

# The selectivity of $\alpha$ -adrenoceptor agonists for the human $\alpha$ 1A, $\alpha$ 1B, and $\alpha$ 1D-adrenoceptors

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## Abstract

Highly selective drugs offer a way to minimize side-effects. For agonist ligands, this could be through highly selective affinity or highly selective efficacy, but this requires careful measurements of intrinsic efficacy. The  $\alpha$ 1-adrenoceptors are important clinical targets, and  $\alpha$ 1-agonists are used to manage hypotension, sedation, attention deficit hypersensitivity disorder (ADHD), and nasal decongestion. With 100 years of drug development, there are many structurally different compounds with which to study agonist selectivity. This study examined 62  $\alpha$ -agonists at the three human  $\alpha$ 1-adrenoceptor ( $\alpha$ 1A,  $\alpha$ 1B, and  $\alpha$ 1D) stably expressed in CHO cells. Affinity was measured using whole-cell  $^3\text{H}$ -prazosin binding, while functional responses were measured for calcium mobilization, ERK1/2-phosphorylation, and cAMP accumulation. Efficacy ratios were used to rank compounds in order of intrinsic efficacy. Adrenaline, noradrenaline, and phenylephrine were highly efficacious  $\alpha$ 1-agonists at all three receptor subtypes. A61603 was the most selective agonist and its very high  $\alpha$ 1A-selectivity was due to selective  $\alpha$ 1A-affinity (>660-fold). There was no evidence of Gq-calcium versus ERK-phosphorylation biased signaling at the  $\alpha$ 1A,  $\alpha$ 1B, or  $\alpha$ 1D-adrenoceptors. There was little evidence for  $\alpha$ 1A calcium versus cAMP biased signaling, although there were suggestions of calcium versus cAMP bias the  $\alpha$ 1B-adrenoceptor. Comparisons of the rank order of ligand intrinsic efficacy suggest little evidence for selective intrinsic efficacy between the compounds, with perhaps the exception of dobutamine which may have some  $\alpha$ 1D-selective efficacy. There seems plenty of scope to develop affinity selective and intrinsic efficacy selective drugs for the  $\alpha$ 1-adrenoceptors in future.

## KEYWORDS

agonist selectivity, calcium, cAMP, ERK1/2-phosphorylation,  $\alpha$ -adrenoceptor

**Abbreviations:** cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; HBS, Hepes buffered saline; sfm, serum free media.

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## 1 | INTRODUCTION

Highly selective drugs are a prime goal in drug development because high-target receptor selectivity is expected to maximize clinical effectiveness while minimizing side-effects.<sup>1</sup> For antagonist drugs, this solely involves evaluating the affinity (ability of the ligand to bind to the receptor). However, for agonists, there are two properties that need to be evaluated: affinity and efficacy (ability of the receptor-ligand complex to induce a response).<sup>1-4</sup> A highly potent agonist could achieve this potency through high affinity or through high efficacy. Thus agonists can be highly selective due to highly selective affinity, or highly selective efficacy (where the compound could bind to several different receptors, but only activate one) or a mixture of both.<sup>2,5</sup>

Agonist efficacy depends on several factors. Tissue and assay-dependent factors include receptor number, receptor-effector coupling efficiency, effector response measured, assay response window, and any desensitization that occurs within the timeframe of the measurement. This makes direct comparisons of potency ( $EC_{50}$ ) impossible across systems. Ligand/receptor factors are the innate ability of a certain receptor–ligand complex to induce a response and depend upon the chemical interaction between ligand and receptor. This, termed “intrinsic efficacy”, is a measure of efficacy at the molecular/single ligand–receptor level<sup>1,6</sup> and is a more accurate measure of true ligand efficacy than either potency or maximal response.<sup>7</sup> A good way to compare the intrinsic efficacy of ligands is to remove tissue/assay factors and measure responses from individual receptor subtypes for many agonists in parallel in a null background. This allows the ligand's intrinsic efficacy to be ranked (e.g. by “efficacy ratios”  $K_D/EC_{50}$ <sup>1,3,4,6</sup>) and thus compared across different receptors. Previous studies using this have found that some agonists are highly selective purely because of a highly selective binding profile, and not because of any intrinsic efficacy selectivity (e.g. salmeterol at  $\beta$ 2-adrenoceptors).<sup>8</sup>

The  $\alpha$ 1-adrenoceptors ( $\alpha$ 1A,  $\alpha$ 1B, and  $\alpha$ 1D, Alexander et al., 2019/2020) are Gq-coupled GPCRs expressed in a wide range of tissues including blood vessels, heart, kidney, spleen, liver, brain, and lower urinary tract.<sup>9-14</sup> Whereas  $\alpha$ -adrenoceptor antagonists ( $\alpha$ -blockers) are used to treat hypertension and benign prostatic hyperplasia,  $\alpha$ -agonists are used to manage hypotension and sedation in intensive care settings (e.g. phenylephrine,<sup>15</sup> dexmedetomidine, and clonidine<sup>16</sup>), for ADHD (attention deficit hypersensitivity disorder e.g. guanfacine<sup>17,18</sup>), for muscle spasm and spasticity (e.g. tizanidine<sup>19</sup>) but are probably most widely as over-the-counter nasal decongestants (e.g. oxymetazoline and xylometazoline<sup>20,21</sup>). Thus there are many structurally different  $\alpha$ -adrenoceptor agonists with which to study agonist selectivity and determine how that is achieved.

In addition to  $\alpha$ 1-adrenoceptor-Gq-PLC-calcium signaling, the  $\alpha$ 1-adrenoceptors have also been shown to stimulate other signaling cascades.<sup>14,22,23</sup> Some recent studies have suggested that biased signaling can occur via the  $\alpha$ 1A-adrenoceptor. Isoprenaline was thought to have  $\alpha$ 1A-cAMP biased signaling.<sup>24</sup> Oxymetazoline

was initially thought to have ERK1/2-phosphorylation bias.<sup>25</sup> It was later confirmed that the “biased” responses were occurring via a different receptor although phenylephrine and methoxamine ERK1/2-phosphorylation bias and A61603 cAMP bias were proposed.<sup>26</sup> However the best way to determine whether a certain ligand is indeed an outlier inducing biased-signaling is to examine many ligands in parallel rather than just a few.<sup>1</sup>

Many  $\alpha$ 1-agonist studies examine only a few ligands, study just one receptor, or use receptors from different species, making comparing intrinsic efficacy difficult (e.g. 24–27). The aim of this study was to examine the selectivity of a large range of agonists for the human  $\alpha$ 1A,  $\alpha$ 1B, and  $\alpha$ 1D-adrenoceptors, with specific aims to identify whether agonists were selective due to selective affinity or selective intrinsic efficacy. Additionally, as several different agonist responses were examined, ligands with bias toward one signaling cascade over another would also be identified.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

<sup>3</sup>H-prazosin, <sup>3</sup>H-adenine, Microscint 20, Ultima Gold XR scintillation fluid and the Surefire Alphascreen pERK1/2 kit were from PerkinElmer. <sup>14</sup>C-cAMP was from Hartmann Analytic. Fluo-4AM and pluronic F-127 were from Invitrogen. Gibco foetal bovine serum was from Fischer Scientific. All other reagents were from Sigma-Aldrich. A list of the ligands studied with the source and supplier code is given in Table S1.

### 2.2 | Ligand selection

Commercially available ligands with known  $\alpha$ -adrenoceptor agonist activity from the literature were investigated. In addition, several ligands generally considered to be antagonists were investigated (taken from [28]) if they were found to have agonist activity at one or more  $\alpha$ 1-adrenoceptors. Brimonidine and UK14304 were purchased from different suppliers and as they appeared very different in solution (brimonidine was clear whereas UK14304 was bright yellow) are reported separately. Medetomidine (racemate) and its active isomer dexmedetomidine (increasingly used in intensive care units) are also reported separately.

### 2.3 | Cell culture

CHO-K1 (RIDD: CVCL\_0214) stably expressing the human  $\alpha$ 1A-adrenoceptor, human  $\alpha$ 1B-adrenoceptor, or human  $\alpha$ 1D-adrenoceptor (full length) were used.<sup>28</sup>

In addition, the parental CHO cell line without any transfected receptors was also used. Cells were grown in Dulbecco's modified Eagle's medium nutrient mix F12 (DMEM/F12) containing 10%

foetal calf serum (FCS) and 2 mM L-glutamine in a 37°C humidified 5% CO<sub>2</sub>; 95% air atmosphere.

