

ORIGINAL ARTICLE



The affinity and selectivity of α -adrenoceptor antagonists, antidepressants, and antipsychotics for the human α 1A, α 1B, and α 1D-adrenoceptors

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Abstract

α 1-adrenoceptor antagonists are widely used for hypertension (eg, doxazosin) and benign prostatic hypertrophy (BPH, eg, tamsulosin). Some antidepressants and antipsychotics have been reported to have α 1 affinity. This study examined 101 clinical drugs and laboratory compounds to build a comprehensive understanding of α 1-adrenoceptor subtype affinity and selectivity. [³H]prazosin whole-cell binding was conducted in CHO cells stably expressing either the full-length human α 1A, α 1B, or α 1D-adrenoceptor. As expected, doxazosin was a high-affinity nonselective α 1-antagonist although other compounds (eg, cyclazosin, 3-MPPI, and ARC239) had higher affinities. Several highly α 1A-selective antagonists were confirmed (SNAP5089 had over 1700-fold α 1A selectivity). Despite all compounds demonstrating α 1 affinity, only BMY7378 had α 1D selectivity and no α 1B-selective compounds were identified. Phenoxybenzamine (used in pheochromocytoma) and dibenamine had two-component-binding inhibition curves at all three receptors. Incubation with sodium thiosulfate abolished the high-affinity component suggesting this part is receptor mediated. Drugs used for hypertension and BPH had very similar α 1A/ α 1B/ α 1D-adrenoceptor pharmacological profiles. Selective serotonin reuptake inhibitors (antidepressants) had poor α 1-adrenoceptor affinity. Several tricyclic antidepressants (eg, amitriptyline) and antipsychotics (eg, chlorpromazine and risperidone) had high α 1-adrenoceptor affinities, similar to, or higher than, α blockers prescribed for hypertension and BPH, whereas others had poor α 1 affinity (eg, protriptyline, sulpiride, amisulpiride, and olanzapine). The addition of α blockers for the management of hypertension or BPH in people already taking tricyclic antidepressants and certain antipsychotics may not be beneficial. Awareness of the α -blocking potential of different antipsychotics may affect the choice of drug for those with delirium where additional hypotension (eg, in sepsis) may be detrimental.

KEYWORDS

affinity, antidepressant, antipsychotic, benign prostatic hypertrophy, hypertension, α antagonist

Abbreviations: BPH, benign prostatic hypertrophy; CHO, Chinese hamster ovary; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

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1 | INTRODUCTION

The α -adrenoceptors are expressed in a wide range of tissues including blood vessels, kidney, spleen, liver, brain, and lower urinary tract.¹⁻³ There are three subtypes: α 1A, α 1B, and α 1D-adrenoceptors.¹⁻⁴ All are present in blood vessels, and whilst α 1A and α 1D are both important in smooth muscle contraction (and control of blood pressure), the role of the α 1B-adrenoceptors is less certain.^{2,3,5,6}

α -adrenoceptor antagonists (α blockers) were first used to reduce systemic blood pressure with dibenamine, phentolamine, and phenoxybenzamine used in the diagnosis and management of pheochromocytoma, an adrenal catecholamine-secreting tumor.^{7,8} While phenoxybenzamine is still important for pheochromocytoma, longer acting, nonselective α 1-antagonists were developed (doxazosin, terazosin, indoramin, and prazosin) and remain important in the management of resistant hypertension.

α blockers are also used in benign prostatic hypertrophy (BPH) where α 1A blockade induces prostate and lower urinary tract smooth muscle relaxation, improving urinary flow.⁹ Phenoxybenzamine was the first α blocker to be used in BPH¹⁰ although its α 2 effects limited its use.¹¹ The nonselective α 1-antagonists doxazosin, terazosin, indoramin, and prazosin were used effectively for BPH, but caused hypotension, particularly postural hypotension, and required dose titration to manage this problematic side effect.^{9,12} Selective α 1A-antagonists were developed, hoping to minimize hypotension by reducing α 1B-antagonism.^{11,13} Tamsulosin, alfuzosin, and silodosin were developed as prostate-specific (α 1A selective) drugs and are used without dose titration.⁹ Despite reports of "better tolerability,"^{11,14} alfuzosin is reported to be a nonselective α 1-antagonist and tamsulosin to have equal α 1A- and α 1D-adrenoceptor affinity,^{15,16} suggesting they may be pharmacologically indistinguishable from drugs used for hypertension. Indeed, tamsulosin (the most commonly prescribed α blocker for BPH) is associated with increased hypotension, falls, and fractures.^{12,13,17} Although effective for BPH, silodosin appears to have more sexual side effects, whereas its cardiovascular effects remain uncertain.¹⁸

α 1-adrenoceptors are the most abundant adrenoceptors in the brain and modulate neurotransmitter release.³ Many antidepressants prevent the reuptake of neurotransmitters (serotonin and noradrenaline), and therefore increase synaptic neurotransmitter concentration. However, several antidepressants have significant α 1-adrenoceptor affinity.¹⁹⁻²¹ This high affinity is seen in brain homogenates.²² In theory these two effects (increased neurotransmitter presence, but receptor blockade) could cancel each other out.²⁰ However, antidepressants cause hypotension, particularly postural hypotension (up to 58% users^{23,24}). Not surprisingly therefore, antidepressant use is associated with twice the risk of falls.²⁵

Several antipsychotics (neuroleptics) bind to α 1-adrenoceptors in blood vessels and brain homogenates.^{6,26,27} Many antipsychotics

cause postural hypotension,^{28,29} and again, rates are high (eg, 48% taking risperidone²⁴) including postural hypotension in those taking long-term antipsychotics (77%³⁰). Interestingly, the degree of postural hypotension seen with several antipsychotics correlates well with the α 1A-adrenoceptor affinity.²⁹ Antipsychotic drug use is also associated with falls and hip fractures and regular use is associated with twice the risk of falls (even after controlling for other risks^{31,32}).

There are many studies examining the affinity of α 1-adrenoceptor ligands. Many are older studies before the identification of the three subtypes and many are in whole tissue where multiple subtypes will be present. Most studies only report the two or three ligands under investigation. Here we aimed to investigate the subtype selectivity of a wide range of α -antagonists including those used in hypertension, BPH, antidepressants, antipsychotics as well as laboratory compounds. Human α 1A, α 1B, and α 1D-adrenoceptors were expressed in intact mammalian cells, in order to build a comprehensive and directly comparable picture of α 1-subtype selectivity in living cells.

2 | METHODS

2.1 | Materials

A list of all of the compounds studied, together with the source and supplier code from which it was purchased, is given in Table S1. White-sided view plates were from Greiner Bio-one, Kremsmunster, Austria; and [3H]prazosin, Microscint 20, and scintillation fluid from PerkinElmer (Buckinghamshire, UK). Fetal 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 DNA of the human α 1A-adrenoceptor, human α 1B-adrenoceptor (DNAs from Guthrie DNA Resource Centre), or human α 1D-adrenoceptor (full-length DNA from Andre Pupo³³; using Lipofectamine and Optimem according to the manufacturers' instructions. Transfected cells were selected for 3 weeks using resistance to neomycin (at 1mg/ml). Single clones from each transfection were then isolated by dilution cloning giving rise to the stable cell lines CHO- α 1A, CHO- α 1B, and CHO- α 1D.

2.3 | Cell culture

CHO cells were grown in Dulbecco's modified Eagle's medium nutrient mix F12 (DMEM/F12) containing 10% fetal calf serum and 2 mmol/L 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.

2.4 | [3H]prazosin binding—saturation binding

The K_D value for [3H]prazosin was determined in each cell line by saturation binding. [3H]prazosin was diluted in serum-free media. Media were removed from each well and replaced with either 100 μ L serum-free media (total binding) or 100 μ L 20 μ mol/L tamsulosin (α 1A and α 1B) or 200 μ mol/L tamsulosin (α 1D) to determine nonspecific binding. [3H]prazosin was then added to the wells (quadruplicates per condition, 1 in 2 dilution in well), and the plates incubated for 2 hour at 37°C in a humidified 5% CO₂; 95% air atmosphere. After 2 hours, the cells were washed twice by the addition and removal of 2 \times 200 μ L cold (4°C) phosphate-buffered saline. 100 μ L Microscint 20 was added to each well and a white base applied to the plate to convert the wells into white-sided/white-bottomed wells. Plates were left at room temperature for at least 6 hours before being counted on a Topcount (PerkinElmer), with a counting time of 2 minutes per well.

2.5 | [3H]prazosin whole-cell binding—competition binding

Ligands were serially diluted in serum-free media (DMEM/F12 containing 2 mmol/L L-glutamine only) to twice their final required concentration. Media was removed from each well of the 96-well view plate and 100 μ L ligand added to triplicate wells. This was immediately followed by the addition of 100 μ L [3H]prazosin (diluted in serum-free media) and the cells incubated for 2 hours at 37°C (5% CO₂, humidified atmosphere). After 2 hours the plates were washed as above. Cells were inspected under a light microscope to ensure cells were still present 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 nonspecific binding (6 wells/plate) determined by the presence of 10 μ mol/L tamsulosin (α 1A and α 1B) or 100 μ mol/L tamsulosin (α 1D) was defined in every plate.

Sodium thiosulfate reacts with 2-chloroethylamines in a 1:1 stoichiometry to inactivate the ethyleniminium ions generated in solution (see Discussion). Sodium thiosulfate had no effect on [3H]prazosin binding up to concentrations of 10 mmol/L. Therefore, to ensure that all ethyleniminium ions were inactivated, sodium thiosulfate was used in excess, with a final well concentration of 1 mmol/L. When used, competing ligands were serially diluted in serum-free media (just as above) in the absence and presence of thiosulfate and both dilution series were then incubated for 30 minutes at 37°C (5% CO₂, humidified atmosphere). Media was then removed from the cells and competing ligand (in the presence or absence of thiosulfate)

added to the wells immediately followed by [3H]prazosin (thus thiosulfate was present with the competing ligand for 30 minutes before addition to the cells, and then throughout the 2-hour incubation with cells at 1 mmol/L).

