1A}-AR affinities and some subtype selectivity through in silico database screening [30]. Current 5-HT_{1A}-R pharmacophores are restricted in multiple aspects of their design. Training sets are limited to derivatives of only one class of chemical compounds, typically arylpiperazine based ligands [31–33], thus limiting their predictive power for other classes of compounds that may show 5-HT_{1A}-R affinity. Additionally, no pharmacophores for the α_1-ARs or 5-HT_{1A}-R at present have been designed to include areas that a ligand cannot occupy for steric reasons (excluded volumes).

Therefore our aim is to construct 5-HT_{1A}-R pharmacophore models that will identify multiple classes of chemical compounds with 5-HT_{1A}-R affinity. Secondly, we aim to validate the predictive power of these pharmacophores by determining in vitro the 5-HT_{1A}-R, α_{1A}-AR and α_{1B}-AR affinities of novel compounds selected from in silico screening and profiling with these pharmacophores.

Materials and methods

Materials

Test compounds were obtained from the Developmental Therapeutics Program of the National Cancer Institute (USA) (compounds (**48**), (**51**)–(**57**)) and Tripos, Inc. (England) (compounds (**49**) and (**50**)). All test compounds were solubilized in dimethyl sulfoxide (DMSO) at 10 mM concentration and stored at −80 °C. 5-methylurapidil, BMY7378 hydrochloride, phentolamine methanesulfonate and serotonin hydrochloride were purchased from Sigma-Aldrich (St. Louis, Missouri). Tamsulosin hydrochloride was supplied by Tocris Bioscience (Bristol, UK). [^{3}H]-OH-DPAT (226 Ci mmol^{-1}) was purchased from GE Healthcare (Uppsala, Sweden) and [^{3}H]prazosin (85 Ci mmol^{-1}) from Perkin Elmer (Waltham, Massachusetts). Chemicals used in buffered solutions were from Ajax Finechem (Cheltenham, Australia) and were of the highest purity available.

Pharmacophore modelling

Training sets for the two pharmacophore models were derived from published reports containing 5-HT_{1A}-R agonists or 5-HT_{1A}-R antagonists. Compounds were chosen from studies where K_i values were obtained from radioligand competition assays using human recombinant receptors expressed in cell lines. All compounds were built using ChemBioDraw Ultra version 12 (CambridgeSoft, www.cambridgesoft.com) and imported into Discovery Studio Client (DS) versions 2–3.5 (Accelrys Inc., San Diego, CA, USA), or sketched directly in DS. Conformers of each

compound were generated as described earlier [29] in DS using the 'BEST' conformational search and a 20 kcal/mol energy threshold as recommended for pharmacophore development [34].

Hypotheses were generated using the HypoGen algorithm, which allows a maximum of five different features in pharmacophore generation. The features chosen were hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), general hydrophobic (HY), hydrophobic aromatic (Har) and the positive ionisable feature (PI). The latter describes a feature such as a basic nitrogen, likely to be protonated at physiological pH, and thus capable of forming an ionic interaction. For each training set ten hypotheses were generated with and without considering excluded volumes.

Ligand mapping and database screening

Test compounds were mapped to the resulting best pharmacophores following conformer generation as described above. In order to attain a predicted affinity value, each compound was allowed to miss one pharmacophore feature in its mapping.

The NCI2000 database containing 238,819 compounds was screened against the α_{1A}-AR pharmacophore using the DS protocol 'Search 3D database' to generate a hit list of compounds fitting onto the α_{1A}-AR pharmacophore. The hits obtained from the NCI2000 database screening were subsequently profiled against the α_{1A}, α_{1B}, α_{1D}-AR, 5-HT_{1A}-R agonist and 5-HT_{1A}-R antagonist pharmacophores using the 'Ligand Profiler' protocol in order to retrieve compounds which specifically fit only to the α_{1A}-AR pharmacophore. The 'Ligand Profiler' gives a FitValue of each compound to the respective pharmacophore between 0.0 and 1.0, where 1.0 means 100 % fit. After obtaining FitValues, we considered only compounds which had high FitValues for the α_{1A}-AR pharmacophore and zero FitValues for the α_{1B}-AR, α_{1D}-AR, 5-HT_{1A}-R agonist and 5-HT_{1A}-R antagonist pharmacophores.

Expression of cloned genes in COS-1 cells and membrane preparation

COS-1 cells (American Type Culture Collection, Manassas, VA) were maintained using standard culturing techniques. Transient expression of human 5-HT_{1A}-R and α_1-AR cDNA obtained from the Missouri S&T cDNA Resource Center (www.cdna.org) in COS-1 cells was achieved using diethylaminoethane-dextran (DEAE-dextran) methodology as previously described [35]. Membrane preparations were made from COS-1 cells transiently expressing the receptor of interest as previously described [36] and protein concentration was determined by the Bradford method [37].

Radioligand binding experiments

Receptor binding assays were performed as previously described [30].

5-HT_{1A}-R binding

Ligands and membrane preparations were suspended in 50 mM Tris–HCl and 4 mM $CaCl_2$, pH 7.4. In saturation experiments, 5-HT_{1A}-R containing membrane was incubated with varying concentrations of [^{3}H]-OH-DPAT (0.5–16 nM). Competition experiments contained membrane incubated with [^{3}H]-OH-DPAT (1 nM) and increasing concentrations of test compounds. Nonspecific binding was defined as binding in the presence of 10 μM serotonin.

α_1-AR binding

Ligands and membrane preparations were suspended in 20 mM HEPES, 1.4 mM EGTA and 12.5 mM $MgCl_2$, pH 7.4. For saturation experiments, α_{1A} and α_{1B}-ARs were incubated with varying concentrations of [^{3}H]prazosin (0.125–16 nM). In competition experiments, α_{1A} and α_{1B}-ARs were incubated with 0.2 nM [^{3}H]prazosin and increasing concentrations of test compounds. Nonspecific binding was defined as binding in the presence of 100 μM phentolamine.

Data analysis

In generating pharmacophore hypotheses, Catalyst analyses statistical validity using three parameters: the correlation (R^2) between real and estimated affinities of compounds; the configuration cost, which describes the total hypothesis space and should have a value less than 17 to ensure all possibilities have been considered; and thirdly the difference in total costs between generated hypotheses and the null hypothesis. The null cost is the cost of a hypothesis that would give no correlation between the predicted and experimental activities [38]. Costs are expressed in units of bits.

