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

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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, 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 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 (81 nM) is only 9-fold greater than its α2C-affinity. MK-912 is the highest-affinity, most α2C-selective antagonist (0.15 nM α2C-affinity) although its α2C-selectivity is only 13-fold greater than at α2A. There are no truly α2B-selective antagonists. A few α-ligands with significant β-affinity were detected, for example, nafopidil 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 (9 nM) and paliperidone (14 nM) have the highest α2C-adrenoceptor affinity however this is only 5-fold selective over α2A, and both have a higher affinity for α1A (2 nM and 4 nM, 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
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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INTRODUCTION

The α2-antagonist yohimbine, obtained from the African Corynanthe yohimb tree (Pausinystalia yohimbe), has been in clinical use as an aphrodisiac for over a century. 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 and can indeed bind yohimbine from tree bark. The α2-antagonist idozaxan, 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. This led to the development of RX821002, a 2-methyl congener of idozaxan, in the 1980s which retained high α2-adrenoceptor affinity but without imidazoline receptor affinity (although 5-HT receptor interactions still occur).

α2-Adrenoceptors are subdivided into α2A, α2B, and α2C-subtypes. With receptors being present in the heart, blood vessels, and kidney, 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 the brain also have clinical roles in anaesthesia and psychiatric treatments with both pre- and post-synaptic effects on neurotransmission. α2-adrenoceptors are widely expressed and are important for blood pressure, sedation, analgesia, platelet aggregation, and hypothermia. 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. α2A-adrenoceptor antagonism may be important in sepsis (administration of the α2A-antagonist BRL44408 reduced pro-inflammatory cytokines, TNF-α and IL-6 and increased survival in a rat model of sepsis) and potentially clinically relevant α2A-mirtazapine-induced reversal of analgesia. 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). The expression and effects of the α2B-adrenoceptors appear very minor in the brain. The α2C-adrenoceptor is involved in catecholamine release in adrenal chromaffin cells and in the brain process of startle and stress responses.

α2-adrenoceptors form 10% of all brain adrenoceptors but appear particularly prevalent in the striatum and hippocampus. For certain antipsychotics (e.g., clozapine), α2C-antagonism, in addition to dopamine D2 blockade, is thought to be beneficial in the management of schizophrenia and α2C-antagonism may be helpful in improving cognition in dementia. 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.

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, for example, human platelet or cortex for α2A versus neonatal rat lung for α2B. 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). Prazosin is the opposite with 15–20-fold high affinity for rat/mouse kidney receptors than human/rabbit/dog α2A-adrenoceptors. 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, 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 300 nM to a few thousand nM, to 16000 nM. 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. 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 p1 and β2-adrenoceptors (included here, and α1-adrenoceptors) this study explores the affinity and selectivity of ligands across the human adrenoceptors commonly targeted for cardiovascular, urological, and CNS effects.

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 side 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 α2A-adrenoceptor, human α2B-adrenoceptor or human...
**Table 1** Log _K_ₐ values obtained from inhibition of [³H]-RX281002 or [³H]-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.

|                | [³H]-RX281002 as radioligand | [³H]-rauwolscine as radioligand |
|----------------|-------------------------------|---------------------------------|
|                | _α₂A_ | _α₂B_ | _α₂C_ | _α₂A_ | _α₂B_ | _α₂C_ |
| **MK912**      | -8.76 ± 0.05 | 5     | -8.23 ± 0.11 | 5     | -10.00 ± 0.15 | 4     |
| **yohimbine**  | -8.58 ± 0.03 | 5     | -7.66 ± 0.05 | 5     | -7.88 ± 0.10 | 5     |
| **RX281002**   | -8.23 ± 0.02 | 5     | -7.67 ± 0.04 | 5     | -7.28 ± 0.07 | 5     |
| **WB4101**     | -7.58 ± 0.05 | 6     | -6.88 ± 0.05 | 6     | -5.84 ± 0.13 | 6     |
| **BRL44408**   | -7.24 ± 0.06 | 6     | -5.59 ± 0.06 | 6     | -6.32 ± 0.09 | 6     |
| **carvedilol** | -6.58 ± 0.04 | 5     | -6.46 ± 0.05 | 5     | -7.46 ± 0.14 | 5     |
| **ARC239**     | -5.99 ± 0.06 | 4     | -7.29 ± 0.14 | 4     | -7.18 ± 0.05 | 4     |
| **chlorpromazine** | -5.57 ± 0.11 | 6     | -6.63 ± 0.11 | 6     | -6.02 ± 0.16 | 6     |
| **prazosin**   | -5.41 ± 0.03 | 6     | -6.34 ± 0.03 | 6     | -6.48 ± 0.09 | 6     |
| **JP1302**     | -5.22 ± 0.04 | 5     | -5.22 ± 0.04 | 5     | -6.57 ± 0.26 | 5     |
| **labetalol**  | -4.63 ± 0.04 | 5     | -4.99 ± 0.07 | 5     | -5.42 ± 0.05 | 5     |
| **JP1302**     | -4.62 ± 0.07 | 5     | -4.71 ± 0.08 | 5     | -5.27 ± 0.04 | 5     |

**Note:** apparent the maximum concentration of competing ligand inhibited most but not all of specific binding. An IC₅₀ 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_ₐ was calculated.

**2.5 | [³H]-rauwolscine, [³H]-RX281002, and [³H]-CGP1217/whole cell competition binding**

Affinity was assessed using the whole cell binding method of [31]. Ligands were diluted in fm 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 CHO-β-adrenoceptor DNA (DNAs from Guthrie DNA Resource Centre) to the wells. 20 μl of the 96-well plate were removed from the triplicate wells and added to a white-sided/white-bottomed plate. 100 μl Microscint 20 was added to each well and a transparent top seal applied to the plate. Plate was kept at room temperature in the dark. In 2 dilution and the wells incubated at 37°C humidified 1% CO₂, 9% air atmosphere for 2 h. After 2 h, the wells were washed twice by the addition of 2 X 200 μl cold (4°C) 5% CO₂, 95% air atmosphere. The radioligands were diluted to twice the final concentration in serum-free media (DMEM/F12 containing 2 mM glutamine in a 37°C humidified 5% CO₂, 95% air atmosphere and 2 μM I-glutamine). Media was removed from each well and replaced with either 100 μl cold or 20 μl yohimbine. When [³H]-rauwolscine (used) or 20 μM yohimbine with [³H]-RX281002 was used in this experiment, non-specific binding (NSB) was determined by the addition and removal of 2 X 200 μl cold (4°C) 5% CO₂, 95% air atmosphere for at least 6 h before being counted on a TopCount (Packard). The _K_ₐ 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 (DMEM/F12 containing 2 mM glutamine in a 37°C humidified 1% CO₂, 95% air atmosphere) and grown to confluence. Cells were always grown in the absence of any antibiotics. Macrophages contamination was immediately been monitored within the laboratory (negative) but cell lines were not tested routinely with each experiment.