[3H]prazosin concentrations were determined from taking the average of triplicate 50 μ L samples of each [3H]prazosin concentration used and counted on a PerkinElmer Scintillation counter and were in the range from 0.22 to 1.40 nmol/L.

All experiments have been conducted in intact living mammalian cells expressing human α 1A or α 1B or α 1D-adrenoceptors. Unlike membrane-binding studies, physiological levels of intracellular endogenous GTP will therefore always have been present. Although it should not make much difference for antagonists, the receptors (and therefore measurements taken) in this living system are therefore more akin to how drugs bind in people, than studies conducted in membrane preparations.

2.6 | Data analysis

In all cases where a K_D value is stated, increasing concentrations of the competing ligand fully inhibited the specific binding of [3H]prazosin (unless otherwise annotated in the tables).

The following equation was then fitted to the data using Graphpad Prism 7 and the IC₅₀ was then determined as the concentration required to inhibit 50% of the specific binding.

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

where [A] is the concentration of the competing ligand and IC₅₀ is the concentration at which half of the specific binding of [3H]prazosin has been inhibited.

From the IC₅₀ value, the known concentration of [3H]prazosin and the known K_D for [3H]prazosin 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:

$$K_D = \frac{IC_{50}}{1 + \left(\frac{[3H]prazosin}{K_D[3H]prazosin} \right)}$$

In some cases, the maximum concentration of competing ligand was not able to inhibit all of the specific binding. Where no inhibition of [3H]prazosin binding was seen, even with maximum concentration of competing ligand possible, “no binding” is given in the tables. Where the inhibition produced by the maximum concentration of the competing ligand was 50% or less, an IC₅₀ could not be determined and thus a K_D value not calculated. This is shown in the tables as IC₅₀ > top concentration used (ie, IC₅₀ > 100 μ mol/L means that 100 μ mol/L inhibited some but less than 50% of the specific binding). In cases where the competing ligand caused a substantial (greater than 60%, but not 100%) inhibition of specific binding, an IC₅₀ value was determined by extrapolating the curve to nonspecific levels and assuming that a

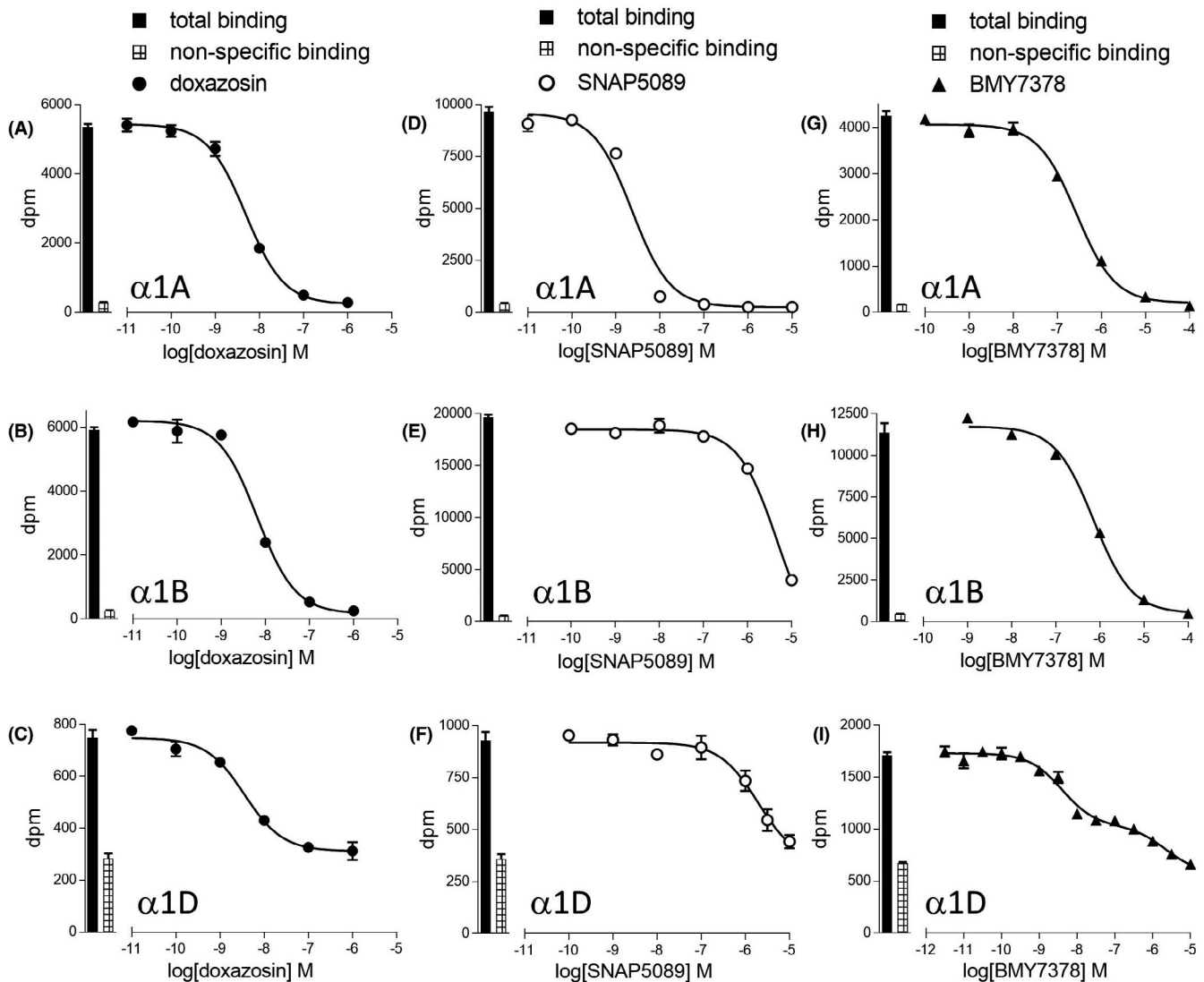


FIGURE 1 Inhibition of [³H]prazosin binding to whole cells by doxazosin (A–C), SNAP5089 (D–F) or BMY7378 (G–I) to CHO- α 1A cells (A, D, G), CHO- α 1B cells (B, E, H), or CHO- α 1D cells (C, F, I). Bars represent total [³H]prazosin binding and nonspecific binding was determined in the presence of 10 μ mol/L tamsulosin (CHO- α 1A and CHO- α 1B) or 100 μ mol/L tamsulosin (CHO- α 1D). The concentration of [³H]prazosin was (A) 0.31 nmol/L, (B) 0.31 nmol/L, (C) 0.70 nmol/L, (D) 0.68 nmol/L, (E) 0.68 nmol/L, (F) 0.60 nmol/L, (G) 0.24 nmol/L, (H) 0.42 nmol/L, and (I) 1.25 nmol/L. Data points are mean \pm SE mean of triplicate determinations

greater concentration would have resulted in 100% inhibition. These values are given as apparent K_D values in the tables.

For some ligands, the inhibition of [³H]prazosin binding was best described by a two-component curve, using the equation below:

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

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.

Selectivities are given as a ratio of the K_D values for the different receptors.

3 | RESULTS

Saturation binding yielded a K_D value for [³H]prazosin of 0.71 nmol/L \pm 0.07 (1552 \pm 166 fmol/mg protein, $n = 11$) at the human α 1A-adrenoceptor, 0.87nM \pm 0.11 (4350 \pm 317 fmol/mg protein, $n = 12$) at the human α 1B-adrenoceptor, and 1.90 \pm 0.31 nmol/L (417 \pm 48 fmol/mg protein, $n = 9$) at the full-length human α 1D-adrenoceptor. As the lower expression of the α 1D-receptor meant that a larger proportion of the experimental window was nonspecific binding, the affinity of prazosin was also determined by competing prazosin with [³H]prazosin. The log K_D values obtained were -9.07 ± 0.04 (=0.85 nmol/L, $n = 9$) at the α 1A-adrenoceptor, -8.74 ± 0.06 (=1.82 nmol/L, $n = 8$) at the α 1B-adrenoceptor, and -9.07 ± 0.23 (=0.85 nmol/L, $n = 10$) at the α 1D-adrenoceptor.

TABLE 1 Log K_D values and selectivity ratios of α -antagonists binding to the human $\alpha1A$, $\alpha1B$ and $\alpha1D$ -adrenoceptors. Values represent mean \pm SE 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, SNAP5089 has 1778 fold higher affinity for the $\alpha1A$ -adrenoceptor than the $\alpha1B$ -adrenoceptor. Compounds are arranged in order of $\alpha1A$ -selectivity