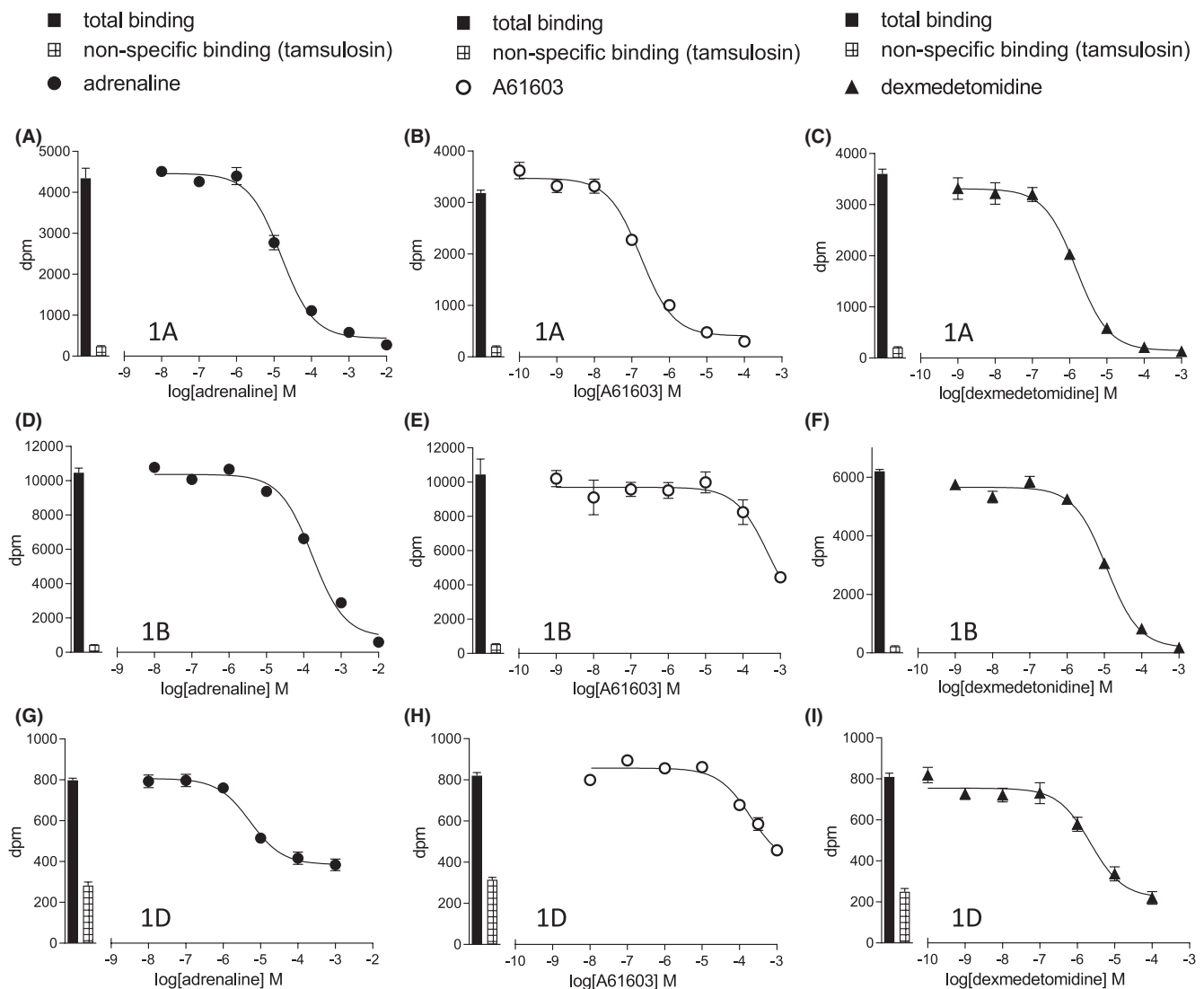
## 2.4 | <sup>3</sup>H-prazosin whole-cell radioligand binding

Cells were grown to confluence in white-sided 96-well view plates and whole-cell binding studies were conducted as previously described<sup>28</sup> in a total well volume of 200 µl per well. Cells were incubated with <sup>3</sup>H-prazosin and competing ligand in 200 µl for 2 h in serum-free media (sfm) at 37°C and plates counted using a Topcount (2 min per well) after a minimum of 6 h in the dark at room temperature. Total binding and non-specific binding (tamsulosin 10 µM for α1A and α1B, and 100 µM for α1D—see<sup>28</sup> for

full data and explanation) were determined in every plate. <sup>3</sup>H-prazosin concentrations were determined from the average of triplicate 50 µl samples of each <sup>3</sup>H-prazosin concentration used and were in the range of 0.21 to 1.41 nM. *K<sub>D</sub>* values were calculated from IC<sub>50</sub> values using the Cheng-Prusoff equation (see below).

## 2.5 | Intracellular free calcium mobilization

Cells were grown to confluence in black-sided 96-well view plates, and calcium measurements were made using a FlexStation 3 at 37°C. Cells were loaded for 45 min at 37°C with Fluo-4AM/pluronic-F127 in sfm containing 25 mM probenecid. Cells were



**FIGURE 1** Inhibition of <sup>3</sup>H-prazosin binding to whole cells to CHO-α1A cells (A-C), CHO-α1B cells (D-F), or CHO-α1D cells (G-I) by adrenaline (A, D, G), A61603 (B, E, H) or dexmedetomidine (C, F, I). Bars represent total <sup>3</sup>H-prazosin binding and non-specific binding, determined in the presence of 10 µM tamsulosin (CHO-α1A and CHO-α1B) or 100 µM tamsulosin (CHO-α1D). The concentration of <sup>3</sup>H-prazosin was (A) 0.34 nM, (B) 0.20 nM, (C) 0.22 nM, (D) 0.42 nM, (E) 0.44 nM, (F) 0.22 nM, (G) 0.58 nM, (H) 0.58 nM and (I) 0.57 nM. Data points are mean ± SEM of triplicate determinations

washed twice with  $2 \times 200 \mu\text{l}$  HEPES-buffered saline (HBS, containing  $2 \mu\text{M}$   $\text{CaCl}_2$ ).  $80 \mu\text{l}$  HBS was then added to each well and the plate put into the Flexstation. Agonist ligands were diluted to five times final concentration of HBS in round bottomed 96-well compound plates and put in the Flexstation. The Flexstation robotics added  $20 \mu\text{l}$  agonist ligand from the compound plate into the existing  $80 \mu\text{l}$  HBS in the cell plate (1:5 dilution). Basal and maximum responses (defined by  $10 \mu\text{M}$  ionomycin) were determined in each plate. Calcium mobilization was followed for 120–200 s per well. The data were plotted as the maximum value obtained for calcium mobilization over the basal value obtained for that well before the addition of ligand.

## 2.6 | ERK1/2-phosphorylation

Extracellular signal-regulated kinases (ERK1/2) activation was measured using a Surefire Alphascreen pERK1/2 kit as per manufacturer's instructions. Cells were grown to confluence in clear-sided 96-well plates, then double serum starved by washing the cells twice with  $100 \mu\text{l}$  sfm before incubating in a further (third)  $100 \mu\text{l}$  sfm for 24 h before experimentation. Agonists in  $20 \mu\text{l}$  sfm were added to the well (contained about  $80 \mu\text{l}$  after some evaporation over 24 h, thus approximately a 1:5 dilution in wells) and incubated for 2–4 min (at  $37^\circ\text{C}$ ). Responses were initially studied at 2, 5, 10, and 15 min after addition of agonist. Responses retained a similar pattern (with regards to  $\text{EC}_{50}$  value and proportion of the of the positive control response— $10 \mu\text{M}$  PDBu); however, the response window was greatest at 2 and 5 min and thus all data reported here are following 2–4 min agonist incubation. Reagents were then removed,  $20 \mu\text{l}$  lysis buffer added to each well, and ERK1/2-phosphorylation measured using the Alphascreen kit as per manufacturer's instructions. After a minimum of 2 h in the dark, the plates were read on an Envision plate reader using standard Alphascreen settings. Basal and maximum ERK1/2-phosphorylation (as determined by  $10 \mu\text{M}$  PDBu, Phorbol 12,13-dibutyrate) was measured in each plate.

## 2.7 | $^3\text{H}$ -cAMP accumulation

Cells were grown to confluence in clear-sided 48-well plates and  $^3\text{H}$ -cAMP accumulation was measured as previously described.<sup>5</sup> Following a  $^3\text{H}$ -adenine load, cells were washed and incubated in sfm containing  $1 \text{ mM}$  IBMX ( $500 \mu\text{l}$  per well). Agonist (in  $5 \mu\text{l}$ ) was added and the cells were incubated for 5 h at  $37^\circ\text{C}$ . Basal and response to  $10 \mu\text{M}$  forskolin were determined in every plate. Where used to examine Gi-coupled responses, basal cAMP was augmented by  $10 \mu\text{M}$  forskolin and inhibition of this forskolin-induced response was examined. In these cases, forskolin was added to the wells 10 min after the addition of agonist. The assay was terminated with  $50 \mu\text{l}$  concentrated HCl per well and  $^3\text{H}$ -nucleotides separated by column chromatography.<sup>5</sup>

## 2.8 | Data analysis

All pharmacological data were plotted using Graphpad Prism7.