**2.4 | [³H]-rauwolscine and [³H]-RX281002 whole cell saturation binding**

CHO cells were grown in Dulbecco's modiﬁed Eagle's medium nutrient mixture F12 (DMEM/F12 containing 10% fetal calf serum and 2 mM L-glutamine in a 37°C humidified 5% CO₂, 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. Macrophages contamination was immediately been monitored within the laboratory (negative) but cell lines were not tested routinely with each experiment.
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 ³H-prazosin binding seen
with dibenamine and phenoxybenzamine at the α₁-adrenoceptors,
sodium thioulsphate, which reacts with the ethylenimininium ions,
was used in dibenamine and phenoxybenzamine experiments, in ex-
cess, as in Ref. 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, physio-
logical 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 recep-
tor subtypes. Thus, if one compound is a partial agonist at one recep-
tor 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:

\[
\text{Specific binding} = B_{\text{max}} \times K_d \left( \left[ ^3H - \text{radioligand} \right] + K_d \right)
\]

where \( B_{\text{max}} \) is the maximum specific binding, \( K_d \) is the dissociation con-
stant of the radioligand and \( [^3H\text{-radioligand}] \) 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\text{50} was then determined as the concentration required to inhibit
50% of the specific binding.

\[
\% \text{ Specific binding} = 100 - \left( 100 \times \frac{|A|}{(|A| + \text{IC}_{50})} \right)
\]

where \( |A| \) is the concentration of the competing ligand and IC\text{50} is the
concentration at which half of the specific binding of radioligand that
has been inhibited.

From the IC\text{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:

\[
K_d \text{ competing ligand} = \frac{\text{IC}_{50}}{1 + \left( \frac{[^3H - \text{radioligand}]}{K_d[^3H - \text{radioligand}]} \right)}
\]

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 com-
peting ligand was 50% or less, an IC\text{50} could not be determined and
thus a \( K_d \) value not calculated. This is shown in the tables as IC\text{50}+top
concentration used (i.e., IC\text{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\text{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-rawolscine binding (e.g.,
Figure 2B) in which case a two-component curve was used, using
the equation below:

\[
\% \text{ specific binding} = \frac{|A|N}{(|A| + \text{IC}_{50}1) + \frac{|A|(100−N)}{(|A| + \text{IC}_{50}2)}}
\]

where \( |A| \) is the concentration of the competing ligand, IC\text{50}1 and IC\text{50}2
are the respective IC\text{50} values for the two components and N is the
percentage of the response occurring through the first component
(IC\text{50}1). \( K_d \) values were calculated from IC\text{50} values as above.

Radioligand concentrations were determined from taking the av-
ge 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 expression in these cell
lines and concerns about depletion of the free radioligand in the
binding assays, depletion was monitored. Free radioligand deple-
tion of 20% was encountered (resulting in a potential inaccuracy of
0.04 log units in the stated \( K_d \) values). Ligand depletion of a maxi-
mum 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\text{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, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.

3 | RESULTS

3.1 | 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.). 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 ± 0.24 nM (5830 ± 853 fmol/mg protein, n=7), in CHO-α2B cells of 7.87 ± 0.78 nM (13102 ± 2805 fmol/mg protein, n=9) and in CHO-α2C cells of 0.76 ± 0.07 nM (1379 ± 98 fmol/mg protein, n = 9). For $^3$H-RX821002 saturation-binding studies, the values were $K_d$ 4.73 ± 0.42 nM (4584 ± 667 fmol/mg protein, n=8) in CHO-α2A cells, 17.96 ± 1.41 nM (11326 ± 3531 fmol/mg protein, n = 6) in CHO-α2B cells and 3.60 ± 0.24 nM (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.

3.2 | 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. 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. Sodium thiosulphate reacts with the ethyleniminium ions preventing them interacting with α-adrenoceptors. 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$^3$).

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.

3.3 | 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 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, 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).

4 | 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.$^11,14,15,20$

4.1 | Selectivity between α2A, α2B, and α2C-adrenoceptors

Yohimbine and RX821002 were confirmed as high affinity antagonists at all three subtypes. Both compounds had a lower affinity at α2B-adrenoceptors than at α2A or α2C, in keeping with some other studies (both in cell lines, and in tissues.$^7,30,39,40$ Other compounds with high affinity at all 3 subtypes were: atipamezole and RS7994B and should thus be regarded as non-selective α2-ligands. Lisuride has a high affinity across many different receptor subtypes.$^{41,42}$

BRL44408 (65 nM at α2A) was the most α2A-adrenoceptor selective ligand in keeping with however although it was 60-fold selective for α2A over α2B, BRL44408’s selectivity for α2A
over \( \alpha_2 \)-adrenoceptors was only 9-fold. Although S32212 and ARC239 were 15- to 21-fold selective for the \( \alpha_2 \)-adrenoceptor, their \( \alpha_2 \)-versus \( \alpha_2 \)-selectivity of 43 and 65 over \( \alpha_2 \)- and \( \alpha_2 \)-receptors, respectively, in keeping with\(^{21,24,28,43,44} \) and thus there are no \( \alpha_2 \)-selective ligands. Within the \( \alpha_2 \)-adrenoceptors, JP1302 was the overall most \( \alpha_2 \)-selective ligand with an \( \alpha_2 \)-selectivity of 43 and 65 over \( \alpha_2 \) and \( \alpha_2 \)-receptors, respectively, in keeping with\(^{20} \) however its affinity (120 nM at \( \alpha_2 \)) was a little lower than previously reported (16-28 nM\(^{20} \)).

