

ORIGINAL ARTICLE

The affinity and selectivity of α -adrenoceptor antagonists, antidepressants and antipsychotics for the human α 2A, α 2B, and α 2C-adrenoceptors and comparison with human α 1 and β -adrenoceptors

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Funding information

University of Nottingham; Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES)-University of Nottingham Programme in Drug Discovery, Grant/Award Number: CAPES 88881.141267/2017-1

Abstract

α 2-Adrenoceptors, subdivided into α 2A, α 2B, and α 2C subtypes and expressed in heart, blood vessels, kidney, platelets and brain, are important for blood pressure, sedation, analgesia, and platelet aggregation. Brain α 2C-adrenoceptor blockade has also been suggested to be beneficial for antipsychotic action. However, comparing α 2-adrenoceptor subtype affinity is difficult due to significant species and methodology differences in published studies. Here, ^3H -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 α 2A, α 2B, or α 2C-adrenoceptors, using an identical method to β and α 1-adrenoceptor measurements, thus allowing direct human receptor comparisons. Yohimbine, RX821002, RS79948, and atipamezole are high affinity non-selective α 2-antagonists. BRL44408 was the most α 2A-selective antagonist, although its α 1A-affinity (81 nM) is only 9-fold greater than its α 2C-affinity. MK-912 is the highest-affinity, most α 2C-selective antagonist (0.15 nM α 2C-affinity) although its α 2C-selectivity is only 13-fold greater than at α 2A. There are no truly α 2B-selective antagonists. A few α -ligands with significant β -affinity were detected, for example, nafopidil where its clinical α 1A-affinity is only 3-fold greater than off-target β 2-affinity. Antidepressants (except mirtazapine) and first-generation antipsychotics have higher α 1A than α 2-adrenoceptor affinity but poor β -affinity. Second-generation antipsychotics varied widely in their α 2-adrenoceptor affinity. Risperidone (9 nM) and paliperidone (14 nM) have the highest α 2C-adrenoceptor affinity however this is only 5-fold selective over α 2A, and both have a higher affinity for α 1A (2 nM and 4 nM, respectively). So, despite a century of yohimbine use, and decades of α 2-subtype studies, there remains plenty of scope to develop α 2-subtype selective antagonists.

KEYWORDS

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

Abbreviations: BPH, benign prostatic hyperplasia; CHO, Chinese hamster ovary; sfm, serum free media =DMEM/F12 containing 2mM L-glutamine.

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1 | INTRODUCTION

The $\alpha 2$ -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) $\alpha 2$ -effects and potential local effects as $\alpha 2A$, $\alpha 2B$, and $\alpha 2C$ -adrenoceptors are expressed in human corpus cavernosum,^{1,2} and can indeed bind yohimbine from tree bark.³ The $\alpha 2$ -antagonist idazoxan, developed in 1970s, is selective for $\alpha 2$ over $\alpha 1$ -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 $\alpha 2$ -adrenoceptor affinity but without imidazoline receptor affinity (although 5-HT receptor interactions still occur^{6,7}).

$\alpha 2$ -Adrenoceptors are subdivided into $\alpha 2A$, $\alpha 2B$, and $\alpha 2C$ -subtypes. With receptors being present in the heart, blood vessels, and kidney,⁸ $\alpha 2$ -adrenoceptors are important in blood pressure control (an interplay between $\alpha 1$, $\alpha 2$, and **β -adrenoceptors**) and including central and peripheral $\alpha 2$ -effects. In addition, many $\alpha 2$ -adrenoceptors present in the brain also have clinical roles in anaesthesia and psychiatric treatments⁹ with both pre- and post-synaptic effects on neurotransmission.¹⁰⁻¹³

$\alpha 2A$ -adrenoceptors are widely expressed and are important for blood pressure, sedation, analgesia, platelet aggregation, and hypothermia.^{14,15} In the brain, 90% of all $\alpha 2$ -adrenoceptors are of the $\alpha 2A$ subtype and they are highly expressed in the prefrontal cortex where activation increases cognitive function.^{16,17} $\alpha 2A$ -adrenoceptor antagonism may be important in sepsis (administration of the $\alpha 2A$ - antagonist BRL44408 reduced pro-inflammatory cytokines, TNF- α and IL-6 and increased survival in a rat model of sepsis¹⁸) and potentially clinically relevant $\alpha 2A$ -mirtazapine-induced reversal of analgesia.¹⁹ The roles of the **$\alpha 2B$ -adrenoceptors** are less clear. $\alpha 2B$ -adrenoceptors are involved in blood pressure control (activation causes a hypertensive response related to renal salt balance.¹⁴ The expression and effects of the $\alpha 2B$ -adrenoceptors appear very minor in the brain.¹⁷ The **$\alpha 2C$ -adrenoceptor** is involved in catecholamine release in adrenal chromaffin cells¹⁵ and in the brain process of startle and stress responses.¹⁴ $\alpha 2C$ -adrenoceptors form 10% of all brain adrenoceptors but appear particularly prevalent in the striatum and hippocampus.¹⁶ For certain antipsychotics (e.g., clozapine), $\alpha 2C$ -antagonism, in addition to dopamine D2 blockade, is thought to be beneficial in the management of schizophrenia^{12,13,17} and $\alpha 2C$ -antagonism may be helpful in improving cognition in dementia.¹² However, a lack of subtype selective $\alpha 2$ -adrenoceptor ligands has impaired understanding and knowledge of $\alpha 2$ -subtype expression and $\alpha 2$ -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 $\alpha 2$ -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, for example, human platelet or cortex for $\alpha 2A$ versus neonatal

rat lung for $\alpha 2B$.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 $\alpha 2A$ -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 $\alpha 2A$ -adrenoceptors.^{4,25} Overall, it appears that the human $\alpha 2$ -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 $\alpha 2A$ -adrenoceptors range 50-fold, from 300 nM^{21,28} to a few thousand nM,^{23,24,29} to 16000 nM.⁶ Differences in affinity have also been attributed to technique. A 5-fold difference in ³H-rauwolscine affinity, and 4-fold difference in ³H-RX821002 and ³H-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 $\alpha 2A$, $\alpha 2B$, or $\alpha 2C$ -adrenoceptor. Furthermore, as these measurements were determined using an identical technique in human $\beta 1$ and $\beta 2$ -adrenoceptors (included here, and^{31,32}) and $\alpha 1$ -adrenoceptors,³³ this study explores the affinity and selectivity of ligands across the human adrenoceptors commonly targeted for cardiovascular, urological and CNS effects.

2 | MATERIALS AND METHODS

2.1 | 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. ³H-rauwolscine (a stereoisomer of yohimbine, specific activity 82.9), ³H-RX821002 (specific activity 36.5), ³H-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).

2.2 | Cell lines

CHO-K1 (RIDD: CVCL_0214) were stably transfected with the human $\alpha 2A$ -adrenoceptor, human $\alpha 2B$ -adrenoceptor or human

TABLE 1 Log K_D values obtained from inhibition of ^3H -RX821002 or ^3H -rauwolscine binding to the human $\alpha 2\text{A}$, $\alpha 2\text{B}$, and $\alpha 2\text{C}$ -adrenoceptors in living cells. Values represent mean \pm s.e. mean of n separate experiments. Compounds are arranged in order of $\alpha 2\text{A}$ -affinity.

