The affinity and selectivity of α-adrenoceptor antagonists, antidepressants and antipsychotics for the human α2A, α2B and α2C-adrenoceptors and comparison with human α1 and β-adrenoceptors.

Richard G.W. Proudman, Juliana Akinaga, Jillian G. Baker

Cell Signalling Research Group,
Division of Physiology, Pharmacology and Neuroscience,
School of Life Sciences,
C Floor Medical School,
Queen's Medical Centre,
University of Nottingham,
Nottingham,
NG7 2UH,
UK.

Running title: α2A, α2B, α2C-adrenoceptor antagonist selectivity

Cell Signalling Research Group,
Division of Physiology, Pharmacology and Neuroscience,
School of Life Sciences,
C Floor Medical School,
Queen's Medical Centre,
University of Nottingham,
Nottingham,
NG7 2UH,
UK.

Address correspondence to: Prof Jillian G Baker
Tel: 0115 8230085
Email: jillian.baker@nottingham.ac.uk

Number of text pages = 35 final (i.e. non red-pen) version

Number of Tables = 5

Number of Figures = 4

Number of references = 50

Abstract = 250 words

Introduction = 758 words

Discussion = 1481 words

Supplementary data

Table 1 = all ligands arranged in alphabetical order with suppliers and individual ligand codes, α2A, α2B, α2C, β1 and β2-affnities

Table 2 – all ligands arranged in order of α2A-affinity with selectivity ratios between α2-adrenoceptors.

Abbreviations

CHO, Chinese hamster ovary

BPH, benign prostatic hyperplasia

sfm, serum free media = DMEM/F12 containing 2mM L-glutamine

Abstract

α2-adrenoceptors, subdivided into α2A, α2B and α2C subtypes and expressed in heart, blood vessels, kidney, platelets and brain, are important for blood pressure, sedation, analgesia and platelet aggregation. Brain α2C-adrenoceptor blockade has also been suggested to be beneficial for antipsychotic action. However, comparing α2-adrenoceptor subtype affinity is difficult due to significant species and methodology differences in published studies. Here, ^{3}H-rauwolscine whole cell binding was used to determine the affinity and selectivity of 99 α-antagonists (including antidepressants and antipsychotics) in CHO cells expressing human α2A, α2B or α2C-adrenoceptors, using an identical method to β and α1-adrenoceptor measurements, thus allowing direct human receptor comparisons. Yohimbine, RX821002, RS79948 and atipamezole are high affinity non-selective α2-antagonists. BRL44408 was the most α2A-selective antagonist, although its α1A-affinity (81nM) is only 9-fold greater than its α2C-affinity. MK-912 is the highest-affinity, most α2C-selective antagonist (0.15nM α2C-affinity) although its α2C-selectivity is only 13-fold greater than at α2A. There are no α2B-selective antagonists. A few α-ligands with significant β-affinity were detected, e.g. naftopidil where its clinical α1A-affinity is only 3-fold greater than off-target β2-affinity. Antidepressants (except mirtazapine) and first generation antipsychotics have higher α1A than α2-adrenoceptor affinity but poor β-affinity. Second generation antipsychotics varied widely in their α2-adrenoceptor affinity. Risperidone (9nM) and paliperidone (14nM) have the highest α2C-adrenoceptor affinity however this is only 5-fold selective over α2A, and both have higher affinity for α1A (2nM and 4nM respectively). So, despite a century of yohimbine use, and decades of α2-subtype studies, there remains plenty of scope to develop α2-subtype selective antagonists.

Keywords

α-adrenoceptor, antagonist, affinity, selectivity, hypertension, antipsychotic, antidepressant

Introduction.

The α2-antagonist yohimbine, obtained from the African Corynanthe yohimbe tree (*Pausinystalia johimbe*), has been in clinical use as an aphrodisiac for over a century [1-2]. It has been used for erectile dysfunction and increases many sexual behaviours through central (CNS) α2-effects and potential local effects as α2A, α2B and α2C-adrenoceptors are expressed in human corpus cavernosum [1-2], and can indeed bind yohimbine from tree bark [3]. The α2-antagonist idazoxan, developed in 1970s, is selective for α2 over α1-adrenoceptors, but also binds to other imidazoline binding sites which limits its usefulness in tissue or animal studies [4-5]. This led to the development of RX821002, a 2-methyl congener of idazoxan, in the 1980s which retained high α2-adrenoceptor affinity but without imidazoline receptor affinity (although 5-HT receptor interactions still occur [6-7]).

α2-adrenoceptors are subdivided into α2A, α2B and α2C-subtypes. With receptors being present in heart, blood vessels and kidney [8], α2-adrenoceptors are important in blood pressure control (an interplay between α1, α2 and β-adrenoceptors) and including central and peripheral α2-effects. In addition, many α2-adrenoceptors present in brain also have clinical roles in anaesthesia and psychiatric treatments [9] with both pre- and post-synaptic effects on neurotransmission [10-13].

α2A-adrenoceptors are widely expressed and are important for blood pressure, sedation, analgesia, platelet aggregation and hypothermia [14-15]. In the brain, 90% of all α2-adrenoceptors are of the α2A subtype and they are highly expressed in the prefrontal cortex where activation increases cognitive function [16.17]. α2A-adrenoceptor antagonism may be important in sepsis (administration of the α2A-selective antagonist BRL44408 reduced pro-inflammatory cytokines, TNF-α and IL-6 and increased survival in a rat model of sepsis [18]) and potentially clinically relevant α2A-mirtazapine-induced reversal of analgesia [19]. The roles of the α2B-adrenoceptors are less clear. α2B-adrenoceptors are involved in blood pressure control (activation causes a hypertensive response related to renal salt balance [14]. The expression and effects of the α2B-adrenoceptors appear very minor in brain [17]. The α2C-adrenoceptor is involved in catecholamine release in adrenal chromaffin cells [15] and in the brain process of startle and stress responses [14]. α2C-adrenoceptors form 10% of all brain adrenoceptors but appear particularly prevalent in the striatum and hippocampus [16].

For certain antipsychotics (e.g. clozapine), α2C-antagonsim, in addition to dopamine D2 blockade, is thought to be beneficial in the management of schizophrenia [12-13, 17] and α2C-antagonism may be helpful in improving cognition in dementia [12]. However, a lack of subtype selective α2-adrenoceptor ligands has impaired understanding and knowledge of α2-subtype expression and α2-subtype function, with much information coming from knockout mice, with subtype adaptation problems that this brings [12-15, 17, 20].

Determining the affinity and selectivity between different α2-adrenoceptor antagonists has been difficult due to significant variability both within individual, and between different existing studies. Many older studies (pre-cloned receptors) used different tissue preparations from different species as examples of subtype-selective tissue e.g. human platelet or cortex for α2A vs neonatal rat lung for α2B [21-23]. However, there are significant species differences. Differences of up to 30-fold for the affinity of several ligands (including yohimbine and its stereoisomer rauwolscine) for α2A-adrenoceptors have been reported for human/pig (higher affinity) vs rat/guinea pig (lower affinity) [23-34]. Prazosin is the opposite with 15-20-fold high affinity for rat/mouse kidney receptors than human/rabbit/dog α2A-adrenoceptors [4, 25]. Overall, it appears that the human α2-adrenoceptors have more similarity to those of pig, dog and rabbit than those of rat, mouse and guinea pig [6-7, 26-27], which adds further caution with extrapolating from knock-out mice studies to human clinical relevance of drug actions.

In addition, substantial differences are reported for affinity measurements of single ligands at single subtypes. Reports of prazosin affinity at human α2A-adrenoceptors range 50-fold, from 300nM [21, 28] to a few thousand nM [23-24, 29], to 16000nM [6]. Differences in affinity have also been attributed to technique. A 5-fold difference in ^{3}H-rauwolscine affinity, and 4-fold difference in ^{3}H-RX821002 and ^{3}H-atipamezole affinity was found with different buffers [30]. Thus, previously reported differences in affinity are likely to be due to several explanations: species is very important but techniques (cloned receptor vs whole tissue, membrane vs whole cell, different buffers) are also important and make direct comparison of studies difficult.

This study therefore measured the affinity and selectivity of a wide range of α-antagonists (including antidepressants and antipsychotics) in living CHO cells expressing the human α2A, α2B or α2C-adrenoceptor. Furthermore, as these measurements were determined using

an identical technique in human β1 and β2-adrenoceptors (included here, and [31-32]) and α1-adrenoceptors [33], this study explores the affinity and selectivity of ligands across the human adrenoceptors commonly targeted for cardiovascular and CNS effects.

Methods.

Materials.

All compounds, together with the supplier and catalogue number are given in alphabetical order in Supplementary Data Table 1. White sided view plates were from Greiner Bio-one, Kremsmunster, Austria. ^{3}H-rauwolscine (a stereoisomer of yohimbine, specific activity 82.9), ^{3}H-RX821002 (specific activity 36.5), ^{3}H-CGP12177 (specific activity 37.7), Microscint 20 and Ultima Gold XL scintillation fluid were from PerkinElmer (Buckinghamshire, UK). Foetal calf serum was from Gibco (Thermo-Fisher), Lipofectamine and OPTIMEM were from Life Technologies, Thermo-Fisher, Massachusetts USA. All other cell culture reagents were from Sigma Chemicals (Poole, Dorset, UK).

Cell lines

CHO-K1 (RIDD: CVCL_0214) were stably transfected with the human α2A-adrenoceptor, human α2B-adrenoceptor or human α2C-adrenoceptor DNA (DNAs from Guthrie DNA Resource Centre) using Lipofectaime and Optimem according to the manufacturers instructions. Following 3 weeks selection using resistance to neomycin (at 1mg/ml), single clones from each transfection were isolated by dilution cloning. Thus stable cell lines CHO-α2A, CHO-α2B and CHO-α2C were created. CHO lines stable expressing the human β1 or β2-adrenoceptor were also used [31].