Nonlinear regression analysis of saturation and competition binding assay data was performed using the curve fitting program GraphPad Prism (San Diego, CA, USA). The K_i values for each compound tested were produced by transformation of the program-calculated IC_{50} value (concentration of ligand resulting in 50 % inhibition of radioligand binding) via the Cheng-Prusoff equation $K_i = IC_{50}/1 + ([L]/K_D)$; where [L] is 0.2 nM [^{3}H]prazosin (ARs) or

1 nM [^{3}H]-OH-DPAT (5-HT$_{1A}$-R) and K_D is the dissociation constant of L. The competitive binding data for each ligand was tested for both one- and two-site binding. A one-site binding model was determined as the appropriate form of analysis for all binding data. Statistically significant differences ($p < 0.01$) between the affinities of all compounds for the α_1-AR subtypes and the 5-HT$_{1A}$-R were determined using one way ANOVA and the Student–Newman–Keuls multiple comparison test.

Results

Pharmacophore training set construction

Compounds were chosen such that each training set contained high chemical diversity and affinity values ranging over 3.5 orders of magnitude (Table 1). This was in order to ensure representation of the known classes of chemical compounds that interact with each receptor in each training set. In total, 46 compounds were gathered from the literature and classified into two specific training sets: 5-HT$_{1A}$-R agonists (Table 2) and 5-HT$_{1A}$-R antagonists (Table 3).

Pharmacophore generation

Pharmacophore hypotheses were constructed for each training set using the features general hydrophobic (HY), hydrophobic aromatic (Har), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and ionic interactions (PI). The ten top-ranked hypotheses were first generated without considering steric hindrance, and then were re-generated to consider the impact of steric hindrance by allowing for the inclusion of up to 10 excluded volume features (EX). Each EX represents a sphere in the vicinity of the ligand where additional ligand bulk leads to a lower affinity. Consideration of these "steric clashes" can improve the accuracy of a hypothesis to distinguish between active and inactive agents [39]. The most statistically valid pharmacophore models with EXs were selected.

A basic amine (PI feature) is known to be essential for the binding of compounds to both the 5-HT$_{1A}$-R and α_1-ARs [40–42], therefore the inclusion of a PI feature was forced in hypothesis generation. This was previously [29] found to improve correlation, particularly for high affinity compounds, and was considered a valid approach for generating the best pharmacophore models.

For 5-HT$_{1A}$-R agonist pharmacophore generation the highest ranked hypothesis was a model consisting of 1 PI, 1 HBA, 3 HY features and 7 EXs (Fig. 1a). The statistical parameters associated with pharmacophore generation are provided in Table 1. With a configuration cost of 15, a correlation of 0.85 between the actual and the predicted affinities of the training set compounds, and a difference between the null and the hypothesis cost of 75, there is a >90 % statistical probability that this hypothesis represents a real correlation with biological activity [38].

The highest ranked hypothesis for the 5-HT$_{1A}$-R antagonist pharmacophore consisted of 1 PI, 1 HBA, 1 HY and 1 Har features and 5 EXs (Fig. 1b). The configuration cost was 14, the correlation 0.83 and the cost differential 41 (Table 1), indicating a 75 % statistical probability that this hypothesis represents a real correlation with biological activity [38].

Ligand pharmacophore mappings

Using the pharmacophores, known α_1-AR ligands with 5-HT$_{1A}$-R affinity were mapped and affinities predicted. For consistency and to enable comparisons between predictions, these mappings are performed in a slightly different way to the way ligands are mapped and predicted during the pharmacophore generation runs. Based on their known K_i values, the affinities of BMY7378, 5-methylurapidil and tamsulosin are generally underpredicted in these mapping runs (Table 4). It should be acknowledged that 5-methylurapidil was part of the 5-HT$_{1A}$-R agonist training set, and tamsulosin was part of the 5-HT$_{1A}$-R antagonist training set, and their predicted affinities are much higher when using the correct pharmacophore (agonist vs. antagonist pharmacophore) for mapping. Tamsulosin is correctly predicted to have the highest affinity for the α_{1A}-AR. The 5-HT$_{1A}$-R agonist BMY7378 is predicted to display similar affinity by the agonist and the antagonist pharmacophores.

Three novel compounds previously identified by α_1-AR pharmacophores (compounds (**48**)–(**50**)) [29], and

Table 1 Summary of pharmacophore statistical parameters

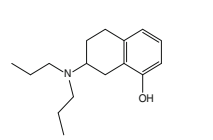
Pharmacophore	Training set compounds	Range of affinity, K_i (nM)	Correlation (R^2)[a]	Cost difference[b]
5-HT$_{1A}$-R agonist	24	0.03–50,000	0.85	75
5-HT$_{1A}$-R antagonist	22	0.2–10,000	0.83	41

[a] Refers to the correlation between predicted and actual affinity of compounds in the training set, for the highest ranked hypothesis

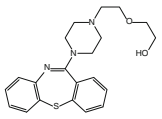
[b] Refers to the difference between the cost of the highest ranked hypothesis and the null cost

Table 2 Structure and composition of 5-HT_{1A} agonist pharmacophore training set

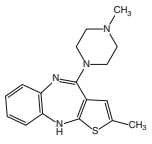
Compound scaffolds



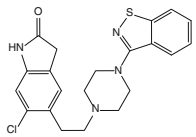
(R) 8-OH-DPAT



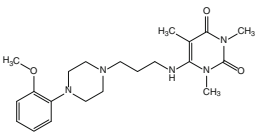
Quetiapine



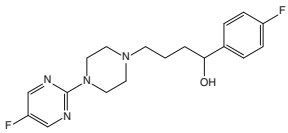
Olanzapine



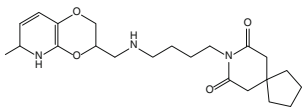
Ziprasidone



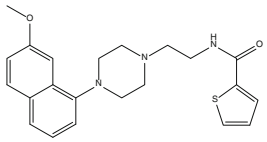
5-Methylurapidil



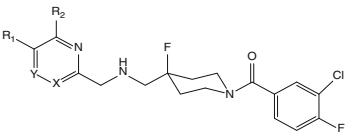
(±)BMY14802



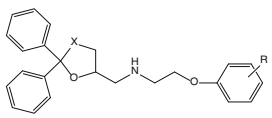
JB788



S14671



(A)



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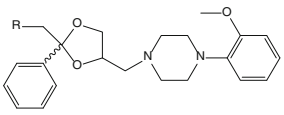
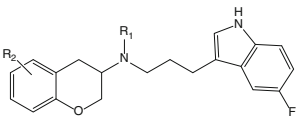
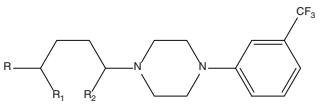