Ligand	Log K_D $\alpha1A$	n	Log K_D $\alpha1B$	n	Log K_D $\alpha1D$	n	$\alpha1A$ vs $\alpha1B$	n	$\alpha1A$ vs $\alpha1D$	$\alpha1B$ vs $\alpha1D$	$\alpha1A$ vs $\alpha1B$	$\alpha1A$ vs $\alpha1D$	$\alpha1B$ vs $\alpha1D$
α -antagonists													
SNAP5089	-8.89 \pm 0.03	6	-5.64 \pm 0.04 ^{app}	6	-5.65 \pm 0.09 ^{app}	5	1778	5	1738	1.0			
Silodosin	-9.61 \pm 0.08	9	-6.50 \pm 0.09	9	-6.94 \pm 0.16	9	1288	9	468	2.8			
RS100329	-9.60 \pm 0.05	10	-6.67 \pm 0.07	7	-7.63 \pm 0.17	6	851	6	93	9.1			
					-5.13 \pm 0.15								
					70.5 \pm 3.0% site 1								
Niguldipine	-9.24 \pm 0.11	9	-6.33 \pm 0.08	5	-5.92 \pm 0.06	6	813	6	2089	2.6			
5-methyl-urapidil	-8.23 \pm 0.05	5	-6.06 \pm 0.04	5	-5.61 \pm 0.07	5	148	5	417	2.8			
Lisuride	-7.94 \pm 0.06	5	-6.07 \pm 0.04	5	-6.93 \pm 0.11	7	74	7	10	7.2			
Benoxathian	-9.08 \pm 0.05	6	-7.32 \pm 0.03	6	-7.91 \pm 0.10	7	58	7	15	3.9			
					-5.62 \pm 0.19								
					62.5 \pm 3.4% Site 1								
Anisodamine	-5.21 \pm 0.03	5	-3.45 \pm 0.04 ^{app}	5	-4.21 \pm 0.05	5	58	5	10	5.8			
RS17053	-8.33 \pm 0.09	11	-6.61 \pm 0.09	11	-6.84 \pm 0.13	6	52	6	31	1.7			
Urapidil	-7.21 \pm 0.02	5	-5.50 \pm 0.07	7	-6.37 \pm 0.10	6	51	6	6.9	7.4			
2-MPMDQ	-9.06 \pm 0.07	6	-7.37 \pm 0.04	6	-9.01 \pm 0.16	8	49	8	1.1	4.4			
					-5.66 \pm 0.29								
					64.0 \pm 2.1% site1								
WB4104	-9.03 \pm 0.04	10	-7.39 \pm 0.05	7	-8.63 \pm 0.11	9	44	9	2.5	17			
					-5.96 \pm 0.09								
					59.6 \pm 3.2% site 1								
Indoramin	-8.43 \pm 0.07	5	-6.82 \pm 0.04	5	-6.29 \pm 0.07	5	41	5	138	3.4			
Phentolamine	-8.15 \pm 0.08	8	-6.55 \pm 0.05	5	-6.84 \pm 0.11	6	40	6	20	1.9			
					-4.64 \pm 0.14								
					60.5 \pm 3.8% site 1								
Tamsulosin	-9.67 \pm 0.06	17	-8.12 \pm 0.04	15	-9.18 \pm 0.08	13	35	13	3.1	11			
					-5.67 \pm 0.15								
					54.6 \pm 3.7% site 1								
Amitraz	-5.52 \pm 0.05	5	Log IC_{50} > -4	6	-5.08 \pm 0.05	5	>33	5	2.8	>12			
Labetolol	-7.33 \pm 0.04	7	-5.91 \pm 0.03	7	-6.12 \pm 0.07	6	26	6	16	1.6			
Domperidone	-6.85 \pm 0.12	6	-5.50 \pm 0.05	5	-5.98 \pm 0.05	5	22	5	7.4	3.0			

(Continues)

TABLE 1 (Continued)

Ligand	Log K_D α 1A	n	Log K_D α 1B	n	Log K_D α 1D	n	α 1A	vs	α 1B	α 1D	vs	α 1A	vs	α 1B	vs	α 1D
Dibenzamine	-7.91 ± 0.06	15	-6.57 ± 0.07	14	-7.37 ± 0.15	9	22			3.5						6.3
	-5.32 ± 0.08		-4.66 ± 0.06		-5.00 ± 0.14											
	83.0 ± 1.8% site 1		67.6 ± 2.6% site 1		47.8 ± 3.2% site 1											
Atipamezole	-5.99 ± 0.03	5	-4.68 ± 0.08	6	-5.33 ± 0.04	5	20			4.6						4.5
	-6.76 ± 0.03	5	-5.46 ± 0.05	5	-7.30 ± 0.16	7	20			3.5						6.9
2-PMDQ					-5.50 ± 0.25											
	-8.19 ± 0.09	5	-6.95 ± 0.05	6	61.2 ± 5.5% site 1	9	17			1.7						3.0
					-8.42 ± 0.12											
BRL44408					-5.61 ± 0.12											
	-5.92 ± 0.09	9	-4.68 ± 0.07	9	57.6 ± 2.8% site 1	5	17			7.2						2.4
ARC239	-9.35 ± 0.08	8	-8.15 ± 0.07	9	-8.74 ± 0.12	7	16			4.1						3.9
					-5.42 ± 0.21											
Efaroxan	-5.47 ± 0.03	5	-4.27 ± 0.07	5	60.5 ± 1.4% site 1	5	16			3.2						5.0
	-7.66 ± 0.11	9	-6.49 ± 0.07	6	-8.12 ± 0.18	8	15			2.9						4.3
Naftopidil					-6.05 ± 0.13											
	-7.97 ± 0.03	6	-6.82 ± 0.06	6	48.8 ± 4.5% site 1	7	14			8.1						1.7
SKF86466	-6.06 ± 0.05	5	-4.93 ± 0.05	5	-7.06 ± 0.11	5	13			7.9						1.7
	-5.78 ± 0.06	5	-4.65 ± 0.06	5	-5.33 ± 0.23	6	13			2.8						4.8
RX821002	-6.51 ± 0.09	6	-5.46 ± 0.06	6	-5.31 ± 0.13	7	11			1.6				1.4		1.5
	-9.57 ± 0.06	6	-8.59 ± 0.03	6	-9.76 ± 0.15	7	9.5									1.5
3-MPPI					-6.93 ± 0.17											
					66.7 ± 3.4% site 1											
S32212	-5.90 ± 0.06	5	-4.92 ± 0.02 ^{app}	5	-5.69 ± 0.13 ^{app}	5	9.5			1.6						5.9
Promethazine	-7.00 ± 0.10	11	-6.06 ± 0.05	10	-5.75 ± 0.07	5	8.7			18				2.0		
AH11110A	-6.48 ± 0.03	5	-5.65 ± 0.09	5	-4.98 ± 0.06	5	6.8			32				4.7		
Yohimbine	-6.23 ± 0.03	5	-5.44 ± 0.05	5	-6.20 ± 0.08	8	6.2			1.1						5.8
	-5.67 ± 0.07	5	-4.88 ± 0.03	5	-5.23 ± 0.11	5	6.2			2.8						2.2
Idazoxan	-8.73 ± 0.06	5	-7.96 ± 0.07	5	-7.31 ± 0.15 ^{early plateau}	9	5.9			26				4.5		
	-8.45 ± 0.12	12	-7.69 ± 0.06	13	-8.43 ± 0.19	10	5.8			1.0						5.5
Bromocriptine					-5.42 ± 0.08											
					39.1 ± 2.0% site 1											
Phenoxybenzamine	-6.02 ± 0.08															
	77.7 ± 5.2% site 1		67.5 ± 2.5% site 1													

(Continues)

TABLE 1 (Continued)

Ligand	Log K_D $\alpha1A$	n	Log K_D $\alpha1B$	n	Log K_D $\alpha1D$	n	$\alpha1A$	vs	$\alpha1B$	$\alpha1A$	vs	$\alpha1D$	vs	$\alpha1B$	$\alpha1D$	vs	$\alpha1D$
RS79948	-5.75 ± 0.05	5	-4.99 ± 0.05	5	-6.07 ± 0.11	6	5.8					2.1					12
JP1302	-6.21 ± 0.04	5	-5.46 ± 0.02	5	-5.58 ± 0.09	5	5.6			4.3							1.3
Imiloxan	-4.60 ± 0.05	5	$IC_{50} > -4$	5	-5.02 ± 0.07	5	>4.0					2.6					>10
HEAT	-8.57 ± 0.06	5	-8.04 ± 0.04	5	-8.11 ± 0.18	8	3.4			2.9					1.2		
					-5.15 ± 0.26												
					64.1 ± 4.1% site 1												
Carvedilol	-8.35 ± 0.06	12	-7.84 ± 0.06	6	-7.87 ± 0.12	7	3.2			3.0							1.1
A80426	-6.57 ± 0.05	5	-6.08 ± 0.02	5	-6.07 ± 0.07	5	3.1			3.1							1.0
Rec15-2615	-8.26 ± 0.10	6	-7.79 ± 0.09	6	-7.89 ± 0.07	7	3.0			2.3							1.3
Spiroxatrine	-6.86 ± 0.06	5	-6.41 ± 0.06	5	-7.86 ± 0.13	6	2.8					10					28
					-5.84 ± 0.31												
					61.9 ± 4.0% site 1												
BMY7378	-6.61 ± 0.05	5	-6.23 ± 0.05	6	-8.60 ± 0.13	9	2.4					98					234
					-5.93 ± 0.37												
					57.7 ± 2.6% site 1												
Prazosin	-9.07 ± 0.04	9	-8.74 ± 0.06	8	-9.07 ± 0.23	10	2.1					1.0					2.1
Alfuzosin	-7.82 ± 0.11	8	-7.56 ± 0.08	6	-7.66 ± 0.11	6	1.8			1.4							1.3
Cyclazosin	-8.89 ± 0.06	7	-8.68 ± 0.08	5	-9.87 ± 0.06	7	1.6					9.5					15
					-7.44 ± 0.10												
					56.8 ± 3.4% site 1												
Doxazosin	-8.58 ± 0.09	6	-8.46 ± 0.05	8	-8.33 ± 0.13	11	1.3			1.8				1.3			
Terazosin	-7.93 ± 0.05	6	-7.95 ± 0.05	6	-7.71 ± 0.13	7			1.7					1.7			
β -blockers																	
Carazolol	-6.57 ± 0.03	5	-4.68 ± 0.05	6	-5.04 ± 0.08 ^{app}	5	78			34							2.3
SDZ21009	-5.24 ± 0.07	6	$Log IC_{50} > -4$	6	-5.09 ± 0.18	6	>17			1.4							>12
CGP12177	-5.14 ± 0.05	6	$Log IC_{50} > -4$	5	-4.20 ± 0.11	5	>14			8.7							>1.6
Bucindolol	-7.57 ± 0.07	5	-6.46 ± 0.04	5	-6.45 ± 0.09	5	13			13							1.0
IC118551	-5.23 ± 0.03	5	-4.20 ± 0.06 ^{app}	5	-4.96 ± 0.03	5	11			1.9							5.8
CGP20712A	-4.93 ± 0.10	5	$Log IC_{50} > -4$	5	-4.96 ± 0.07 ^{app}	5	>8.5					1.1					>9.1
Propranolol	-4.91 ± 0.02	6	-3.98 ± 0.04 ^{app}	6	-4.89 ± 0.09	5	8.5					1.0					8.1
Cyanopindolol	-5.59 ± 0.05	8	-4.91 ± 0.09	7	-5.40 ± 0.07	5	4.8			1.5							3.1

^{app}The maximum concentration of competing ligand inhibited most but not all of specific binding (as in Figure 1E). 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 $app K_D$ was calculated.