Cell culture

CHO cells were grown in Dulbecco's modified Eagle's medium nutrient mix F12 (DMEM/F12) containing 10% foetal calf serum and 2mM L-glutamine in a 37°C humidified 5% CO_2 : 95% air atmosphere. Cells were seeded into white-sided, clear bottomed 96-well view plates and grown to confluence. Cells were always grown in the absence of any antibiotics. Mycoplasma contamination has intermittently been monitored within the laboratory (negative) but cell lines were not tested routinely with each experiment.

^{3}H-rauwolscine and ^{3}H-RX821002 whole cell saturation binding

The K_D value for both radioligands was determined in each cell line by saturation binding. The radioligands were diluted to twice the final concentration in serum free media (sfm DMEM/F12 containing 2mM L-glutamine). Media was removed from each well and replaced with either 100µl sfm (total binding) or 100µl 20µM RX821002 (when ^{3}H-rauwolscine used)

or 20μM yohimbine (when ^{3}H-RX821002 used) in sfm to determine non-specific binding. 100μl radioligand was then added to the wells (quadruplicates per condition = 1 in 2 dilution in well), and the plates incubated at 37°C (humidified 5% CO_2 : 95% air atmosphere) for 2 hours. After 2 hrs, the cells were washed twice by the addition and removal of 2 x 200μl cold (4°C) phosphate buffered saline. A white base was applied to the plate to convert the wells into white-sided/white-bottomed wells, 100μl Microscint 20 was added to each well and a transparent topseal applied to the plates. Plates were left at room temperature in the dark for at least 6 hours before being counted on a Topcount (PerkinElmer, 2 minute count per well).

^{3}H-rauwolscine, ^{3}H-RX821002 and ^{3}H-CGP12177 whole cell competition binding

Affinity was assessed using the whole cell binding method of [31]. Ligands were diluted in sfm to twice their final concentration. Media was removed from each well and 100μl ligand added to triplicate wells. This was immediately followed by the addition of 100μl radioligand (diluted in sfm) and the cells incubated for 2 hours at 37°C (5% CO_2, humidified atmosphere), after which the plates were washed as above. Cells were inspected under a light microscope to ensure cells were still adherent after the wash and before the addition of Microscint 20. In a few cases, high concentrations of competing ligand caused the cells to round up and be washed off the plates. These concentrations were excluded from the analysis. Total binding (6 wells/plate) and non-specific binding (6 wells/plate (determined by the presence of 10μM yohimbine or 10μM RX821002 in sfm) was defined in every plate.

Given the two-component inhibition of ^{3}H-prazosin binding seen with dibenamine and phenoxybenzamine at the α1-adrenoceptors, sodium thiosulphate, which reacts with the ethyleniminium ions, was used in dibenamine and phenoxybenzamine experiments, in excess, as in [33].

Thus all studies in human β, α1 and α2-adrenoceptors have been conducted in intact living mammalian cells using the same method. The only differences between the experiments are the radioligand, the ligand used to define non-specific binding and the transfected receptor. As all experiments were conducted in living cells, physiological levels of intracellular endogenous GTP will always have been present and potentially are therefore more akin to how drugs bind in people, rather than studies conducted in membrane preparations. There is theoretically a potential difference in affinity measurement if compounds have a different

intrinsic efficacy for different receptor subtypes. Thus, if one compound is a partial agonist at one receptor subtype but an inverse agonist at another, a different receptor state is induced upon binding to the receptor. This may therefore affect how the compound and radioligand compete for the receptor, which in turn could theoretically affect affinity measurements. As this study was aimed at studying antagonists, this effect is likely to be minimal.

Data analysis

Saturation curves for specific radioligand binding were plotted using the following equation in GraphPad Prism 7:

$$\text{Specific binding} = \frac{B_{max} \times K_D}{([^3\text{H-radioligand}] + K_D)}$$

where B_{max} is the maximum specific binding, K_D is the dissociation constant of the radioligand and [^{3}H-radioligand] is the concentration of the radioligand.

In all cases where a K_D value is stated, increasing concentrations of the competing ligand fully inhibited the specific binding of the radioligand (unless otherwise annotated in the tables). The following equation was then fitted to the data using Graphpad Prism 7 and the IC_{50} was then determined as the concentration required to inhibit 50% of the specific binding

$$\text{\% specific binding} = 100 - \frac{(100 \times [A]}{([A] + IC_{50})}$$

where [A] is the concentration of the competing ligand and IC_{50} is the concentration at which half of the specific binding of radioligand that has been inhibited.

From the IC_{50} value, the known concentration of radioligand and the known radioligand K_D for at each receptor, a K_D (concentration at which half the receptors are bound by the competing ligand) value was calculated using the Cheng-Prusoff equation:

$$K_D \text{ competing ligand} = \frac{IC_{50}}{1 + ([^3\text{H-radioligand}]/K_D\ ^3\text{H-radioligand})}$$

In some cases the maximum concentration of competing ligand was not able to inhibit all of the specific binding. Where no inhibition of radioligand binding was seen, even with maximum concentration of competing ligand possible, “no binding” is given in the tables. Where the inhibition produced by the maximum concentration of the competing ligand was

50% or less, an IC_{50} could not be determined and thus a K_D value not calculated. This is shown in the tables as IC_{50}>top concentration used (i.e. IC_{50}>100µM means that 100µM inhibited some but less than 50% of the specific binding). In cases where the competing ligand caused a substantial (greater than 50%, but not 100%) inhibition of specific binding, an IC_{50} value was determined by extrapolating the curve to non-specific levels and assuming that a greater concentration would have resulted in 100% inhibition. These values are given as apparent K_D values in the tables.

For some ligands, a one-component sigmoidal fit was visually not a good fit for the inhibition of ^{3}H- rauwolscine binding (e.g. Figure 2b) in which case a two-component curve was used, using the equation below:

$$\% \text{ specific binding} = \frac{[A].N}{([A] + IC_{50}1)} + \frac{[A].(100-N)}{([A] + IC_{50}2)}$$

where [A] is the concentration of the competing ligand, $IC_{50}1$ and $IC_{50}2$ are the respective IC_{50} values for the two components and N is the percentage of the response occurring through the first component ($IC_{50}1$). K_D values were calculated from IC_{50} values as above.

Radioligand concentrations were determined from taking the average of triplicate 50µl samples of each radioligand concentration used and counted on a PerkinElmer Scintillation counter.
Selectivity ratios are given as a ratio of the K_D values for the different receptors.

In view of the higher level of receptor expressions in these cell lines and concerns about depletion of the free radioligand in the binding assays, depletion was monitored. Free radioligand depletion of 20% was encountered (resulting in a potential inaccuracy of 0.04 log units in the stated K_D values). Ligand depletion of a maximum of 25-33% were noted in occasional experiments. This results in a potential inaccuracy of 0.06 to 0.08 log units in the stated K_D value of the competing ligands. However, as radioligand depletion would not have been constant through the displacement curve, with only half the depletion at IC_{50} (i.e. usually therefore an error of 0.02 log units for the calculated K_D value), this is within experimental error and does not substantially affect the results. Data are therefore plotted and K_D values calculated assuming no radioligand depletion.

Results

Evaluation of ^{3}H-rauwolscine and ^{3}H-RX821002 for whole cell binding

^{3}H-rauwolscine and ^{3}H-RX821002 have previously been used for membrane binding studies in both cell lines and with human tissue (e.g. [20-21, 30, 34-35]. However, given the reported differences in off target affinity, both radioligands were investigated for their suitability for studying radioligand binding in whole living cells. Saturation binding yielded a K_D value for ^{3}H-rauwolscine in CHO-α2A cell of 2.79 ± 0.24nM (5830 ± 853 fmol/mg protein, n=7), in CHO-α2B cells of 7.87 ± 0.78nM (13102 ± 2805 fmol/mg protein, n=9) and in CHO-α2C cells of 0.76 ± 0.07nM (1379 ± 98 fmol/mg protein, n=9). For ^{3}H-RX821002 saturation binding studies, the values were K_D 4.73 ± 0.42nM (4584 ± 667 fmol/mg protein, n=8) in CHO-α2A cells, 17.96 ± 1.41nM (11326 fmol/mg protein, n=6) in CHO-α2B cells and 3.60 ± 0.24nM (798 ± 143 fmol/mg protein, n=6) in CHO-α2C cells. Several ligands were investigated in competition studies using both radioligands and very similar results were obtained (Table 1). Thus both ^{3}H-rauwolscine and ^{3}H-RX821002 are good ligands for whole cell studies in living CHO cells with transfected human α2-adrenoceptors. ^{3}H-rauwolscine was chosen for all further studies as its affinity was slightly higher at all three receptors.

Affinity and selectivity of ligands at α2-adrenoceptors

The affinity and selectivity of a large range of α-adrenoceptor antagonists was evaluated (Figure 1; Table 2). It is clear that there are few α2-subtype selective ligands. Dibenamine and phenoxybenzamine inhibited ^{3}H-rauwolscine binding in a manner best described by a two-component response in CHO-α2B cells for both compounds and for phenoxybenzamine in CHO-α2C cells (Figure 2, Table 2) in a manner similar to that seen in the α1-adrenoceptors [33]. The responses in CHO-α2A cells and for dibenamine in CHO-α2C cells were too low affinity for a second component to be clearly determined. Dibenamine and phenoxybenzamine both contain a nitrogen mustard group, which cyclises to form ethyleniminium ions [36]. Sodium thiosulphate reacts with the ethyleniminium ions preventing them interacting with α-adrenoceptors [36]. Preincubation with sodium thiosulphate abolished the higher affinity components and reduced the affinity of both ligands at all three receptors a follows: dibenamine -4.59 ± 0.08 n=5, -4.64 ± 0.07 n=5 and -4.64 ± 0.11 n=5 for α2A, α2B and α2C respectively; and for phenoxybenzamine -4.71 ± 0.13 n=5, -4.86 ± 0.08 n=5 and -4.96 ± 0.10 n=5 for α2A, α2B and α2C respectively and are therefore similar to the second component response. The higher affinity K_D values in Table 2 are

therefore highly likely to be the affinity of the ligand interacting with the receptor (as in [33]).