(C)



(D)



(E)

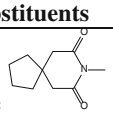
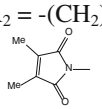
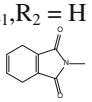
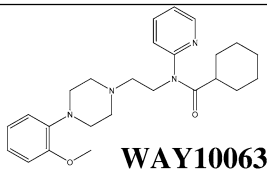
Compound, [Reference]	K_i (nM)	Scaffold, Substituents	Compound, [Reference]	K_i (nM)	Scaffold, Substituents	Compound, [Reference]	K_i (nM)	Scaffold, Substituents
(*R*) 8-OH-DPAT **(1)** [63]	1.3	–	F15599 **(9)** [64]	2.69	**A** X= N Y = CH R_1=CH_3 R_2=H	**(17)** [55]	1.25	**C** R= 
Quetiapine **(2)** [65]	250	–	F13714 **(10)** [64]	0.04	**A** X=Y=CH R_1= CH_3 R_2 = -NHMe	**(18)** [55]	11.2	**C** R= 
Olanzapine **(3)** [65]	1637	–	**(11)** [66]	7.4	**A** X=Y=CH R_1= CH_2OH R_2=H	**(19)** [67]	15.8	**D** (±) R_1= *n*-Pr R_2= 5-OCH_3
Ziprasidone **(4)** [65]	1.24	–	**(12)** [66]	66	**A** X=CH Y=N R_1=CH_3 R_2=H	**(20)** [67]	210	**D** (±) R_1= *n*-Pr R_2= 4,5- 
5-Methyl urapidil **(5)** [46]	0.06	–	**(13)** [66]	10 000	**A** X=Y=CH R_1= COOH R_2=H	**(21)** [67]	50 000	**D** (±) R_1= H R_2 = 4,5- 
BMY14802 **(6)** [65]	62.8	–	**(14)** [52]	1.02	**B** X= S R= 2-OCH_3	**(22)** [68]	0.30	**E** R_1-R_2 = -$(CH_2)_2$- R = 
JB788 **(7)** [69]	0.80	–	**(15)** [52]	53.7	**B** X= O R= 2,6-diOCH_3	**(23)** [68]	1.34	**E** R_1-R_2 = -$(CH_2)_2$- R = 
S14671 **(8)** [65]	0.03	–	**(16)** [52]	1000	**B** X= O R= 4-C_6H_5	**(24)** [68]	34	**E** R_1,R_2 = H R = 

Table 3 Structure and composition of 5-HT_{1A} antagonist pharmacophore training set

Compound scaffolds



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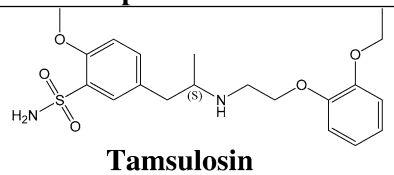
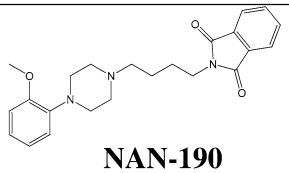


Tamsulosin



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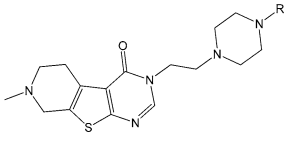
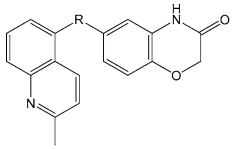
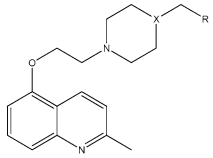


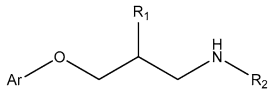
(A)



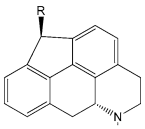
(B)



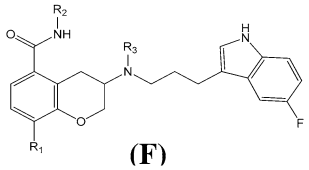
(C)



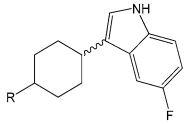
(D)



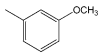
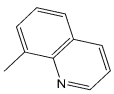
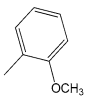
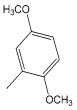
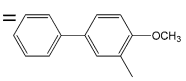
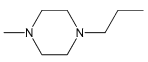
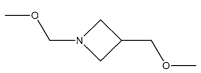
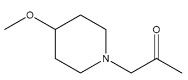
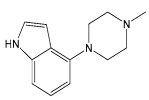
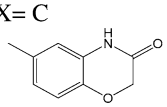
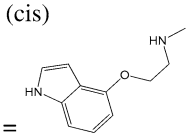
(E)



(F)



(G)

Compound, [Reference]	K_i (nM)	Scaffold, substituents	Compound,[Reference]	K_i (nM)	Scaffold, substituents
WAY100635 **(25)** [70]	2.2	–	**(36)** [71]	13	**C** X= N R = 
Tamsulosin **(26)** [72]	0.79	–	**(37)** [71]	320	**C** X= N R_2 = 
NAN-190 **(27)** [73]	2.14	–	**(38)** [74]	1.3	**D** Ar = 4-Indole R_1 =  R_2 = cyclohexyl
(28) [75]	0.2	**A** R= 	**(39)** [74]	94	**D** Ar = 4-Indole R_1 = H R_2 = cyclopentyl
(29) [75]	0.7	**A** R = 	**(40)** [74]	3000	**D** Ar = 5-Indole (±)R_1 = OH R_2 = cyclohexyl
(30) [75]	24	**A** R = 	**(41)** [63]	31	**E** R = CH_3
(31) [75]	428	**A** R = 	**(42)** [63]	1210	**E** R = OH
(32) [76]	0.25	**B** R = 	**(43)** [10]	0.93	**F** R_1 = H R_2 = H R_3 = Pr
(33) [76]	51	**B** R = 	**(44)** [10]	196	**F** R_1 = F R_2 = Pr R_3 = cyclobutyl
(34) [76]	10 000	**B** R = 	**(45)** [77]	37	**G** (cis) R = 
(35) [71]	0.32	**C** X= C R = 	**(46)** [77]	147	**G** (cis) R = 