^{early plateau}Bromocriptine did not fully inhibit specific binding at the $\alpha1D$ -adrenoceptor. The inhibition curve reached a plateau of maximal inhibition of binding at $71.0\% \pm 3.4\%$ inhibition of specific binding ($n = 9$).

app, apparent.

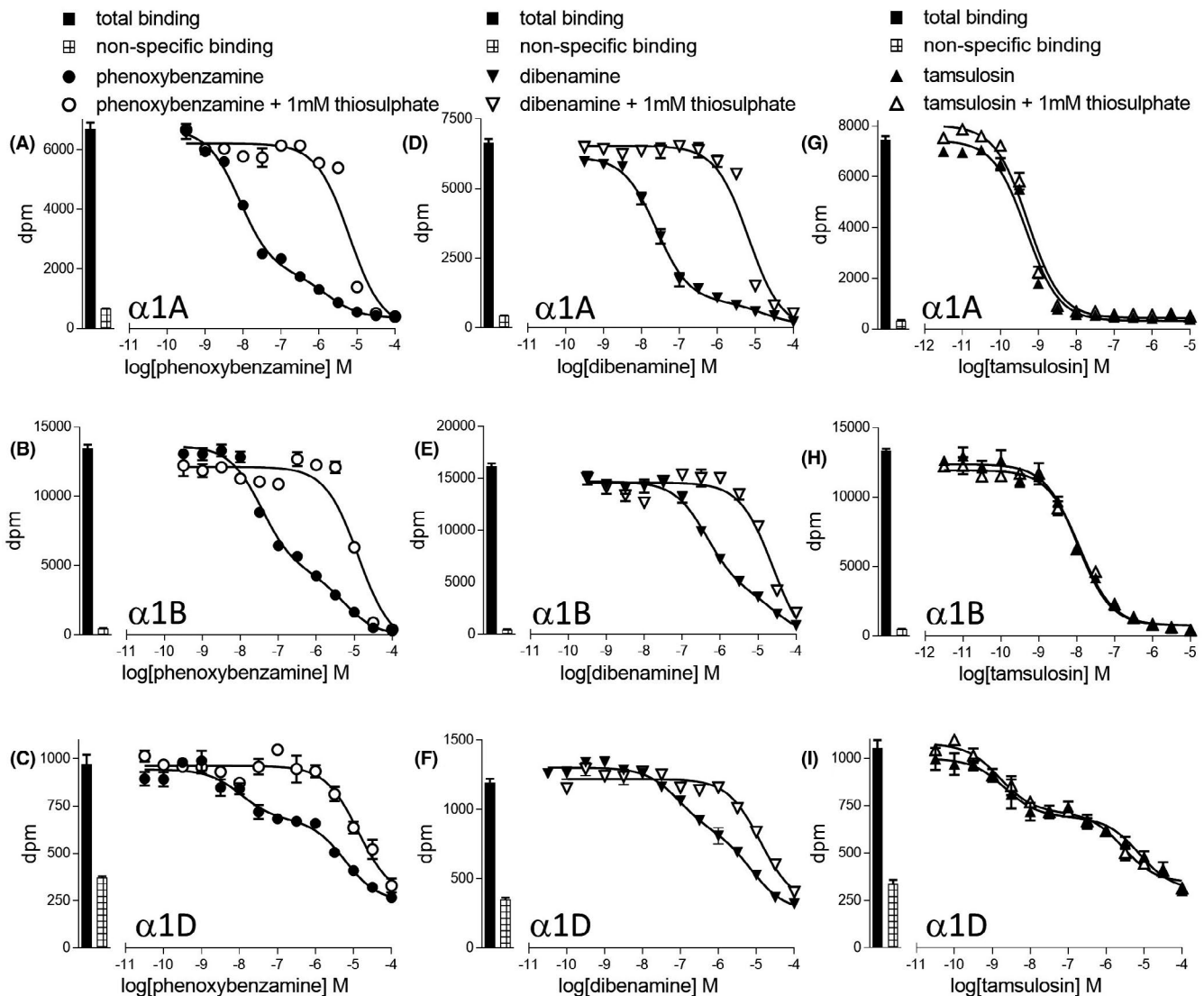


FIGURE 2 Inhibition of $[^3\text{H}]$ prazosin binding to whole cells by phenoxybenzamine (A–C), dibenamine (D–F) or tamsulosin (G–I) to CHO- α 1A cells (A, D, G), CHO- α 1B cells (B, E, H), or CHO- α 1D cells (C, F, I). Bars represent total $[^3\text{H}]$ prazosin binding and nonspecific binding was determined in the presence of 10 $\mu\text{mol/L}$ tamsulosin (CHO- α 1A and CHO- α 1B) or 100 $\mu\text{mol/L}$ tamsulosin (CHO- α 1D). The concentration of $[^3\text{H}]$ prazosin was (A) 0.48 nmol/L, (B) 0.48 nmol/L, (C) 0.86 nmol/L, (D) 0.58 nmol/L, (E) 0.56 nmol/L, (F) 1.49 nmol/L, (G) 0.56 nmol/L, (H) 0.58 nmol/L, and (I) 1.49 nmol/L. Data points are mean \pm SE mean of triplicate determinations

These values are all within twofold of the value obtained from saturation studies. The values from saturation studies were used for further K_D calculations. A lower receptor expression level for the full-length α 1D-adrenoceptors is a common finding^{15,33} and reports suggest truncation of the N-terminus results in higher receptor expressions.^{33–35}

Doxazosin, a commonly used α blocker in the treatment of hypertension, inhibited all three receptors with high affinity (log K_D –8.58, –8.46, and –8.33 at the α 1A, α 1B, and α 1D-adrenoceptor, respectively, Figure 1, Table 1). Of all the compounds studied, SNAP 5089 had the highest receptor selectivity, being over 1700-fold selective for the α 1A-adrenoceptor (Figure 1, Table 1). No compound was found to have α 1B-adrenoceptor selectivity. The ability of BMY7378 to inhibit $[^3\text{H}]$ prazosin binding was best described by a two-component curve with the high-affinity component (log K_D

–8.60 at the α 1D-adrenoceptor) giving it 98- and 234-fold selectivity for the α 1D-adrenoceptor over the α 1A and α 1B-adrenoceptors, respectively (Figure 1, Table 1). Several compounds had affinities of less than 0.25nM, including ligands with α 1A selectivity (silodosin, RS100329, and tamsulosin), cyclazosin with slight α 1D selectivity, and nonselective 3-MPPI (Table 1).

Two compounds were best described by a two-component-binding inhibition curve at all three receptors—phenoxybenzamine and dibenamine (Figure 2, Table 1). Both of these are *N,N*-disubstituted-2-chloroethylamines. Preincubation of phenoxybenzamine and dibenamine with sodium thiosulfate before addition to the cells yielded a single-component-binding inhibition (Figure 2, Table 2), whereby the high-affinity-binding component of the parent curve had been abolished. Sodium thiosulfate had no effect on the binding of tamsulosin (Figure 2, Table 2). At the α 1D-adrenoceptor, several

TABLE 2 Log K_D values of phenoxybenzamine, dibenamine and tamsulosin binding to the human $\alpha1A$, $\alpha1B$ and $\alpha1D$ -adrenoceptors obtained in the absence and presence of 1 mmol/L sodium thiosulphate (Figure 2). Values represent mean \pm SE mean of n separate experiments

	Control				+1 mmol/L sodium thiosulphate			
	Log K_D site 1	Log K_D site 2	% site 1	n	Log K_D site 1	Log K_D site 2	% site 1	n
CHO-$\alpha1A$								
Phenoxybenzamine	-8.45 \pm 0.12	-6.02 \pm 0.08	77.7 \pm 5.2	12	-5.43 \pm 0.07			7
Dibenamine	-7.91 \pm 0.06	-5.32 \pm 0.08	83.0 \pm 1.8	15	-5.16 \pm 0.10			7
Tamsulosin	-9.67 \pm 0.06			17	-9.75 \pm 0.16			7
CHO-$\alpha1B$								
Phenoxybenzamine	-7.69 \pm 0.06	-5.57 \pm 0.06	67.5 \pm 2.5	13	-5.18 \pm 0.05			6
Dibenamine	-6.57 \pm 0.07	-4.66 \pm 0.06	67.6 \pm 2.6	14	-4.85 \pm 0.05			6
Tamsulosin	-8.12 \pm 0.04			15	-8.13 \pm 0.08			6
CHO-$\alpha1D$								
Phenoxybenzamine	-8.43 \pm 0.19	-5.42 \pm 0.08	39.1 \pm 2.0	10	-4.93 \pm 0.10			5
Dibenamine	-7.37 \pm 0.15	-5.00 \pm 0.14	47.8 \pm 3.2	9	-4.74 \pm 0.09			5
Tamsulosin	-9.18 \pm 0.08	-5.67 \pm 0.15	54.6 \pm 3.7	13	-9.11 \pm 0.12	-5.60 \pm 0.08	44.0 \pm 2.8	7