	^3H -RX821002 as radioligand						^3H -rauwolscine as radioligand					
	Log K_D $\alpha 2\text{A}$	Log K_D $\alpha 2\text{B}$	Log K_D $\alpha 2\text{C}$	Log K_D $\alpha 2\text{A}$	Log K_D $\alpha 2\text{B}$	Log K_D $\alpha 2\text{C}$	Log K_D $\alpha 2\text{A}$	Log K_D $\alpha 2\text{B}$	Log K_D $\alpha 2\text{C}$	Log K_D $\alpha 2\text{A}$	Log K_D $\alpha 2\text{B}$	Log K_D $\alpha 2\text{C}$
MK912	-8.76 \pm 0.05	-8.23 \pm 0.11	-10.00 \pm 0.15	4	8	-8.71 \pm 0.05	8	-8.16 \pm 0.10	8	-9.82 \pm 0.11	9	
yohimbine	-8.58 \pm 0.03	-7.66 \pm 0.05	-8.78 \pm 0.10	5	5	-8.48 \pm 0.07	5	-7.66 \pm 0.10	5	-8.52 \pm 0.05	5	
RX821002	-8.23 \pm 0.02	-7.67 \pm 0.04	-8.28 \pm 0.07	5	5	-8.10 \pm 0.07	5	-7.45 \pm 0.06	5	-8.14 \pm 0.02	5	
WB4101	-7.58 \pm 0.05	-6.88 \pm 0.05	-8.24 \pm 0.13	6	6	-7.55 \pm 0.05	6	-6.77 \pm 0.05	6	-8.17 \pm 0.05	6	
BRL44408	-7.24 \pm 0.05	-5.59 \pm 0.05	-6.32 \pm 0.09	6	6	-7.19 \pm 0.04	7	-5.41 \pm 0.04	7	-6.22 \pm 0.07	7	
carvedilol	-6.58 \pm 0.04	-6.46 \pm 0.05	-7.46 \pm 0.14	5	5	-6.54 \pm 0.02	5	-6.31 \pm 0.02	5	-7.32 \pm 0.05	5	
ARC239	-5.99 \pm 0.06	-7.29 \pm 0.14	-7.18 \pm 0.05	4	4	-5.99 \pm 0.06	5	-7.32 \pm 0.14	6	-7.25 \pm 0.14	5	
chlorpromazine	-5.57 \pm 0.11 ^{app}	-6.63 \pm 0.11	-6.02 \pm 0.16	6	6	-5.65 \pm 0.13 ^{app}	6	-6.60 \pm 0.12	6	-5.93 \pm 0.11	6	
prazosin	-5.41 \pm 0.03	-6.34 \pm 0.03	-6.48 \pm 0.09	6	6	-5.33 \pm 0.05	6	-6.17 \pm 0.05	6	-6.59 \pm 0.04	6	
JP1302	-5.22 \pm 0.04	-5.22 \pm 0.04	-6.57 \pm 0.26	5	5	-5.29 \pm 0.04	5	-5.11 \pm 0.05	5	-6.92 \pm 0.13	5	
labetalol	-4.63 \pm 0.04 ^{app}	-4.99 \pm 0.07 ^{app}	-5.42 \pm 0.05	5	5	-4.62 \pm 0.07 ^{app}	5	-4.71 \pm 0.08 ^{app}	5	-5.27 \pm 0.04	5	

Note: ^{app} 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.

$\alpha 2\text{C}$ -adrenoceptor DNA (DNAs from Guthrie DNA Resource Centre) using Lipofectamine and Optimem according to the manufacturer's instructions. Following 3 weeks selection using resistance to neomycin (at 1 mg/ml), single clones from each transfection were isolated by dilution cloning. Thus stable cell lines CHO- $\alpha 2\text{A}$, CHO- $\alpha 2\text{B}$, and CHO- $\alpha 2\text{C}$ were created. CHO lines stable expressing the human $\beta 1$ or $\beta 2$ -adrenoceptor were also used.³¹

2.3 | 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.

2.4 | ^3H -rauwolscine and ^3H -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 2 mM L-glutamine). Media was removed from each well and replaced with either 100 μl sfm (total binding) or 100 μl , 20 μM RX821002 (when ^3H -rauwolscine used) or 20 μM yohimbine (when ^3H -RX821002 used) in sfm to determine non-specific binding. 100 μl 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 h. After 2 h, the cells were washed twice by the addition and removal of 2 \times 200 μl cold (4°C) phosphate-buffered saline. A white base was applied to the plate to convert the wells into white-sided/white-bottomed plates, 100 μl Microscint 20 was added to each well and a transparent top seal applied to the plates. Plates were left at room temperature in the dark for at least 6 h before being counted on a Topcount (PerkinElmer, 2-min count per well).

2.5 | ^3H -rauwolscine, ^3H -RX821002, and ^3H -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 100 μl ligand added to triplicate wells. This was immediately followed by the addition of 100 μl radioligand (diluted in sfm) and the cells incubated for 2 h 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 10 μ M yohimbine or 10 μ M RX821002 in sfm) was defined in every plate.

Given the two-component inhibition of ^3H -prazosin binding seen with dibenamine and phenoxybenzamine at the α 1-adrenoceptors, sodium thiosulphate, which reacts with the ethyleniminium ions, was used in dibenamine and phenoxybenzamine experiments, in excess, as in Ref.³³

Thus all studies in human β , α 1, and α 2-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.

2.6 | Data analysis

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

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

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

$$\% \text{ Specific binding} = 100 - (100 \times [A] / ([A] + \text{IC}_{50}))$$

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:

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

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 a 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 $\text{IC}_{50} > \text{top}$ concentration used (i.e., $\text{IC}_{50} > 100\mu\text{M}$ means that 100 μM 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 ^3H -rauwolscine binding (e.g., Figure 2B) in which case a two-component curve was used, using the equation below:

$$\% \text{ specific binding} = \frac{[A].N}{([A] + \text{IC}_{501})} + \frac{[A].(100-N)}{([A] + \text{IC}_{502})}$$

where $[A]$ is the concentration of the competing ligand, IC_{501} and IC_{502} are the respective IC_{50} values for the two components and N is the percentage of the response occurring through the first component (IC_{501}). K_D values were calculated from IC_{50} values as above.

Radioligand concentrations were determined from taking the average of triplicate 50 μ l 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 expression 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, or up to 0.04 log units in the worst cases), 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.

Nomenclature of Targets and Ligands.

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,³⁴ and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.³⁵

3 | RESULTS

3.1 | Evaluation of ³H-rauwolscine and ³H-RX821002 for whole cell binding

³H-rauwolscine and ³H-RX821002 have previously been used for membrane binding studies in both cell lines and with human tissue (e.g.,^{20,21,30,36,37}). 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 ³H-rauwolscine in CHO- α 2A cell of 2.79 ± 0.24 nM (5830 ± 853 fmol/mg protein, $n=7$), in CHO- α 2B cells of 7.87 ± 0.78 nM (13102 ± 2805 fmol/mg protein, $n=9$) and in CHO- α 2C cells of 0.76 ± 0.07 nM (1379 ± 98 fmol/mg protein, $n = 9$). For ³H-RX821002 saturation-binding studies, the values were K_D 4.73 ± 0.42 nM (4584 ± 667 fmol/mg protein, $n=8$) in CHO- α 2A cells, 17.96 ± 1.41 nM (11326 ± 3531 fmol/mg protein, $n = 6$) in CHO- α 2B cells and 3.60 ± 0.24 nM (798 ± 143 fmol/mg protein, $n=6$) in CHO- α 2C cells. Several ligands were investigated in competition studies using both radioligands and very similar results were obtained (Table 1). Thus both ³H-rauwolscine and ³H-RX821002 are good ligands for whole cell studies in living CHO cells with transfected human α 2-adrenoceptors. ³H-rauwolscine was chosen for all further studies as its affinity was slightly higher at all three receptors.