## 2.9 | Whole-cell binding

The affinity of  $^3\text{H}$ -prazosin has previously been determined from saturation binding in these cell lines.<sup>28</sup> The affinity of competing ligands was determined from a one-site sigmoidal response curve where the  $\text{IC}_{50}$  is the concentration required to inhibit 50% of the specific binding of the  $^3\text{H}$ -prazosin, A is the concentration of the competing ligand and NS is the non-specific binding (Equation 1).

$$\% \text{ uninhibited binding} = 100 - \frac{(100 \times A)}{(A + \text{IC}_{50})} + \text{NS}. \quad (1)$$

The affinity ( $K_D$  value) of the competing ligand was then calculated from the  $\text{IC}_{50}$  using the Cheng-Prusoff equation (Equation 2) where [ $^3\text{H}$ -prazosin] is the concentration of  $^3\text{H}$ -prazosin in that experiment and  $K_D$   $^3\text{H}$ -prazosin is the  $K_D$  value of the radioligand.

$$K_D = \frac{\text{IC}_{50}}{1 + ([^3\text{H} - \text{prazosin}]/K_D \text{ } ^3\text{H} - \text{prazosin})}. \quad (2)$$

## 2.10 | Functional experiments

Agonist responses were usually best described by a one-site sigmoidal concentration response curve (Equation 3) where  $E_{\text{max}}$  is the maximum response, [A] is the agonist concentration and  $\text{EC}_{50}$  is the concentration of agonist that produces 50% of the maximal response

$$\text{Response} = \frac{E_{\text{max}} \times [A]}{\text{EC}_{50} + [A]}. \quad (3)$$

Some responses were best described by a two-component response (e.g. Figure 3). Here a two-component response curve was used (Equation 4)

$$\% \text{ maximum stimulation} = \frac{[A] \cdot N}{(\text{EC}_{150} + [A])} + \frac{[A] \cdot (100 - N)}{(\text{EC}_{250} + [A])}, \quad (4)$$

where  $N$  is the percentage of site 1, [A] is the concentration of agonist, and  $\text{EC}_{150}$  and  $\text{EC}_{250}$  are the respective  $\text{EC}_{50}$  values (or  $\text{IC}_{50}$  values) for the two agonist sites. For the data in Tables 2 and 3, the log  $\text{EC}_{50}$  quote for ERK1/2-phosphorylation is that of the initial stimulatory part of the response.

## 2.11 | Efficacy ratios

Efficacy ratios were calculated by dividing the  $K_D$  value by the  $\text{EC}_{50}$  value for each ligand as per method of Furchgott.<sup>6</sup>

**TABLE 1** Log  $K_D$  values of  $\alpha$ -agonists binding to the human  $\alpha1A$ ,  $\alpha1B$  and  $\alpha1D$ -adrenoceptors. Values represent mean  $\pm$  SEM of  $n$  separate experiments. Selectivity ratios are also given where a ratio of 1 demonstrates no selectivity for one receptor subtype over another. Thus, A61603 has 661-fold higher affinity for the  $\alpha1A$ -adrenoceptor than the  $\alpha1B$ -adrenoceptor. Compounds are arranged in order of  $\alpha1A$ -binding selectivity

Ligand	Log $K_D$ values determined from $^3H$ -prazosin whole cell binding			Affinity selectivity ratio		
	$\alpha1A$	$\alpha1B$	$\alpha1D$	$\alpha1A$ vs $\alpha1B$	$\alpha1A$ vs $\alpha1D$	$\alpha1B$ vs $\alpha1D$
A61603	-6.82 $\pm$ 0.09	IC <sub>50</sub> > -4	-3.92 $\pm$ 0.11	>661	794	>1.2
RWJ52353	-5.28 $\pm$ 0.08	IC <sub>50</sub> > -3	-4.30 $\pm$ 0.12	>190	9.6	>20
PF3774076	-6.89 $\pm$ 0.03	-4.74 $\pm$ 0.04 <sup>apparent</sup>	-5.24 $\pm$ 0.18	141	44.7	3.2
Oxymethazoline	-7.19 $\pm$ 0.07	-5.17 $\pm$ 0.05	-5.28 $\pm$ 0.04	105	81.3	1.3
Lisuride <sup>a</sup>	-7.94 $\pm$ 0.06	-6.07 $\pm$ 0.04	-6.93 $\pm$ 0.11	74.1	10.2	7.2
Xylometazoline	-6.94 $\pm$ 0.05	-5.16 $\pm$ 0.04	-5.23 $\pm$ 0.04	60.3	51.3	1.2
Dihydroergotamine	-8.62 $\pm$ 0.08	-6.92 $\pm$ 0.08	-7.19 $\pm$ 0.16 <sup>early plateau</sup>	50.1	26.9	1.9
2-MPMDQ <sup>a</sup>	-9.06 $\pm$ 0.07	-7.37 $\pm$ 0.04	-9.01 $\pm$ 0.16 -5.66 $\pm$ 0.29 64.0 $\pm$ 2.1% site1	49.0	1.1	43.7
Allyphenylne	-6.79 $\pm$ 0.04	-5.11 $\pm$ 0.05	-5.85 $\pm$ 0.05	47.9	8.7	5.5
Methoxamine	-4.63 $\pm$ 0.10	IC <sub>50</sub> > -3	-3.82 $\pm$ 0.08	>42.7	6.5	>6.6
ST-91	-5.94 $\pm$ 0.01	-4.39 $\pm$ 0.04	-5.24 $\pm$ 0.09	35.5	5.0	7.1
Amitraz	-5.52 $\pm$ 0.05	IC <sub>50</sub> > -4	-5.08 $\pm$ 0.05	>33.1	2.8	>12.0
Guanfacine	-5.33 $\pm$ 0.02	-3.87 $\pm$ 0.06	-4.93 $\pm$ 0.09	28.8	2.5	11.5
Labetolol <sup>a</sup>	-7.33 $\pm$ 0.04	-5.91 $\pm$ 0.03	-6.12 $\pm$ 0.07	26.3	16.2	1.6
Fenoterol	-5.29 $\pm$ 0.04	-3.91 $\pm$ 0.10	-4.35 $\pm$ 0.05	24.0	8.7	2.8
Buspirone	-6.02 $\pm$ 0.03	-4.65 $\pm$ 0.03	-5.90 $\pm$ 0.11	23.4	1.3	17.8
Formoterol	-5.82 $\pm$ 0.04	-4.47 $\pm$ 0.04	-5.15 $\pm$ 0.09	22.4	4.7	4.8
$\alpha$ -Methylnorepinephrine	-4.32 $\pm$ 0.04	IC <sub>50</sub> > -3	-4.84 $\pm$ 0.14 <sup>early plateau</sup>	>20.9	3.3	>69.2
Atipamezole <sup>a</sup>	-5.99 $\pm$ 0.03	-4.68 $\pm$ 0.08	-5.33 $\pm$ 0.04	20.4	4.6	4.5
Isoprenaline	-4.07 $\pm$ 0.08	-2.80 $\pm$ 0.05 <sup>apparent</sup>	-3.96 $\pm$ 0.05	18.6	1.3	14.5
Synephrine	-4.26 $\pm$ 0.04	IC <sub>50</sub> > -3	IC <sub>50</sub> > 3.5	>18.2	>5.8	>3
Xylazine	-4.48 $\pm$ 0.06	-3.22 $\pm$ 0.05	-4.56 $\pm$ 0.19	18.2	1.2	21.9
BRL44408 <sup>a</sup>	-5.92 $\pm$ 0.09	-4.68 $\pm$ 0.07	-5.06 $\pm$ 0.05	17.4	7.2	2.4
2-PMDQ <sup>a</sup>	-8.19 $\pm$ 0.09	-6.95 $\pm$ 0.05	-8.42 $\pm$ 0.12 -5.61 $\pm$ 0.12 57.6 $\pm$ 2.8% site 1	17.4	1.7	29.5
Detomidine	-6.85 $\pm$ 0.07	-5.65 $\pm$ 0.02	-6.01 $\pm$ 0.11	15.8	6.9	2.3

(Continues)

TABLE 1 (Continued)