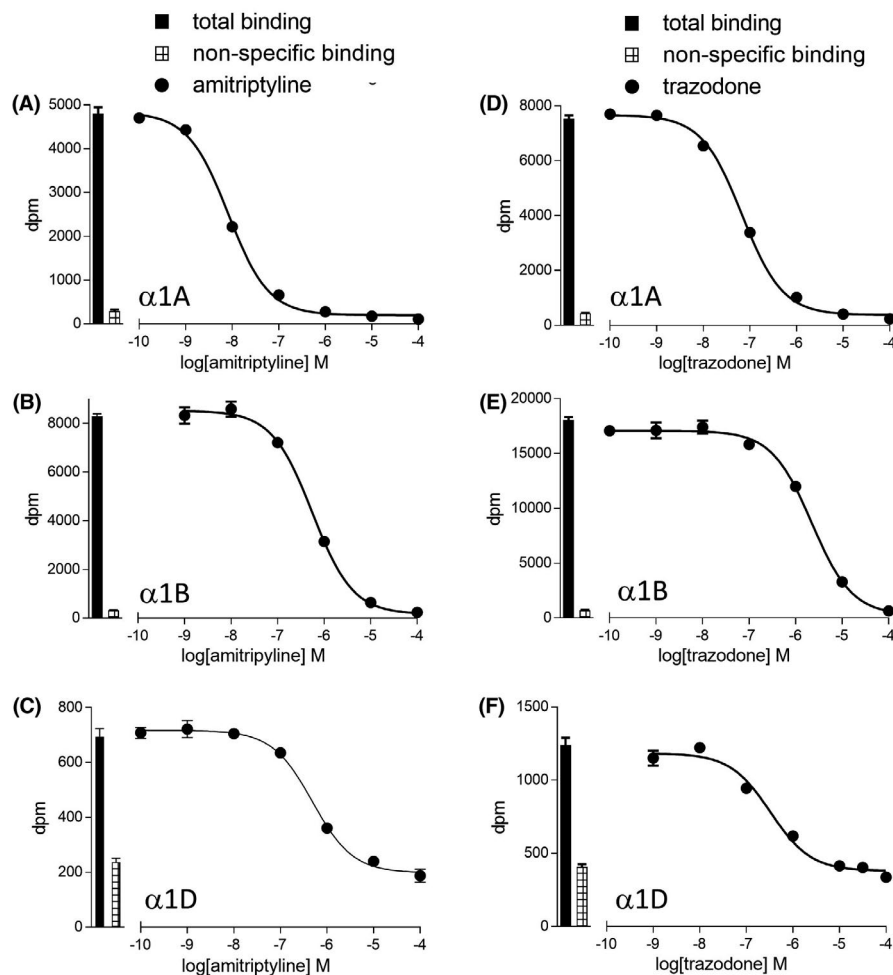


FIGURE 3 Inhibition of $[3H]$ prazosin binding to whole cells by two commonly prescribed antidepressants amitriptyline (A–C) or trazodone (D–F) to CHO- $\alpha1A$ cells (A, D), CHO- $\alpha1B$ cells (B, E), or CHO- $\alpha1D$ cells (C, F). Bars represent total $[3H]$ prazosin binding and nonspecific binding was determined in the presence of 10 μ mol/L tamsulosin (CHO- $\alpha1A$ and CHO- $\alpha1B$) or 100 μ mol/L tamsulosin (CHO- $\alpha1D$). The concentration of $[3H]$ prazosin was a) 0.39 nmol/L, (B) 0.45 nmol/L, (C) 0.57 nmol/L, (D) 0.66 nmol/L, (E) 0.45 nmol/L, and (F) 0.66 nmol/L. Data points are mean \pm SE mean of triplicate determinations

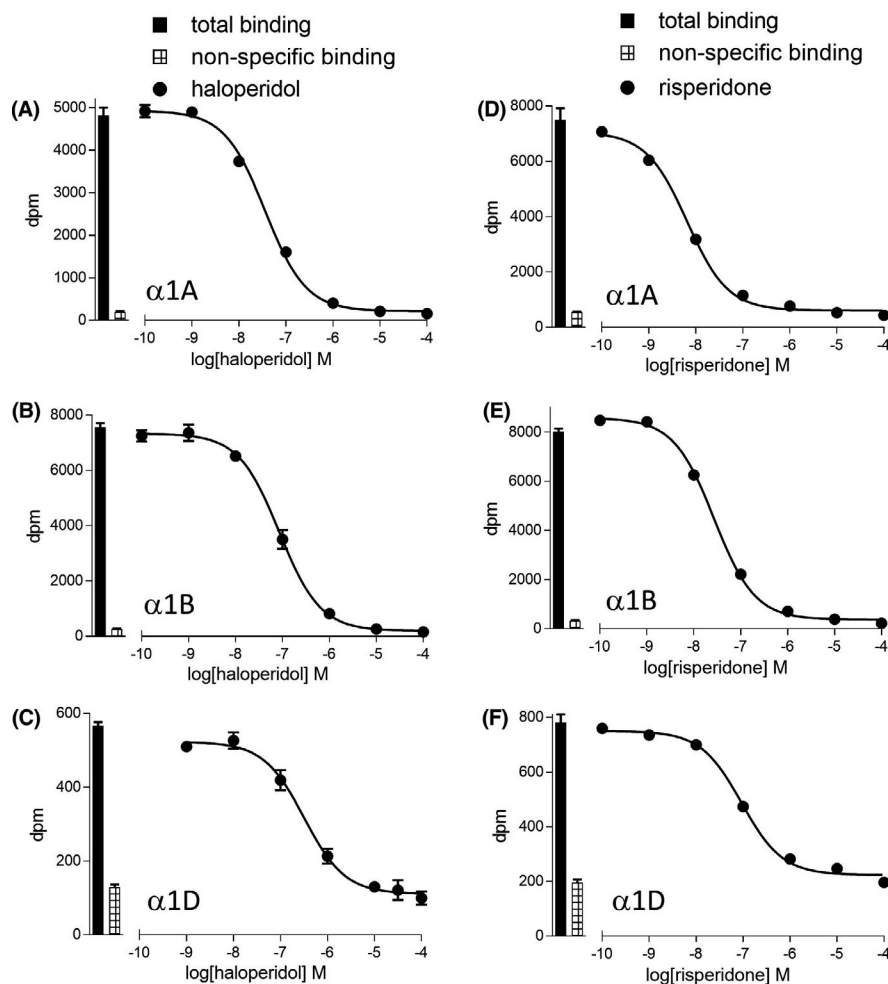


FIGURE 4 Inhibition of [3H]prazosin binding to whole cells by two commonly prescribed antipsychotics haloperidol (A–C) or risperidone (D–F) to CHO- α 1A cells (A, D), CHO- α 1B cells (B, E) or CHO- α 1D cells (C, F). Bars represent total [3H]prazosin binding and nonspecific binding was determined in the presence of 10 μ mol/L tamsulosin (CHO- α 1A and CHO- α 1B) or 100 μ mol/L tamsulosin (CHO- α 1D). The concentration of [3H]prazosin was (A) 0.39 nmol/L, (B) 0.39 nmol/L, (C) 0.53 nmol/L, (D) 0.82 nmol/L, (E) 0.45 nmol/L, and (F) 0.66 nmol/L. Data points are mean \pm SE mean of triplicate determinations

other ligands were best described by a two-component-binding inhibition curve. Just as with tamsulosin (Figure 2), preincubation with sodium thiosulfate had no effect on either component of any of these other two-component ligands.

The affinity of several antidepressants and antipsychotics was then examined. Several of these were found to have high α 1-adrenoceptor affinity (Figures 3 and 4, Table 3 and 4). Risperidone (previously suggested to have α 1B selectivity,^{4,36} had slight α 1A selectivity, in keeping with the findings of³⁷. There have also been discrepancies in the affinity of olanzapine: Richelson and Souder²⁷ found it to have high affinity (44nM for α 1-adrenoceptor) and Nourain et al,²⁹ had conflicting data with low rat α 1-adrenoceptor affinity, but significant hypotension in rats. However, here, olanzapine had low affinity, in keeping with³⁸ and the findings of³⁹ where olanzapine was described as having low postural hypotension potential. WB4104, was also initially thought to have α 1B selectivity,⁴⁰ however, it had higher and equal affinity for α 1A and α 1D-adrenoceptors (in keeping with^{41,42}).

Tables combining all of these ligands are presented in Supplementary Data. Table S1 has the ligands arranged in alphabetical order (together with their suppliers and individual ligand codes). Table S2 has the ligands organized in order of α 1A affinity.

4 | DISCUSSION

Dibenamine, phentolamine, and phenoxybenzamine were the first clinical α blockers⁴³ and phenoxybenzamine is still used in the management of pheochromocytoma, particularly during surgery where catastrophic catecholamine release can cause hypertensive crises and arrhythmias.⁷ Both phenoxybenzamine and dibenamine are *N,N*-disubstituted-2-chloroethylamines containing a nitrogen mustard group. Both compounds were best described by a two-component-binding inhibition curve at all three α 1-adrenoceptors (Figure 2, Table 1). In aqueous solution at physiological pH, the nitrogen mustard group cyclizes to form ethyleniminium ions.⁴⁴ These highly reactive, unstable ions are pharmacologically active and covalently bind to a cysteine in transmembrane 3 of the α adrenoceptors, giving these compounds their “irreversible” properties.⁴³ Phenoxybenzamine has a longer duration of action in clinical studies than phentolamine⁷ and hence its continued use in pheochromocytoma (although similar outcomes have been reported with doxazosin, terazosin, and prazosin,^{45–47} Sodium thiosulfate also rapidly reacts with the ethyleniminium ions thus prevents them from interacting with α adrenoceptors.⁴⁴ Pretreatment with intravenous sodium thiosulfate prevented dibenamine binding to α adrenoceptors (in cats,⁴⁸ and pretreatment with sodium thiosulfate prevented the

TABLE 3 Log K_D values and selectivity ratios of antidepressants binding to the human $\alpha1A$, $\alpha1B$ and $\alpha1D$ -adrenoceptors. Values represent mean \pm SE 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, amitriptyline has 93 fold higher affinity for the $\alpha1A$ -adrenoceptor than the $\alpha1B$ -adrenoceptor. Compounds are arranged in order of $\alpha1A$ -selectivity