3.2 | Affinity and selectivity of ligands at α 2-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 α 2-subtype selective ligands. Dibenamine and phenoxybenzamine inhibited ³H-rauwolscine binding in a manner best described by a two-component response in CHO- α 2B cells for both compounds and for phenoxybenzamine in CHO- α 2C cells (Figure 2, Table 2) in a manner similar to that seen in the α 1-adrenoceptors.³³ The responses in CHO- α 2A cells and for dibenamine in CHO- α 2C 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.³⁸ Sodium thiosulphate reacts with the ethyleniminium ions preventing them interacting with α -adrenoceptors.³⁸ 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 α 2A, α 2B, and α 2C, respectively; and for phenoxybenzamine -4.71 ± 0.13 $n = 5$, -4.86 ± 0.08 $n = 5$, and -4.96 ± 0.10 $n = 5$ for α 2A, α 2B, and α 2C, 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 in³³).

Given the more recent suggestions of α 2C affinity being important for antipsychotic drug actions, the affinity and selectivity of antidepressants (Table 3) and antipsychotics (Figure 3; Table 4) were examined.

3.3 | Affinity and selectivity of ligands at β 1 and β 2-adrenoceptors

Given that drug interactions at α 1, α 2, β 1, and β 2-adrenoceptors affect blood pressure control, and that the affinity of these ligand has been assessed in comparative assays in α 1 and α 2 receptors, the affinity of ligands was also evaluated in CHO cells stably expressing the human β 1 or β 2-adrenoceptor using ³H-CGP12177 whole cell binding (Figure 3; Table 5).

Tables combining all ligands are presented in Supplementary Data. Supplementary Data Table 1 has the ligands arranged in alphabetical order (with suppliers and individual ligand codes, α 2A, α 2B, α 2C, β 1, and β 2 affinity). Supplementary Data Table 2 has all ligands organised in order of α 2A affinity (α 2A, α 2B, α 2C affinities, and selectivities).

4 | DISCUSSION

One aim of this study was to determine the selectivity of a range of ligands at the human α 2-adrenoceptors and this study confirmed previous comments that there are few α 2-subtype selective ligands.^{11,14,15,20}

4.1 | Selectivity between α 2A, α 2B, and α 2C-adrenoceptors

Yohimbine and RX821002 were confirmed as high affinity antagonists at all three subtypes. Both compounds had a lower affinity at α 2B-adrenoceptors than at α 2A or α 2C, in keeping with some other studies (both in cell lines,^{24,29} and in tissues.^{7,30,39,40} Other compounds with high affinity at all 3 subtypes were: atipamezole^{30,39} and RS79948²⁷ and should thus be regarded as non-selective α 2-ligands. Lisuride has a high affinity across many different receptor subtypes.^{41,42}

BRL44408 (65 nM at α 2A) was the most α 2A-adrenoceptor selective ligand in keeping with^{22,24,26,43} however although it was 60-fold selective for α 2A over α 2B, BRL44408's selectivity for α 2A

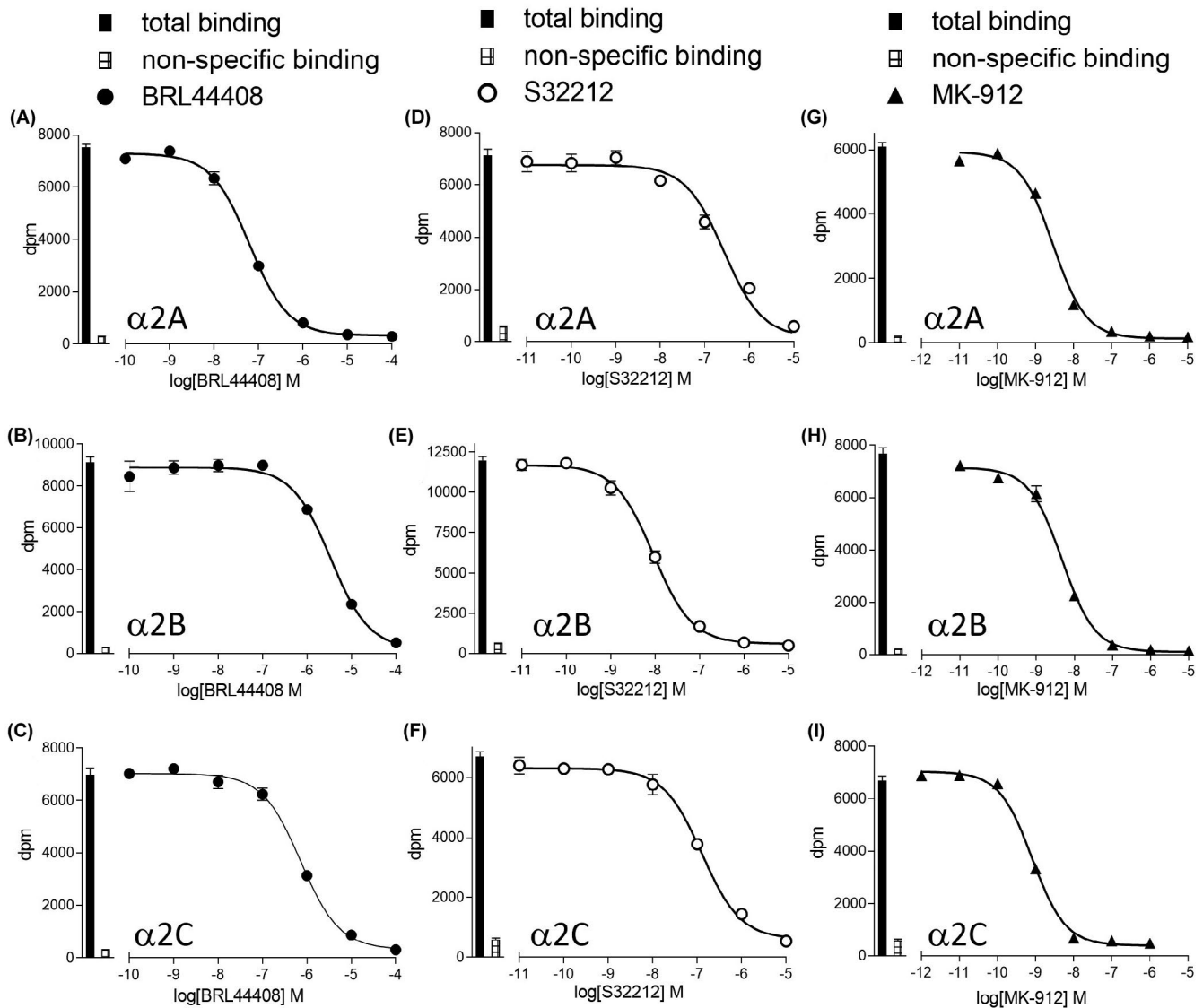


FIGURE 1 Inhibition of ^3H -rauwolscine binding to whole cells by BRL44408 (A–C), S32212 (D–F) or MK-912 (G–I) to CHO- $\alpha 2\text{A}$ cells (A, D, G), CHO- $\alpha 2\text{B}$ cells (B, E, H) or CHO- $\alpha 2\text{C}$ cells (C, F, I). Bars represent total ^3H -rauwolscine and non-specific binding (determined in the presence of $10\mu\text{M}$ RX821002). The concentration of ^3H -rauwolscine was (A) 0.99 nM , (B) 0.99 nM , (C) 0.99 nM , (D) 0.88 nM , (E) 0.88 nM , (F) 0.88 nM , (G) 0.86 nM , (H) 0.86 nM , and (I) 0.88 nM . Data points are mean \pm s.e. mean of triplicate determinations