MK-912 was the highest affinity ligand (0.15 nM at \( \alpha_2 \)) and also had some \( \alpha_2 \)-selectivity (having 13 and 46-fold higher \( \alpha_2 \)-affinity than \( \alpha_2 \) or \( \alpha_2 \)-selectively) again in keeping with previous studies.\(^{24,26,27,43,43} \)

Prazosin had higher affinity for \( \alpha_2 \) (257 nM) and \( \alpha_2 \) (676 nM) than \( \alpha_2 \) (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 \( \beta \)-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 transfectant 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, \( \alpha_1 \), \( \alpha_1 \), and \( \alpha_1 \)-adrenoceptor subtypes from\(^{33} \) 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

| Ligand       | Log K<sub>α2A</sub> | n | Log K<sub>α2B</sub> | n | Log K<sub>α2C</sub> | n | Selectivity ratios | α2A vs α2B | α2B vs α2C |
|--------------|---------------------|---|--------------------|---|---------------------|---|-------------------|-----------|-----------|
| BRL44408     | -7.19 ± 0.04        | 7 | -5.41 ± 0.04       | 7 | -6.22 ± 0.07       | 7 |                    |           |           |
| bucindol      | -5.96 ± 0.06        | 5 | -7.17 ± 0.02       | 5 | -7.75 ± 0.03       | 5 |                    |           |           |
| linsosin     | -6.05 ± 0.04        | 5 | -6.33 ± 0.04       | 5 | -6.31 ± 0.03       | 5 |                    |           |           |
| alfuzosin     | -6.84 ± 0.07        | 5 | -6.79 ± 0.04       | 5 | -6.42 ± 0.05       | 5 |                    |           |           |
| yohimbine     | -8.18 ± 0.05        | 5 | -7.41 ± 0.04       | 5 | -7.66 ± 0.02       | 5 |                    |           |           |
| lidocaine     | -7.17 ± 0.04        | 5 | -6.93 ± 0.05       | 6 | -6.67 ± 0.05       | 6 |                    |           |           |
| WB41010      | -7.74 ± 0.08        | 6 | -7.75 ± 0.05       | 6 | -6.52 ± 0.06       | 6 |                    |           |           |
| A80426       | -8.19 ± 0.05        | 6 | -8.50 ± 0.04       | 5 | -7.65 ± 0.04       | 5 |                    |           |           |
| ephedramine   | -6.63 ± 0.05        | 5 | -7.41 ± 0.05       | 5 | -7.06 ± 0.04       | 5 |                    |           |           |
| atropine      | -8.92 ± 0.04        | 5 | -8.51 ± 0.04       | 5 | -8.48 ± 0.09       | 5 |                    |           |           |
| RX821022      | -8.10 ± 0.07        | 5 | -7.8 ± 0.04        | 6 | -6.65 ± 0.08       | 6 |                    |           |           |
| sulpiride     | -8.18 ± 0.06        | 5 | -7.8 ± 0.04        | 6 | -6.65 ± 0.08       | 6 |                    |           |           |
| doxazosin     | -7.26 ± 0.03        | 5 | -6.99 ± 0.05       | 5 | -6.69 ± 0.06       | 5 |                    |           |           |
| phentolamine   | -6.71 ± 0.05        | 5 | -6.96 ± 0.05       | 5 | -6.69 ± 0.06       | 5 |                    |           |           |
| RX100219      | -6.20 ± 0.01        | 5 | -6.71 ± 0.05       | 5 | -6.47 ± 0.04       | 5 |                    |           |           |
| lisuride      | -7.00 ± 0.02        | 5 | -7.82 ± 0.05       | 5 | -7.41 ± 0.04       | 5 |                    |           |           |
| BMY7378       | -5.40 ± 0.03        | 5 | -6.51 ± 0.02       | 5 | -6.42 ± 0.07       | 5 |                    |           |           |
| carvediol     | -5.24 ± 0.02        | 5 | -6.54 ± 0.02       | 5 | -6.31 ± 0.02       | 5 |                    |           |           |
| SKF84466      | -6.9 ± 0.05         | 5 | -6.72 ± 0.05       | 5 | -6.53 ± 0.05       | 5 |                    |           |           |
| QP3902        | -5.29 ± 0.04        | 5 | -6.29 ± 0.05       | 5 | -6.17 ± 0.047      | 5 |                    |           |           |
| IC<sub>50</sub> 4  | -6.67 ± 0.05PP      | 5 | -6.17 ± 0.047      | 5 | -6.9 ± 0.05        | 5 |                    |           |           |
| TQF144076     | -5.59 ± 0.04        | 5 | -5.29 ± 0.04       | 5 | -5.26 ± 0.01       | 5 |                    |           |           |
| AH11102       | -4.70 ± 0.04        | 5 | -4.70 ± 0.04       | 5 | -4.81 ± 0.03PP     | 5 |                    |           |           |