Ligand	Log K_D $\alpha1A$	n	Log K_D $\alpha1B$	n	Log K_D $\alpha1D$	n	$\alpha1A$	vs	$\alpha1B$	$\alpha1A$	vs	$\alpha1D$	$\alpha1B$	vs	$\alpha1D$
Noradrenaline and serotonin reuptake inhibitors															
Tricyclic antidepressants															
Amitriptyline	-8.19 \pm 0.02	9	-6.22 \pm 0.05	9	-6.25 \pm 0.05	5	93		$\alpha1B$	87			$\alpha1B$		1.1
Dosulepin	-7.11 \pm 0.04	5	-5.28 \pm 0.11	7	-5.58 \pm 0.05	5	68		$\alpha1A$	34			$\alpha1B$		2.0
Clomipramine	-8.12 \pm 0.10	9	-6.34 \pm 0.07	9	-6.15 \pm 0.09	5	60		$\alpha1A$	93			$\alpha1B$	1.5	
Imipramine	-7.47 \pm 0.04	6	-5.76 \pm 0.05	6	-5.89 \pm 0.05	5	51		$\alpha1A$	38			$\alpha1B$		1.3
Norclomipramine	-7.52 \pm 0.08	11	-5.84 \pm 0.04	12	-5.84 \pm 0.06	5	48		$\alpha1A$	48			$\alpha1B$	1.0	
Nortriptyline	-7.74 \pm 0.03	6	-6.07 \pm 0.07	5	-5.81 \pm 0.05	5	47		$\alpha1A$	85			$\alpha1B$	1.8	
Doxepin	-7.74 \pm 0.04	5	-6.18 \pm 0.03	5	-6.27 \pm 0.11	6	36		$\alpha1A$	30			$\alpha1B$		1.2
Desipramine	-7.07 \pm 0.05	6	-5.57 \pm 0.05	5	-5.46 \pm 0.07	5	32		$\alpha1A$	41			$\alpha1B$	1.3	
Lofepramine	-6.94 \pm 0.06	6	-5.44 \pm 0.07	6	-5.37 \pm 0.04	5	32		$\alpha1A$	37			$\alpha1B$	1.2	
Trimipramine	-7.37 \pm 0.08	5	-6.10 \pm 0.06	5	-5.99 \pm 0.05	5	19		$\alpha1A$	24			$\alpha1B$	1.3	
Protriptyline	-6.67 \pm 0.03	5	-5.57 \pm 0.02	5	-5.46 \pm 0.12	5	13		$\alpha1A$	16			$\alpha1B$	1.3	
Tetracyclic antidepressants															
Mirtazepine	-6.36 \pm 0.02	5	-5.36 \pm 0.03	5	-5.94 \pm 0.05	5	10		$\alpha1A$	2.6			$\alpha1B$		3.8
Other noradrenaline and serotonin reuptake inhibitors															
Duloxetine	-5.65 \pm 0.05	5	-4.71 \pm 0.03 ^{app}	5	-5.58 \pm 0.12	7	8.7		$\alpha1A$	1.2			$\alpha1B$		7.4
Venlafaxine	-3.69 \pm 0.02 ^{app}	5	No binding to 1 mmol/L	5	-4.38 \pm 0.16	7	>4.9		$\alpha1A$	>4.9			$\alpha1B$	4.9	>24
Noradrenaline reuptake inhibitors															
Reboxetine	-4.91 \pm 0.08	5	Log IC_{50} > -4	5	-4.67 \pm 0.12	5	>8.1		$\alpha1A$	1.7			$\alpha1B$		>4.7
Selective serotonin reuptake inhibitors (SSRI)															
Fluvoxamine	-6.10 \pm 0.03	5	Log IC_{50} > -4	5	-4.97 \pm 0.03	5	>126		$\alpha1A$	14			$\alpha1B$		>9.3
Citalopram	-5.95 \pm 0.06	4	Log IC_{50} > -4	4	-4.91 \pm 0.11	5	>89		$\alpha1A$	11			$\alpha1B$		>8.1
Fluoxetine	-5.45 \pm 0.04	5	-4.41 \pm 0.06	5	-4.90 \pm 0.13	5	11		$\alpha1A$	3.5			$\alpha1B$		3.1
Paroxetine	-5.59 \pm 0.09	5	Log IC_{50} > -5	5	-5.63 \pm 0.13	5	>3.9		$\alpha1A$			1.1	$\alpha1B$		>4.3
Sertraline	-5.72 \pm 0.04	5	-5.45 \pm 0.05	5	-5.61 \pm 0.04	5	1.9		$\alpha1A$	1.3			$\alpha1B$		1.4
Serotonin reuptake inhibitors															
Vortioxetine	-6.32 \pm 0.05	5	-5.42 \pm 0.02	5	-5.43 \pm 0.08	5	7.9		$\alpha1A$	7.8			$\alpha1B$		1.0
Trazodone	-7.33 \pm 0.04	6	-6.56 \pm 0.07	6	-6.38 \pm 0.15	7	5.9		$\alpha1A$	8.9			$\alpha1B$	1.5	
Metalonin agonist															
Agomelatine	-4.57 \pm 0.11 ^{app}	5	No binding to 100 μ mol/L	5	IC_{50} > -4.5	6	>3.7		$\alpha1A$	1.1			$\alpha1B$		>3.2

^{app}The maximum concentration of competing ligand inhibited most but not all of specific binding (as in Figure 1E). 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 app K_D was calculated.

app, apparent.

TABLE 4 Log K_D values and selectivity ratios of antipsychotics binding to the human $\alpha1A$, $\alpha1B$ and $\alpha1D$ -adrenoceptors. Values represent mean \pm SE 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, chlorpromazine has 13 fold high affinity for the $\alpha1A$ -adrenoceptor than the $\alpha1B$ -adrenoceptor. Compounds are arranged in order of $\alpha1A$ -selectivity

Ligand	Log K_D $\alpha1A$	n	Log K_D $\alpha1B$	n	Log K_D $\alpha1D$	n	$\alpha1A$ vs $\alpha1B$	$\alpha1A$ vs $\alpha1D$	$\alpha1B$ vs $\alpha1D$	$\alpha1A$ vs $\alpha1B$	$\alpha1A$ vs $\alpha1D$	$\alpha1B$ vs $\alpha1D$
First generation antipsychotics												
Sulpiride	-4.50 \pm 0.07	5	IC ₅₀ > 3	5	-3.66 \pm 0.09 ^{app}	5	>32	6.9				>4.6
Chlorpromazine	-8.94 \pm 0.06	5	-7.84 \pm 0.05	5	-8.00 \pm 0.08 -5.91 \pm 0.20 56.0 \pm 5.0% site 1	6	13	8.7				1.4
Flupenthixol	-8.35 \pm 0.05	5	-7.47 \pm 0.07	5	-6.96 \pm 0.12	7	7.6		25			3.2
Trifluoperazine	-7.75 \pm 0.03	5	-6.88 \pm 0.07	5	-6.36 \pm 0.08	5	7.4		25			3.3
Prochlorperazine	-7.61 \pm 0.12	4	-6.88 \pm 0.06	5	-6.53 \pm 0.13	8	5.4		12			2.2
Perphenazine	-8.15 \pm 0.09	5	-7.43 \pm 0.08	5	-7.86 \pm 0.10 -6.03 \pm 0.19 53.3 \pm 4.1% site 1	5	5.2		1.9			2.7
Pimozide	-7.44 \pm 0.16	5	-6.79 \pm 0.05	5	-5.95 \pm 0.08	6	4.5		31			6.9
Haloperidol	-7.70 \pm 0.03	5	-7.21 \pm 0.07	6	-6.42 \pm 0.06	5	3.1		19			6.2
Second generation antipsychotics												
Amisulpiride	-5.05 \pm 0.04	5	No binding to 100 μ mol/L	5	-4.55 \pm 0.08 ^{app}	5	11		3.2			3.5
Ziprasidone	-8.73 \pm 0.05	7	-7.70 \pm 0.07	8	-7.20 \pm 0.09	5	11		34			3.2
Paliperidone	-8.36 \pm 0.09	5	-7.36 \pm 0.08	5	-7.47 \pm 0.10 -5.57 \pm 0.21 57.6 \pm 4.9% site 1	6	10		7.8			1.3
Sertindole	-9.27 \pm 0.09	9	-8.28 \pm 0.11	8	-6.93 \pm 0.12	8	9.8		219			22
Risperidone	-8.74 \pm 0.06	7	-7.77 \pm 0.05	7	-7.14 \pm 0.07	6	9.3		40			4.3
Clozapine	-8.27 \pm 0.04	5	-7.39 \pm 0.07	5	-6.41 \pm 0.05	5	7.6		72			9.5
Quetiapine	-7.89 \pm 0.10	5	-7.21 \pm 0.04	5	-6.48 \pm 0.10	5	4.8		26			5.4
Lurasidone	-7.80 \pm 0.11	5	-7.17 \pm 0.09	5	-8.19 \pm 0.10 -5.92 \pm 0.06 24.8 \pm 3.1% site 1	7	4.3		2.5			10
Aripiprazole	-7.32 \pm 0.07	6	-6.69 \pm 0.03	6	-6.15 \pm 0.11	5	4.3		15			3.5
Olanzapine	-6.61 \pm 0.11	7	-6.00 \pm 0.10	10	-5.86 \pm 0.06	5	4.1		5.6			1.4

^{app}The maximum concentration of competing ligand inhibited most but not all of specific binding (as in Figure 1E). 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 app K_D was calculated.

app, apparent.

harmful interactions of the chemical weapon mustard gas in humans. Here, preincubation of phenoxybenzamine or dibenamine with sodium thiosulfate yielded single-component-binding inhibition curves (Figure 2, Table 2). Abolishment of the high-affinity-binding component suggests that it was due to specific α 1-adrenoceptor interaction. The K_D values of the low-affinity components were very similar to those obtained in the presence of thiosulfate, suggesting that this component is a non-orthosteric site or non-receptor-mediated effect.

Several other ligands were found to have a [3H]prazosin inhibition best described a two-component curve at the α 1D-adrenoceptor, including tamsulosin (and hence why 100 μ M was used to define nonspecific binding in CHO- α 1D cells, rather than 10 μ M used in α 1A and α 1B cells), and the only α 1D-selective ligand, BMY7378. As expected for these nonmustard compounds, preincubation with sodium thiosulfate had no effect on binding. The reason for the second component is therefore unknown. Affinity (K_D value) obtained for the high-affinity component the α 1D-adrenoceptor has been used to determine receptor selectivity.