over $\alpha 2\text{C}$ -adrenoceptors was only 9-fold. Although S32212 and ARC239 were 15- to 21-fold selective for the $\alpha 2\text{B}$ over the $\alpha 2\text{A}$ -adrenoceptor, their $\alpha 2\text{B}$ versus $\alpha 2\text{C}$ is marginal (less than 5-fold), in keeping with^{21,24,28,29,43,44} and thus there are no $\alpha 2\text{B}$ -selective ligands. Within the $\alpha 2$ -adrenoceptors, JP1302 was the overall most $\alpha 2\text{C}$ -selective ligand with an $\alpha 2\text{C}$ -selectivity of 43 and 65 over $\alpha 2\text{A}$ and $\alpha 2\text{B}$ respectively, in keeping with²⁰ however its affinity (120 nM at $\alpha 2\text{C}$) was a little lower than previously reported ($16\text{--}28\text{ nM}$ ²⁰). MK-912 was the highest affinity ligand overall (0.15 nM at $\alpha 2\text{C}$) and also had some $\alpha 2\text{C}$ -selectivity (having 13 and 46-fold higher $\alpha 2\text{C}$ -affinity than $\alpha 2\text{A}$ or $\alpha 2\text{B}$ respectively) again in keeping with previous studies.^{24,26,27,43}

Prazosin had higher affinity for $\alpha 2\text{C}$ (257 nM) and $\alpha 2\text{B}$ (676 nM) than $\alpha 2\text{A}$ (4678 nM), and thus the pattern of affinity at these three 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).

4.2 | Selectivity across $\alpha 1$, $\alpha 2$ and β -adrenoceptors

Given that the affinity values determined in this study were using an identical technique to affinity values determined in the human $\alpha 1$ and $\beta 1$ and $\beta 2$ -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 ($\alpha 2$, $\beta 1$, and $\beta 2$ reported here, $\alpha 1\text{A}$, $\alpha 1\text{B}$, and $\alpha 1\text{D}$ -adrenoceptor subtypes from³³ and $\beta 1$, $\beta 2$, and $\beta 3$ from^{31,32}). The findings of these studies are therefore discussed as a whole, in comparison with other literature findings.

TABLE 2 Log K_D values obtained from inhibition of 3H -rauwolscine binding by adrenoceptor antagonists to the human α_2A , α_2B , and α_2C -adrenoceptors in living cells. Values represent mean \pm 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 α_2A than the α_2B -adrenoceptor. Compounds are arranged in order of α_2A -selectivity.

Ligand	Affinity measurements			Selectivity ratios					
	Log K_D α_2A	n	Log K_D α_2B	n	Log K_D α_2C	n	α_2A vs α_2B	α_2A vs α_2C	α_2B vs α_2C
BRL 44408	-7.19 \pm 0.04	7	-5.41 \pm 0.04	7	-6.22 \pm 0.07	7	60.3	9.3	6.5
benoxathian	-7.17 \pm 0.02	5	-5.96 \pm 0.06	5	-7.75 \pm 0.03	5	16.2	3.8	61.7
tamsulosin	-6.33 \pm 0.04	5	-5.31 \pm 0.04	5	-6.41 \pm 0.03	5	10.5	1.2	12.6
alfuzosin	-5.56 \pm 0.04	5	-4.62 \pm 0.05	5	-6.14 \pm 0.04	5	8.7	3.8	33.1
2-MPMDQ	-6.79 \pm 0.04	5	-5.94 \pm 0.09	5	-7.50 \pm 0.02	5	7.1	5.1	36.3
yohimbine	-8.48 \pm 0.07	5	-7.66 \pm 0.10	5	-8.52 \pm 0.05	5	6.6	1.1	7.2
idazoxan	-7.17 \pm 0.04	5	-6.39 \pm 0.05	5	-7.16 \pm 0.03	5	6.0	1.0	5.9
WB4104	-7.55 \pm 0.05	6	-6.77 \pm 0.05	6	-8.17 \pm 0.05	6	6.0	4.2	25.1
A80426	-7.24 \pm 0.08	6	-6.52 \pm 0.06	6	-7.46 \pm 0.07	6	5.2	1.7	8.7
eforaxan	-7.58 \pm 0.05	5	-6.88 \pm 0.07	5	-7.44 \pm 0.04	5	5.0	1.4	3.6
2-PMDDQ	-6.83 \pm 0.05	5	-6.14 \pm 0.08	5	-7.07 \pm 0.02	5	4.9	1.7	8.5
atipamezole	-8.50 \pm 0.08	5	-7.85 \pm 0.04	5	-8.48 \pm 0.09	5	4.5	1.0	4.3
RX 821002	-8.10 \pm 0.07	5	-7.45 \pm 0.06	5	-8.14 \pm 0.02	5	4.5	1.1	4.9
sunepitron	-7.28 \pm 0.04	6	-6.65 \pm 0.08	6	-8.11 \pm 0.04	6	4.3	6.8	28.8
doxazosin	-5.35 \pm 0.04	6	-4.74 \pm 0.07 ^{app}	6	-6.24 \pm 0.02	6	4.1	7.8	31.6
phentolamine	-7.26 \pm 0.03	5	-6.69 \pm 0.05	5	-6.92 \pm 0.04	5	3.7	2.2	1.7
MK-912	-8.71 \pm 0.05	8	-8.16 \pm 0.10	8	-9.82 \pm 0.11	9	3.6	12.9	45.7
RS17053	-6.20 \pm 0.11	5	-5.65 \pm 0.07	5	-6.35 \pm 0.08	5	3.5	1.4	5.0
RS100329	-7.00 \pm 0.03	5	-6.47 \pm 0.04	5	-7.82 \pm 0.03	5	3.4	6.6	22.4
lisuride	-8.99 \pm 0.05	5	-8.52 \pm 0.05	5	-9.27 \pm 0.05	5	3.0	1.9	5.6
BMY7378	-5.30 \pm 0.03	5	-4.98 \pm 0.09 ^{app}	5	-6.26 \pm 0.01	5	2.1	9.1	19.1
RS79948	-8.93 \pm 0.03	5	-8.57 \pm 0.03	5	-9.36 \pm 0.04	5	2.3	2.7	6.2
carvedilol	-6.54 \pm 0.02	5	-6.31 \pm 0.02	5	-7.32 \pm 0.05	5	1.7	6.0	10.2
JP1302	-5.29 \pm 0.04	5	-5.11 \pm 0.05	5	-6.92 \pm 0.13	5	1.5	42.7	64.6
SKF86466	-6.29 \pm 0.05	5	-6.17 \pm 0.047	5	-6.39 \pm 0.04	5	1.3	1.3	1.7
3-MPPI	-6.67 \pm 0.05 ^{ep}	5	IC ₅₀ >-4	5	-7.01 \pm 0.03 ^{ep}	5		2.2	
PF3774076	-5.59 \pm 0.04	6	IC ₅₀ >-4	6	-5.29 \pm 0.09	6		2.0	
Rec15-2615	-5.53 \pm 0.12 ^{app}	6	IC ₅₀ >-4.5	6	-6.56 \pm 0.13	6		10.7	
AH11110A	-4.70 \pm 0.04 ^{app}	5	IC ₅₀ >-4	5	-4.86 \pm 0.03 ^{app}	5		1.4	