Values represent the 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.
| 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     | $-5.49 \pm 0.06^{\text{app}}$ | 6   | $IC_{50} > 5$ | 6   | $-6.12 \pm 0.06^{\text{app}}$ | 6   |                      | 4.3   |                      |
| 5-methyl-urapidil | $-5.18 \pm 0.05$ | 5   | $-5.17 \pm 0.05$ | 5   | $-5.81 \pm 0.07$ | 5   | 1.0                     | 4.3   | 4.4                   |
| SNAP5089      | $IC_{50} > 5$ | 5   | $IC_{50} > 5$ | 5   | $-5.65 \pm 0.06$ | 5   |                      | 1.1   | 4.2                   | 3.7   |
| anisodamine   | $IC_{50} > 3$ | 5   | $IC_{50} > 3$ | 5   | $-3.56 \pm 0.07^{\text{app}}$ | 5   |                      | 1.2   | 4.5                   | 3.6   |
| 2-niguldipine | $IC_{50} > 5$ | 5   | $-5.48 \pm 0.11$ | 5   | $-6.07 \pm 0.11$ | 5   | 3.9                     |               |
| naftapidil    | $-6.55 \pm 0.09$ | 5   | $-6.60 \pm 0.07$ | 5   | $-7.17 \pm 0.08$ | 5   |                      | 1.3   | 6.2                   | 4.6   |
| labetolol     | $-4.62 \pm 0.07^{\text{app}}$ | 5   | $-4.71 \pm 0.08^{\text{app}}$ | 5   | $-5.27 \pm 0.04$ | 5   |                      | 1.6   | 4.9                   | 3.1   |
| ifenprodil    | $-6.01 \pm 0.05$ | 5   | $-6.14 \pm 0.06$ | 5   | $-6.80 \pm 0.05$ | 5   |                      | 1.9   | 7.1                   | 3.6   |
| domperidone   | $-5.09 \pm 0.06^{\text{app}}$ | 6   | $-5.29 \pm 0.07$ | 6   | $-5.78 \pm 0.08$ | 6   |                      | 1.9   | 4.0                   | 2.1   |
| urapidil      | $-5.49 \pm 0.05$ | 5   | $-5.78 \pm 0.08$ | 5   | $-6.34 \pm 0.05$ | 5   |                      | 2.1   | 4.7                   | 2.2   |
| HEAT          | $-7.45 \pm 0.04$ | 5   | $-7.72 \pm 0.11$ | 5   | $-8.05 \pm 0.19$ | 5   |                      | 2.2   | 15.1                  | 6.8   |
| indoramin     | $-5.13 \pm 0.03^{\text{app}}$ | 6   | $-5.46 \pm 0.05$ | 6   | $-5.80 \pm 0.05$ | 6   |                      | 4.0   | 2.5                   | 1.6   |
| cicalazosin   | $-5.00 \pm 0.03$ | 5   | $-5.35 \pm 0.13$ | 5   | $-6.18 \pm 0.02$ | 5   |                      | 4.3   | 2.4                   | 1.8   |
| imiloxan      | $-5.88 \pm 0.03$ | 6   | $-6.48 \pm 0.05$ | 6   | $-6.27 \pm 0.03$ | 6   |                      | 4.7   | 1.1                   | 5.1   |
| dibenamine    | $-5.80 \pm 0.06$ | 10  | $-6.43 \pm 0.06$ | 10  | $-6.44 \pm 0.07$ | 10  |                      | 5.2   | 4.9                   | 1.1   |
| promethazine  | $-5.58 \pm 0.07$ | 5   | $-6.25 \pm 0.06$ | 5   | $-5.54 \pm 0.05$ | 5   |                      | 6.9   | 18.2                  | 2.6   |
| phenoxybenzamine | $-5.72 \pm 0.10$ | 10  | $-6.44 \pm 0.11$ | 10  | $-6.41 \pm 0.11$ | 10  |                      | 7.9   | 12.3                  | 1.5   |
| prazosin      | $-5.33 \pm 0.05$ | 6   | $-6.17 \pm 0.05$ | 6   | $-6.59 \pm 0.04$ | 6   |                      | 7.9   | 58.9                  | 7.4   |
| terazosin     | $-5.18 \pm 0.03$ | 5   | $-6.08 \pm 0.05$ | 5   | $-6.27 \pm 0.08$ | 5   |                      | 15.1  | 3.6                   | 4.2   |
| spiroxatrine  | $-6.97 \pm 0.03$ | 6   | $-7.87 \pm 0.07$ | 6   | $-8.74 \pm 0.04$ | 6   |                      | 21.4  | 18.2                  | 1.2   |
| S32212        | $-6.62 \pm 0.13$ | 8   | $-7.80 \pm 0.10$ | 8   | $-7.18 \pm 0.10$ | 8   |                      | 3.9   | 21.4                  | 1.4   |
| ARC239        | $-5.99 \pm 0.06$ | 5   | $-7.32 \pm 0.14$ | 6   | $-7.25 \pm 0.14$ | 5   |                      | 5.5   | 3.9                   | 21.4  |
| β-blockers    |                      |     |                      |     |                      |     |                      | 5.5   | 3.9                   | 21.4  |
| cyanopindolol | $-5.56 \pm 0.10$ | 5   | $-4.82 \pm 0.10^{\text{app}}$ | 5   | $-6.15 \pm 0.07$ | 5   |                      | 1.5   | 1.4                   | 2.1   |
| bucindolol    | $-5.81 \pm 0.05$ | 5   | $-5.63 \pm 0.06$ | 5   | $-5.95 \pm 0.04$ | 5   |                      | 1.0   |                      |
| ICI18551      | $-5.03 \pm 0.03$ | 5   | $IC_{50} > 4$ | 5   | $-5.05 \pm 0.04$ | 5   |                      | 1.0   |                      |
| SDZ21009      | $-4.86 \pm 0.07^{\text{app}}$ | 6   | $IC_{50} > 4$ | 6   | $IC_{50} > 4.5$ | 6   |                      | 1.4   |                      |
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.

\[ \text{TABLE 2 (Continued)} \]

| Ligand     | \( \log K_a \) \( \alpha_{2A} \) | n | \( \log K_a \) \( \alpha_{2B} \) | n | \( \log K_a \) \( \alpha_{2C} \) | n |
|------------|-------------------------------|---|-------------------------------|---|-------------------------------|---|
| Carazolol  | \(-4.66 \pm 0.06^{\text{app}}\) | 6 | No bind to 1mM               | 5 | \(-5.17 \pm 0.03\)              | 5 |
| CGP12177   | \(-4.66 \pm 0.05^{\text{app}}\) | 6 | No bind to 1mM               | 5 | \(-5.17 \pm 0.03\)              | 5 |

Note:\footnote{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 \( K_a \) was calculated.

\[ \text{FIGURE 2} \] Inhibition of \(^{3}\text{H}-\text{rauwolscine}\) binding to whole cells by dibenamine following pre-incubation of dibenamine with sfm or 1mM thiosulphate to \( \text{CHO-}\alpha_{2A} \) cells (A), \( \text{CHO-}\alpha_{2B} \) cells (B), or \( \text{CHO-}\alpha_{2C} \) cells (C). Bars represent total \(^{3}\text{H}-\text{rauwolscine}\) binding and non-specific binding as determined in the presence of 10 \( \mu\text{M}\) RX821002. The concentration of \(^{3}\text{H}-\text{rauwolscine}\) was 0.74 nM in all cases. Data points are mean \( \pm \text{s.e.}\) mean of triplicate determinations.
TABLE 3 Log $K_D$ values of antidepressants binding to the human $\alpha_2$A, $\alpha_2$B and $\alpha_2$C-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, clomipramine has 2.5-fold higher affinity for the $\alpha_2$B than the $\alpha_2$A-adrenoceptor. Compounds are arranged in order of $\alpha_2$A-selectivity.