Given the more recent suggestions of α2C affinity being important for antipsychotic drug actions, the affinity and selectivity of antidepressants (Table 3) and antipsychotics (Figure 3; Table 4) were examined.

Affinity and selectivity of ligands at β1 and β2-adrenoceptors

Given that drug interactions at α1, α2, β1 and β2-adrenoceptors affect blood pressure control, and that the affinity of these ligand has been assessed in comparative assays in α1 and α2 receptors, the affinity of ligands was also evaluated in CHO cells stably expressing the human β1 or β2-adrenoceptor using ^{3}H-CGP12177 whole cell binding (Figure 3; Table 5).

Tables combing all ligands are presented in Supplementary Data. Supplementary Data Table 1 has the ligands arranged in alphabetical order (with suppliers and individual ligand codes, α2A, α2B, α2C, β1 and β2 affinity). Supplementary Data Table 2 has all ligands organised in order of α2A affinity (α2A, α2B, α2C affinities and selectivities).

Discussion

One aim of this study was to determine the selectivity of a range of ligands at the human α2-adrenoceptors and this study confirmed previous comments that there are few α2-subtype selective ligands [11, 14-15, 20].

Selectivity between α2A, α2B and α2C-adrenoceptors

Yohimbine and RX821002 were confirmed as high affinity antagonists at all 3 subtypes. Both compounds had lower affinity at α2B-adrenoceptors than at α2A or α2C, in keeping with some other studies (both in cell lines [24, 29], and in tissues [7, 30, 37-38]. Other compounds with high affinity at all 3 subtypes were: atipamezole [30, 37] and RS79948 [27] and should thus be regarded as non-selective α2-antagonists. Lisuride has high affinity across many different receptor subtypes [39-40].

BRL44408 (65nM at α2A) was the most α2A-adrenoceptor selective ligand in keeping with [22, 24, 26, 41] however although it was 60-fold selective for α2A over α2B, BRL44408's selectivity for α2A over α2C-adrenoceptors was only 9-fold. Although S32212 and ARC239 were 15-21-fold selective for the α2B over the α2A-adrenoceptor, their α2B vs α2C is marginal (less than 5 fold), in keeping with [21, 24, 28-29, 41-42] and thus there are no α2B-selective ligands. Within the α2-adrenoceptors, JP1302 was the overall most α2C-selective ligand with an α2C-selectivity of 43 and 65 over α2A and α2B respectively, in keeping with [20] however its affinity (120nM at α2C) was a little lower than previously reported (16-28nM [20]). MK-912 was the highest affinity ligand overall (0.15nM at α2C) and also had some α2C-selectivity (having 13 and 46-fold higher α2C-affinity than α2A or α2B respectively) again in keeping with previous studies [24, 26-27, 41].

Prazosin had higher affinity for α2C (257nM) and α2B (676nM) than α2A (4678nM), and thus the pattern of affinity at these 3 subtypes was similar to some other studies of human receptors [24, 29-30] even if the absolute values have varied considerably (see Introduction for details).

Selectivity across α1, α2 and β-adrenoceptors

Given that the affinity values determined in this study were using an identical technique to affinity values determined in the human α1 and β1 and β2-adrenoceptors (the only difference

was transfected receptor, radioligand and ligand used for non-specific binding), a second aim of this study was to compare affinities between the human adrenoceptors (α2, β1 and β2 reported here, α1A, α1B and α1D-adrenoceptor subtypes from [33] and β1, β2 and β3 from [31-32]. The findings of these studies are therefore discussed as a whole, in comparison with other literature findings.

SNAP5089, silodosin and niguldipine are indeed highly α1A-selective antagonists (>500 selectivity over α2 or β1 or β2-adrenoceptors), and BMY7378 has ~100-fold α1D-selectivity. BRL44408 is the best α2A selective antagonist although its affinity for α2A is only a modest 9-fold greater its α2C affinity. MK-912 is the best α2C-antagonist (0.15nM α2C-affntiy) although again its α2C selectivity is only modest (13-fold greater than α2A). JP1302 (α2C affinity 120nM) has an α1A-adrenoceptor affinity of 617nM, only 5-fold less, so is not a truly α2C-selective ligand. CGP20712A (β1) and ICI118551 (β2) are also highly selective antagonists with minimal α-affinity. There are no truly α1B or α2B selective antagonists. Figure 4 shows the affinity (log K_D values) of most selective ligand and each adrenoceptor subtype (i.e. BRL44408 for α2A, S32212 for α2B and MK-912 for α2C) along with the single most selective antagonists at the other adrenoceptors and demonstrates that the α2-adrenoceptors fall behind α1 and β with regards to availability of highly subtype-selective ligands.

Silodosin (used for benign prostatic hyperplasia BPH) and naftopidil (used especially in Japan for BPH and ureteral stone expulsion [43], have significant β2-adrenoceptor affinity (~30nM). Silodosin is highly α1A-selective (0.25nM) giving a >100-fold selectivity window compared to the other adrenoceptors. Naftopidil, however is not selective, with α1A and β2 affinities only 3-fold apart and thus potentially increasing the risk of bronchospasm in those with asthma. Likewise, there is little evidence here to support SKF86466 being an α2-selective antagonist [44-46]. The affinity of SKF86466 for the β2-adrenoceptor (250nM) is similar to the highest α-adrenoceptor affinity (407nM at α2C). This may well be a species issue (see introduction) with previous studies being conducted in rodents [44-46], however others suggest a human α2A-affinity of 13nM [23].

Labetolol and carvedilol are often usually referred to as dual α/β-blockers (e.g. [47]. Labetolol (affinities of β2 6-9nM, β1 11-23nM and α1A 47nM) has very poor affinity at α1B, α1D, α2A, α2B, α2C and β3-adrenoceptors and thus reasonable affinity at only 1 out of 6 α-

adrenoceptors. A β/α1A-antagonist would be a more accurate description. Likewise, carvedilol with affinities for β2 of 0.1-0.4nM, β1 of 0.6-1.8nM and β3 of 5nM also has highest α-affinity for α1A (4nM) over α1B or α1D (14nM) or α2-adrenoceptors (48-490nM), so with affinities up to 1000-fold different across the 9 different adrenoceptors should not be considered a pan α/β-blocker. The lack of affinity of other β-blockers for the α-adrenoceptors may also be expected [48].

Antidepressants and antipsychotics

Given the considerable CNS expression of α2A and α2C-adrenoceptors, and that many antidepressants and antipsychotics have high α1A-affinity, a third aim of this study was to compare the affinity of antidepressants and antipsychotics across the adrenoceptors.

The antidepressants generally had poor α2-adrenoceptor affinity, considerably lower affinity than that seen for the tricyclic antidepressant affinities at the α1A-adrenoceptor. The antidepressant mirtazapine is a slight outsider with the highest α2-affinity of the antidepressants studied here, and higher than α1A-affinity. It has been associated with antinociceptive properties attributed to α2-adrenoceptors in mice [19, 49]. Mirtazepine (α2A-affinity 158nM) and α2C 110nM), had similar affinity to the α2-antagonist idazoxan and similar values to those obtained in human α2A receptors (79-126nM) in [49], who also reported lower affinity at human α1 and unmeasurable affinity at human β1 or β2-adrenoceptors. Of note, [49] also reported similar values for mirtazapine for human and rat receptors, whereas [17] suggest ~10-fold higher α2-affinity in what appears to be data gathered from mice.

Interestingly, many tricyclic antidepressant had a slight α2B-selectivity, something not seen with most α-ligands (Table 2), with the most potent (amitriptyline) having an α2B-affinity (76nM) only 10-fold lower than that at α1A-adrenoceptor. Vortioxetine was the only antidepressant with any significant β-adrenoceptor affinity and the only to have β-adrenoceptor affinity greater than α-adrenoceptor affinity (178nM for the β2-adrenoceptor).

α2C-adrenoceptor affinity has previously been suggested to have added benefits for the clinical actions of certain antipsychotics [17, 50]. Here, first generation antipsychotics had lower affinity for the α2-adrenoceptors than α1-adrenoceptors, and had little selectivity for

α2C over the other α2-subtypes. For example, chlorpromazine had affinities of α2A 2239nM, α2B 251nM, α2C 1175nM, whereas its α1A-adrenoceptor affinity is 1nM. There is however huge heterogeneity even between studies of human α2-adrenoceptors. Chlorpromazine affinities range from α2A 78nM, α2B 4.8nM and α2C 41nM (^{3}H-rauwloscine membrane binding from human receptors expressed in COS cells [28], α2A 396-535nM (^{3}H-yohimbine membrane binding using human colonic cancer cells and human platelets [21], α2A 600nM, α2B 43nM and α2C 260nM (^{3}H-RX821002 membrane binding for human receptors expressed in CHO cells [29], α2A 1008nM, α2B 34nM and α2C 85nM (^{3}H-RX821002 membrane binding to human receptors expressed in mouse cells [30], α2A 2245nM (^{3}H-RX821002 membrane binding to human platelets [23], to α2A 4169nM and α2C 1413nM (antagonism of agonist responses living CHO cells expressing the human α2-adrenoceptor [50]).