α 1-adrenoceptor antagonists (α blockers, especially doxazosin) have been used for hypertension for decades. Doxazosin had high affinity for all three subtypes, similar to previous [3H]prazosin-binding studies.⁴¹ Terazosin and prazosin were also nonselective ligands (as in⁴¹) as was phentolamine. Indoramin, (licensed for hypertension), had an α 1A selectivity of 40-fold (similar to⁵⁰). Of the α blockers that are used for the treatment of BPH in the UK, alfuzosin was nonselective, whereas tamsulosin with its α 1A vs α 1B selectivity of 35-fold, was equipotent at α 1A and α 1D receptors (as in 15,16,51). Thus, drugs used for hypertension and BPH include nonselective α 1 blockers and those with up to 40-fold α 1A selectivity. It would therefore be expected that drugs like tamsulosin and alfuzosin, licensed for BPH, are likely to have as much of an effect on blood pressure as α blockers intentionally prescribed for hypertension. Several other high-affinity non/poorly selective ligands were also identified that have higher affinity than doxazosin, for example, cyclazosin, 3-MPPI, and ARC239.

Carvedilol (commonly used in heart failure) is considered a dual α/β blocker. Carvedilol was nonselective, with high affinity at all three α 1-adrenoceptors, however the α 1A affinity (log K_D of -8.35) was still 10-fold less than that for the β 2 adrenoceptor.⁵² Labetolol (used in hypertension particularly in pregnancy, and intravenously in hypertensive emergencies), is also considered a dual α/β blocker. Labetolol has lower affinity than carvedilol for all β ⁵² and α 1-adrenoceptors (log K_D -7.33 at α 1A), but very poor affinity for the α 1B and α 1D-adrenoceptors. Labetolol should be considered a β 1/ β 2/ α 1A blocker rather than dual pan α 1 and β blocker. Given these dual α/β ligands, the affinity of a few β blockers with very high β -adrenoceptor affinity were examined (Table 1). With the exception of bucindolol, the affinity was poor at all three α 1-adrenoceptors, confirming their β selectivity. Although the affinity of bucindolol was reasonably high (log K_D at α 1A -7.57), this is 54-fold and 263-fold lower than that for the human β 1 and β 2 adrenoceptor, respectively.⁵³

The most selective ligand detected here was SNAP5089, with 1700-fold selectivity for the α 1A over the α 1B or α 1D-adrenoceptors. Other α 1A-selective ligands were silodosin, RS100329, and niguldipine (in keeping with^{50,54}). As well as tamsulosin, several ligands had higher affinity for the α 1A and α 1D receptors than the α 1B—for example, 2-MPMDQ, MK-912, 2-PMDQ, and ifenprodil. BMY7378 was the only compound with substantial α 1D selectivity (the 100- to 200-fold selectivity is similar to^{6,20,41,50,55}). No α 1B-selective ligand was identified. To pharmacologically infer the presence of α 1B-adrenoceptors in cells or tissues, several different compounds with different patterns of selectivity would be required, for example, SNAP5089, doxazosin, 2-MPMDQ, and BMY7378.

Several tricyclic antidepressants (TCA) had significant affinity for the α 1-adrenoceptors. Amitriptyline, clomipramine, doxepin, and nortriptyline have similar α 1-adrenoceptor affinities and selectivities to α blockers prescribed for hypertension or BPH. Thus patients taking these TCAs should be considered to be α blocked and are at risk from postural hypotension (as in²³). Furthermore, the addition of an α blocker for concomitant hypertension or BPH may not have any additional clinical benefit and may actually cause significant postural problems. Other TCA had lower affinity, for example, protriptyline and lofepramine and would therefore be expected to have less effect on blood pressure. The selective serotonin reuptake inhibitors (SSRIs) had very poor affinity for any of the α adrenoceptors and are therefore less likely to have significant α 1-mediated hypotension.

Several antipsychotics (including first-generation chlorpromazine and flupenthixol and second-generation sertindole, risperidone, and clozapine) had high α 1-adrenoceptor affinity. The very high affinity of sertindole (and 300-fold selectivity for α 1A over α 1D-adrenoceptors) was similar to previous reports.^{6,29} The degree of α 1A-adrenoceptor affinity observed here correlates well with the rankings for observations in rats.²⁹ The high α 1A affinity of sertindole, risperidone, and ziprasidone (log K_D -9.3 to -8.7) is similar to studies,³⁸ including in brain tissue,²⁷ and similar to (or even higher than) that for many drugs used to treat hypertension. The high rate of postural hypotension observed with these drugs^{31,32} is therefore not surprising. A similar hypotensive effect would be expected with other antipsychotics α 1 affinities equal or greater than that for α 1 blockers used for hypertension, for example, chlorpromazine, flupenthixol, perphenazine, paliperidone, quetiapine, and lurasidone. Aripiprazole had lower α 1 affinity (in keeping with⁵⁶ and indeed has a relative lack of reported postural hypotension in clinical studies⁵⁷). However, sulpiride and amisulpiride would be expected to have even less hypotensive effect.

Thus, the high α 1 affinity and selectivity profile of many antipsychotics is comparable to the α 1 blockers intentionally prescribed for hypertension. Equivalent reductions in blood pressure are a likely very common side effect. These drugs are used to manage schizophrenia where their effect on blood pressure in agitated patients is less likely to be an issue. However, antipsychotics are also widely used to manage delirium in sick patients including the intensive care

unit^{58,59} and in palliative case^{60,61} even though recent studies have questioned their effectiveness.⁶² Delirium is common in older unwell patients who are more likely to be suffering from conditions with lower blood pressure such as sepsis. In these cases, the choice of antipsychotic may well be important in order not to exacerbate already low or labile blood pressures. This study suggests that sulpiride, amisulpiride, aripiprazole, and olanzapine should have the least effect on blood pressure.

In conclusion, there are several highly α 1A-selective antagonists (eg, SNAP5089), and one α 1D-selective antagonist (BMY7378), however no α 1B-selective ligand has been identified. The drugs used for hypertension and BPH have a very similar pharmacological profile in terms of α 1-adrenoceptor subtype affinity and selectivity. Several antidepressants and antipsychotics have high α 1-adrenoceptor affinities, similar to, or even greater than, those seen for α blockers prescribed for hypertension and BPH. The addition of further α blockers for the management of hypertension or BPH in these patients may not be beneficial. The excellent correlation between the affinity values determined from this cell studies with the affinities measured in blood vessels, brain tissue, and whole animals (including humans) means that many, but not all antipsychotics and antidepressant may cause significant peripheral α -adrenoceptor blockade and associated hypotension. Finally, awareness of the α -blocking potential of certain, but not all antipsychotics may affect the choice of drug used for the management of delirium in the intensive care unit where additional α blockade and blood pressure lowering in a sick patient may be detrimental.

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DISCLOSURE

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

AUTHORS CONTRIBUTIONS

JGB designed the research study. ASP contributed the α 1D DNA and aided discussions. RGWP and JGB performed the research. JGB analyzed the data. JGB and ASP wrote the paper.

ETHICAL STATEMENT

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

DATA SHARING

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

OPEN RESEARCH BADGES



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REFERENCES

- Bylund DB. Subtypes of alpha 1- and alpha 2-adrenergic receptors. *FASEB J*. 1992;6:832-839.
- Docherty JR. Subtypes of functional alpha1-adrenoceptor. *Cell Mol Life Sci*. 2010;67:405-417.
- Piasecki MT, Perez DM. Alpha1-adrenergic receptors: new insights and directions. *J Pharmacol Exp Ther*. 2001;298:403-410.
- Ford AP, Williams TJ, Blue DR, Clarke DE. Alpha 1-adrenoceptor classification: sharpening Occam's razor. *Trends Pharmacol Sci*. 1994;15:167-170.
- Akinaga J, García-Sáinz JA, Pupo SA. Updates in the function and regulation of α 1 -adrenoceptors. *Br J Pharmacol*. 2019;176:2343-2357.
- Ipsen M, Zhang Y, Dragsted N, Han C, Mulvany MJ. The antipsychotic drug sertindole is a specific inhibitor of alpha1A-adrenoceptors in rat mesenteric small arteries. *Eur J Pharmacol*. 1997;336:29-35.
- Ross EJ, Prichard BN, Kaufman L, Robertson AI, Harries BJ. Preoperative and operative management of patients with pheochromocytoma. *Br Med J*. 1967;1:91-98.
- Spear HC, Griswold D. The use of dibenamine in pheochromocytoma; report of a case. *N Engl J Med*. 1948;239:736-739.
- Hollingsworth JM, Wilt TJ. Lower urinary tract symptoms in men. *BMJ*. 2014;349:g4474.
- Caine M, Perlberg S, Meretyk S. A placebo-controlled double-blind study of the effect of phenoxybenzamine in benign prostatic obstruction. *Br J Urol*. 1978;50:551-554.
- Lowe FC. Role of the newer alpha, -adrenergic-receptor antagonists in the treatment of benign prostatic hyperplasia-related lower urinary tract symptoms. *Clin Ther*. 2004;26:1701-1713.
- Bird ST, Delaney JA, Brophy JM, Etmann M, Skeldon SC, Hartzema AG. Tamsulosin treatment for benign prostatic hyperplasia and risk of severe hypotension in men aged 40–85 years in the United States: risk window analyses using between and within patient methodology. *BMJ*. 2013;347:f6320.
- Welk B, McArthur E, Fraser LA, et al. The risk of fall and fracture with the initiation of a prostate-selective α antagonist: a population based cohort study. *BMJ*. 2015;351:h5398.
- Michel MC. The forefront for novel therapeutic agents based on the pathophysiology of lower urinary tract dysfunction: alpha blockers in the treatment of male voiding dysfunction - how do they work and why do they differ in tolerability? *J Pharmacol Sci*. 2010;112:151-157.
- Kenny BA, Miller AM, Williamson IJ, O'Connell J, Chalmers DH, Naylor AM. Evaluation of the pharmacological selectivity profile of alpha 1 adrenoceptor antagonists, at prostatic alpha 1 adrenoceptors: binding, functional and in vivo studies. *Br J Pharmacol*. 1996;118:871-878.
- Quaresma BMCS, Pimenta AR, Santos da Silva AC, et al. Revisiting the pharmacodynamic uroselectivity of α 1-adrenergic receptor antagonists. *J Pharmacol Exp Ther*. 2019;371:106-112.