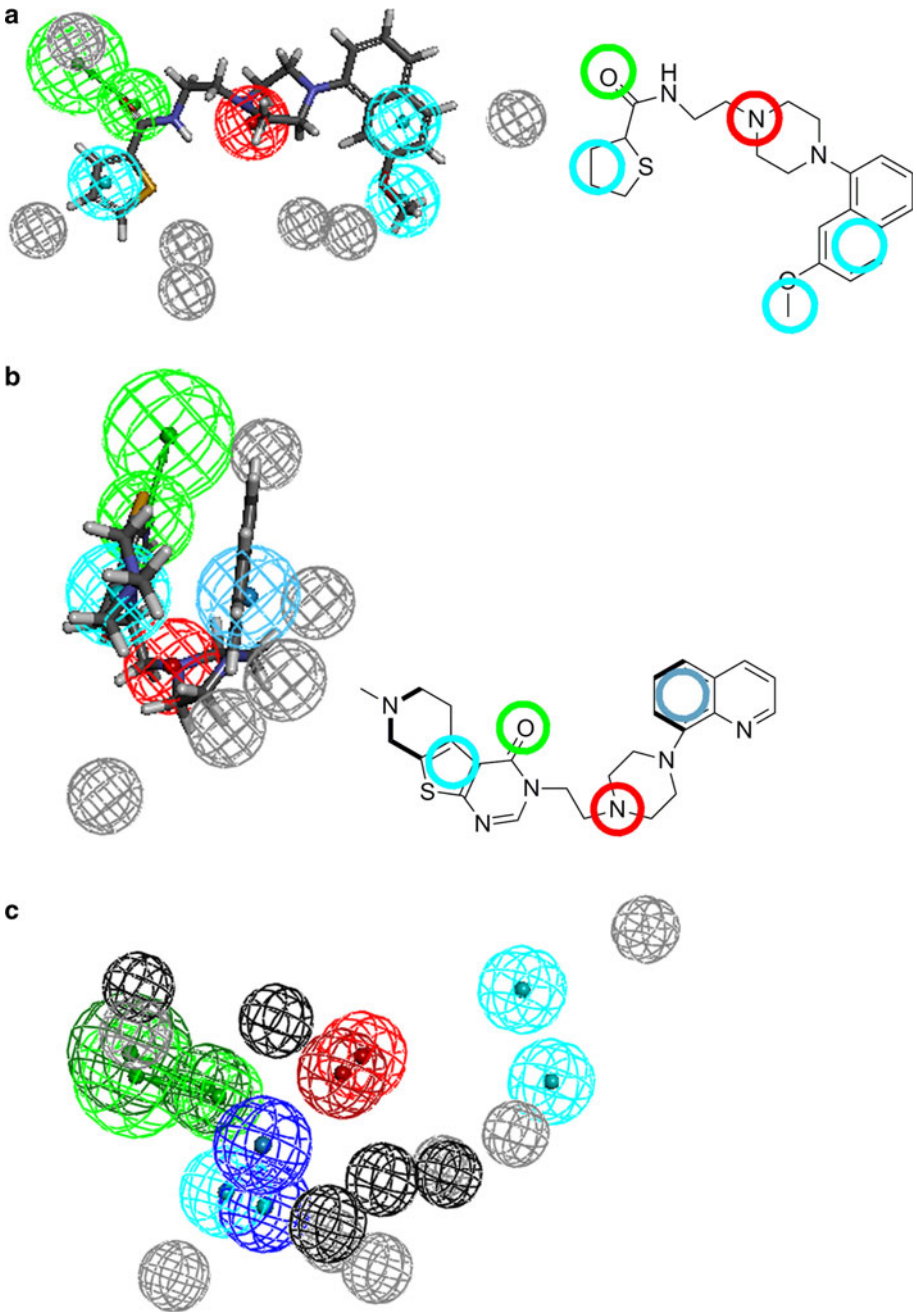
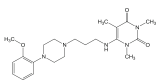
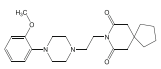
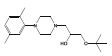
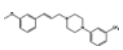


Fig. 1 5-HT_{1A}-agonist (**a**) and 5-HT_{1A}-antagonist (**b**) pharmacophore models and comparison (**c**). The three dimensional pharmacophore is shown on the *left*, and a 2D representation on the *right* in **a** and **b**. The most active ligand in each training set has been mapped on to the respective pharmacophore using stick representation. The atoms are colour coded as follows: carbon (*grey*), hydrogen (*white*), oxygen (*red*), nitrogen (*blue*). The *red spheres* represent the location which a positive ionisable group (basic nitrogen) should occupy, the *green spheres* map the projection path of a hydrogen bond acceptor. The *cyan spheres* represent general hydrophobic groups and the *light blue sphere* represents aromatic hydrophobic groups. The *grey spheres* represent excluded volumes that a ligand cannot occupy. The two pharmacophores have been superimposed in panel **c**. Some features of the antagonist pharmacophore have been recoloured for clarity: excluded volumes are black, the hydrogen bond acceptor is *dark green*, the positive ionisable group is *dark red*, and the hydrophobic and aromatic hydrophobic features are *dark blue*

confirmed in vitro to have α_1-AR affinity at rodent receptors [30] were predicted by all pharmacophores to have affinity for the human receptors (Table 4). In all cases, the lowest affinity was predicted for the α_{1B}-AR, which mirrors the observed $\alpha_{1A/D}$-AR selectivity over α_{1B}-AR (for rodent α_1-ARs). These compounds had been selected for this potential, and had not previously been mapped to the 5-HT_{1A}-R pharmacophores, where they are predicted to display similar, or even better affinities than at the α_{1A}-AR.

The predicted and experimentally determined selectivities of the compounds described in Table 4 are presented in Table 5. Selectivity for α_{1A} over α_{1B}-AR is correctly predicted for tamsulosin (**26**), for (**48**) and for (**50**), although the magnitude of this selectivity is not predicted correctly for (**50**). Compound (**49**) is predicted to show threefold selectivity for α_{1A} over α_{1B}-AR, but this was not observed experimentally (similar affinity for the two rodent receptors). The 35-fold and twofold selectivities of (**5**) and (**47**) for the α_{1A} over α_{1B}-AR are not predicted correctly. Selectivity for α_{1A}-AR over 5-HT_{1A}-R is predicted correctly for (**26**), and for 5-HT_{1A}-R over α_{1A}-AR for (**5**) and (**47**).