Ligand	Log K <sub>D</sub> values determined from <sup>3</sup> H-prazosin whole cell binding				Affinity selectivity ratio				
	α1A	n	α1B	n	α1D	n	α1A vs α1B	α1A vs α1D	α1B vs α1D
ARC239 <sup>a</sup>	-9.35 ± 0.08	8	-8.15 ± 0.07	9	-8.74 ± 0.12 -5.42 ± 0.21 60.5 ± 1.4% site 1	7	15.8	4.1	3.9
Eforaxan <sup>a</sup>	-5.47 ± 0.03	5	-4.27 ± 0.07	5	-4.97 ± 0.06	5	15.8	3.2	5.0
Adrenaline	-5.09 ± 0.07	9	-3.94 ± 0.09	10	-5.19 ± 0.14	9	14.1	1.3	17.8
CGP 12177 <sup>a</sup>	-5.14 ± 0.05	6	IC <sub>50</sub> >-4	5	-4.20 ± 0.11	5	>13.8	8.7	>1.6
Sunepitriion <sup>a</sup>	-5.78 ± 0.06	5	-4.65 ± 0.06	5	-5.33 ± 0.23	6	13.5	2.8	4.8
Tizanidine	-5.46 ± 0.02	5	-4.35 ± 0.05	5	-5.41 ± 0.12	5	12.9	1.1	11.5
Ephedrine	-4.07 ± 0.06	5	IC <sub>50</sub> >-3	5	-3.57 ± 0.07 <sup>apparent</sup>	5	>11.7	3.2	>3.7
Metaraminol	-4.07 ± 0.02	5	IC <sub>50</sub> >-3	5	-4.25 ± 0.09	5	>11.7	1.5	>17.8
Cirazoline	-6.17 ± 0.09	9	-5.10 ± 0.06	8	-5.53 ± 0.04	5	11.7	4.4	2.7
Moxonidine	-4.54 ± 0.03	6	-3.47 ± 0.10	7	-3.96 ± 0.05	5	11.7	3.8	3.1
para-amino-clonidine	-6.23 ± 0.03	6	-5.17 ± 0.06	5	-5.39 ± 0.16	6	11.5	6.9	1.7
Guanabenz	-6.48 ± 0.04	5	-5.45 ± 0.04	5	-6.02 ± 0.04	5	10.7	2.9	3.7
Noradrenaline	-4.81 ± 0.10	7	-3.79 ± 0.09	9	-5.48 ± 0.18	8	10.5	4.7	49.0
R-phenylephrine	-4.87 ± 0.05	5	-3.87 ± 0.05	6	-4.65 ± 0.11	6	10.0	1.7	6.0
Etilefrine	-3.99 ± 0.08	6	IC <sub>50</sub> >-3	5	-4.45 ± 0.07	5	>9.8	2.9	>28.1
Salmeterol	-6.11 ± 0.06	5	-5.13 ± 0.04	5	-5.77 ± 0.08	6	9.5	2.2	4.4
3-MPPI <sup>a</sup>	-9.57 ± 0.06	6	-8.59 ± 0.03	6	-9.76 ± 0.15 -6.93 ± 0.17 66.7 ± 3.4% site 1	7	9.5	1.5	14.8
BHT920	-4.70 ± 0.04	5	-3.73 ± 0.04	5	-4.49 ± 0.07	5	9.3	1.6	5.8
Clonidine	-6.06 ± 0.02	5	-5.13 ± 0.01	5	-5.59 ± 0.11	5	8.5	3.0	2.9
Dobutamine	-6.34 ± 0.09	8	-5.44 ± 0.04	6	-5.36 ± 0.11	6	7.9	9.5	1.2
Salbutamol	-3.84 ± 0.06	7	IC <sub>50</sub> >-3	5	-3.87 ± 0.11	5	>6.9	1.1	>7.4
Dexmedetomidine	-5.88 ± 0.06	5	-5.04 ± 0.03	5	-5.91 ± 0.05	5	6.9	1.3	9.3
Naphazoline	-6.54 ± 0.05	6	-5.74 ± 0.07	7	-5.69 ± 0.12	6	6.3	7.1	1.1
Idazoxan <sup>a</sup>	-5.67 ± 0.07	5	-4.88 ± 0.03	5	-5.23 ± 0.11	5	6.2	2.8	2.2
Medetomidine	-5.63 ± 0.05	5	-4.84 ± 0.03	5	-5.67 ± 0.09	5	6.2	1.1	6.8
T-CG 1000	-5.96 ± 0.05	5	-5.18 ± 0.06	5	-5.91 ± 0.12	7	6.0	1.1	5.4
Tetrahydrozoline	-5.93 ± 0.04	6	-5.22 ± 0.06	6	-5.34 ± 0.10	5	5.1	3.9	1.3
Dopamine	-3.60 ± 0.06	7	-2.89 ± 0.09 <sup>apparent</sup>	7	-4.09 ± 0.03	5	5.1	3.1	15.8

(Continues)

TABLE 1 (Continued)

Ligand	Log $K_D$ values determined from $^3\text{H}$ -prazosin whole cell binding				Affinity selectivity ratio				
	$\alpha 1A$	n	$\alpha 1B$	n	$\alpha 1D$	n	$\alpha 1A$ vs $\alpha 1B$	$\alpha 1A$ vs $\alpha 1D$	$\alpha 1B$ vs $\alpha 1D$
Brimonidine	$-5.36 \pm 0.04$	5	$-4.68 \pm 0.04$	5	$-5.27 \pm 0.06$	5	4.8	1.2	3.9
UK14304	$-5.53 \pm 0.04$	5	$-4.89 \pm 0.06$	5	$-5.36 \pm 0.10$	5	4.4	1.5	3.0
BHT933	$-3.60 \pm 0.06^{\text{apparent}}$	5	No binding	5	$-3.70 \pm 0.18^{\text{apparent}}$	5	>4.0	1.3	>5.0
Rilmenidine	$-4.49 \pm 0.04$	6	$IC_{50} > -4$	10	$-4.73 \pm 0.07$	5	>3.1	1.7	>5.4
Octopamine	$-3.44 \pm 0.07$	7	$IC_{50} > -3$	6	$IC_{50} > -3$	5	>2.8	>2.8	1.0
BMY7378 <sup>a</sup>	$-6.61 \pm 0.05$	5	$-6.23 \pm 0.05$	6	$-8.60 \pm 0.13$ $-5.93 \pm 0.37$ $57.7 \pm 2.6\%$ site 1	9	2.4	97.7	234
Chloroethylclonidine	$-5.43 \pm 0.05$	5	$-5.35 \pm 0.07$	5	$-5.50 \pm 0.06$	5	1.2	1.2	1.4
Midodrine	$IC_{50} > -3$	5	No binding	5	$IC_{50} > -3$	5			
Methyldopa	No binding	5	No binding	5	No binding	5			

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

<sup>early plateau</sup>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 at the  $\alpha 1D$ -adrenoceptor was for 72.3%  $\pm$  3.8% for dihydroergotamine and 55.3%  $\pm$  5.0% for  $\alpha$ -methylnorepinephrine.

<sup>a</sup>Data from Proudman et al., 2020. For some ligands, the binding curve obtained for inhibition of  $^3\text{H}$ -prazosin specific binding at the  $\alpha 1D$  receptor was best described by a two-component inhibition curve. Here the  $K_D$  value for the first component (higher affinity) and second component (lower affinity) is given with the % of the response at the first component. For further details and example graphs see Proudman et al., 2020.



**TABLE 2** Log  $K_D$  values from  $^3\text{H}$ -prazosin whole-cell binding (from Table 1), log  $\text{EC}_{50}$  values, and % ionomycin maximal responses obtained from intracellular calcium mobilization and intrinsic efficacy ratios ( $K_D/\text{EC}_{50(\text{calcium})}$ ) obtained from CHO- $\alpha 1\text{A}$  cells. The log  $\text{EC}_{50}$  values obtained from ERK1/2-phosphorylation (and % PDBu responses), and those obtained from cAMP accumulation (and % forskolin maximum response), and cAMP accumulation in the presence of forskolin (with fold increase of that response) are also given