| Ligand                  | Affinity measurements | Selectivity ratios |
|-------------------------|-----------------------|--------------------|
|                         | Log $K_D$ $\alpha_2$A | n                  | Log $K_D$ $\alpha_2$B | n                  | Log $K_D$ $\alpha_2$C | n                  | $\alpha_2$A vs $\alpha_2$B | $\alpha_2$A vs $\alpha_2$C | $\alpha_2$B vs $\alpha_2$C |
| **Tricyclic antidepressants** |                       |                    |                      |                    |                      |                    |                              |                              |                            |
| clomipramine            | $-5.71 \pm 0.07^{app}$ | 5                  | $-6.10 \pm 0.13$    | 5                  | $-5.80 \pm 0.02^{app}$ | 5                  | 2.5                          | 1.2                          | 2.0                          |
| protriptyline           | $-5.00 \pm 0.05$      | 5                  | $-5.39 \pm 0.13$    | 5                  | $-5.26 \pm 0.07$      | 5                  | 2.5                          | 1.8                          | 1.3                          |
| norclomipramine         | $-5.29 \pm 0.09^{app}$ | 6                  | $-5.74 \pm 0.04^{app}$ | 6                  | $-5.80 \pm 0.07^{app}$ | 6                  | 2.8                          | 3.2                          | 1.1                          |
| trimipramine            | $-5.67 \pm 0.03$      | 5                  | $-6.22 \pm 0.05$    | 5                  | $-6.37 \pm 0.03$      | 5                  | 3.5                          | 5.0                          | 1.4                          |
| nortriptyline           | $-5.65 \pm 0.05$      | 5                  | $-6.38 \pm 0.02$    | 5                  | $-6.19 \pm 0.08$      | 5                  | 5.4                          | 3.5                          | 1.5                          |
| desipramine             | $-5.04 \pm 0.06$      | 5                  | $-5.78 \pm 0.04$    | 5                  | $-5.52 \pm 0.03$      | 5                  | 5.5                          | 3.0                          | 1.8                          |
| lofepramine             | $-4.86 \pm 0.04^{app}$ | 5                  | $-5.60 \pm 0.08$    | 5                  | $-5.28 \pm 0.06$      | 5                  | 5.5                          | 2.6                          | 2.1                          |
| doxepin                 | $-5.69 \pm 0.12$      | 5                  | $-6.67 \pm 0.05$    | 5                  | $-6.60 \pm 0.07$      | 5                  | 9.5                          | 2.2                          | 4.3                          |
| dosulepin               | $-5.16 \pm 0.06$      | 5                  | $-6.20 \pm 0.06$    | 5                  | $-5.63 \pm 0.11$      | 5                  | 11.0                         | 3.0                          | 3.7                          |
| imipramine              | $-5.25 \pm 0.04$      | 5                  | $-6.36 \pm 0.08$    | 5                  | $-5.89 \pm 0.03$      | 5                  | 12.9                         | 4.4                          | 3.0                          |
| amitriptyline           | $-5.86 \pm 0.05^{app}$ | 5                  | $-7.12 \pm 0.05$    | 5                  | $-6.67 \pm 0.09$      | 5                  | 18.2                         | 6.5                          | 2.8                          |
| **Tetracyclic antidepressants** |                       |                    |                      |                    |                      |                    |                              |                              |                            |
| mirtazepine             | $-6.80 \pm 0.05$      | 5                  | $-6.09 \pm 0.06$    | 5                  | $-6.96 \pm 0.03$      | 5                  | 5.1                          | 1.4                          | 7.4                          |
| **other noradrenaline and serotonin reuptake inhibitors** |                       |                    |                      |                    |                      |                    |                              |                              |                            |
| duloxetine              | $-5.43 \pm 0.06$      | 5                  | $-5.31 \pm 0.09$    | 5                  | $-5.67 \pm 0.06$      | 5                  | 1.3                          | 1.7                          | 2.3                          |
| venlafaxine             | $-3.46 \pm 0.03^{app}$ | 5                  | IC$_{50}>3$         | 5                  | IC$_{50}>0.11^{app}$ | 5                  | 1.9                          |                              |                              |
| **Noradrenaline reuptake inhibitors** |                       |                    |                      |                    |                      |                    |                              |                              |                            |
| reboxetine              | IC$_{50}>4$           | 5                  | IC$_{50}>4$         | 5                  | IC$_{50}>4$           | 5                  | IC$_{50}>4$                  | 5                            |                              |
| fluvoxamine             | $-4.81 \pm 0.04^{app}$ | 6                  | $-4.37 \pm 0.08^{app}$ | 5                  | $-4.82 \pm 0.07^{app}$ | 6                  | 2.8                          | 1.0                          | 2.8                          |
| sertraline              | $-5.67 \pm 0.07^{app}$ | 6                  | $-5.62 \pm 0.11^{app}$ | 6                  | $-5.64 \pm 0.05^{app}$ | 6                  | 1.1                          | 1.1                          | 1.0                          |
| fluoxetine              | $-4.70 \pm 0.10^{app}$ | 5                  | $-4.99 \pm 0.03$    | 5                  | $-4.79 \pm 0.07^{app}$ | 5                  | 1.9                          | 1.2                          | 1.6                          |
| citalopram              | IC$_{50}>4$           | 5                  | 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                  | IC$_{50}>5$                  | 5                            |                              |
| **Serotonin reuptake inhibitors** |                       |                    |                      |                    |                      |                    |                              |                              |                            |
| vortioxetine            | $-5.63 \pm 0.06^{app}$ | 6                  | $-5.32 \pm 0.04^{app}$ | 6                  | $-5.84 \pm 0.05$      | 6                  | 2.0                          | 1.6                          | 3.3                          |
| trazadone               | $-6.17 \pm 0.08$      | 5                  | $-5.96 \pm 0.07$    | 5                  | $-6.69 \pm 0.04$      | 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 $\alpha_2$A selective antagonist although its affinity for $\alpha_2$A is only a modest 9-fold greater its $\alpha_2$C affinity. MK-912 is the best $\alpha_2$C-antagonist (0.15 nM $\alpha_2$C-affinity) although again its $\alpha_2$C selectivity is only modest (13-fold greater than $\alpha_2$A). JP1302 ($\alpha_2$C affinity 120 nM) has an $\alpha_1$A-adrenoceptor affinity of 617 nM, only 5-fold less, so is not a truly $\alpha_2$C-selective ligand. CGP20712A ($\beta_1$) and ICI118551 ($\beta_2$) are also highly selective antagonists with minimal $\alpha$-affinity. There are no truly $\alpha_1$B or $\alpha_2$B selective antagonists. Figure 4 shows the affinity (log $K_D$ values) of the most selective ligand at each adrenoceptor subtype (i.e., BRL44408 for $\alpha_2$A, S32212 for $\alpha_2$B and MK-912 for $\alpha_2$C) along with the single most selective antagonists at the other adrenoceptors and demonstrates that the $\alpha_2$-adrenoceptors fall behind $\alpha_1$ and $\beta$ 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) have significant $\beta_2$-adrenoceptor affinity (~30 nM). Silodosin is highly $\alpha_1$-selective (0.25 nM) giving a >100-fold selectivity window compared to the other adrenoceptors. Naftopidil, however, is not selective, with $\alpha_1$A and $\beta_2$ affinities only 3-fold apart and thus potentially increasing the risk of bronchospasm in...
FIGURE 3  Inhibition of $^3$H-rauwolscine (α2A, α2B, and α2C) or $^3$H-CGP12177 (β1 and β2) 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) 0.54 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 ± s.e.mean of triplicate determinations.
## Table 4: Log $K_D$ values of antipsychotics binding to the human $\alpha_2A$, $\alpha_2B$, and $\alpha_2C$-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 $\alpha_2A$-selectivity.