The second generation antipsychotics had a wide range of affinity for the α2-adrenoceptors, with risperidone (9nM, α2C) and paliperidone (14nM α2C) having the highest affinity (in keeping with other human α2-adrenoceptor studies [50]), to >1000nM affinity for olazepine and amisulpiride. Even for risperidone and paliperidone, the α2C affinity is lower than that seen at the α1A-adrenoceptor and once again α2A vs α2C-selectivity was very marginal. Clozepine, which has been particularly noted for α2C-affinity [12-13, 17] had an α2C-affinity of 135nM, compared to its α1A-affinity of 5.4nM measured under identical conditions. This α2C affinity is similar to that measured in intact CHO cells expressing human receptors (54nM [50], but poorer than that reported in membrane radioligand binding studies (6.5nM [21, 29]).

Conclusion

This study, using identical methods to previous α1 and β-adrenoceptor studies, allows comparison of ligand affinity, and thus selectivity, between the α and β-adrenoceptor subtypes. Overall, there is huge variety in the literature for the affinity of α2 ligands (more so than for α1 or β), and for which species differences appear to play a significant role, but technique may also be important. Whilst selective antagonists exist for α1A, α1D, β1 and β2-adrenoceptor, there are few selective α2-adrenoceptor ligands and for those that do exist (BRL44408 for α1A and MK-912 for α2C) only have small windows of selectivity. Antidepressants (with the exception of mirtazapine) and first generation antipsychotics have

higher α1A than α2-adrenoceptor affinity. Second generation antipsychotic varied widely in their α2-adrenoceptor affinity however this study does not lend much support for an important role for α2C-selective action for certain antipsychotics. Clearly, however, even after a century of yohimbine use, there remains plenty of scope to develop selective α2-antagonists.

Authorship contributions

JGB designed the research study. RGWP, JA and JGB performed the research. JGB analysed the data. JGB wrote the paper.

Acknowledgements

We thank June McCulloch for her technical assistance. This was funded by the School of Life Sciences, University of Nottingham, and by a Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES)-University of Nottingham Programme in Drug Discovery (CAPES 88881.141267/2017-1).

Conflicts of interest

JGB has been on the Scientific Advisory Board for CuraSen Therapeutics since 2019.

Data sharing

Further information and requests for data and reagents should be directed to and will be fulfilled by the corresponding author, Jillian Baker. Please contact jillian.baker@nottingham.ac.uk

Ethical statement

No animals, human tissue, human volunteers or patients were used in this study.

References

[1] Morales A. (2001) Yohimbine in erectile dysfunction: would an orphan drug ever be properly assessed? World J Urol. 19(4): 251-5. doi: 10.1007/s003450000182.

[2] Tam SW, Worcel M, Wyllie M. (2001) Yohimbine: a clinical review. Pharmacol Ther. 91(3):215-43. doi: 10.1016/s0163-7258(01)00156-5.

[3] Sperling H, Lorenz A, Krege S, Arndt R, Michel MC (2002) An extract from the bark of Aspidosperma quebracho blanco binds to human penile alpha-adrenoceptors. J Urol. 168(1): 160-3.

[4] Michel MC, Brodde OE, Schnepel B, Behrendt J, Tschada R, Motulsky HJ, Insel PA. (1989) [3H]idazoxan and some other alpha 2-adrenergic drugs also bind with high affinity to a nonadrenergic site. Mol Pharmacol. 35(3):324-30.

[5] Brown CM, MacKinnon AC, McGrath JC, Spedding M, Kilpatrick AT. (1990) Alpha 2-adrenoceptor subtypes and imidazoline-like binding sites in the rat brain. Br J Pharmacol. 803-9. doi: 10.1111/j.1476-5381.1990.tb13010.x.

[6] Miralles A, Olmos G, Sastre M, Barturen F, Martin I, Garcia-Sevilla JA. (1993) Discrimination and pharmacological characterization of I2-imidazoline sites with [3H]idazoxan and alpha-2 adrenoceptors with [3H]RX821002 (2-methoxy idazoxan) in the human and rat brains. J Pharmacol Exp Ther. 264(3):1187-97.

[7] Clarke RW, Harris J. (2002) RX 821002 as a tool for physiological investigation of alpha(2)-adrenoceptors. CNS Drug Rev. 8(2):177-92. doi: 10.1111/j.1527-3458.2002.tb00222.x.

[8] Pettinger WA, Jackson EK (2020) α 2-Adrenoceptors: Challenges and Opportunities-Enlightenment from the Kidney. Cardiovasc Ther doi: 10.1155/2020/2478781.

[9] Calzada BC, Artiñano AA (2001) Alpha-adrenoceptor subtypes. Pharmacol Res 44(3): 195-208. doi: 10.1006/phrs.2001.0857.

[10] Trendelenburg AU, Philipp M, Meyer A, Klebroff W, Hein L and Starke K (2003) All three α2-adrenoceptor types serve as autoreceptors in postganglionic neurons. Naunyn-Schmiedeberg's Arch Pharmacol 368:504-512.

[11] Philipp M, Brede M, Hein L. (2002) Physiological significance of alpha(2)-adrenergic receptor subtype diversity: one receptor is not enough. Am J Physiol Regul Integr Comp Physiol. 283(2):R287-95. doi: 10.1152/ajpregu.00123.2002.

[12] Langer SZ (2015) α2-Adrenoceptors in the treatment of major neuropsychiatric disorders Trends Pharmacol Sci 36(4): 196-202. doi: 10.1016/j.tips.2015.02.006.

[13] Uys MM, Shahid M, Harvey BH. (2017) Therapeutic Potential of Selectively Targeting the α2C-Adrenoceptor in Cognition, Depression, and Schizophrenia-New Developments and Future Perspective. Front Psychiatry. 8:144. doi: 10.3389/fpsyt.2017.00144.

[14] Kable JW, Murrin LC, Bylund DB. (2000) In vivo gene modification elucidates subtype-specific functions of alpha(2)-adrenergic receptors. J Pharmacol Exp Ther. 293(1):1-7.

[15] Knaus AE, Muthig V, Schickinger S, Moura E, Beetz N, Gilsbach R, Hein L. (2007) Alpha2-adrenoceptor subtypes--unexpected functions for receptors and ligands derived from gene-targeted mouse models. Neurochem Int. 51(5):277-81. doi: 10.1016/j.neuint.2007.06.036.

[16] Bücheler MM, Hadamek K, Hein L. (2002) Two alpha(2)-adrenergic receptor subtypes, alpha(2A) and alpha(2C), inhibit transmitter release in the brain of gene-targeted mice. Neuroscience. 109(4):819-26. doi: 10.1016/s0306-4522(01)00531-0.

[17] Brosda J, Jantschak F, Pertz HH. (2014) α2-Adrenoceptors are targets for antipsychotic drugs. Psychopharmacology 231(5): 801-12. doi: 10.1007/s00213-014-3459-8.

[18] Zhang F, Wu R, Qiang X, Zhou M, Wang P. (2010) Antagonism of alpha2A-adrenoceptor: a novel approach to inhibit inflammatory responses in sepsis. J Mol Med (Berl). 88(3):289-96. doi: 10.1007/s00109-009-0555-z.

[19] Neyama H, Dozono N, Uchida H, Ueda H. (2020) Mirtazapine, an α2 Antagonist-Type Antidepressant, Reverses Pain and Lack of Morphine Analgesia in Fibromyalgia-Like Mouse Models. J Pharmacol Exp Ther. 375(1):1-9. doi: 10.1124/jpet.120.265942.

[20] Sallinen J, Höglund I, Engström M, Lehtimäki J, Virtanen R, Sirviö J, Wurster S, Savola JM, Haapalinna A. (2007) Pharmacological characterization and CNS effects of a novel highly selective alpha2C-adrenoceptor antagonist JP-1302. Br J Pharmacol. 150(4):391-402. doi: 10.1038/sj.bjp.0707005.

[21] Bylund DB, Ray-Prenger C, Murphy TJ. (1988) Alpha-2A and alpha-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype. J Pharmacol Exp Ther. 245(2):600-7.

[22] Young P, Berge J, Chapman H, Cawthorne MA. (1989) Novel alpha 2-adrenoceptor antagonists show selectivity for alpha 2A- and alpha 2B-adrenoceptor subtypes. Eur J Pharmacol. 168(3):381-6. doi: 10.1016/0014-2999(89)90801-7.

[23] Renouard A, Widdowson PS, Millan MJ. (1994) Multiple alpha 2 adrenergic receptor subtypes. I. Comparison of [3H]RX821002-labeled rat R alpha-2A adrenergic receptors in cerebral cortex to human H alpha2A adrenergic receptor and other populations of alpha-2 adrenergic subtypes. J Pharmacol Exp Ther. 270(3):946-57.

[24] Uhlén S, Porter AC, Neubig RR. (1994) The novel alpha-2 adrenergic radioligand [3H]-MK912 is alpha-2C selective among human alpha-2A, alpha-2B and alpha-2C adrenoceptors. J Pharmacol Exp Ther. 271(3):1558-65.

[25] Neylon CB, Summers RJ. (1985) [3H]-rauwolscine binding to alpha 2-adrenoceptors in the mammalian kidney: apparent receptor heterogeneity between species. Br J Pharmacol. 85(2): 349-59. doi: 10.1111/j.1476-5381.1985.tb08868.x.

[26] Wikberg-Matsson A, Wikberg JE, Uhlén S. (1995) Identification of drugs subtype-selective for alpha 2A-, alpha 2B-, and alpha 2C-adrenoceptors in the pig cerebellum and kidney cortex. Eur J Pharmacol. 284(3):271-9. doi: 10.1016/0014-2999(95)00354-n.

[27] Uhlén S, Dambrova M, Näsman J, Schiöth HB, Gu Y, Wikberg-Matsson A, Wikberg JE. (1998) [3H]RS79948-197 binding to human, rat, guinea pig and pig alpha2A-, alpha2B- and alpha2C-adrenoceptors. Comparison with MK912, RX821002, rauwolscine and yohimbine. Eur J Pharmacol. 343(1):93-101. doi: 10.1016/s0014-2999(97)01521-5.