(Continues)

TABLE 2 (Continued)

Ligand	Affinity measurements				Selectivity ratios				
	Log K_D $\alpha 2A$	n	Log K_D $\alpha 2B$	n	Log K_D $\alpha 2C$	n	$\alpha 2A$ vs $\alpha 2B$	$\alpha 2A$ vs $\alpha 2C$	$\alpha 2B$ vs $\alpha 2C$
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
dibenamidine	-5.80 ± 0.06	10	-6.43 ± 0.06 -4.64 ± 0.07 $60.9 \pm 3.4\%$ 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 $51.4 \pm 3.3\%$ site 1	10	-6.41 ± 0.11 -4.71 ± 0.13 $74.1 \pm 4.1\%$ 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	

TABLE 2 (Continued)

Ligand	Affinity measurements			Selectivity ratios		
	Log K_D $\alpha 2A$	Log K_D $\alpha 2B$	n	$\alpha 2A$ vs $\alpha 2B$	$\alpha 2A$ vs $\alpha 2C$	$\alpha 2B$ vs $\alpha 2C$
carazolol	-4.66 ± 0.06^{app}	$IC_{50} > -4$	6		1.0	
CGP121177	$IC_{50} > -3$	No bind to 1mM	5			
CGP20712A	$IC_{50} > -4$	$IC_{50} > -4$	5			

Note: ^{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 $75.6 \pm 0.9\%$ at $\alpha 2A$ and $87.1 \pm 1.5\%$ at $\alpha 2C$

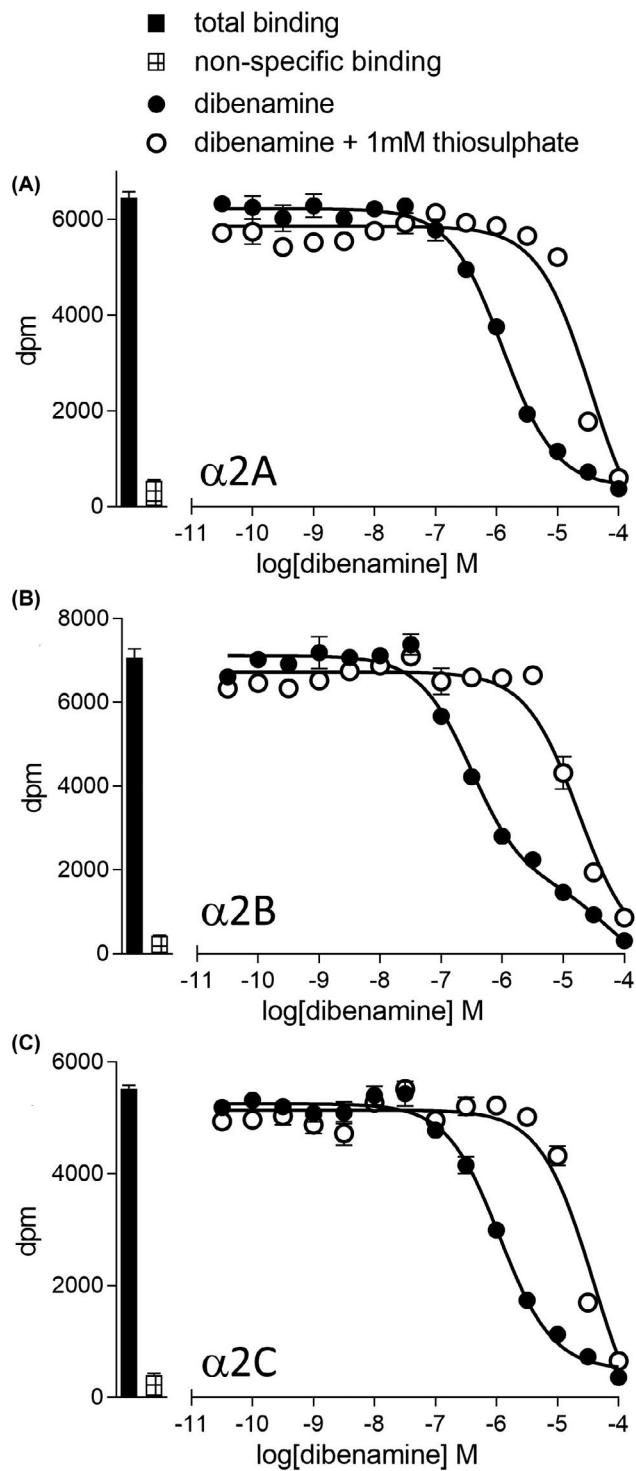


FIGURE 2 Inhibition of 3H -rauwolscine binding to whole cells by dibenamine following pre-incubation of dibenamine with sfm or 1mM thiosulphate to CHO- $\alpha 2A$ cells (A), CHO- $\alpha 2B$ cells (B), or CHO- $\alpha 2C$ cells (C). Bars represent total 3H -rauwolscine binding and non-specific binding as determined in the presence of $10 \mu M$ RX821002. The concentration of 3H -rauwolscine was $0.74 nM$ in all cases. Data points are mean \pm s.e. mean of triplicate determinations.

SNAP5089, silodosin and niguldipine are indeed highly $\alpha 1A$ -selective antagonists (>500 selectivity over $\alpha 2$ or $\beta 1$ or $\beta 2$ -adrenoceptors), and BMY7378 has ~ 100 -fold $\alpha 1D$ -selectivity.

TABLE 3 Log K_D values of antidepressants binding to the human α_2A , α_2B and α_2C -adrenoceptors. Values represent mean \pm 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, clomipramine has 2.5-fold higher affinity for the α_2B than the α_2A -adrenoceptor. Compounds are arranged in order of α_2A -selectivity.

ligand	Affinity measurements						Selectivity ratios		
	Log K_D α_2A	n	Log K_D α_2B	n	Log K_D α_2C	n	α_2A vs α_2B	α_2A vs α_2C	α_2B vs α_2C
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
venlafaxine	-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 ± 0.08^{app}	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

Note: 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.