| 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 $\alpha_2C$ |
| First-generation antipsychotics |
| sulpiride    | $-4.50 \pm 0.02$      | $-4.37 \pm 0.06$    | $-4.67 \pm 0.07$    | 5                         | 1.3                       | 1.5                       | 2.0                       |
| haloperidol  | $-5.38 \pm 0.06$      | $-5.53 \pm 0.10$    | $-5.77 \pm 0.05$    | 5                         | 1.4                       | 2.5                       | 1.7                       |
| flupenthixol | $-6.10 \pm 0.12$      | $-6.28 \pm 0.13$    | $-6.88 \pm 0.14$    | 5                         | 1.5                       | 6.0                       | 4.0                       |
| pimozide     | $-5.76 \pm 0.12^{op}$ | $-6.30 \pm 0.10$    | $-6.84 \pm 0.05$    | 5                         | 3.5                       | 12.0                      | 3.5                       |
| trifluoperazine | $-5.60 \pm 0.05$    | $-6.22 \pm 0.12$    | $-6.20 \pm 0.06$    | 5                         | 4.2                       | 4.0                       | 1.0                       |
| prochlorperazine | $-5.78 \pm 0.02^{op}$ | $-6.46 \pm 0.11$    | $-6.31 \pm 0.09$    | 6                         | 4.8                       | 3.4                       | 1.4                       |
| chlorpromazine | $-5.65 \pm 0.13^{op}$ | $-6.60 \pm 0.12$    | $-5.93 \pm 0.11$    | 6                         | 8.9                       | 1.9                       | 4.7                       |
| perphenazine | $-6.00 \pm 0.06$      | $-7.16 \pm 0.05$    | $-6.83 \pm 0.04$    | 5                         | 14.5                      | 6.8                       | 2.1                       |
| Second-generation antipsychotics |
| amisulpiride | $-5.11 \pm 0.09^{op}$ | $-4.69 \pm 0.13^{op}$ | $-5.57 \pm 0.07$    | 5                         | 2.6                       | 2.9                       | 7.6                       |
| aripiprazole | $-6.68 \pm 0.08$      | $-5.54 \pm 0.08$    | $-7.23 \pm 0.14$    | 6                         | 1.4                       | 3.5                       | 4.9                       |
| sertindole   | $-5.95 \pm 0.06$      | $-5.81 \pm 0.07$    | $-6.17 \pm 0.03$    | 5                         | 1.4                       | 1.7                       | 2.3                       |
| olanzapine   | $-5.59 \pm 0.05$      | $-5.47 \pm 0.06$    | $-5.86 \pm 0.02$    | 5                         | 1.3                       | 1.9                       | 2.5                       |
| paliperidone | $-7.12 \pm 0.04$      | $-7.26 \pm 0.05$    | $-7.84 \pm 0.03$    | 5                         | 1.4                       | 5.2                       | 3.8                       |
| risperidone  | $-7.30 \pm 0.09$      | $-7.47 \pm 0.08$    | $-8.04 \pm 0.03$    | 5                         | 1.5                       | 5.5                       | 3.7                       |
| ziprasidone  | $-6.36 \pm 0.11$      | $-6.59 \pm 0.08$    | $-6.77 \pm 0.08$    | 5                         | 1.7                       | 2.6                       | 1.5                       |
| clozapine    | $-5.86 \pm 0.08^{op}$ | $-6.20 \pm 0.05$    | $-6.87 \pm 0.08$    | 5                         | 2.2                       | 10.2                      | 4.7                       |
| lurasidone   | $-6.67 \pm 0.05$      | $-7.36 \pm 0.06$    | $-7.34 \pm 0.03$    | 5                         | 4.9                       | 4.7                       | 1.0                       |
| quetiapine   | $-5.81 \pm 0.08$      | $-6.72 \pm 0.08$    | $-6.66 \pm 0.03$    | 5                         | 8.1                       | 7.1                       | 1.1                       |

Note: $^{op}$ = 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 $\alpha_2A$.

| those with asthma. Likewise, there is little evidence here to support SKF86466 being an $\alpha_2$-selective antagonist.46-48 The affinity of SKF86466 for the $\beta_2$-adrenoceptor (250 nM) is similar to the highest $\alpha$-adrenoceptor affinity (407 nM at $\alpha_2C$). This may well be a species issue (see introduction) with previous studies being conducted in rodents,46-48 however others suggest a human $\alpha_2A$-affinity of 13 nM.23

Labetolol and carvedilol are often usually referred to as dual $\alpha/\beta$-blockers (e.g.,49). Labetolol (affinities of $\beta_2$ 6-9 nM, $\beta_1$ 11-23 nM, and $\alpha_1A$ 47 nM) has very poor affinity at $\alpha_1B$, $\alpha_1D$, $\alpha_2A$, $\alpha_2B$, $\alpha_2C$, and $\beta_3$-adrenoceptors and thus reasonable affinity at only 1 out of 6 $\alpha$-adrenoceptors. A $\beta/\alpha_1A$-antagonist would be a more accurate description. Likewise, carvedilol with affinities for $\beta_2$ of 0.1-0.4 nM, $\beta_1$ of 0.6-1.8 nM, and $\beta_3$ of 5 nM also has highest $\alpha$-affinity for $\alpha_1A$ (4 nM) over $\alpha_1B$ or $\alpha_1D$ (14 nM) or $\alpha_2$-adrenoceptors (48-490 nM), so with affinities up to 1000-fold different across the 9 different adrenoceptors should not be considered a pan $\alpha/\beta$-blocker. The lack of affinity of other $\beta$-blockers for the $\alpha$-adrenoceptors may also be expected.50

### 4.3 Antidepressants and antipsychotics

Given the considerable CNS expression of $\alpha_2A$ and $\alpha_2C$-adrenoceptors, and that many antidepressants and antipsychotics have high $\alpha_1A$-affinity, a third aim of this study was to compare the affinity of antidepressants and antipsychotics across the adrenoceptors.