[28] Bylund DB, Blaxall HS, Iversen LJ, Caron MG, Lefkowitz RJ, Lomasney JW (1992) Pharmacological characteristics of alpha 2-adrenergic receptors: comparison of pharmacologically defined subtypes with subtypes identified by molecular cloning Mol Pharmacol. 42(1):1-5.

[29] Laurila JM, Wissel G, Xhaard H, Ruuskanen JO, Johnson MS, Scheinin M. (2011) Involvement of the first transmembrane segment of human alpha(2) -adrenoceptors in the subtype-selective binding of chlorpromazine, spiperone and spiroxatrine. Br J Pharmacol. 164(5):1558-72. doi: 10.1111/j.1476-5381.2011.01520.x.

[30] Halme M, Sjöholm B, Savola JM, Scheinin M. (1995) Recombinant human alpha 2-adrenoceptor subtypes: comparison of [3H]rauwolscine, [3H]atipamezole and [3H]RX821002 as radioligands. Biochim Biophys Acta. 1266(2):207-14. doi: 10.1016/0167-4889(95)90410-i.

[31] Baker JG. The selectivity of β-adrenoceptor antagonists at the β1, β2 and β3 adrenoceptors. Br. J. Pharmacol. 2005 144: 317-322

[32] Baker JG. The selectivity of β-adrenoceptor agonists at the human β1, β2 and β3 adrenoceptors. Br. J. Pharmacol. 2010 160: 148-161

[33] Proudman RGW, Pupo AS, Baker JG. (2020) The affinity and selectivity of α-adrenoceptor antagonists, antidepressants, and antipsychotics for the human α1A, α1B, and α1D-adrenoceptors. Pharmacol Res Perspect. 8(4):e00602. doi: 10.1002/prp2.602.

[34] Lawhead RG, Blaxall HS, Bylund DB. (1992) Alpha-2A is the predominant alpha-2 adrenergic receptor subtype in human spinal cord. Anesthesiology 77(5):983-91. doi: 10.1097/00000542-199211000-00022.

[35] Erdbrügger W, Raulf M, Otto T and Michel MC (1995) Does [3H]2-methoxy-idazoxan (RX 821002) detect more alpha-2-adrenoceptor agonist high-affinity sites than [3H]rauwolscine? A comparison of nine tissues and cell lines. J Pharmacol Exp Ther 273:1287-1294.

[36] Graham JD (1957) The ethyleneiminium ion as the active species in 2-haloalkylamine compounds. Br J Pharmacol. 2: 489-497.

[37] Haapalinna A, Viitamaa T, MacDonald E, Savola JM, Tuomisto L, Virtanen R, Heinonen E. (1997) Evaluation of the effects of a specific alpha 2-adrenoceptor antagonist, atipamezole, on alpha 1- and alpha 2-adrenoceptor subtype binding, brain neurochemistry

and behaviour in comparison with yohimbine. Naunyn Schmiedebergs Arch Pharmacol. 356(5):570-82. doi: 10.1007/pl00005092.

[38] Pihlavisto M, Scheinin M. (1999) Functional assessment of recombinant human alpha(2)-adrenoceptor subtypes with cytosensor microphysiometry. Eur J Pharmacol. 385(2-3):247-53. doi: 10.1016/s0014-2999(99)00715-3.

[39] Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A. (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther. 303(2):791-804. doi: 10.1124/jpet.102.039867.

[40] Newman-Tancredi A, Cussac D, Audinot V, Nicolas JP, De Ceuninck F, Boutin JA, Millan MJ. (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. II. Agonist and antagonist properties at subtypes of dopamine D(2)-like receptor and alpha(1)/alpha(2)-adrenoceptor. J Pharmacol Exp Ther. 303(2):805-14. doi: 10.1124/jpet.102.039875.

[41] Uhlén S, Muceniece R, Rangel N, Tiger G, Wikberg JE. (1995) Comparison of the binding activities of some drugs on alpha 2A, alpha 2B and alpha 2C-adrenoceptors and non-adrenergic imidazoline sites in the guinea pig. Pharmacol Toxicol.76(6):353-64. doi: 10.1111/j.1600-0773.1995.tb00161.x.

[42] Millan MJ, Mannoury la Cour C, Chanrion B, Dupuis DS, Di Cara B, Audinot V, Cussac D, Newman-Tancredi A, Kamal M, Boutin JA, Jockers R, Marin P, Bockaert J, Muller O, Dekeyne A, Lavielle G. (2012) S32212, a novel serotonin type 2C receptor inverse agonist/α2-adrenoceptor antagonist and potential antidepressant: I. A mechanistic characterization. J Pharmacol Exp Ther. 340(3):750-64. doi: 10.1124/jpet.111.187468.

[43] Li J, Tang Z, Gao L, Li J, Qin F, Yuan J (2017) Efficacy and Safety of Naftopidil in the Medical Expulsion Therapy for Distal Ureteral Stone: A Systematic Review and Meta-Analysis. J Endourol 31(5):427-437. doi: 10.1089/end.2016.0486.

[44] Hieble JP, DeMarinis RM, Fowler PJ, Matthews WD. (1986) Selective alpha-2 adrenoceptor blockade by SK&F 86466: in vitro characterization of receptor selectivity. J Pharmacol Exp Ther. 236(1):90-6.

[45] Roesler JM, McCafferty JP, DeMarinis RM, Matthews WD, Hieble JP. (1986) Characterization of the antihypertensive activity of SK&F 86466, a selective alpha-2 antagonist, in the rat. J Pharmacol Exp Ther. 236(1):1-7.

[46] Schafers RF, Elliott HL, Howie CA, Reid JL. (1990) An evaluation of the alpha-adrenoceptor antagonism produced by SK&F 86466 in healthy normotensive males. Br J Clin Pharmacol. 30(6): 884-8. doi: 10.1111/j.1365-2125.1990.tb05455.x.

[47] Wong GWK, Laugerotte A, Wright JM (2015) Blood pressure lowering efficacy of dual alpha and beta blockers for primary hypertension Cochrane Database Syst Rev 2015(8):CD007449. doi: 10.1002/14651858.CD007449.pub2.

[48] Michel MC (2020) α1-adrenoceptor activity of β-adrenoceptor ligands - An expected drug property with limited clinical relevance. Eur J Pharmacol. 889: 173632. doi: 10.1016/j.ejphar.2020.173632.

[49] Millan MJ, Gobert A, Rivet JM, Adhumeau-Auclair A, Cussac D, Newman-Tancredi A, Dekeyne A, Nicolas JP, Lejeune F. (2000) Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic, but not serotonergic, transmission by blockade of alpha2-adrenergic and serotonin2C receptors: a comparison with citalopram. Eur J Neurosci. 12(3):1079-95. doi: 10.1046/j.1460-9568.2000.00982.x.

[50] Kalkman HO, Loetscher E. (2003) alpha2C-Adrenoceptor blockade by clozapine and other antipsychotic drugs. Eur J Pharmacol. 462(1-3):33-40. doi: 10.1016/s0014-2999(03)01308-6.

Table 1
Log K_D values obtained from inhibition of ^{3}H-RX821002 or ^{3}H-rauwolscine binding to the human α2A, α2B and α2C-adrenoceptors in living cells. Values represent mean ± s.e.mean of n separate experiments. Compounds are arranged in order of α2A-affinity.

	^{3}H-RX821002 as radioligand							^{3}H-rauwolscine as radioligand					
	Log K_D α2A		Log K_D α2B		Log K_D α2C			Log K_D α2A		Log K_D α2B		Log K_D α2C	
MK912	-8.76 ± 0.05	5	-8.23 ± 0.11	5	-10.00 ± 0.15	4		-8.71 ± 0.05	8	-8.16 ± 0.10	8	-9.82 ± 0.11	9
yohimbine	-8.58 ± 0.03	5	-7.66 ± 0.05	5	-8.78 ± 0.10	5		-8.48 ± 0.07	5	-7.66 ± 0.10	5	-8.52 ± 0.05	5
RX821002	-8.23 ± 0.02	5	-7.67 ± 0.04	5	-8.28 ± 0.07	5		-8.10 ± 0.07	5	-7.45 ± 0.06	5	-8.14 ± 0.02	5
WB4101	-7.58 ± 0.05	6	-6.88 ± 0.05	6	-8.24 ± 0.13	6		-7.55 ± 0.05	6	-6.77 ± 0.05	6	-8.17 ± 0.05	6
BRL44408	-7.24 ± 0.05	6	-5.59 ± 0.05	6	-6.32 ± 0.09	6		-7.19 ± 0.04	7	-5.41 ± 0.04	7	-6.22 ± 0.07	7
carvedilol	-6.58 ± 0.04	5	-6.46 ± 0.05	5	-7.46 ± 0.14	5		-6.54 ± 0.02	5	-6.31 ± 0.02	5	-7.32 ± 0.05	5
ARC239	-5.99 ± 0.06	4	-7.29 ± 0.14	4	-7.18 ± 0.05	4		-5.99 ± 0.06	5	-7.32 ± 0.14	6	-7.25 ± 0.14	5
chlorpromazine	-5.57 ± 0.11^{app}	6	-6.63 ± 0.11	6	-6.02 ± 0.16	6		-5.65 ± 0.13^{app}	6	-6.60 ± 0.12	6	-5.93 ± 0.11	6
prazosin	-5.41 ± 0.03	6	-6.34 ± 0.03	6	-6.48 ± 0.09	6		-5.33 ± 0.05	6	-6.17 ± 0.05	6	-6.59 ± 0.04	6
JP1302	-5.22 ± 0.04	5	-5.22 ± 0.04	5	-6.57 ± 0.26	5		-5.29 ± 0.04	5	-5.11 ± 0.05	5	-6.92 ± 0.13	5
labetalol	-4.63 ± 0.04^{app}	5	-4.99 ± 0.07^{app}	5	-5.42 ± 0.05	5		-4.62 ± 0.07^{app}	5	-4.71 ± 0.08^{app}	5	-5.27 ± 0.04	5

^{apparent} the maximum concentration of competing ligand inhibited most but not all of specific binding. An IC_{50} was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible. Thus an apparent K_D was calculated.