CHO- $\alpha 1\text{A}$	$^3\text{H}$ -prazosin binding		Intracellular calcium release		Log efficacy ratio	ERK1/2-phosphorylation			cAMP accumulation			cAMP accumulation (in presence of forskolin)		
	Log $K_D$	Log $\text{EC}_{50}$	% ionomycin	n		$K_D/\text{EC}_{50}$	Log $\text{EC}_{50}$	% PDBu	n	Log $\text{EC}_{50}$	% forskolin	n	Log $\text{EC}_{50}$	Fold increase
Adrenaline	-5.09	-9.09 ± 0.12	58.9 ± 3.7	10	4.00	-7.74 ± 0.19 <sup>a</sup>	71.3 ± 7.7	5	-5.63 ± 0.08	164.0 ± 7.3	5	-6.08 ± 0.16	2.65 ± 0.09	5
Noradrenaline	-4.81	-8.61 ± 0.09	62.2 ± 3.0	9	3.80	-7.49 ± 0.09 <sup>a</sup>	84.2 ± 8.4	6	-5.46 ± 0.13	167.3 ± 13.3	5	-6.13 ± 0.13	2.73 ± 0.09	5
A61603	-6.82	-10.32 ± 0.06	61.0 ± 3.8	7	3.50	-9.92 ± 0.09 <sup>a</sup>	74.8 ± 4.5	8	-8.05 ± 0.04	142.7 ± 8.7	5	-9.21 ± 0.07	2.19 ± 0.14	5
R-phenylephrine	-4.87	-8.34 ± 0.09	58.3 ± 2.9	7	3.47	-7.87 ± 0.10 <sup>a</sup>	84.5 ± 4.5	9	-5.58 ± 0.08	132.5 ± 4.5	5	-7.02 ± 0.09	2.44 ± 0.12	5
Methoxamine	-4.63	-8.06 ± 0.05	55.9 ± 2.3	8	3.43	-7.56 ± 0.14 <sup>a</sup>	81.4 ± 4.5	6	-5.31 ± 0.05	120.0 ± 3.7	5	-6.40 ± 0.09	2.19 ± 0.09	5
$\alpha$ -Methylnorepinephrine	-4.32	-7.69 ± 0.07	65.2 ± 2.6	9	3.37	-6.86 ± 0.12 <sup>a</sup>	87.5 ± 6.3	8	-5.09 ± 0.06	166.8 ± 7.4	5	-5.74 ± 0.10	2.49 ± 0.05	5
ST-91	-5.94	-9.08 ± 0.11	64.6 ± 2.6	8	3.14	-8.70 ± 0.06	79.5 ± 5.2	6	-6.80 ± 0.05	108.2 ± 12.5	5	-7.86 ± 0.14	2.17 ± 0.10	5
Metaraminol	-4.07	-7.21 ± 0.05	66.9 ± 3.2	8	3.14	-7.01 ± 0.12	83.9 ± 4.1	6	100 $\mu\text{M}$	122.7 ± 9.8	5	-6.09 ± 0.11	2.54 ± 0.11	5
Etilefrine	-3.99	-7.11 ± 0.11	58.1 ± 2.1	9	3.12	-6.84 ± 0.05	72.8 ± 7.0	7	100 $\mu\text{M}$	82.7 ± 5.5	5	-5.89 ± 0.08	2.17 ± 0.08	5
Cirazoline	-6.17	-9.18 ± 0.11	58.2 ± 2.0	10	3.01	-9.02 ± 0.08	80.8 ± 3.6	10	-6.91 ± 0.07	108.9 ± 6.5	5	-7.97 ± 0.06	2.38 ± 0.15	5
Octopamine	-3.44	-6.10 ± 0.04	57.5 ± 2.1	8	2.66	-5.38 ± 0.14	81.2 ± 6.8	6	100 $\mu\text{M}$	18.0 ± 1.5	5	100 $\mu\text{M}$	1.89 ± 0.06	5
Dopamine	-3.60	-6.16 ± 0.06	57.9 ± 4.1	7	2.56	-5.75 ± 0.07	82.2 ± 5.0	5	1 mM	104.4 ± 7.4	5	-4.57 ± 0.15	2.75 ± 0.11	5
Para-amino-clonidine	-6.23	-8.70 ± 0.09	52.0 ± 2.8	10	2.47	-8.40 ± 0.10	84.5 ± 4.9	6	-6.76 ± 0.03	26.6 ± 3.2	5	-7.36 ± 0.05	1.89 ± 0.05	5
Synephrine	-4.26	-6.66 ± 0.06	55.4 ± 1.9	7	2.40	-6.22 ± 0.11	87.4 ± 8.6	7	100 $\mu\text{M}$	25.4 ± 3.4	5	-5.15 ± 0.08	2.12 ± 0.05	5
Naphazoline	-6.54	-8.93 ± 0.09	50.9 ± 2.6	7	2.39	-8.39 ± 0.07	90.9 ± 4.4	9	-6.57 ± 0.04	57.9 ± 5.7	5	-7.38 ± 0.04	2.18 ± 0.05	5
Midodrine	>-3	-5.23 ± 0.15	52.0 ± 1.7	10	>2.23	100 $\mu\text{M}$	87.7 ± 11.0	6	ND	ND	5	ND	ND	5
Oxymethazoline	-7.19	-9.31 ± 0.10	54.5 ± 2.5	9	2.12	-8.97 ± 0.18 <sup>b</sup>	92.8 ± 2.7	6	-7.18 ± 0.05	43.9 ± 5.2	5	-7.93 ± 0.13	2.33 ± 0.13	5
Medetomidine	-5.63	-7.69 ± 0.09	57.2 ± 1.8	8	2.06	-7.50 ± 0.14	85.8 ± 3.3	5	-6.19 ± 0.03	46.3 ± 5.2	5	-6.90 ± 0.06	2.12 ± 0.09	5
Clonidine	-6.06	-8.11 ± 0.07	51.6 ± 1.2	8	2.05	-7.78 ± 0.12	85.7 ± 4.7	6	-6.16 ± 0.03	33.8 ± 3.2	5	-7.15 ± 0.09	2.28 ± 0.08	5
Dobutamine	-6.34	-8.38 ± 0.11	31.3 ± 3.6	14	2.04	-7.36 ± 0.10	85.5 ± 4.3	5	-6.06 ± 0.11	11.9 ± 1.6	5	-6.18 ± 0.11	1.98 ± 0.14	5
Tizanidine	-5.46	-7.44 ± 0.09	53.5 ± 1.6	10	1.98	-7.43 ± 0.10	82.1 ± 3.9	6	-5.78 ± 0.06	65.6 ± 6.5	5	-6.91 ± 0.09	2.36 ± 0.10	5
Moxonidine	-4.54	-6.51 ± 0.18	59.3 ± 4.5	7	1.97	-6.85 ± 0.08	78.0 ± 7.2	5	ND	ND	5	ND	ND	5
Guanfacine	-5.33	-7.28 ± 0.10	56.8 ± 2.2	7	1.95	-7.46 ± 0.05	87.4 ± 2.3	7	-5.54 ± 0.09	41.1 ± 2.3	5	-6.81 ± 0.13	2.22 ± 0.05	5
Dexmedetomidine	-5.88	-7.79 ± 0.10	57.3 ± 2.8	8	1.91	-7.86 ± 0.12	81.7 ± 4.4	5	-6.29 ± 0.05	57.6 ± 5.7	5	-7.07 ± 0.08	2.13 ± 0.09	5
Tetrahydrozoline	-5.93	-7.83 ± 0.12	52.4 ± 4.5	5	1.90	-7.50 ± 0.05	83.3 ± 7.1	6	-6.10 ± 0.07	34.7 ± 1.3	5	-6.79 ± 0.07	2.03 ± 0.08	5
Allylphenylamine	-6.79	-8.53 ± 0.11	58.6 ± 3.0	8	1.74	-8.80 ± 0.10	79.3 ± 3.5	5	-7.05 ± 0.05	82.4 ± 5.5	5	-8.10 ± 0.08	2.38 ± 0.08	5
Xylometazoline	-6.94	-8.60 ± 0.13	64.4 ± 2.7	6	1.66	-8.42 ± 0.10 <sup>b</sup>	98.8 ± 5.1	6	-6.90 ± 0.06	32.4 ± 5.7	5	-7.52 ± 0.13	2.00 ± 0.09	5

(Continues)



TABLE 2 (Continued)