BRL44408 is the best α_2A selective antagonist although its affinity for α_2A is only a modest 9-fold greater its α_2C affinity. MK-912 is the best α_2C -antagonist (0.15 nM α_2C -affinity) although again its α_2C selectivity is only modest (13-fold greater than α_2A). JP1302 (α_2C affinity 120 nM) has an α_1A -adrenoceptor affinity of 617 nM, only 5-fold less, so is not a truly α_2C -selective ligand. CGP20712A (β_1) and ICI118551 (β_2) are also highly selective antagonists with minimal α -affinity. There are no truly α_1B or α_2B selective antagonists. Figure 4 shows the affinity (log K_D values) of the most selective ligand at each adrenoceptor subtype (i.e., BRL44408 for α_2A , S32212 for α_2B and MK-912 for

α_2C) along with the single most selective antagonists at the other adrenoceptors and demonstrates that the α_2 -adrenoceptors fall behind α_1 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,⁴⁵) have significant β_2 -adrenoceptor affinity (~30 nM). Silodosin is highly α_1A -selective (0.25 nM) giving a >100-fold selectivity window compared to the other adrenoceptors. Naftopidil, however is not selective, with α_1A and β_2 affinities only 3-fold apart and thus potentially increasing the risk of bronchospasm in

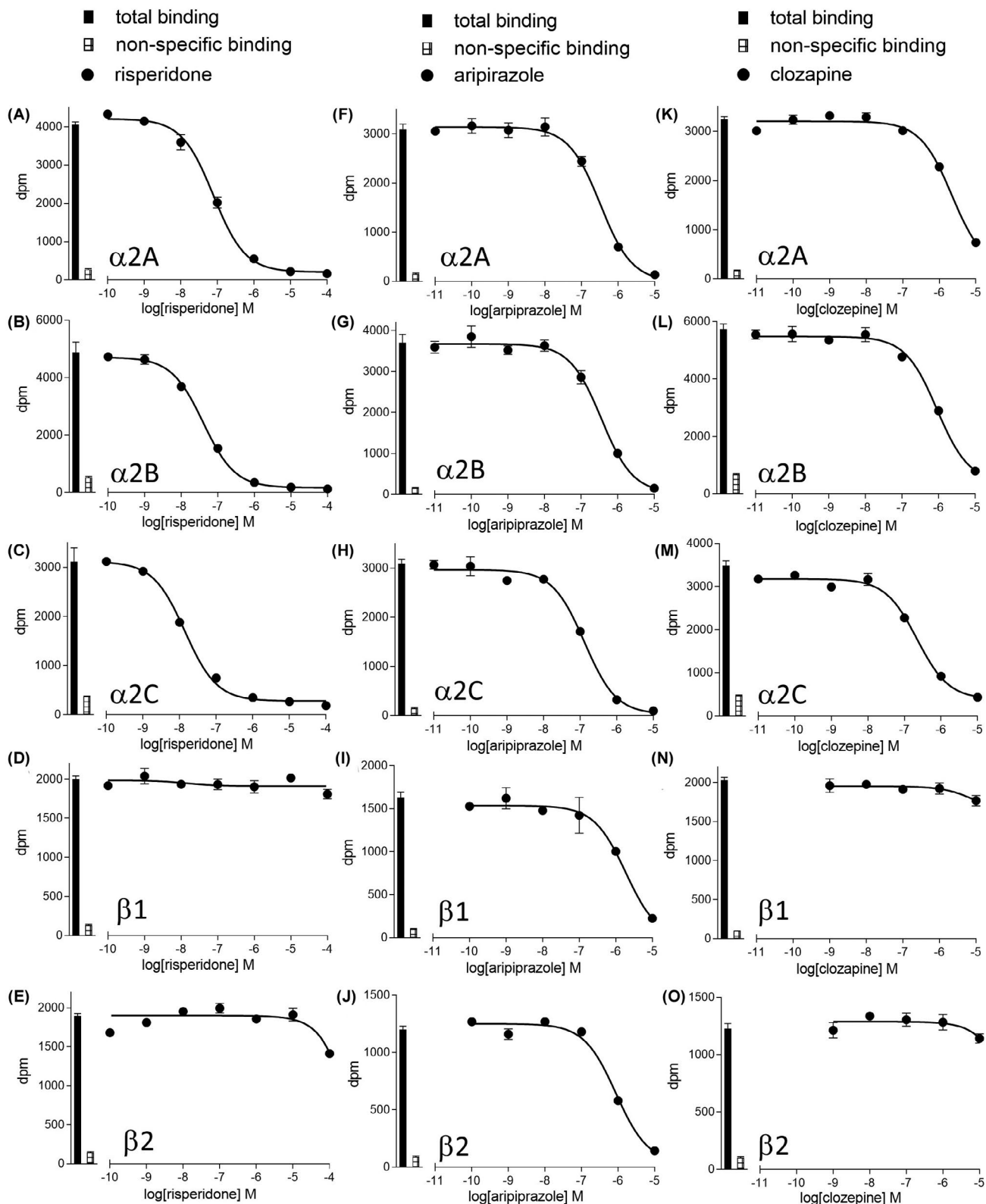


FIGURE 3 Inhibition of ^3H -rauwolscine ($\alpha 2A$, $\alpha 2B$, and $\alpha 2C$) or ^3H -CGP12177 ($\beta 1$ and $\beta 2$) binding to whole cells by (A–E) risperidone, (F–J) aripiprazole and (K–O) clozapine to CHO- $\alpha 2A$ cells, CHO- $\alpha 2B$ cells, CHO- $\alpha 2C$ cells, CHO- $\beta 1$ cells, CHO- $\beta 2$ cells. Bars represent total radioligand binding and non-specific binding as determined in the presence of $10\mu\text{M}$ RX821002 ($\alpha 2A$, $\alpha 2B$, and $\alpha 2C$ cells) or $10\mu\text{M}$ propranolol ($\beta 1$ and $\beta 2$ cells). The concentration of radioligand was (A) 0.54 nM , (B) 0.54 nM , (C) 0.54 nM , (D) 0.77 nM , (E) 1.00 nM , (F) 0.50 nM , (G) 0.50 nM , (H) 0.50 nM , (I) 0.72 nM , (J) 0.72 nM , (K) 0.50 nM , (L) 0.54 nM , (M) 0.54 nM , (N) 0.94 nM and (O) 0.72 nM . Data points are mean \pm s.e. mean of triplicate determinations

TABLE 4 Log K_D values of antipsychotics binding to the human α_2A , α_2B , and α_2C -adrenoceptors. Values represent mean \pm 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 α_2A -selectivity.