The antidepressants generally had poor $\alpha_2$-adrenoceptor affinity, considerably lower affinity than that seen for the tricyclic antidepressant affinities at the $\alpha_1A$-adrenoceptor. The antidepressant mirtazapine is a slight outsider with the highest $\alpha_2$-affinity of the antidepressants studied here, and higher than $\alpha_1A$-affinity. It has been associated with antinociceptive properties attributed to $\alpha_2$-adrenoceptors in mice.19,51 Mirtazapine ($\alpha_2A$-affinity 158 nM and $\alpha_2C$ 110 nM), had similar affinity to the $\alpha_2$-antagonist idazoxan and similar values to those obtained in human $\alpha_2A$ receptors (79–126 nM) in,52 who also reported lower affinity at human $\alpha_1$ and unmeasurable affinity at human $\beta_1$ or $\beta_2$-adrenoceptors. Of note,53 also reported similar values for mirtazapine for human and rat receptors,
### TABLE 5 Log K<sub>D</sub> values of ligands binding to the human β1 and β2-adrenoceptors as measured by <sup>3</sup>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 Tables 2, 3, and 4 for ease of comparison. Supplementary Table 1 has these ligands, alongside the α2-data, presented in alphabetical order.

| Ligand       | Affinity measurements |  |  |  |  |
|--------------|-----------------------|---|---|---|---|
|              | Log K<sub>D</sub> β1  | n | Log K<sub>D</sub> β2 | n |
| α-antagonists|                       |   |                           |   |
| BRL44408     | No binding to −3      | 5 | No binding to −3          | 5 |
| benoxathian  | −4.55 ± 0.03<sup>app</sup> | 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<sup>app</sup> | 5 |
| 2-MPMDQ      | IC<sub>50</sub> < −5  | 6 | IC<sub>50</sub> < −5      | 6 |
| yohimbine    | No binding to −4      | 5 | No binding to −4          | 5 |
| idazoxan     | IC<sub>50</sub> < −3  | 5 | IC<sub>50</sub> < −3      | 5 |
| WB4104       | IC<sub>50</sub> < −4  | 5 | IC<sub>50</sub> < −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 to −4      | 5 | IC<sub>50</sub> < −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<sup>app</sup> | 5 |
| sunepitron   | IC<sub>50</sub> < −3  | 5 | IC<sub>50</sub> < −3      | 5 |
| doxazosin    | −4.72 ± 0.06<sup>app</sup> | 5 | −5.57 ± 0.01              | 6 |
| MK-912       | IC<sub>50</sub> < −4  | 6 | IC<sub>50</sub> < −4      | 6 |
| phenotamine  | IC<sub>50</sub> < −4  | 6 | IC<sub>50</sub> < −4      | 6 |
| RS17053      | −5.44 ± 0.04          | 6 | −6.42 ± 0.06              | 6 |
| RS100329     | IC<sub>50</sub> < −3  | 5 | −4.77 ± 0.07              | 5 |
| lisuride     | −6.03 ± 0.06          | 5 | −7.48 ± 0.04              | 5 |
| BMY7378      | IC<sub>50</sub> < −4  | 9 | IC<sub>50</sub> < −4      | 9 |
| RS79948      | −3.84 ± 0.05          | 5 | IC<sub>50</sub> < −3      | 5 |
| carvedilol   | −9.20 ± 0.05          | 8 | −9.98 ± 0.06              | 8 |
| JP1302       | IC<sub>50</sub> < −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<sub>50</sub> < −4      | 5 |
| PF3774076    | No binding to −4      | 5 | No binding to −4          | 5 |
| Rec15-2615   | IC<sub>50</sub> < −4  | 5 | IC<sub>50</sub> < −4      | 5 |
| AH11110A     | −6.23 ± 0.07          | 6 | −6.36 ± 0.07              | 6 |
| silodosin    | IC<sub>50</sub> < −5  | 6 | −7.52 ± 0.10              | 6 |
| 5-methyl-urapidil | −6.12 ± 0.04         | 5 | −5.00 ± 0.07              | 5 |
| SNAP5089     | IC<sub>50</sub> < −5  | 5 | IC<sub>50</sub> < −5      | 5 |
| anisodamine  | no binding to −3      | 9 | no binding to −3          | 9 |
| 2-niguldipine| IC<sub>50</sub> < −4  | 5 | IC<sub>50</sub> < −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<sub>50</sub> < −5  | 5 | IC<sub>50</sub> < −5      | 5 |
| domperidone  | IC<sub>50</sub> < −4  | 5 | IC<sub>50</sub> < −4      | 5 |
| urapidil     | −5.32 ± 0.06          | 5 | −5.00 ± 0.02              | 5 |
| HEAT         | IC<sub>50</sub> < −4.5 | 5 | IC<sub>50</sub> < −4      | 5 |
| indoramin    | −4.73 ± 0.10<sup>app</sup> | 5 | −5.27 ± 0.11<sup>app</sup> | 5 |