Table 4 5-HT_{1A} and α_1-AR pharmacophore predicted and experimentally determined affinity of experimental compounds

Compound	Structure	Pharmacophore predicted affinity K_i (nM)				Experimental affinities K_i (nM)[d]		
		5-HT_{1A}-R agonist	5-HT_{1A}-R antagonist	α_{1A}-AR	α_{1B}-AR	5-HT_{1A}-R [Reference]	α_{1A}-AR [Reference]	α_{1B}-AR [Reference]
5-Methylurapidil (**5**)		0.65	64	105	77	0.06 [46]	0.98 [78]	33.9 [78]
BMY7378 (**47**)		25	20	40	8.5	1.26 [14]	380 [14]	708 [14]
Tamsulosin (**26**)		11	2.8	2.4	10	0.79 [72]	0.13 [72]	1.92 [72]
NSC 134750[a,b] (**48**)		(R) 1,100 (S) 53	(R) 460 (S) 420	(R) 1,100 (S) 1,300	(R) 2,100 (S) 1,700	N/A	2,000[e] [30]	11,800[e] [30]
Tripos 01572[a,c] (**49**)		(R) 0.32 (S) 0.63	(R) 110 (S) 200	(R) 5.7 (S) 8.9	(R) 19 (S) 27	N/A	1,170[e] [30]	1,420[e] [30]
Tripos 09931[c] (**50**)		1.5	65	13	62,000	N/A	490[e] [30]	4,590[e] [30]

[a] Unknown stereochemistry. Both enantiomers were mapped to the pharmacophores. Values for both enantiomers are given

[b] NSC refers to the National Institute of Cancer database code

[c] Tripos database code

[d] References indicated in square brackets

[e] Determined using rodent receptors

Table 5 Predicted and experimentally determined selectivities of experimental compounds

Compound	Pharmacophore predicted selectivity (fold)[d]			Experimental selectivity (fold)[d]	
	α_{1A}-AR over α_{1B}-AR	α_{1A}-AR over 5-HT_{1A}-R agonist	α_{1A}-AR over 5-HT_{1A}-R antagonist	α_{1A}-AR over α_{1B}-AR	α_{1A}-AR over 5-HT_{1A}-R
5-Methylurapidil (**5**)	0.7	0.006	0.6	35	0.06
BMY7378 (**47**)	0.2	0.6	0.5	2	0.003
Tamsulosin (**26**)	4	5	1	15	6
NSC 134750[a,b] (**48**)	(R) 2	(R) 1	(R) 0.4	6	
	(S) 1	(S) 0.04	(S) 0.3		
Tripos 01572[a,c] (**49**)	(R) 3	(R) 0.06	(R) 20	1	
	(S) 3	(S) 0.07	(S) 20		
Tripos 09931[c] (**50**)	5,000	0.12	5	9	

[a] Unknown stereochemistry. Both enantiomers were mapped to the pharmacophores. Values for both enantiomers are given

[b] NSC refers to the National Institute of Cancer database code

[c] Tripos database code

[d] 1/ratio of K_i values presented in Table 4

Database screening and compound selection

Our published α_{1A}-AR pharmacophore [29] was used to search the NCI2000 database, containing 238,819 compounds, yielding 7,539 hits (3.2 % of the database). The 'Ligand Profiler' protocol was then used in order to select compounds which showed a good fit on the α_{1A}-AR pharmacophore and did not fit onto any of the other pharmacophores (α_{1A}, α_{1B}, α_{1D} -AR, 5-HT_{1A}-R agonist and 5-HT_{1A}-R antagonist pharmacophores). Using this method, it was determined that there were 168 unique α_{1A}-AR hits with fits of higher than 0.7 (0.07 % of the whole database).

Table 6 Ligand Profiler results

NSC code	Relative fit to α_{1A}-AR pharmacophore	MW	Comments
276751	0.944	429	No screening data available; novel structure; requested
152009	0.911	599	Excluded: too large
135759	0.903	459	No screening data available; novel structure; requested
111066	0.900	489	No screening data available. Excluded: highly similar to 135759

Compounds were selected from the top, in order of α_{1A}-AR pharmacophore fit value, taking into account structural novelty, as well as size. Table 6 illustrates this process for the top four hits from Ligand Profiler, with the next three unique hits in the list also excluded based on their similarity to hits already selected. Figure 2 provides the structures of the selected compounds, and indicates which ones were available to be tested, as well as their NSC code and relative fit value to the α_{1A}-AR pharmacophore. A reference describing the synthesis of (**52**) for an unrelated use was retrieved [43]. No further references to any of the compounds were found. The identity of each tested hit was confirmed and its purity estimated from liquid chromatography/mass spectrometry (LCMS).

Screening with previous, preliminary pharmacophores (data not shown) had retrieved a further three compounds, and their structures and details are provided in Fig. 3. Despite their lower Ligand Profiler fits to the α_{1A}-AR pharmacophore, it was decided to test these three compounds in vitro, as well. (**55**) in particular is an analogue of our previous lead compound (**48**) and appeared to be very pure. Two references were found for (**56**), dealing with Hsp90 inhibitors and with its synthesis. This compound is structurally novel as an α_1-AR ligand, although its 8-aminoquinoline unit is related to the amino-substituted quinazoline unit found in prazosin and related α_1-AR antagonists, and a 4-aminoquinoline unit is found in one unique class of dimeric α_1-AR ligands [44]. All three compounds, (**55**)–(**57**), did not fit onto any of the other pharmacophores (α_{1A}, α_{1B}, α_{1D}-AR, 5-HT_{1A}-R agonist and 5-HT_{1A}-R antagonist pharmacophores).

Evaluation of compound affinity at recombinant human α_{1A} and α_{1B}-ARs and 5-HT_{1A}-R

Compounds were evaluated for ligand binding characteristics on membrane-expressed α_{1A}-ARs ($K_D = 0.432 \pm$