Ligand	Affinity measurements						Selectivity ratios		
	Log K_D α_2A	n	Log K_D α_2B	n	Log K_D α_2C	n	α_2A vs α_2B	α_2A vs α_2C	α_2B vs α_2C
First-generation antipsychotics									
sulpiride	-4.50 \pm 0.02	5	-4.37 \pm 0.06	5	-4.67 \pm 0.07	5	1.3	1.5	2.0
haloperidol	-5.38 \pm 0.06	5	-5.53 \pm 0.10	5	-5.77 \pm 0.05	5	1.4	2.5	1.7
flupenthixol	-6.10 \pm 0.12	5	-6.28 \pm 0.13	5	-6.88 \pm 0.14	5	1.5	6.0	4.0
pimozide	-5.76 \pm 0.12 ^{ep}	5	-6.30 \pm 0.10	5	-6.84 \pm 0.05	5	3.5	12.0	3.5
trifluoperazine	-5.60 \pm 0.05	5	-6.22 \pm 0.12	5	-6.20 \pm 0.06	5	4.2	4.0	1.0
prochlorperazine	-5.78 \pm 0.02 ^{app}	6	-6.46 \pm 0.11	6	-6.31 \pm 0.09	6	4.8	3.4	1.4
chlorpromazine	-5.65 \pm 0.13 ^{app}	6	-6.60 \pm 0.12	6	-5.93 \pm 0.11	6	8.9	1.9	4.7
perphenazine	-6.00 \pm 0.06	6	-7.16 \pm 0.05	6	-6.83 \pm 0.04	5	14.5	6.8	2.1
Second-generation antipsychotics									
amisulpiride	-5.11 \pm 0.09 ^{app}	5	-4.69 \pm 0.13 ^{app}	5	-5.57 \pm 0.07	5	2.6	2.9	7.6
aripirazole	-6.68 \pm 0.08	5	-6.54 \pm 0.08	6	-7.23 \pm 0.14	5	1.4	3.5	4.9
sertindole	-5.95 \pm 0.06	5	-5.81 \pm 0.07	5	-6.17 \pm 0.03	5	1.4	1.7	2.3
olanzapine	-5.59 \pm 0.05	5	-5.47 \pm 0.06	5	-5.86 \pm 0.02	5	1.3	1.9	2.5
paliperidone	-7.12 \pm 0.04	5	-7.26 \pm 0.05	5	-7.84 \pm 0.03	5	1.4	5.2	3.8
risperidone	-7.30 \pm 0.09	5	-7.47 \pm 0.08	5	-8.04 \pm 0.03	5	1.5	5.5	3.7
ziprasidone	-6.36 \pm 0.11	5	-6.59 \pm 0.08	5	-6.77 \pm 0.08	5	1.7	2.6	1.5
clozapine	-5.86 \pm 0.08 ^{app}	5	-6.20 \pm 0.05	5	-6.87 \pm 0.08	5	2.2	10.2	4.7
lurasidone	-6.67 \pm 0.05	5	-7.36 \pm 0.06	5	-7.34 \pm 0.03	5	4.9	4.7	1.0
quetiapine	-5.81 \pm 0.08	5	-6.72 \pm 0.08	5	-6.66 \pm 0.03	5	8.1	7.1	1.1

Note: ^{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 $79.1 \pm 6.0\%$ at α_2A .

those with asthma. Likewise, there is little evidence here to support SKF86466 being an α_2 -selective antagonist.⁴⁶⁻⁴⁸ The affinity of SKF86466 for the β_2 -adrenoceptor (250 nM) is similar to the highest α -adrenoceptor affinity (407 nM at α_2C). This may well be a species issue (see introduction) with previous studies being conducted in rodents,⁴⁶⁻⁴⁸ however others suggest a human α_2A -affinity of 13 nM.²³

Labetolol and carvedilol are often usually referred to as dual α/β -blockers (e.g.,⁴⁹). Labetolol (affinities of β_2 6-9 nM, β_1 11-23 nM, and α_1A 47 nM) has very poor affinity at α_1B , α_1D , α_2A , α_2B , α_2C , and β_3 -adrenoceptors and thus reasonable affinity at only 1 out of 6 α -adrenoceptors. A β/α_1A -antagonist would be a more accurate description. Likewise, carvedilol with affinities for β_2 of 0.1-0.4 nM, β_1 of 0.6-1.8 nM, and β_3 of 5 nM also has highest α -affinity for α_1A (4 nM) over α_1B or α_1D (14 nM) or α_2 -adrenoceptors (48-490 nM), 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.⁵⁰

4.3 | Antidepressants and antipsychotics

Given the considerable CNS expression of α_2A and α_2C -adrenoceptors, and that many antidepressants and antipsychotics have high α_1A -affinity, a third aim of this study was to compare the affinity of antidepressants and antipsychotics across the adrenoceptors.

The antidepressants generally had poor α_2 -adrenoceptor affinity, considerably lower affinity than that seen for the tricyclic antidepressant affinities at the α_1A -adrenoceptor. The antidepressant mirtazapine is a slight outsider with the highest α_2 -affinity of the antidepressants studied here, and higher than α_1A -affinity. It has been associated with antinociceptive properties attributed to α_2 -adrenoceptors in mice.^{19,51} Mirtazapine (α_2A -affinity 158 nM) and α_2C 110 nM), had similar affinity to the α_2 -antagonist idazoxan and similar values to those obtained in human α_2A receptors (79-126 nM) in,⁵¹ who also reported lower affinity at human α_1 and unmeasurable affinity at human β_1 or β_2 -adrenoceptors. Of note,⁵¹ also reported similar values for mirtazapine for human and rat receptors,

TABLE 5 Log K_D values of ligands binding to the human β_1 and β_2 -adrenoceptors as measured by ^3H -CGP12177 whole cell binding. Values represent mean \pm s.e.mean of n separate experiments. Ligands are arranged by class and presented in the same order as those in Tables 2, 3, and 4 for ease of comparison. Supplementary Table 1 has these ligands, alongside the α_2 -data, presented in alphabetical order.

Ligand	Affinity measurements			
	Log K_D β_1	n	Log K_D β_2	n
α -antagonists				
BRL44408	No binding to -3	5	No binding to -3	5
benoxathian	$-4.55 \pm 0.03^{\text{app}}$	5	-5.08 ± 0.06	5
tamsulosin	-6.26 ± 0.06	5	-6.08 ± 0.05	5
alfuzosin	No binding	5	$-4.18 \pm 0.09^{\text{app}}$	5
2-MPMDQ	$\text{IC}_{50} > -5$	6	$\text{IC}_{50} > -5$	6
yohimbine	No binding to -4	5	No binding to -4	5
idazoxan	$\text{IC}_{50} > -3$	5	$\text{IC}_{50} > -3$	5
WB4104	$\text{IC}_{50} > -4$	5	$\text{IC}_{50} > -4$	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	$\text{IC}_{50} > -4$	5
atipamezole	No binding to -4.5	5	No binding to -4.5	5
RX821002	-4.55 ± 0.05	5	$-3.95 \pm 0.11^{\text{app}}$	5
sunepitron	$\text{IC}_{50} > -3$	5	$\text{IC}_{50} > -3$	5
doxazosin	$-4.72 \pm 0.06^{\text{app}}$	5	-5.57 ± 0.01	6
MK-912	$\text{IC}_{50} > -4$	6	$\text{IC}_{50} > -4$	6
phentolamine	$\text{IC}_{50} > -4$	6	$\text{IC}_{50} > -4$	6
RS17053	-5.44 ± 0.04	6	-6.42 ± 0.06	6
RS100329	$\text{IC}_{50} > -3$	5	-4.77 ± 0.07	5
lisuride	-6.03 ± 0.06	5	-7.48 ± 0.04	5
BMV7378	$\text{IC}_{50} > -4$	9	$\text{IC}_{50} > -4$	9
RS79948	-3.84 ± 0.05	5	$\text{IC}_{50} > -3$	5
carvedilol	-9.20 ± 0.05	8	-9.98 ± 0.06	8
JP1302	$\text{IC}_{50} > -4$	5	-5.58 ± 0.08	5
SKF86466	-5.92 ± 0.08	6	-6.60 ± 0.07	6
3-MPPI	No binding to -4	5	$\text{IC}_{50} > -4$	5
PF3774076	No binding to -4	5	No binding to -4	5
Rec15-2615	$\text{IC}_{50} > -4$	5	$\text{IC}_{50} > -4$	5
AH11110A	-6.23 ± 0.07	6	-6.36 ± 0.07	6
silodosin	$\text{IC}_{50} > -5$	6	-7.52 ± 0.10	6
5-methyl-urapidil	-6.12 ± 0.04	5	-5.00 ± 0.07	5
SNAP5089	$\text{IC}_{50} > -5$	5	$\text{IC}_{50} > -5$	5
anisodamine	no binding to -3	9	no binding to -3	9
2-niguldipine	$\text{IC}_{50} > -4$	5	$\text{IC}_{50} > -4$	5
naftapidil	-5.97 ± 0.07	6	-7.45 ± 0.06	6
labetolol	-7.97 ± 0.04	6	-8.21 ± 0.06	6
ifenprodil	$\text{IC}_{50} > -5$	5	$\text{IC}_{50} > -5$	5
domperidone	$\text{IC}_{50} > -4$	5	$\text{IC}_{50} > -4$	5
urapidil	-5.32 ± 0.06	5	-5.00 ± 0.02	5
HEAT	$\text{IC}_{50} \sim -4.5$	5	$\text{IC}_{50} > -4$	5
indoramin	$-4.73 \pm 0.10^{\text{app}}$	5	$-5.27 \pm 0.11^{\text{app}}$	5