17. Oelke M, Gericke A, Michel MC. Cardiovascular and ocular safety of α 1-adrenoceptor antagonists in the treatment of male lower urinary tract symptoms. *Expert Opin Drug Saf.* 2014;13:1187-1197.
18. Jung JH, Kim J, MacDonald R, Reddy B, Kim MH, Dahm P. Silodosin for the treatment of lower urinary tract symptoms in men with benign prostatic hyperplasia. *Cochrane Database Syst Rev.* 2017;11:CD012615.
19. Cusack B, Nelson A, Richelson E. Binding of antidepressants to human brain receptors: focus on newer generation compounds. *Psychopharmacology.* 1994;114:559-565.
20. Nojimoto FD, Mueller A, Hebelar-Barbosa F, et al. The tricyclic antidepressants amitriptyline, nortriptyline and imipramine are weak antagonists of human and rat α 1B-adrenoceptors. *Neuropharmacology.* 2010;59:49-57.
21. van Zwieten PA. Inhibition of the central hypotensive effect of clonidine by trazodone, a novel antidepressant. *Pharmacology.* 1977;15:331-336.
22. Hall H, Sven-Ove Ö. Effects of antidepressant drugs on different receptors in the brain. *Eur J Pharmacol.* 1981;70:393-407.
23. Kopera H. Anticholinergic and blood pressure effects of mianserin, amitriptyline and placebo. *Br J Clin Pharmacol.* 1978;5:29S-34S.
24. Poon IO, Braun U. High prevalence of orthostatic hypotension and its correlation with potentially causative medications among elderly veterans. *J Clin Pharm Ther.* 2005;30:173-178.
25. Thapa PB, Gideon P, Cost TW, Milam AB, Ray WA. Antidepressants and the risk of falls among nursing home residents. *N Engl J Med.* 1998;339:875-882.
26. Richelson E, Nelson A. Antagonism by neuroleptics of neurotransmitter receptors of normal human brain in vitro. *Eur J Pharmacol.* 1984;103:197-204.
27. Richelson E, Souder T. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. *Life Sci.* 2000;68:29-39.
28. Buckley NA, Sanders P. Cardiovascular adverse effects of antipsychotic drugs. *Drug Saf.* 2000;23:215-228.
29. Nourian Z, Mow T, Muftic D, et al. Orthostatic hypotensive effect of antipsychotic drugs in Wistar rats by in vivo and in vitro studies of α 1-adrenoceptor function. *Psychopharmacology.* 2008;199:15-27.
30. Silver H, Kogan H, Zlotogorski D. Postural hypotension in chronically medicated schizophrenics. *J Clin Psychiatry.* 1990;51:459-462.
31. Ray WA, Griffin MR, Schaffner W, Baugh DK, Melton LJ 3rd. Psychotropic drug use and the risk of hip fracture. *N Engl J Med.* 1987;316:363-369.
32. Thapa PB, Gideon P, Fought RL, Ray WA. Psychotropic drugs and risk of recurrent falls in ambulatory nursing home residents. *Am J Epidemiol.* 1995;142:202-211.
33. Pupo AS, Uberti MA, Minneman KP. N-terminal truncation of human α 1D-adrenoceptors increases expression of binding sites but not protein. *Eur J Pharmacol.* 2003;462:1-8.
34. Hague C, Chen Z, Pupo AS, Schulte NA, Toews ML, Minneman KP. The N terminus of the human α 1D-adrenergic receptor prevents cell surface expression. *J Pharmacol Exp Ther.* 2004;309:388-397.
35. Kountz TS, Lee KS, Aggarwal-Howarth S, et al. Endogenous N-terminal domain cleavage modulates α 1D-adrenergic receptor pharmacodynamics. *J Biol Chem.* 2016;291:18210-18221.
36. Sleight AJ, Koek W, Bigg DC. Binding of antipsychotic drugs at α 1A- and α 1B-adrenoceptors: risperidone is selective for the α 1B-adrenoceptors. *Eur J Pharmacol.* 1993;238:407-410.
37. Eltze M. In functional experiments, risperidone is selective, not for the B, but for the A subtype of α 1-adrenoceptors. *Eur J Pharmacol.* 1996;295:69-73.
38. Schmidt AW, Lebel LA, Howard HR Jr, Zorn SH. Ziprasidone: a novel antipsychotic agent with a unique human receptor binding profile. *Eur J Pharmacol.* 2001;425:197-201.
39. Beasley CM Jr, Hamilton SH, Crawford AM, et al. Olanzapine versus haloperidol: acute phase results of the international double-blind olanzapine trial. *Eur Neuropsychopharmacol.* 1997;7:125-137.
40. Fumagalli L, Pallavicini M, Budriesi R, et al. Affinity and activity profiling of unichiral 8-substituted 1,4-benzodioxane analogues of WB4101 reveals a potent and selective α 1B-adrenoceptor antagonist. *Eur J Med Chem.* 2012;58:184-191.
41. Kenny BA, Chalmers DH, Philpott PC, Naylor AM. Characterization of an α 1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. *Br J Pharmacol.* 1995;115:981-986.
42. Schwinn DA, Johnston GI, Page SO, et al. Cloning and pharmacological characterization of human α -1 adrenergic receptors: sequence corrections and direct comparison with other species homologues. *J Pharmacol Exp Ther.* 1995;272:134-142.
43. Frang H, Cockcroft V, Karskela T, Scheinin M, Marjamäki A. Phenoxybenzamine binding reveals the helical orientation of the third transmembrane domain of adrenergic receptors. *J Biol Chem.* 2001;276:31279-31284.
44. Graham JD. The ethyleneiminium ion as the active species in 2-haloalkylamine compounds. *Br J Pharmacol.* 1957;12:489-497.
45. Buitenwerf E, Osinga TE, Timmers HJLM, et al. Efficacy of α -blockers on hemodynamic control during pheochromocytoma resection - a randomized controlled trial. *J Clin Endocrinol Metab.* 2019;105:dgz188. <https://doi.org/10.1210/clinem/dgz188>
46. Liu C, Lv Q, Chen X, et al. Preoperative selective vs non-selective α -blockade in PPGL patients undergoing adrenalectomy. *Endocr Connect.* 2017;6:830-838.
47. Malec K, Miśkiewicz P, Witkowska A, et al. Comparison of phenoxybenzamine and doxazosin in perioperative management of patients with pheochromocytoma. *Kardiol Pol.* 2017;75:1192-1198.
48. Graham JD, Lewis GP. The role of the cyclic ethyleneiminium ion in the pharmacological activity of the 2-haloethylamines. *Br J Pharmacol.* 1954;9:68-75.
49. McKinley MD, McKinley FR, McGown LE. Thiosulfate as an antidote to mustard poisoning: a review of the literature. 1982 Institute report 127, Division of Research Support, Letterman Army Institute of Research, Presidio of San Francisco, Ca, 94129.
50. Ford AP, Arredondo NF, Blue DR Jr, et al. RS-17053 (N-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro- α , α -dimethyl-1H-indole-3-ethanamine hydrochloride), a selective α 1A-adrenoceptor antagonist, displays low affinity for functional α 1-adrenoceptors in human prostate: implications for adrenoceptor classification. *Mol Pharmacol.* 1996;49:209-215.
51. Silva RO, de Oliveira AS, Nunes Lemes LF, et al. Synthesis and structure-activity relationships of novel arylpiperazines as potent antagonists of α 1-adrenoceptor. *Eur J Med Chem.* 2016;122:601-610.
52. Baker JG. The selectivity of β -adrenoceptor antagonists at the β 1, β 2 and β 3 adrenoceptors. *Br J Pharmacol.* 2005;144:317-322.
53. Baker JG. The selectivity of β -adrenoceptor agonists at the human β 1, β 2 and β 3 adrenoceptors. *Br J Pharmacol.* 2010;160:148-161.
54. Williams TJ, Blue DR, Daniels DV, et al. In vitro α 1-adrenoceptor pharmacology of Ro 70-0004 and RS-100329, novel α 1A-adrenoceptor selective antagonists. *Br J Pharmacol.* 1999;127:252-258.
55. Goetz AS, King HK, Ward SD, True TA, Rimele TJ, Saussy DL Jr. BMY 7378 is a selective antagonist of the D subtype of α 1-adrenoceptors. *Eur J Pharmacol.* 1995;272:R5-R6.
56. Goodnick PJ, Jerry JM. Aripiprazole: profile on efficacy and safety. *Expert Opin Pharmacother.* 2002;3:1773-1781.
57. Keck PE Jr, McElroy SL. Aripiprazole: a partial dopamine D2 receptor agonist antipsychotic. *Expert Opin Investig Drugs.* 2003;12:655-662.
58. Burry L, Hutton B, Williamson DR, et al. Pharmacological interventions for the treatment of delirium in critically ill adults. *Cochrane Database Syst Rev.* 2019;9:CD011749.

59. Nikoioe R, Neufeld KJ, Oh ES, et al. Antipsychotics for treating delirium in hospitalized adults: a systematic review. *Ann Intern Med.* 2019;171:485. <https://doi.org/10.7326/M19-1860>
60. Gaertner J, Eychmueller S, Leyhe T, Bueche D, Savaskan E, Schlögl M. Benzodiazepines and/or neuroleptics for the treatment of delirium in palliative care?—a critical appraisal of recent randomized controlled trials. *Ann Palliat Med.* 2019;8:504-515.
61. Skelton L, Guo P. Evaluating the effects of the pharmacological and nonpharmacological interventions to manage delirium symptoms in palliative care patients: systematic review. *Curr Opin Support Palliat Care.* 2019;13:384-391.
62. Girard TD, Exline MC, Carson SS, et al. Haloperidol and ziprasidone for treatment of delirium in critical illness. *N Engl J Med.* 2018;379:2506-2516.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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