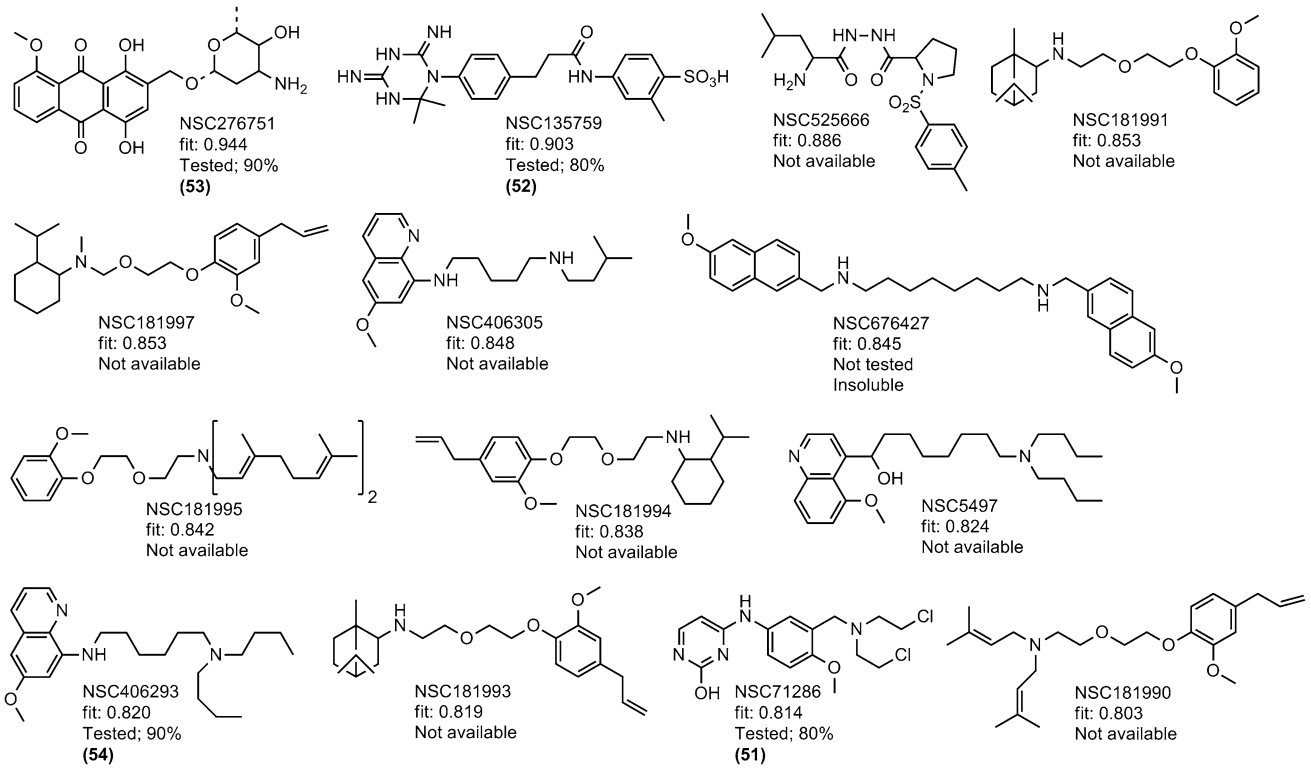


Fig. 2 Structures of hits selected for testing. Fit refers to the relative fit to the α_{1A}-AR pharmacophore. Percentages refer to estimated compound purity. See text for details

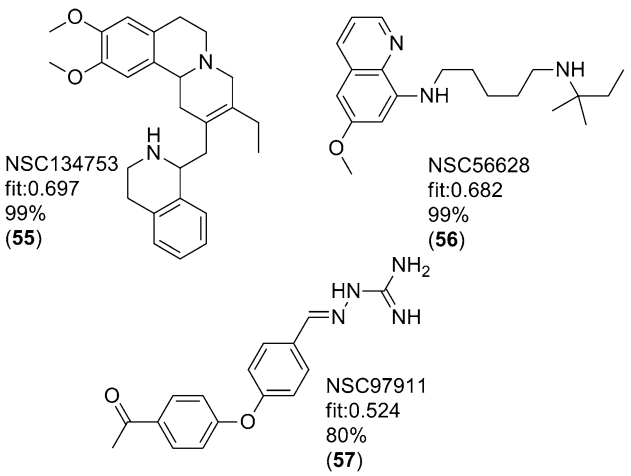


Fig. 3 Structures of additional tested compounds. Fit refers to the relative fit to the α_{1A}-AR pharmacophore. Percentages refer to estimated compound purity. See text for details

0.187 nM, n = 3) and α_{1B}-ARs (K_D = 0.162 ± 0.045 nM, n = 3) in competition radioligand binding studies using [^{3}H]-prazosin and membrane-expressed 5-HT$_{1A}$-R (K_D = 1.40 ± 0.07 nM, n = 3) using [^{3}H]-OH-DPAT. All curves displayed sigmoidal binding, with a Hill slope for all curves not significantly different from 1 ($p < 0.05$).

The affinity values obtained for 5-methylurapidil, BMY7378 and tamsulosin fell within the range of reported values in the literature [14, 45–47], and the documented α_{1A}-AR selectivity of 5-methylurapidil (over α_{1B}-AR) was also observed (Table 7). Three compounds ((**48**), (**49**) and (**50**)) previously identified using our α_1-AR subtype selective pharmacophores [30] were tested on human α_{1A} and α_{1B}-ARs and 5-HT$_{1A}$-R. (**48**) exhibited low micromolar affinity for human α_{1A} and α_{1B}-ARs (Table 7), consistent with values obtained using rat receptors [30]; however the α_{1A}-AR selectivity seen with rodent receptors was reduced [30]. Both (**48**) and (**49**) exhibited low micromolar affinity ($K_i < 3$ μm) for the 5-HT$_{1A}$-R, similar to their affinity for the rat and/or human α_{1A}-AR (Tables 4, 7). Moderate affinity was observed for (**50**) (K_i 22 μM) at the 5-HT$_{1A}$-R, whereas it displayed relatively higher affinity at the rat α_{1A}-AR (K_i 490 nM). In terms of selectivity, (**48**) and (**49**) were predicted to display a small preference for the α_{1A}-AR over the α_{1B}-AR, with a 5,000-fold preference predicted for (**50**) (Table 5). This was verified experimentally for (**48**) (Table 7). Predictions of selectivity between α_{1A}-AR and 5-HT$_{1A}$-R are much more difficult to assess for these three compounds, because they vary widely between the 5-HT$_{1A}$-R agonist and antagonist pharmacophores (Table 5).

The hit compounds identified by the screen described above ((**51**)–(**54**)), as well as with the preliminary pharmacophores ((**55**)–(**57**)), all had some affinity for the α_{1A}-AR, with (**51**) (K_i = 192 nM) and (**57**) (K_i = 209 nM) binding with nanomolar affinity. All seven hit compounds identified ((**51**)–(**57**)) were chosen because their relative fits in the Ligand Profiler screen were zero for the α_{1B}-AR and 5-HT$_{1A}$-R. However, their affinity at the α_{1B}-AR was similar to that at the α_{1A}-AR. Affinity at the 5-HT$_{1A}$-R was also generally similar, however, compound (**57**) displayed statistically significant (about 40 fold), $p < 0.05$) preference for the α_{1A}-AR over the 5-HT$_{1A}$-R, compound (**53**) about tenfold preference, and compounds (**52**) and (**54**) displayed around fourfold preference for the α_{1A}-AR over the 5-HT$_{1A}$-R. On the other hand, compounds (**55**) and (**56**) display statistically significant preferences for the 5-HT$_{1A}$-R over the α_{1A}-AR.