Table 2

Log K_D values obtained from inhibition of ^{3}H-rauwolscine binding by adrenoceptor antagonists to the human α2A, α2B and α2C-adrenoceptors in living cells. Values represent mean ± s.e.mean of n separate experiments. Selectivity ratios are also given where a ratio of 1 demonstrates no selectivity for a given receptor subtype over another. Thus BRL44408 has 60 fold higher affinity for the α2A than the α2B-adrenoceptor. Compounds are arranged in order of α2A-selectivity.

	Affinity measurements						Selectivity ratios			
ligand	Log K_D α2A	n	Log K_D α2B	n	Log K_D α2C	n	α2A vs B	α2A vs α2C	α2B vs α2C	
BRL 44408	-7.19 ± 0.04	7	-5.41 ± 0.04	7	-6.22 ± 0.07	7	60.3	9.3		6.5
benoxathian	-7.17 ± 0.02	5	-5.96 ± 0.06	5	-7.75 ± 0.03	5	16.2		3.8	61.7
tamsulosin	-6.33 ± 0.04	5	-5.31 ± 0.04	5	-6.41 ± 0.03	5	10.5		1.2	12.6
alfuzosin	-5.56 ± 0.04	5	-4.62 ± 0.05	5	-6.14 ± 0.04	5	8.7		3.8	33.1
2-MPMDQ	-6.79 ± 0.04	5	-5.94 ± 0.09	5	-7.50 ± 0.02	5	7.1		5.1	36.3
yohimbine	-8.48 ± 0.07	5	-7.66 ± 0.10	5	-8.52 ± 0.05	5	6.6		1.1	7.2
idazoxan	-7.17 ± 0.04	5	-6.39 ± 0.05	5	-7.16 ± 0.03	5	6.0	1.0		5.9
WB4104	-7.55 ± 0.05	6	-6.77 ± 0.05	6	-8.17 ± 0.05	6	6.0		4.2	25.1
A80426	-7.24 ± 0.08	6	-6.52 ± 0.06	6	-7.46 ± 0.07	6	5.2		1.7	8.7
eforaxan	-7.58 ± 0.05	5	-6.88 ± 0.07	5	-7.44 ± 0.04	5	5.0	1.4		3.6
2-PMDQ	-6.83 ± 0.05	5	-6.14 ± 0.08	5	-7.07 ± 0.02	5	4.9		1.7	8.5
atipamezole	-8.50 ± 0.08	5	-7.85 ± 0.04	5	-8.48 ± 0.09	5	4.5	1.0		4.3
RX 821002	-8.10 ± 0.07	5	-7.45 ± 0.06	5	-8.14 ± 0.02	5	4.5		1.1	4.9
sunepitron	-7.28 ± 0.04	6	-6.65 ± 0.08	6	-8.11 ± 0.04	6	4.3		6.8	28.8
doxazosin	-5.35 ± 0.04	6	-4.74 ± 0.07^{app}	6	-6.24 ± 0.02	6	4.1		7.8	31.6
phentolamine	-7.26 ± 0.03	5	-6.69 ± 0.05	5	-6.92 ± 0.04	5	3.7	2.2		1.7
MK-912	-8.71 ± 0.05	8	-8.16 ± 0.10	8	-9.82 ± 0.11	9	3.6		12.9	45.7
RS17053	-6.20 ± 0.11	5	-5.65 ± 0.07	5	-6.35 ± 0.08	5	3.5		1.4	5.0
RS100329	-7.00 ± 0.03	5	-6.47 ± 0.04	5	-7.82 ± 0.03	5	3.4		6.6	22.4
lisuride	-8.99 ± 0.05	5	-8.52 ± 0.05	5	-9.27 ± 0.05	5	3.0		1.9	5.6
BMY7378	-5.30 ± 0.03	5	-4.98 ± 0.09^{app}	5	-6.26 ± 0.01	5	2.1		9.1	19.1
RS79948	-8.93 ± 0.03	5	-8.57 ± 0.03	5	-9.36 ± 0.04	5	2.3		2.7	6.2

carvedilol	-6.54 ± 0.02	5		-6.31 ± 0.02	5		-7.32 ± 0.05	5		1.7			6.0		10.2
JP1302	-5.29 ± 0.04	5		-5.11 ± 0.05	5		-6.92 ± 0.13	5		1.5			42.7		64.6
SKF86466	-6.29 ± 0.05	5		-6.17 ± 0.047	5		-6.39 ± 0.04	5		1.3			1.3		1.7
3-MPPI	-6.67 ± 0.05[ep]	5		IC_{50}>-4	5		-7.01 ± 0.03[ep]	5					2.2		
PF3774076	-5.59 ± 0.04	6		IC_{50}>-4	6		-5.29 ± 0.09	6				2.0			
Rec15-2615	-5.53 ± 0.12[app]	6		IC_{50}>-4.5	6		-6.56 ± 0.13	6					10.7		
AH11110A	-4.70 ± 0.04[app]	5		IC_{50}>-4	5		-4.86 ± 0.03[app]	5					1.4		
silodosin	-5.49 ± 0.06[app]	6		IC_{50}>-5	6		-6.12 ± 0.06[app]	6					4.3		
5-methyl-urapidil	-5.18 ± 0.05	5		-5.17 ± 0.05	5		-5.81 ± 0.07	5		1.0			4.3		4.4
SNAP5089	IC_{50}>-5	5		IC_{50}>-5	5		-5.65 ± 0.06	5							
anisodamine	IC_{50}>-3	5		IC_{50}>-3	5		-3.56 ± 0.07[app]	5							
2-niguldipine	IC_{50}>-5	5		-5.48 ± 0.11	5		-6.07 ± 0.11	5							3.9
naftapidil	-6.55 ± 0.09	5		-6.60 ± 0.07	5		-7.17 ± 0.08	5			1.1		4.2		3.7
labetolol	-4.62 ± 0.07[app]	5		-4.71 ± 0.08[app]	5		-5.27 ± 0.04	5			1.2		4.5		3.6
ifenprodil	-6.01 ± 0.05	5		-6.14 ± 0.06	5		-6.80 ± 0.05	5			1.3		6.2		4.6
domperidone	-5.09 ± 0.06[app]	6		-5.29 ± 0.07	6		-5.78 ± 0.08	6			1.6		4.9		3.1
urapidil	-5.49 ± 0.05	5		-5.78 ± 0.08	5		-6.34 ± 0.05	5			1.9		7.1		3.6
HEAT	-7.45 ± 0.04	5		-7.72 ± 0.11	5		-8.05 ± 0.19	5			1.9		4.0		2.1
indoramin	-5.13 ± 0.03[app]	6		-5.46 ± 0.05	6		-5.80 ± 0.05	6			2.1		4.7		2.2
cyclazosin	-5.00 ± 0.03	5		-5.35 ± 0.13	5		-6.18 ± 0.02	5			2.2		15.1		6.8
imiloxan	-5.88 ± 0.03	6		-6.48 ± 0.05	6		-6.27 ± 0.03	6			4.0		2.5	1.6	
dibenamine	-5.80 ± 0.06	10		-6.43 ± 0.06 -4.64 ± 0.07 60.9 ± 3.4% site 1	10		-6.18 ± 0.05	10			4.3		2.4	1.8	
promethazine	-5.58 ± 0.07	5		-6.25 ± 0.06	5		-5.54 ± 0.05	5			4.7	1.1		5.1	
phenoxybenzamine	-5.72 ± 0.10	10		-6.44 ± 0.11 -4.89 ± 0.08 51.4 ± 3.3% site 1	10		-6.41 ± 0.11 -4.71 ± 0.13 74.1 ± 4.1% site 1	10			5.2		4.9	1.1	
prazosin	-5.33 ± 0.05	6		-6.17 ± 0.05	6		-6.59 ± 0.04	6			6.9		18.2		2.6
terazosin	-5.18 ± 0.03	5		-6.08 ± 0.05	5		-6.27 ± 0.08	5			7.9		12.3		1.5
spiroxatrine	-6.97 ± 0.03	6		-7.87 ± 0.07	6		-8.74 ± 0.04	6			7.9		58.9		7.4

S32212	-6.62 ± 0.13	8		-7.80 ± 0.10	8		-7.18 ± 0.10	8			15.1		3.6	4.2	
ARC239	-5.99 ± 0.06	5		-7.32 ± 0.14	6		-7.25 ± 0.14	5			21.4		18.2	1.2	
β-blockers															
cyanopindolol	-5.56 ± 0.10	5		-4.82 ± 0.10[app]	5		-6.15 ± 0.07	5		5.5			3.9		21.4
bucindolol	-5.81 ± 0.05	5		-5.63 ± 0.06	5		-5.95 ± 0.04	5		1.5			1.4		2.1
ICI118551	-5.03 ± 0.03	5		IC_{50}>-4	5		-5.05 ± 0.04	5				1.0			
SDZ21009	-4.86 ± 0.07[app]	6		IC_{50}>-4	6		IC_{50}>-4.5	6							
propranolol	-4.85 ± 0.02	5		IC_{50}>-4	5		-4.71 ± 0.06	5				1.4			
carazolol	-4.66 ± 0.06[app]	6		IC_{50}>-4	6		-4.66 ± 0.05[app]	6				1.0			
CGP12177	IC_{50}>-3	5		No bind to 1mM	5		IC_{50}>-3	5							
CGP20712A	IC_{50}>-4	5		IC_{50}>-4	5		-5.17 ± 0.03	5							

[app] = apparent affinity. The maximum concentration of competing ligand inhibited most but not all of specific binding. An IC_{50} was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible. Thus an apparent K_D was calculated.