	<sup>3</sup> H-prazosin binding		Intracellular calcium release		Log efficacy ratio	ERK1/2-phosphorylation		cAMP accumulation		cAMP accumulation (in presence of forskolin)				
	Log K <sub>D</sub>	Log EC <sub>50</sub>	% ionomycin	n		K <sub>D</sub> /EC <sub>50</sub>	Log EC <sub>50</sub>	% PDBu	n	Log EC <sub>50</sub>	% forskolin	n	Log EC <sub>50</sub>	Fold increase
UK14304	-5.53	-7.17 ± 0.07	45.6 ± 1.6	7	1.64	-6.73 ± 0.11	88.7 ± 4.3	5	10 μM	12.0 ± 1.1	5	-5.86 ± 0.07	1.95 ± 0.09	5
Brimonidine	-5.36	-6.93 ± 0.10	47.8 ± 4.1	8	1.57	-6.44 ± 0.08	86.4 ± 3.6	5	-5.24 ± 0.04	17.8 ± 1.7	5	-5.80 ± 0.07	1.96 ± 0.05	5
Isoprenaline	-4.07	-5.58 ± 0.14	42.5 ± 3.7	11	1.51	-5.21 ± 0.12	72.1 ± 10.6	6	-4.09 ± 0.05	16.2 ± 3.0	5	-4.25 ± 0.04	2.22 ± 0.15	5
Xylazine	-4.48	-5.92 ± 0.13	45.1 ± 2.2	9	1.44	-5.69 ± 0.11	78.6 ± 5.8	6	ND					
Ephedrine	-4.07	-5.50 ± 0.11	43.1 ± 1.6	9	1.43	-5.12 ± 0.07	72.7 ± 6.5	7	-3.93 ± 0.09	7.8 ± 1.3	5	-4.15 ± 0.09	1.70 ± 0.10	5
Eforaxan	-5.47	-6.83 ± 0.10	45.0 ± 2.6	6	1.36	-6.12 ± 0.07	76.4 ± 5.2	6	-5.47 ± 0.10	4.0 ± 0.6	5	-5.93 ± 0.13	1.47 ± 0.13	5
BRL 44408	-5.92	-7.24 ± 0.10	36.5 ± 5.4	7	1.32	-7.03 ± 0.14	77.4 ± 4.1	6	-6.27 ± 0.17	4.7 ± 0.5	5	-6.43 ± 0.09	1.62 ± 0.08	5
Fenoterol	-5.29	-6.51 ± 0.09	30.3 ± 7.6	3	1.22	-5.69 ± 0.09	33.8 ± 6.7	6	No response		5	No response		5
Detomidine	-6.85	-8.05 ± 0.09	47.7 ± 1.8	8	1.20	-8.05 ± 0.05	84.2 ± 3.8	6	-6.85 ± 0.05	17.3 ± 1.9	5	-7.44 ± 0.06	1.95 ± 0.13	5
Rilmenidine	-4.49	-5.68 ± 0.07	45.9 ± 3.8	7	1.19	10 μM	89.0 ± 3.9	6	ND					
PF3774076	-6.89	-7.94 ± 0.06	53.7 ± 4.1	5	1.05	-7.80 ± 0.11	83.9 ± 4.5	5	-7.04 ± 0.06	7.3 ± 1.4	5	-7.34 ± 0.07	1.66 ± 0.11	5
CGP 12177	-5.14	-6.18 ± 0.10	37.0 ± 2.8	5	1.04	-6.07 ± 0.12 <sup>b</sup>	86.8 ± 2.6	5	ND					
BHT920	-4.70	-5.68 ± 0.09	39.7 ± 3.3	6	0.98	-5.14 ± 0.05	61.4 ± 5.5	5	ND					
Idazoxan	-5.67	-6.50 ± 0.12	25.9 ± 2.1	10	0.83	-6.21 ± 0.09	64.9 ± 2.4	6	-5.45 ± 0.07	2.6 ± 0.4	5	-5.68 ± 0.18	1.33 ± 0.06	5
Atipamezole	-5.99	-6.61 ± 0.12	44.5 ± 2.6	7	0.62	-7.00 ± 0.08	87.1 ± 5.0	5	-5.93 ± 0.01	14.5 ± 2.6	5	-6.32 ± 0.12	2.00 ± 0.10	5
Labetolol	-7.33	-7.90 ± 0.11	36.9 ± 3.7	7	0.57	-7.51 ± 0.19 <sup>b</sup>	71.3 ± 5.2	5	-7.45 ± 0.09	4.5 ± 0.6	5	-7.39 ± 0.03	1.52 ± 0.08	5
Guanabenz	-6.48	-6.96 ± 0.13	17.8 ± 3.2	8	0.48	-6.69 ± 0.14	68.4 ± 8.6	6	-5.74 ± 0.21	2.1 ± 0.3	5	-6.84 ± 0.12	1.48 ± 0.07	5
BMY7378	-6.61	-7.04 ± 0.13	25.0 ± 3.4	8	0.43	-6.81 ± 0.13	29.0 ± 4.7	5	No response		5	100 μM	1.37 ± 0.04	5
Buspirone	-6.02	-6.43 ± 0.03	46.7 ± 3.0	8	0.41	-6.19 ± 0.08	47.3 ± 10.6	5	-5.38 ± 0.13	3.3 ± 0.06	5	-4.96 ± 0.13	1.93 ± 0.15	5
Sunepitriton	-5.78	-6.17 ± 0.14	30.1 ± 3.0	9	0.39	-5.96 ± 0.12	39.7 ± 8.4	7	ND					
Lisuride	-7.94	-8.19 ± 0.11	26.4 ± 2.4	8	0.25	-7.50 ± 0.18 <sup>b</sup>	75.2 ± 6.4	6	No response		5	No response		5
CHLOROETHYLCLONIDINE	-5.43	-5.57 ± 0.09	38.4 ± 2.8	6	0.14	-6.51 ± 0.14	66.5 ± 6.4	5	ND					
2-PMDQ	-8.19	-7.63 ± 0.13	16.6 ± 1.8	8	-0.56	-8.18 ± 0.12	10.4 ± 3.4	6	No response		5	No response		5
ARC 239	-9.35	-7.99 ± 0.19	20.8 ± 2.0	8	-1.36	-8.36 ± 0.08	29.2 ± 3.8	5	-8.41 ± 0.23	1.2 ± 0.2	5	100 μM	1.45 ± 0.06	5
2-MPMDQ	-9.06	-7.69 ± 0.08	19.7 ± 2.0	7	-1.37	-8.27 ± 0.12	13.6 ± 3.7	5	No response		5	No response		5
Bromocryptine	-8.73	-7.31 ± 0.07	20.0 ± 3.0	6	-1.42	-7.26 ± 0.16	61.2 ± 10.3	5	No response		5	No response		5
3-MPPI	-9.57	-7.79 ± 0.12	21.9 ± 3.6	7	-1.78	-8.25 ± 0.17	15.8 ± 4.4	6	No response		5	No response		5
RWJ52353	-5.28	10 μM	39.4 ± 2.4	5	ND	ND			ND			ND		
Salmeterol	-6.11	10 μM	28.2 ± 2.7	6	ND	ND			ND			ND		

(Continues)

TABLE 2 (Continued)

CHO- $\alpha$ 1A	$^3\text{H}$ -prazosin binding		Intracellular calcium release		Log efficacy ratio	ERK1/2-phosphorylation		cAMP accumulation		cAMP accumulation (in presence of forskolin)		
	Log $K_D$	Log $EC_{50}$	% ionomycin	n		$K_D/EC_{50}$	Log $EC_{50}$	% PDBu	n	Log $EC_{50}$	n	Fold increase
BHT-933	-3.60	100 $\mu\text{M}$	25.5 $\pm$ 3.7	5	ND	ND	ND	ND	ND	ND	ND	ND
T-CG 1000	-5.96	10 $\mu\text{M}$	21.0 $\pm$ 1.9	5	ND	ND	ND	ND	ND	ND	ND	ND
Formoterol	-5.82	10 $\mu\text{M}$	15.0 $\pm$ 4.2	9	-6.84 $\pm$ 0.20	42.0 $\pm$ 8.0	6	No response	5	No response	5	No response
Dihydroergotamine	-8.62	10 $\mu\text{M}$	10.0 $\pm$ 3.1	5	-8.18 $\pm$ 0.13 <sup>b</sup>	57.2 $\pm$ 4.6	7	No response	5	No response	5	No response
Amitraz	-5.52	No response	No response	5	ND	ND	ND	ND	ND	ND	ND	ND
Salbutamol	-3.84	No response	No response	5	100 $\mu\text{M}$	4.3 $\pm$ 1.9	5	ND	ND	ND	ND	ND
Methyldopa	No binding	No response	No response	5	ND	ND	ND	ND	ND	ND	ND	ND

Note: Values represent mean  $\pm$  SEM of  $n$  separate experiments. The ligands are arranged in order of intrinsic efficacy ratio as determined from the calcium response ( $EC_{50}$ ) and binding ( $K_D$ ).

Abbreviations: ND, not determined.

<sup>a</sup>These compounds had a bi-phasic response. Log  $EC_{50}$  and % PDBu given for initial stimulatory part of response.

<sup>b</sup>These compounds stimulate ERK1/2-phosphorylation in parent CHO cells, see Supplementary data Table S1, Figure S1; however, the responses to oxymetazoline, xylometazoline, and labetolol are more than 10-fold more potent than the responses on the untransfected cells, so are likely to be  $\alpha$ 1-adrenoceptor mediated.

$$\log \text{efficacy ratio} = \frac{\log K_D}{\log EC_{50}}. \quad (5)$$

## 2.12 | 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 (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.<sup>29</sup>