Discussion

The discovery of novel, high affinity α_{1A}-AR selective antagonists is essential to the development of more efficacious drugs for urogenital conditions [11, 48, 49]. We have previously constructed pharmacophores that were successfully able to identify α_1-AR subtype selective antagonists suitable for drug development [29, 30]. Currently, clinically used α_{1A}-AR selective antagonists have significant off-target affinity for the 5-HT$_{1A}$-R [22, 46], which contributes to their side effect profile [50]. Therefore, it was now of interest to develop pharmacophores capable of predicting and thus filtering out hit compounds with significant affinity for the 5-HT$_{1A}$-R.

Through constructing structurally diverse training sets, pharmacophores were developed for 5-HT$_{1A}$-R agonists and antagonists, with each exhibiting high correlation in terms of predicted versus actual activity ($R^2 > 0.8$) for the training set compounds. When superimposed on to one another (Fig. 1c), a high degree of similarity is observed between the 5-HT$_{1A}$-R agonist and antagonist pharmacophores. There is close to identical positioning of both the PI and HBA features, with one of the hydrophobic features of the agonist pharmacophore close to the two hydrophobic features of the antagonist pharmacophore and some of the excluded volumes also located in close proximity. However, the agonist pharmacophore extends beyond this cluster of features with two closely spaced hydrophobic features. The features selected in these pharmacophores correspond to the interactions shown to be crucial for 5-HT$_{1A}$-R ligand binding in mutagenesis studies. These essential interactions are the ionic interaction with Asp 3.32[1] (basic amine, positive ionisable feature), a π

[1] Numbers indicate the Ballesteros-Weinstein numbering scheme where the first digit represents the transmembrane helix (TM) number followed by the position relative to the most conserved residue in each TM, assigned number 50. Numbers decrease towards the N-terminus.

Table 7 Experimentally determined affinity and selectivity of compounds at α_{1A}-AR, α_{1B}-AR and 5-HT_{1A}-R

Compound	α_{1A}-AR			α_{1B}-AR			5-HT_{1A}-R			Selectivity (fold)[c]	
	pK_i[a]	K_i[b] (nM)	n	pK_i[a]	K_i[b] (nM)	n	pK_i[a]	K_i[b] (nM)	n	α_{1A}/α_{1B}	α_{1A}/5-HT_{1A}
5-Methylurapidil (**5**)	9.16 ± 0.12	0.7	3	6.84 ± 0.11*	145	3	9.76 ± 0.12	0.2	4	200	0.3
BMY7378 (**47**)	6.24 ± 0.12	590	3	6.78 ± 0.10	168	3	8.52 ± 0.12*	3	4	0.3	0.005
Tamsulosin (**26**)							9.88 ± 0.07	0.1	4		
NSC 134750 (**48**)	5.64 ± 0.08	2,270	3	5.17 ± 0.12	6,700	3	5.68 ± 0.07	2,090	4	3	1
Tripos 01572 (**49**)							5.84 ± 0.10	1,510	4		
Tripos 09931 (**50**)							4.66 ± 0.19	21,900	4		
NSC 71286 (**51**)	6.72 ± 0.19	192	3	5.31 ± 0.30*	4,880	4	6.71 ± 0.09	196	3	26	1
NSC 135759 (**52**)	3.60 ± 0.17	250,000	3	<3	>1 mM	4	NB	NB	3	>4	
NSC 276751 (**53**)	4.79 ± 0.20	16,200	3	4.94 ± 0.10	11,600	4	<4	>100 μM	3	0.7	>6
NSC 406293 (**54**)	5.00 ± 0.10	10,300	4	4.80 ± 0.15	15,800	4	4.39 ± 0.26	40,800	3	2	4
NSC 134753 (**55**)	5.11 ± 0.03	7,730	3	5.56 ± 0.08	2,780	3	6.52 ± 0.19*	303	3	0.4	0.04
NSC 56628 (**56**)	4.47 ± 0.08	33,700	3	4.24 ± 0.10	58,200	3	5.49 ± 0.19*	3,200	4	2	0.1
NSC 97911 (**57**)	6.68 ± 0.20	209	3	6.39 ± 0.25	410	4	5.10 ± 0.19*	7,860	4	2	38

n number of experiments, each performed in triplicate NB indicates no binding detected up to 1 mM

* Significant differences from the α_{1A}-AR ($p < 0.05$)

[a] Log*K*i ± SEM, [b] inhibition constants are the antilog of mean p*K*i. [c] 1/ratio of K_i values

interaction with Phe 6.52 (aromatic or hydrophobic feature) and a hydrogen bond between an acceptor and Ser 5.42 [13, 51].

These features were also observed in previously developed 5-HT_{1A}-R pharmacophores [33, 52]. In the pharmacophore developed by Weber et al. [33], the amine, HBA and aromatic features were arranged in a linear fashion, and this resembles the arrangement we found in our agonist pharmacophore (Fig. 1a) with ligands fitted in a fairly extended conformation. The antagonist pharmacophore we have constructed (Fig. 1b) is much more crowded with ligands fitted in a bent conformation and resembles more that made by Franchini and colleagues [52], using arylpiperazine based ligands. In their pharmacophore the piperazine ring adopts a chair conformation perpendicular to the hydrophobic moiety in order to fit the amine feature. Their pharmacophore also suggests that the HBA is located in close proximity to a hydrophobic group, as occurs in both our pharmacophores. Additionally, their pharmacophore demonstrates the necessity for a second HBA feature, an observation also made in structure–activity studies using arylpiperazine and tetrahydropyridylindole derivatives [53]. Other such studies also suggest that the α_1-AR binding pocket may be more hydrophobic, and the 5-HT_{1A}-R pocket more polar, so that α_1-AR selectivity can be enhanced by removal of charged nitrogen atoms in heterocycles [54], and conversely, increasing the polarity of a ligand has been demonstrated to enhance its 5-HT_{1A}-R selectivity [55]. However, as these studies were restricted in terms of chemical diversity, it could be postulated that not all classes of 5-HT_{1A}-R ligands form this second hydrogen bond in the same fashion, hence a second hydrogen bonding feature was not observed in our pharmacophores.