(Continues)
| Ligand            | Affinity measurements                                      | Log $K_D$ β1 | n  | Log $K_D$ β2 | n  |
|-------------------|-----------------------------------------------------------|--------------|----|--------------|----|
| cyclazosin        | No binding to $-4$                                        | $-5.30 \pm 0.04$ | 6  |              |    |
| imiloxan          | $IC_{50}>-3$                                              |              | 5  |              |    |
| dibenamine        | $-4.60 \pm 0.06^{\text{app}}$                            |              | 5  | $-4.94 \pm 0.10^{\text{app}}$ | 5  |
| promethazine      | $IC_{50}>-4$                                              |              | 10 | $IC_{50}>-4$ | 10 |
| phenoxybenzamine  | $-4.36 \pm 0.10^{\text{app}}$                            |              | 5  |              |    |
| prazosin          | No binding to $-4$                                        | $-5.10 \pm 0.10^{\text{app}}$ | 5  |              |    |
| terazosin         | No binding to $-4$                                        | $-5.10 \pm 0.10^{\text{app}}$ | 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  |
| S-β-cyanopindolol | $-10.39^{\#}$                                             |              |    | $-11.09^{\#}$ |    |
| bucindolol        | $-9.31^{\#}$                                              |              |    | $-9.99^{\#}$ |    |
| ICI118551         | $-6.61 \pm 0.05$                                          |              | 11 | $-9.41 \pm 0.09$ |    |
| SDZ21009          | $-8.94^{\#}$                                              |              |    | $-10.28^{\#}$ |    |
| propranolol       | $-8.16^{*}$                                               |              |    | $-9.08^{*}$ |    |
| carazolol         | $-9.69^{\#}$                                              |              |    | $-10.49^{\#}$ |    |
| CGP12177          | $-9.21^{*}$                                               |              |    | $-9.39^{*}$ |    |
| CGP20712A         | $-8.87 \pm 0.13$                                          |              | 9  | $-5.74 \pm 0.03$ | 10 |
| 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     | $-4.64 \pm 0.13$                                          |              | 5  | $-5.40 \pm 0.08$ |    |
| desipramine       | $IC_{50}>-4$                                              |              | 5  | $-4.93 \pm 0.03^{\text{app}}$ | 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  |
| mirtazepine       | No binding to $-4$                                        |              | 5  | No binding to $-4$ | 5  |
| reboxetine        | $IC_{50}>-4$                                              |              | 10 | $-5.26 \pm 0.06$ | 10 |
| duloxetine        | $IC_{50}>-4.5$                                            | $-6.07 \pm 0.06$ | 11 |              |    |
| venlafaxine       | $-3.80 \pm 0.11^{\text{app}}$                            |              | 5  | $-4.13 \pm 0.13^{\text{app}}$ | 5  |
| Fluoxetine        | $IC_{50}>-4$                                              |              | 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 $K_0$, $\beta 1$  | $n$     | Log $K_0$, $\beta 2$ | $n$ |
| **Serotonin reuptake inhibitors** |                       |         |         |
| vortioxetine          | $-6.37 \pm 0.03$      | 11      | $-6.75 \pm 0.04$  | 11  |
| trazodone             | $IC_{50} > -4$        | 10      | $-5.14 \pm 0.05$  | 10  |
| **First-generation antipsychotics** |                       |         |         |
| sulpiride             | $IC_{50} > -3$        | 10      | $IC_{50} > -3$    | 10  |
| haloperidol           | $IC_{50} > -4$        | 5       | $-4.94 \pm 0.04$  | 5   |
| flupenthixol          | $IC_{50} > -5$        | 10      | $IC_{50} > -5$    | 10  |
| pimozide              | $IC_{50} > -4$        | 10      | $-5.75 \pm 0.06$  | 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  |
| aripiprazole          | $-6.15 \pm 0.04$      | 6       | $-6.68 \pm 0.08$  | 6   |
| sertindole            | $IC_{50} > -5$        | 5       | $IC_{50} > -5$    | 5   |
| olanzapine            | $IC_{50} > -3$        | 4       | $-4.96 \pm 0.05$  | 4   |
| paliperidone          | $IC_{50} > -4.5$      | 10      | $IC_{50} > -4.5$  | 10  |
| risperidone           | No binding to $-4$    | 5       | No binding to $-4$| 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: # from$^{22}$
*from$^{31}$

whereas$^{17}$ suggest ~10-fold higher $\alpha 2$-affinity in what appears to be data gathered from mice.

Interestingly, many tricyclic antidepressants had a slight $\alpha 2B$-selectivity, something not seen with most $\alpha$-ligands (Table 2), with the most potent (amitriptyline) having an $\alpha 2B$-affinity (76 nM) only 10-fold lower than that at the $\alpha 1A$-adrenoceptor. Vortioxetine was the only antidepressant with any significant $\beta$-adrenoceptor affinity and the only to have $\beta$-adrenoceptor affinity greater than $\alpha$-adrenoceptor affinity (178 nM for the $\beta 2$-adrenoceptor).

$\alpha 2C$-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 $\alpha 2$-adrenoceptors than $\alpha 1$-adrenoceptors, and had little selectivity for $\alpha 2C$ over the other $\alpha 2$-subtypes. For example, chlorpromazine had affinities of $\alpha 2A$ 2239 nM, $\alpha 2B$ 251 nM, $\alpha 2C$ 1175 nM, whereas its $\alpha 1A$-adrenoceptor affinity is 1 nM. There is however huge heterogeneity even between studies of human $\alpha 2$-adrenoceptors. Chlorpromazine affinities range from $\alpha 2A$ 78 nM, $\alpha 2B$ 4.8 nM and $\alpha 2C$ 41 nM ($^3$H-rauwolscine membrane binding from human receptors expressed in COS cells,$^{28}$) $\alpha 2A$ 396-535 nM ($^3$H-yohimbine membrane binding using human colonic cancer cells and human platelets,$^{22}$) $\alpha 2A$ 600 nM, $\alpha 2B$ 43 nM, and $\alpha 2C$ 260 nM ($^3$H-RX821002 membrane binding for human receptors expressed in CHO cells,$^{29}$) $\alpha 2A$ 1008 nM, $\alpha 2B$ 34 nM, and $\alpha 2C$ 85 nM ($^3$H-RX821002 membrane binding to human receptors expressed in mouse cells,$^{30}$) $\alpha 2A$ 2245 nM ($^3$H-RX821002 membrane binding to human platelets,$^{23}$) to $\alpha 2A$ 4169 nM and $\alpha 2C$ 1413 nM (agonism of agonist responses living CHO cells expressing the human $\alpha 2$-adrenoceptor$^{52}$).

The second-generation antipsychotics had a wide range of affinity for the $\alpha 2$-adrenoceptors, with risperidone (9 nM, $\alpha 2C$) and paliperidone (14 nM $\alpha 2C$) having the highest affinity (in keeping with other human $\alpha 2$-adrenoceptor studies$^{52}$), to >1000 nM affinity for olanzapine and amisulpiride. Even for risperidone and paliperidone, the $\alpha 2C$ affinity is less potent than that seen at the $\alpha 1A$-adrenoceptor and once again $\alpha 2A$ vs $\alpha 2C$-selectivity was very marginal. Clozapine, which has been particularly noted for $\alpha 2C$-affinity$^{12,13,17}$ had an $\alpha 2C$-affinity of 135 nM, compared to its $\alpha 1A$-affinity of 5.4 nM measured under identical conditions. This $\alpha 2C$ 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$^{21,29}$).
4.4 | 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 variation 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 mirzapine) 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 an 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.

ETHICS STATEMENT

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

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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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