## 3 | RESULTS

### 3.1 | Determination of ligand affinity from $^3\text{H}$ -prazosin whole-cell binding

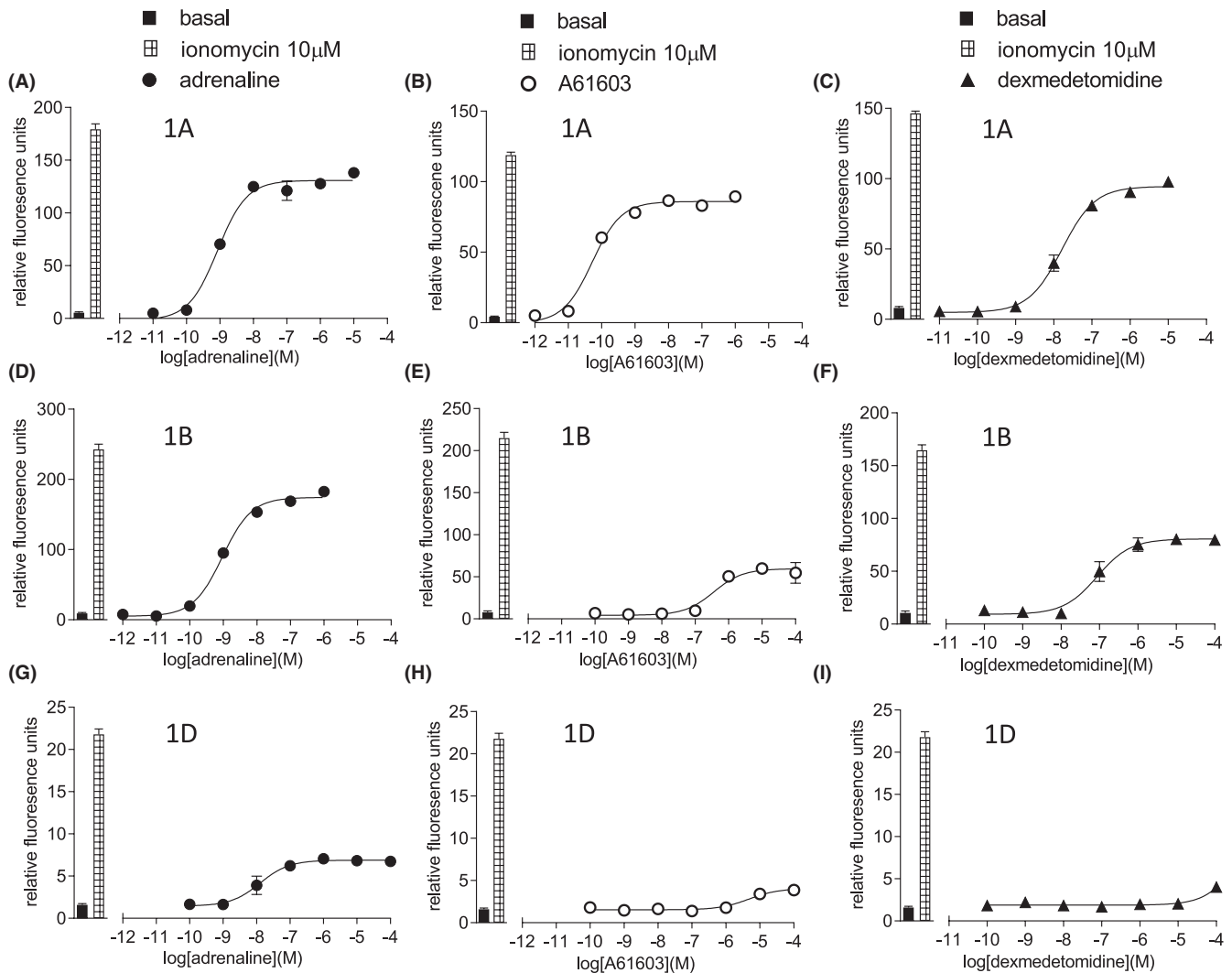
The affinity ( $K_D$ ) for  $^3\text{H}$ -prazosin has previously been determined in these cell lines as 0.71, 0.87, and 1.90 nM for the  $\alpha$ 1A,  $\alpha$ 1B, and  $\alpha$ 1D-adrenoceptor, respectively, with receptor expression levels of 1152fmol/mg protein, 4350fmol/mg protein, and 417fmol/mg protein, respectively.<sup>28</sup> The  $\alpha$ 1D-adrenoceptor is the full-length receptor and is associated with lower levels of expression than either  $\alpha$ 1A or  $\alpha$ 1B-adrenoceptor expression, or an N-terminal truncated  $\alpha$ 1D-adrenoceptor.<sup>30-33</sup> As expected therefore, the window of specific binding was smaller in the CHO- $\alpha$ 1D cells than the CHO- $\alpha$ 1A or CHO- $\alpha$ 1B cells (Figure 1).  $^3\text{H}$ -prazosin whole-cell binding studies yielded an affinity (log  $K_D$ ) for adrenaline of -5.09 in CHO- $\alpha$ 1A cells, -3.94 in CHO- $\alpha$ 1B cells, and -5.19 in CHO- $\alpha$ 1D cells (Table 1, Figure 1). As expected, many agonists had relatively low affinity for the  $\alpha$ 1-adrenoceptors (Table 1, Figure 1). A61603 was the most selective agonist with an  $\alpha$ 1A-adrenoceptor selective binding affinity of over 660-fold (Table 1, Figure 1).

### 3.2 | Free intracellular calcium mobilization

As all three  $\alpha$ 1-adrenoceptors are primarily Gq-coupled receptors, intracellular calcium mobilization was studied. In CHO- $\alpha$ 1A cells, adrenaline stimulated an increase in intracellular calcium (log  $EC_{50}$  = -9.09) that was 58.9% that of the response to 10  $\mu\text{M}$  ionomycin (Table 2, Figure 2). This gave adrenaline an efficacy ratio of 4.00 making it the ligand with the greatest intrinsic efficacy at the  $\alpha$ 1A-adrenoceptor (Table 2). A similar pattern was seen in CHO- $\alpha$ 1B and CHO- $\alpha$ 1D cells (Tables 3 and 4, respectively).

### 3.3 | ERK1/2-phosphorylation

Adrenaline stimulated an increase in ERK1/2-phosphorylation in CHO  $\alpha$ 1A cells that was best described by a two-component response. After an initial increase in ERK1/2-phosphorylation (log  $EC_{50}$



**FIGURE 2** Intracellular calcium mobilization in CHO- $\alpha$ 1A cells (A–C), CHO- $\alpha$ 1B cells (D–F), or CHO- $\alpha$ 1D cells (G–I) in response to adrenaline (A, D, G), A61603 (B, E, H) or dexmedetomidine (C, F, I). Bars represent basal intracellular calcium release and that in response to 10  $\mu$ M ionomycin alone. Data points are mean  $\pm$  SEM of triplicate determinations

–7.74, 71.3% response of 10  $\mu$ M PDBu, Table 2), higher concentrations of adrenaline stimulated a lower total ERK1/2-phosphorylation (Figure 3). This bi-phasic dose–response pattern was seen for several of the ligands (Table 2). In CHO- $\alpha$ 1B cells, adrenaline stimulated a similar bi-phasic ERK1/2-phosphorylation response; however, only a single component response was seen in CHO- $\alpha$ 1D cells (Tables 3 and 4).

### 3.4 | $^3\text{H}$ -cAMP accumulation

Adrenaline stimulated an increase in  $^3\text{H}$ -cAMP accumulation in CHO- $\alpha$ 1A cells (log  $\text{EC}_{50}$  –5.63) that was 164% of the response seen to 10  $\mu$ M forskolin (Figure 4, Table 2). This response is significantly right-shifted when compared with the stimulatory adrenaline-induced calcium mobilization and ERK1/2-phosphorylation responses in these cells. To look for Gi-mediated inhibition of cAMP, the ability of ligands to inhibit forskolin-stimulated cAMP was examined.

In CHO- $\alpha$ 1A cells, adrenaline did not inhibit cAMP accumulation (suggesting no Gi-coupled response, Figure 5, Table 2). However, the stimulatory response was still seen and if anything, augmented, most likely as a result of forskolin augmentation of the Gs-coupled response (as seen in [34, 35]). Responses were also observed in the CHO- $\alpha$ 1B and CHO- $\alpha$ 1D cells (Figures 4 and 5, Tables 3 and 4).

### 3.5 | Responses in parent CHO cells without the transfected receptors

There were no measurable intracellular calcium mobilization dose responses in response to any of the agonists in the parent (untransfected) CHO cells (Table S1). A few compounds had a higher than basal stimulation at the highest concentration only and are given in Table S1. Oxymetazoline, xylometazoline, dihydroergotamine, lisuride, labetalol, and CGP 12177 stimulated ERK1/2-phosphorylation responses in the parent CHO cells (Table S1,

**TABLE 3** Log  $K_D$  values from  $^3\text{H}$ -prazosin whole-cell binding (from Table 1), log  $\text{EC}_{50}$  values, and % ionomycin maximal responses obtained from intracellular calcium mobilization and intrinsic efficacy ratios ( $K_D/\text{EC}_{50, \text{calcium}}$ ) obtained from CHO- $\alpha 1\text{B}$  cells. The log  $\text{EC}_{50}$  values obtained from ERK1/2-phosphorylation (and % PDBu responses), and those obtained from cAMP accumulation (and % forskolin maximum response), and cAMP accumulation in the presence of forskolin (with fold increase of that response) are also given