[ep] = early plateau, the competing ligand did not fully inhibit specific binding and the inhibition curve reached a plateau of maximal inhibition of binding. The specific binding inhibited by 3-MPPI was 75.6 ± 0.9% at α2A and 87.1 ± 1.5% at α2C

Table 3

Log K_D values of antidepressants binding to the human α2A, α2B and α2C-adrenoceptors. Values represent mean ± s.e.mean of n separate experiments. Selectivity ratios are also given, where a ratio of 1 demonstrates no selectivity for a given receptor subtype over another. Thus, clompiramine has 2.5 fold higher affinity for the α2B than the α2A-adrenoceptor. Compounds are arranged in order of α2A-selectivity.

	Affinity measurements								Selectivity ratios					
ligand	Log K_D α2A	n		Log K_D α2B	n		Log K_D α2C	n	α2A vs α2B		α2A vs α2C		α2B vs α2C	
Tricyclic antidepressants														
clomipramine	-5.71 ± 0.07[app]	5		-6.10 ± 0.13	5		-5.80 ± 0.02[app]	5		2.5		1.2	2.0	
protriptyline	-5.00 ± 0.05	5		-5.39 ± 0.13	5		-5.26 ± 0.07	5		2.5		1.8	1.3	
norclomipramine	-5.29 ± 0.09[app]	6		-5.74 ± 0.04[app]	6		-5.80 ± 0.07[app]	6		2.8		3.2		1.1
trimipramine	-5.67 ± 0.03	5		-6.22 ± 0.05	5		-6.37 ± 0.03	5		3.5		5.0		1.4
nortriptyline	-5.65 ± 0.05	5		-6.38 ± 0.02	5		-6.19 ± 0.08	5		5.4		3.5	1.5	
desipramine	-5.04 ± 0.06	5		-5.78 ± 0.04	5		-5.52 ± 0.03	5		5.5		3.0	1.8	
lofepramine	-4.86 ± 0.04[app]	5		-5.60 ± 0.08	5		-5.28 ± 0.06	5		5.5		2.6	2.1	
doxepin	-5.69 ± 0.12	5		-6.67 ± 0.05	5		-6.04 ± 0.07	5		9.5		2.2	4.3	
dosulepin	-5.16 ± 0.06	5		-6.20 ± 0.06	5		-5.63 ± 0.11	5		11.0		3.0	3.7	
imipramine	-5.25 ± 0.04	5		-6.36 ± 0.08	5		-5.89 ± 0.03	5		12.9		4.4	3.0	
amitriptyline	-5.86 ± 0.05[app]	5		-7.12 ± 0.05	5		-6.67 ± 0.09	5		18.2		6.5	2.8	
Tetracyclic antidepressants														
mirtazepine	-6.80 ± 0.05	5		-6.09 ± 0.06	5		-6.96 ± 0.03	5	5.1			1.4		7.4
other noradrenaline and serotonin reuptake inhibitors														
duloxetine	-5.43 ± 0.06	5		-5.31 ± 0.09	5		-5.67 ± 0.06	5	1.3			1.7		2.3
venlafaxime	-3.46 ± 0.03[app]	5		IC_{50}>-3	5		-3.74 ± 0.11[app]	5				1.9		
Noradrenaline reuptake inhibitors														
reboxetine	IC_{50}>-4	5		IC_{50}>-4	5		-4.56 ± 0.07[app]	4						

Selective serotonin reuptake inhibitors (SSRI)																	
fluvoxamine	-4.81 ± 0.04^{app}	6		-4.37 ± 0.08app	5		-4.82 ± 0.07^{app}	6		2.8		1.0			2.8		
sertraline	-5.67 ± 0.07^{app}	6		-5.62 ± 0.11^{app}	6		-5.64 ± 0.05^{app}	6		1.1		1.1		1.0			
fluoxetine	-4.70 ± 0.10^{app}	5		-4.99 ± 0.03	5		-4.79 ± 0.07^{app}	5			1.9		1.2	1.6			
citalopram	IC_{50}>-4	5		IC_{50}>-4	5		IC_{50}>-4	5									
paroxetine	IC_{50}>-5	5		IC_{50}>-5	5		IC_{50}>-5	5									
Serotonin reuptake inhibitors																	
vortioxetine	-5.63 ± 0.06^{app}	5		-5.32 ± 0.04^{app}	6		-5.84 ± 0.05	6		2.0			1.6		3.3		
trazodone	-6.17 ± 0.08	5		-5.96 ± 0.07	5		-6.69 ± 0.04	5		1.6			3.3		5.4		

^{app} = apparent affinity The maximum concentration of competing ligand inhibited most but not all of specific binding. An IC_{50} was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible.

Table 4

Log K_D values of antipsychotics binding to the human α2A, α2B and α2C-adrenoceptors. Values represent mean ± s.e.mean of n separate experiments. Selectivity ratios are also given where a ratio of 1 demonstrates no selectivity for a given receptor subtype over another. Compounds are arranged in order of α2A-selectivity.

	Affinity measurements									Selectivity ratios					
ligand	Log K_D α2A	n		Log K_D α2B	n		Log K_D α2C	n		α2A vs α2B		α2A vs α2C		α2B vs α2C	
First generation antipsychotics															
sulpiride	-4.50 ± 0.02	5		-4.37 ± 0.06	5		-4.67 ± 0.07	5		1.3			1.5		2.0
haloperidol	-5.38 ± 0.06	5		-5.53 ± 0.10	5		-5.77 ± 0.05	5			1.4		2.5		1.7
flupenthixol	-6.10 ± 0.12	5		-6.28 ± 0.13	5		-6.88 ± 0.14	5			1.5		6.0		4.0
pimozide	-5.76 ± 0.12[ep]	5		-6.30 ± 0.10	5		-6.84 ± 0.05	5			3.5		12.0		3.5
trifluoperazine	-5.60 ± 0.05	5		-6.22 ± 0.12	5		-6.20 ± 0.06	5			4.2		4.0	1.0	
prochlorperazine	-5.78 ± 0.02[app]	6		-6.46 ± 0.11	6		-6.31 ± 0.09	6			4.8		3.4	1.4	
chlorpromazine	-5.65 ± 0.13[app]	6		-6.60 ± 0.12	6		-5.93 ± 0.11	6			8.9		1.9	4.7	
perphenazine	-6.00 ± 0.06	6		-7.16 ± 0.05	6		-6.83 ± 0.04	5			14.5		6.8	2.1	
Second generation antipsychotics															
amisulpiride	-5.11 ± 0.09[app]	5		-4.69 ± 0.13[app]	5		-5.57 ± 0.07	5		2.6			2.9		7.6
aripirazole	-6.68 ± 0.08	5		-6.54 ± 0.08	6		-7.23 ± 0.14	5		1.4			3.5		4.9
sertindole	-5.95 ± 0.06	5		-5.81 ± 0.07	5		-6.17 ± 0.03	5		1.4			1.7		2.3
olanzapine	-5.59 ± 0.05	5		-5.47 ± 0.06	5		-5.86 ± 0.02	5		1.3			1.9		2.5
paliperidone	-7.12 ± 0.04	5		-7.26 ± 0.05	5		-7.84 ± 0.03	5			1.4		5.2		3.8
risperidone	-7.30 ± 0.09	5		-7.47 ± 0.08	5		-8.04 ± 0.03	5			1.5		5.5		3.7
ziprasidone	-6.36 ± 0.11	5		-6.59 ± 0.08	5		-6.77 ± 0.08	5			1.7		2.6		1.5
clozapine	-5.86 ± 0.08[app]	5		-6.20 ± 0.05	5		-6.87 ± 0.08	5			2.2		10.2		4.7
lurasidone	-6.67 ± 0.05	5		-7.36 ± 0.06	5		-7.34 ± 0.03	5			4.9		4.7	1.0	
quetiapine	-5.81 ± 0.08	5		-6.72 ± 0.08	5		-6.66 ± 0.03	5			8.1		7.1	1.1	

[app] = apparent affinity. The maximum concentration of competing ligand inhibited most but not all of specific binding. An IC_{50} was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible.

[ep] = early plateau, the competing ligand did not fully inhibit specific binding and the inhibition curve reached a plateau of maximal inhibition of binding. The specific binding inhibited by pimozide was 79.1 ± 6.0% at α2A.

Table 5
Log K_D values of ligands binding to the human β1 and β2-adrenoceptors as measured by ^{3}H-CGP12177 whole cell binding. Values represent mean ± s.e.mean of n separate experiments. Ligands are arranged by class and presented in the same order as those in Table 2, 3 and 4 for ease of comparison. Supplementary Table 1 has these ligands, alongside the α2-data, presented in alphabetical order.