We expand on these previous studies by investigating steric effects, and find large but different sets of excluded volumes for both the agonist and antagonist pharmacophores, as well as very different fitted conformations of ligands to the two pharmacophores. This suggests steric factors may have a large impact on the efficacy of a ligand at the 5-HT_{1A}-R. This observation has been made previously in structure–activity studies, whereby the stereochemistry of a single chiral centre can determine whether a compound acts as an agonist or antagonist at the 5-HT_{1A}-R [56, 57]. Collectively, this suggests that both agonists and antagonists of the 5-HT_{1A}-R are structurally quite similar and of a similar size. This is in marked contrast to α_1-AR ligands, where there is a distinct difference between agonists which are small in comparison to antagonists, which are comparable in size to 5-HT_{1A}-R ligands [14]. However, it also appears from our pharmacophores that, despite their size, the antagonists access a smaller, contracted binding pocket when compared to the agonists. This is in contrast to findings comparing the agonist and antagonist binding pockets in crystal structures of the β_2 adrenoceptor, where the agonist binding pocket is the smaller, more contracted one [58].

To test the ability of the newly constructed pharmacophores to predict 5-HT_{1A}-R affinity, six compounds belonging to a number of structural classes were mapped to each pharmacophore and their affinity predicted (Table 4). These included known α_1-AR ligands with 5-HT_{1A}-R affinity (BMY7378, 5-methylurapidil, tamsulosin), and novel experimental compounds known to have α_1-AR affinity (compounds (**48**)–(**50**)). The in vitro affinity of these compounds is generally not estimated well by the pharmacophores (mostly underestimated, but this is an artefact of the way the predictions were made; see "Methods and results"). The predictions from the 5-HT_{1A}-R agonist and antagonist pharmacophores differ widely for most compounds, except for tamsulosin and BMY7378, with the better fit always to the appropriate pharmacophore for the known compounds. On the other hand, the in vitro affinities of the new hits for the adrenoceptors are mostly overestimated by the pharmacophores. The 5-HT_{1A}-R affinities of these compounds were determined in this work (Table 7), and (**48**) was predicted very well, whereas (**49**) and (**50**) were grossly over predicted. The affinities of (**48**) for the α_1-AR subtypes had previously [30] been determined for rodent receptors, but were found to be similar for human receptors in this work (Tables 4, 7).

Clearly, as was expected and was discussed in our earlier papers [29, 30], the pharmacophores are not able to estimate affinities correctly. They are, however, often able to estimate selectivities and relative affinities (see "Results", Tables 5, 7), but we found them most useful for in silico screening.

The application of ligand-based pharmacophores as a virtual screening tool is more a qualitative approach; whereby if a compound is capable of mapping all pharmacophore features, then there is a significantly high probability that it has micromolar activity. Whilst if more than one feature is missed in mapping, the compound almost certainly has no activity at that target. Previous studies tend to make no mention of predicted values; typical procedure rather is to subject the top hits to single dose testing in vitro at a concentration of $\sim$100 μM and then determine the affinity of those hits that show activity at this level [59, 60]. Furthermore, the affinity of the top hits from a chemical compound database almost never exceed low micromolar range at the target of interest [60–62]. Thus it was somewhat expected that the experimental compounds would not show the predicted nanomolar affinity for the 5-HT_{1A}-R. Also it must be emphasized that these compounds were not designed or chosen to interact with the 5-HT_{1A}-R, rather it is the off-target to their intended target, the α_1-ARs. In the discussion below, we have thus only compared relative fit values of compounds to the pharmacophores (as presented in Figs. 2, 3 and in the "Results" section) to assess predicted selectivities.

We have shown that, as predicted, compound (**48**), previously identified using our α_1-AR subtype selective pharmacophores, cannot discriminate between the 5-HT_{1A}-R and the α_1-ARs in terms of affinity. The structurally related compound (**55**) contains a second tetrahydroisoquinoline group, including a second basic nitrogen. This allows for a variety of different binding modes and potentially accounts for the lack of discrimination observed for this compound. Contrary to predictions, it has higher affinity for the 5-HT_{1A}-R than for the α_{1B}-AR and α_{1A}-AR, which may also be related to the potentially more polar 5-HT_{1A}-R binding pocket discussed above. Three of the remaining new hits ((**52**), (**53**), (**57**)) demonstrate selectivity for α_{1A}-AR over 5-HT_{1A}-R. However, neither of them can discriminate between α_{1A} and α_{1B}-AR. This could potentially be related to the fact that for some of these molecules the positive charge (after protonation) is not concentrated on a single nitrogen, but is "smeared out" through several tautomers ((**52**), (**54**) and (**57**)). Compound (**56**) displays selectivity for 5-HT_{1A}-R, but cannot discriminate between α_{1A} and α_{1B}–AR. The aminoquinoline moiety in (**56**) is quite polar, with the nitrogen in the heterocycle also potentially able to be protonated, and as we suggested above for (**55**), this may explain the high affinity for 5-HT_{1A}-R. The same polarity argument may also explain the high 5-HT_{1A}-R affinity of (**51**). This molecule demonstrates the highest α_{1A}-AR affinity of the test compounds, as well as selectivity over α_{1B}-AR. However, on top of its high 5-HT_{1A}-R affinity (**51**) contains alkylating groups with unacceptable toxicology issues.

The novel scaffold, compact size, rigidity, and lack of groups associated with potential liability in terms of toxicity of (**48**) still makes it a desirable lead for drug development. With the information provided by the new pharmacophores, α_1-AR selectivity may still be achieved for (**48**) via chemical modification. For example, replacement of the methoxy groups not involved in hydrogen bonding with the α_{1A}-AR with methyl groups would reduce polarity, which should decrease 5-HT_{1A}-R affinity.

Conclusions

This study has succeeded in constructing pharmacophores, which through the structural diversity of their training sets define the properties of a ligand needed to bind to the 5-HT_{1A}-R. Virtual screening and profiling, followed by experimental determination of the affinities of selected hits, has demonstrated that our pharmacophores are capable of identifying unique chemical compounds that fit the requirements to bind to our target, the α_{1A}-AR, selectively over the off-target, the 5-HT_{1A}-R. As predicted, our current lead compound exhibits significant 5-HT_{1A}-R affinity. Thus chemical derivatives will need to be synthesized with

increased α_1-AR affinity, and reduced 5-HT_{1A}-R affinity. The insights and knowledge gained through the development of the new 5-HT_{1A}-R pharmacophores will greatly aid in this process, and allow the generation of more efficacious and selective ligands.

Acknowledgments The authors wish to acknowledge the help of Dr Luke Hunter, School of Chemistry, University of New South Wales, in determining the purity and identity of the test compounds, using the Analytical Centre Facilities. J. Chen wishes to acknowledge financial support through a China Scholarship Council scholarship.

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