(Continues)

TABLE 5 (Continued)

Ligand	Affinity measurements			
	Log K _D β1	n	Log K _D β2	n
cyclazosin	No binding to -4	6	-5.30 ± 0.04	6
imiloxan	IC ₅₀ ~ -3	5	no binding to -3	5
dibenzamine	-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
venlafaxine	-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

TABLE 5 (Continued)

Ligand	Affinity measurements			
	Log K _D β1	n	Log K _D β2	n
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
ariprazole	-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

Note: #from³²*from³¹

whereas¹⁷ suggest ~10-fold higher α2-affinity in what appears to be data gathered from mice.

Interestingly, many tricyclic antidepressants had a slight α2B-selectivity, something not seen with most α-ligands (Table 2), with the most potent (amitriptyline) having an α2B-affinity (76 nM) only 10-fold lower than that at the 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 (178 nM for the β2-adrenoceptor).

α2C-Adrenoceptor affinity has previously been suggested to have added benefits for the clinical actions of certain antipsychotics.^{17,52} 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 2239 nM, α2B 251 nM, α2C 1175 nM, whereas its α1A-adrenoceptor affinity is 1 nM. There is however huge heterogeneity even between studies of human α2-adrenoceptors. Chlorpromazine affinities range from α2A 78 nM, α2B 4.8 nM and α2C 41 nM (³H-rauwolscine membrane binding from human receptors expressed in COS cells,²⁸) α2A 396-535 nM (³H-yohimbine membrane binding using human colonic cancer cells

and human platelets,²¹) α2A 600 nM, α2B 43 nM, and α2C 260 nM (³H-RX821002 membrane binding for human receptors expressed in CHO cells,²⁹) α2A 1008 nM, α2B 34 nM, and α2C 85 nM (³H-RX821002 membrane binding to human receptors expressed in mouse cells,³⁰) α2A 2245 nM (³H-RX821002 membrane binding to human platelets,²³) to α2A 4169 nM and α2C 1413 nM (antagonism of agonist responses living CHO cells expressing the human α2-adrenoceptor⁵²).

The second-generation antipsychotics had a wide range of affinity for the α2-adrenoceptors, with risperidone (9 nM, α2C) and paliperidone (14 nM α2C) having the highest affinity (in keeping with other human α2-adrenoceptor studies⁵²), to >1000 nM affinity for olanzapine and amisulpiride. Even for risperidone and paliperidone, the α2C affinity is less potent than that seen at the α1A-adrenoceptor and once again α2A vs α2C-selectivity was very marginal. Clozapine, which has been particularly noted for α2C-affinity^{12,13,17} had an α2C-affinity of 135 nM, compared to its α1A-affinity of 5.4 nM measured under identical conditions. This α2C affinity is similar to that measured in intact CHO cells expressing human receptors (54 nM,⁵² but poorer than that reported in membrane radioligand binding studies (6.5 nM^{21,29}).

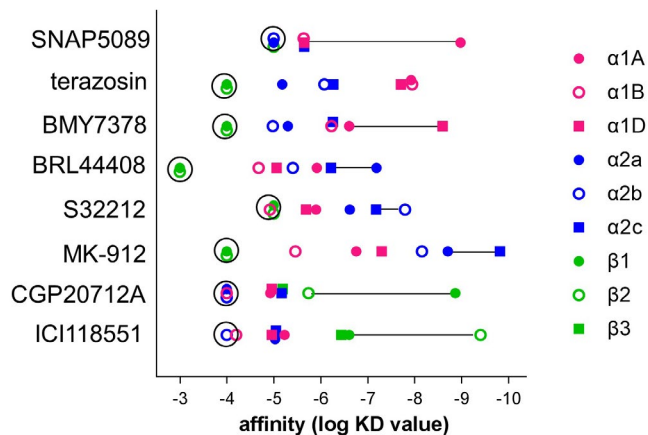


FIGURE 4 Plot of $\log K_D$ values showing the relative selectivity and affinity for the single most selective ligand at each receptor. Thus SNAP5089 is the most $\alpha1A$ -selective ligand and the length of the line represents the selectivity for $\alpha1A$ over the next closest adrenoceptor affinity. Terazosin, although the “most” $\alpha1B$ -selective ligand has no selectivity. The selectivity of the three most selective $\alpha2$ ligands is considerably less than that for $\alpha1A$, $\alpha1D$, $\beta1$, or $\beta2$. 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 $\alpha1$ -adrenoceptors are from.³³ $\beta3$ data are included for CGP20712A and ICI118551 from³¹

4.4 | Conclusion

This study, using identical methods to previous $\alpha1$ and β -adrenoceptor studies, allows comparison of ligand affinity, and thus selectivity, between the α and β -adrenoceptor subtypes. Overall, there is huge variation in the literature for the affinity of $\alpha2$ ligands (more so than for $\alpha1$ or β), and for which species differences appear to play a significant role, but technique may also be important. Whilst selective antagonists exist for $\alpha1A$, $\alpha1D$, $\beta1$, and $\beta2$ -adrenoceptor, there are few selective $\alpha2$ -adrenoceptor ligands and for those that do exist (BRL44408 for $\alpha1A$ and MK-912 for $\alpha2C$) only have small windows of selectivity. Antidepressants (with the exception of mirtazapine) and first-generation antipsychotics have higher $\alpha1A$ than $\alpha2$ -adrenoceptor affinity. Second-generation antipsychotic varied widely in their $\alpha2$ -adrenoceptor affinity, however, this study does not lend much support for an important role for an $\alpha2C$ -selective action for certain antipsychotics. Clearly, however, even after a century of yohimbine use, there remains plenty of scope to develop selective $\alpha2$ -antagonists.

ETHICS STATEMENT

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

ACKNOWLEDGEMENTS

The authors 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.

AUTHOR CONTRIBUTION

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

DATA AVAILABILITY STATEMENT

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.

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How to cite this article: Proudman RGW, Akinaga J, Baker JG. The affinity and selectivity of α -adrenoceptor antagonists, antidepressants and antipsychotics for the human α 2A, α 2B, and α 2C-adrenoceptors and comparison with human α 1 and β -adrenoceptors. *Pharmacol Res Perspect.* 2022;10:e00936. doi:[10.1002/prp2.936](https://doi.org/10.1002/prp2.936)