	Affinity measurements				
ligand	Log K_D β1	n		Log K_D β2	n
α-antagonists					
BRL44408	No binding to -3	5		No binding to -3	5
benoxathian	-4.55 ± 0.03^{app}	5		-5.08 ± 0.06	5
tamsulosin	-6.26 ± 0.06	5		-6.08 ± 0.05	5
alfuzosin	No binding	5		-4.18 ± 0.09^{app}	5
2-MPMDQ	IC_{50}>-5	6		IC_{50}>-5	6
yohimbine	No binding to -4	5		No binding to -4	5
idazoxan	IC_{50}>-3	5		IC_{50}>-3	5
WB4104	IC_{50}>-4	5		IC_{50}>-4	5
A80426	-6.03 ± 0.05	6		-5.88 ± 0.04	6
eforaxan	no binding to -3	5		no binding to -3	5
2-PMDQ	No binding -4	5		IC_{50}>-4	5
atipamezole	No binding to -4.5	5		No binding to -4.5	5
RX821002	-4.55 ± 0.05	5		-3.95 ± 0.11^{app}	5
sunepitron	IC_{50}>-3	5		IC_{50}>-3	5
doxazosin	-4.72 ± 0.06^{app}	5		-5.57 ± 0.01	6
MK-912	IC_{50}>-4	6		IC_{50}>-4	6
phentolamine	IC_{50}>-4	6		IC_{50}>-4	6
RS17053	-5.44 ± 0.04	6		-6.42 ± 0.06	6
RS100329	IC_{50}>-3	5		-4.77 ± 0.07	5
lisuride	-6.03 ± 0.06	5		-7.48 ± 0.04	5
BMY7378	IC_{50}>-4	9		IC_{50}>-4	9
RS79948	-3.84 ± 0.05	5		IC_{50}>-3	5
carvedilol	-9.20 ± 0.05	8		-9.98 ± 0.06	8
JP1302	IC_{50}>-4	5		-5.58 ± 0.08	5
SKF86466	-5.92 ± 0.08	6		-6.60 ± 0.07	6
3-MPPI	No binding to -4	5		IC_{50}>-4	5
PF3774076	No binding to -4	5		No binding to -4	5
Rec15-2615	IC_{50}>-4	5		IC_{50}>-4	5
AH11110A	-6.23 ± 0.07	6		-6.36 ± 0.07	6
silodosin	IC_{50}>-5	6		-7.52 ± 0.10	6
5-methyl-urapidil	-6.12 ± 0.04	5		-5.00 ± 0.07	5
SNAP5089	IC_{50}>-5	5		IC_{50}>-5	5
anisodamine	no binding to -3	9		no binding to -3	9
2-niguldipine	IC_{50}>-4	5		IC_{50}>-4	5
naftapidil	-5.97 ± 0.07	6		-7.45 ± 0.06	6
labetolol	-7.97 ± 0.04	6		-8.21 ± 0.06	6
ifenprodil	IC_{50}>-5	5		IC_{50}>-5	5
domperidone	IC_{50}>-4	5		IC_{50}>-4	5
urapidil	-5.32 ± 0.06	5		-5.00 ± 0.02	5

HEAT	IC_{50} ~-4.5	5		IC_{50}>-4	5
indoramin	-4.73 ± 0.10[app]	5		-5.27 ± 0.11[app]	5
cyclazosin	No binding to -4	6		-5.30 ± 0.04	6
imiloxan	IC_{50}~-3	5		no binding to -3	5
dibenamine	-4.60 ± 0.06[app]	5		-4.94 ± 0.10[app]	5
promethazine	IC_{50}>-4	10		IC_{50}>-4	10
phenoxybenzamine	-4.36 ± 0.10[app]	5		-5.17 ± 0.13[app]	5
prazosin	No binding to -4	6		-5.10 ± 0.10[app]	5
terazosin	No binding to -4	4		No binding to -4	4
spiroxatrine	IC_{50}>-4.5	5		IC_{50}>-4.5	5
S32212	IC_{50}>-5	5		IC_{50}>-5	5
ARC239	IC_{50}>-5	6		IC_{50}>-5	5
β-blockers					
S-cyanopindolol	-10.39#			-11.09#	
bucindolol	-9.31#			-9.99#	
ICI118551	-6.61 ± 0.05	11		-9.41 ± 0.09	10
SDZ21009	-8.94#			-10.28#	
propranolol	-8.16*			-9.08*	
carazolol	-9.69#			-10.49#	
CGP12177	-9.21*			-9.39*	
CGP20712A	-8.87 ± 0.13	9		-5.74 ± 0.03	10
Tricyclic antidepressants					
clomipramine	IC_{50}>-5	7		IC_{50}>-5	7
protriptyline	IC_{50}>-4	5		IC_{50}>-4	5
norclomipramine	IC_{50}>-4.5	10		IC_{50}>-4.5	10
trimipramine	IC_{50}>-4	5		IC_{50}>-4	5
nortriptyline	-4.64 ± 0.13	5		-5.40 ± 0.08	5
desipramine	IC_{50}>-4	5		-4.93 ± 0.03[app]	5
lofepramine	IC_{50}>-4	4		IC_{50}>-4	4
doxepin	IC_{50}>-4	5		IC_{50}>-4	5
dosulepin	IC_{50}>-4	5		IC_{50}>-4	5
imipramine	IC_{50}>-4	5		IC_{50}>-4	5
amitriptyline	IC_{50}>-4	9		IC_{50}>-4	9
Tetracyclic antidepressants					
mirtazepine	No binding to -4	5		No binding to -4	5
other noradrenaline and serotonin reuptake inhibitors					
duloxetine	IC_{50}>-4.5			-6.07 ± 0.06	11
venlafaxime	-3.80 ± 0.11[app]	5		-4.13 ± 0.13[app]	5
Noradrenaline reuptake inhibitors					
reboxetine	IC_{50}>-4	10		-5.26 ± 0.06	10
Selective serotonin reuptake inhibitors (SSRI)					
fluvoxamine	IC_{50}>-4	10		IC_{50}>-4	10

sertraline	IC_{50}>-5	10		IC_{50}>-5	10
fluoxetine	IC_{50}>-4	10		IC_{50}>-4	10
citalopram	No binding to -4	9		No binding to -4	9
paroxetine	IC_{50}>-4.5	10		IC_{50}>-4.5	10
Serotonin reuptake inhibitors					
vortioxetine	-6.37 ± 0.03	11		-6.75 ± 0.04	11
trazodone	IC_{50}>-4	10		-5.14 ± 0.05	10
First generation antipsychotics					
sulpiride	IC_{50}>-3	10		IC_{50}>-3	10
haloperidol	IC_{50}>-4	5		-4.94 ± 0.04^{app}	5
flupenthixol	IC_{50}>-5	10		IC_{50}>-5	10
pimozide	IC_{50}>-4	10		-5.75 ± 0.06	10
trifluoperazine	IC_{50}>-5	10		IC_{50}>-5	10
prochlorperazine	IC_{50}>-5	10		IC_{50}>-5	10
chlorpromazine	IC_{50}>-5	5		IC_{50}>-5	5
perphenazine	IC_{50}>-5	10		IC_{50}>-5	10
Second generation antipsychotics					
amisulpiride	No binding to -4	10		No binding to -4	10
aripirazole	-6.15 ± 0.04	6		-6.68 ± 0.08	6
sertindole	IC_{50}>-5	5		IC_{50}>-5	5
olanzapine	IC_{50}>-3	4		-4.96 ± 0.05	4
paliperidone	IC_{50}>-4.5	10		IC_{50}>-4.5	10
risperidone	No binding to -4	5		IC_{50}>-4	5
ziprasidone	No binding to -4	5		No binding to -4	5
clozapine	IC_{50}>-5	5		IC_{50}>-5	5
lurasidone	IC_{50}>-5	10		IC_{50}>-5	10
quetiapine	IC_{50}>-4	10		IC_{50}>-4	10

#from [32]

*from [31]

Figure Legends

Figure 1

Inhibition of ^{3}H-rauwolscine binding to whole cells by BRL44408 (a-c), S32212 (d-f) or MK-912 (g-i) to CHO-α2A cells (a, d, g), CHO-α2B cells (b, e, h) or CHO-α2C cells (c, f, i). Bars represent total ^{3}H-rauwolscine and non-specific binding (determined in the presence of 10μM RX821002. The concentration of ^{3}H-rauwolscine was a) 0.99nM, b) 0.99nM, c) 0.99nM, d) 0.88nM, e) 0.88nM, f) 0.88nM, g) 0.86nM, h) 0.86nM and i) 0.88nM. Data points are mean ± s.e.mean of triplicate determinations.

Figure 2

Inhibition of ^{3}H-rauwolscine binding to whole cells by dibenamine following pre-incubation of dibenamine with sfm or 1mM thiosulphate to CHO-α2A cells (a), CHO-α2B cells (b) or CHO-α2C cells (c). Bars represent total ^{3}H-rauwolscine binding and non-specific binding as determined in the presence of 10μM RX821002. The concentration of ^{3}H-rauwolscine was 0.74nM in all cases. Data points are mean ± s.e.mean of triplicate determinations.

Figure 3

Inhibition of ^{3}H-rauwolscine (α2A, α2B and α2C cells) or ^{3}H-CGP12177 (β1 and β2 cells) binding to whole cells by a-e) risperidone, f-j) aripiprazole and k-o) clozapine to CHO-α2A cells, CHO-α2B cells, CHO-α2C cells, CHO-β1 cells CHO-β2 cells. Bars represent total radioligand binding and non-specific binding as determined in the presence of 10μM RX821002 (α2A, α2B and α2C cells) or 10μM propranolol (β1 and β2 cells). The concentration of radioligand was a) 0.54nM, b) 0.54nM, c) 0.54nM, d) 0.77nM, e) 1.00nM, f) 0.50nM, g) 0.50nM, h) 0.50nM, i) 0.72nM, j) 0.72nM, k) 0.50nM, l) 0.54nM, m) 0.54nM, n) 0.94nM and o) 0.72nM. Data points are mean ± s.e.mean of triplicate determinations.

Figure 4

Plot of log K_D values showing the relative selectivity and affinity for the single most selective ligand at each receptor. Thus SNAP5089 is the most α1A-selective ligand and the length of the line represents the selectivity for α1A over the next closest adrenoceptor affinity. Terazosin, although the “most” α1B-selective ligand has no selectivity. The selectivity of the 3 most selective α2 ligands is considerably less than that for α1A, α1D, β1 or β2. Compounds

within the black circles represent compounds where the log K_D is greater than the -3, -4 or -5 stated but included here to demonstrate attempts were made measurement. Data for α1-adrenoceptors is from [33]. β3 data is included for CGP20712A and ICI118551 from [31].