CHO- $\alpha 1\text{B}$	$^3\text{H}$ -prazosin binding	Intracellular calcium release			Log efficacy ratio	ERK1/2-phosphorylation			cAMP accumulation (in presence of forskolin)					
		Log $\text{EC}_{50}$	% ionomycin	n		$K_D/\text{EC}_{50}$	Log $\text{EC}_{50}$	% PDBu	n	Log $\text{EC}_{50}$	% forskolin	n	Log $\text{EC}_{50}$	Fold increase
Adrenaline	-3.94	-9.41 ± 0.13	59.8 ± 3.3	9	5.47	-7.60 ± 0.15 <sup>a</sup>	76.6 ± 9.0	6	-5.43 ± 0.02	172.1 ± 11.3	5	-5.60 ± 0.11	3.14 ± 0.09	5
Noradrenaline	-3.79	-9.23 ± 0.10	62.5 ± 1.2	8	5.44	-7.62 ± 0.04 <sup>a</sup>	75.0 ± 8.8	6	-5.46 ± 0.02	155.5 ± 7.4	5	-5.93 ± 0.10	2.99 ± 0.06	5
R-phenylephrine	-3.87	-9.04 ± 0.10	67.0 ± 3.3	6	5.17	-7.84 ± 0.10	77.0 ± 4.8	8	-6.11 ± 0.07	86.2 ± 5.3	5	-7.40 ± 0.11	2.43 ± 0.08	5
$\alpha$ -Methylnorepinephrine	$\text{IC}_{50} > -3$	-8.10 ± 0.11	69.1 ± 2.6	8	>5.10	-6.75 ± 0.13	90.6 ± 4.4	9	100 $\mu\text{M}$	129.7 ± 20.5	5	-5.56 ± 0.05	3.09 ± 0.21	5
Etilefrine	$\text{IC}_{50} > -3$	-7.86 ± 0.14	61.1 ± 2.1	9	>4.86	-6.52 ± 0.11	78.8 ± 6.1	7	100 $\mu\text{M}$	73.4 ± 7.1	5	-6.18 ± 0.13	2.47 ± 0.09	5
Dopamine	-2.89	-7.15 ± 0.08	62.5 ± 2.9	6	4.26	-5.83 ± 0.12	85.8 ± 6.0	7	-4.59 ± 0.08	53.0 ± 7.5	5	-5.26 ± 0.02	3.06 ± 0.14	5
Synephrine	$\text{IC}_{50} > -3$	-7.09 ± 0.12	57.5 ± 1.9	5	>4.09	-5.78 ± 0.08	95.1 ± 8.7	6	100 $\mu\text{M}$	23.2 ± 2.5	5	-5.94 ± 0.09	2.38 ± 0.17	5
Metaraminol	$\text{IC}_{50} > -3$	-6.95 ± 0.11	60.1 ± 3.1	7	>3.95	-5.84 ± 0.15	92.2 ± 3.9	6	100 $\mu\text{M}$	42.0 ± 5.1	5	-5.83 ± 0.08	2.58 ± 0.07	5
ST-91	-4.39	-8.13 ± 0.17	62.0 ± 2.1	11	3.74	-7.33 ± 0.12	83.6 ± 8.9	6	-6.52 ± 0.04	30.4 ± 3.5	5	-7.23 ± 0.07	2.34 ± 0.09	5
Guanfacine	-3.87	-7.57 ± 0.07	59.0 ± 2.4	7	3.70	-6.71 ± 0.14	97.4 ± 2.7	7	-5.79 ± 0.04	39.0 ± 5.8	5	-6.49 ± 0.14	2.67 ± 0.18	5
Methoxamine	$\text{IC}_{50} > -3$	-6.64 ± 0.08	59.1 ± 3.2	7	>3.64	-5.55 ± 0.09	83.8 ± 5.7	7	100 $\mu\text{M}$	24.2 ± 4.6	5	-5.16 ± 0.12	2.42 ± 0.05	5
Isoprenaline	-2.80	-6.16 ± 0.13	53.9 ± 3.3	9	3.36	-5.36 ± 0.10	77.8 ± 8.5	7	ND	ND	ND	ND	ND	ND
Moxonidine	-3.47	-6.49 ± 0.05	57.9 ± 2.1	7	3.02	-6.04 ± 0.15	102.0 ± 6.5	6	ND	ND	ND	ND	ND	ND
Cirazoline	-5.10	-8.05 ± 0.10	53.3 ± 1.5	6	2.95	-6.92 ± 0.11	82.5 ± 8.6	8	-6.92 ± 0.15	11.4 ± 2.4	5	-7.40 ± 0.10	2.09 ± 0.13	5
Octopamine	$\text{IC}_{50} > -3$	-5.91 ± 0.13	54.9 ± 2.9	6	>2.91	-5.11 ± 0.19	76.1 ± 6.8	6	ND	ND	ND	ND	ND	ND
Tizanidine	-4.35	-7.05 ± 0.08	48.8 ± 1.8	12	2.70	-6.54 ± 0.15	57.7 ± 5.3	6	-6.15 ± 0.17	10.7 ± 1.5	5	-6.74 ± 0.11	2.01 ± 0.11	5
Clonidine	-5.13	-7.77 ± 0.14	45.4 ± 2.1	8	2.64	-7.05 ± 0.12	60.6 ± 7.6	6	-6.60 ± 0.20	7.5 ± 0.8	4	-7.35 ± 0.10	1.67 ± 0.16	5
Para-amino-clonidine	-5.17	-7.79 ± 0.05	33.7 ± 2.4	8	2.62	-7.25 ± 0.08	32.8 ± 6.9	6	-7.48 ± 0.43	2.5 ± 0.6	5	-7.31 ± 0.11	1.46 ± 0.04	5
A61603	$\text{IC}_{50} > -4$	-6.52 ± 0.08	35.7 ± 3.7	10	>2.52	-5.75 ± 0.11	54.5 ± 7.9	9	-5.63 ± 0.18	5.3 ± 0.5	5	-6.16 ± 0.09	1.79 ± 0.05	5
Fenoterol	-3.91	-6.27 ± 0.10	38.0 ± 3.2	6	2.36	-5.56 ± 0.20	33.8 ± 7.3	7	ND	ND	ND	ND	ND	ND
Brimonidine	-4.68	-7.01 ± 0.11	35.8 ± 2.4	8	2.33	-6.14 ± 0.08	41.2 ± 6.2	6	-6.89 ± 0.12	4.5 ± 1.6	5	-6.98 ± 0.09	1.58 ± 0.07	5
Naphazoline	-5.74	-8.03 ± 0.10	38.9 ± 2.9	8	2.29	-6.82 ± 0.09	71.1 ± 7.2	9	-7.89 ± 0.17	3.9 ± 0.7	4	-8.26 ± 0.11	1.65 ± 0.07	5
Dexmedetomidine	-5.04	-7.33 ± 0.06	43.6 ± 2.1	8	2.29	-6.88 ± 0.14	55.4 ± 7.4	6	-7.05 ± 0.15	3.7 ± 0.6	5	-7.48 ± 0.10	1.60 ± 0.07	5
UK14304	-4.89	-7.15 ± 0.05	27.5 ± 1.8	5	2.26	-6.50 ± 0.15	33.8 ± 5.0	6	-7.02 ± 0.18	2.7 ± 0.1	5	-7.01 ± 0.15	1.50 ± 0.05	5
Oxymethazoline	-5.17	-7.42 ± 0.11	27.5 ± 3.6	7	2.25	-7.33 ± 0.11 <sup>b</sup>	67.9 ± 11.3	6	-6.66 ± 0.08	1.4 ± 0.4	4	-6.53 ± 0.12	1.20 ± 0.04	5
Salbutamol	$\text{IC}_{50} > -3$	-5.14 ± 0.11	58.4 ± 3.0	5	>2.14	100 $\mu\text{M}$	7.1 ± 3.5	5	ND	ND	ND	ND	ND	ND

(Continues)

TABLE 3 (Continued)

CHO- $\alpha$ 1B	<sup>3</sup> H-prazosin binding	Intracellular calcium release			Log efficacy ratio	ERK1/2-phosphorylation			cAMP accumulation			cAMP accumulation (in presence of forskolin)		
		Log EC <sub>50</sub>	% ionomycin	n		K <sub>D</sub> /EC <sub>50</sub>	Log EC <sub>50</sub>	% PDBu	n	Log EC <sub>50</sub>	% forskolin	n	Log EC <sub>50</sub>	Fold increase
Xylazine	-3.22	-5.33 ± 0.14	30.4 ± 3.9	8	2.11	-5.17 ± 0.14	26.4 ± 5.8	6	ND	ND	ND	ND	ND	ND
Medetomidine	-4.84	-6.93 ± 0.13	43.0 ± 3.4	8	2.09	-6.86 ± 0.06	55.2 ± 5.3	6	-6.94 ± 0.19	4.6 ± 0.9	5	-7.03 ± 0.06	1.52 ± 0.04	5
Formoterol	-4.47	-6.56 ± 0.14	18.6 ± 1.6	9	2.09	No response	No response	6	No response	No response	5	-6.55 ± 0.13	1.27 ± 0.02	5
Idazoxan	-4.88	-6.95 ± 0.12	25.7 ± 3.0	10	2.07	-6.18 ± 0.11	31.3 ± 2.2	5	-6.76 ± 0.23	2.4 ± 0.3	4	-6.84 ± 0.06	1.50 ± 0.04	5
Allyphenylne	-5.11	-7.10 ± 0.07	39.9 ± 1.9	5	1.99	-6.55 ± 0.12	65.3 ± 3.0	6	-6.76 ± 0.12	5.5 ± 0.9	5	-7.29 ± 0.10	1.87 ± 0.07	5
Detomidine	-5.65	-7.49 ± 0.11	33.8 ± 1.7	7	1.84	-7.17 ± 0.09	31.5 ± 5.4	6	-7.90 ± 0.09	2.8 ± 0.6	5	-7.89 ± 0.15	1.57 ± 0.07	5
Buspirone	-4.65	-6.42 ± 0.09	47.8 ± 3.2	5	1.77	-6.05 ± 0.18	47.2 ± 8.6	6	ND	ND	ND	ND	ND	ND
Guanabenz	-5.45	-7.03 ± 0.12	33.7 ± 4.7	8	1.58	-5.86 ± 0.18	44.9 ± 7.9	6	-7.12 ± 0.13	2.3 ± 0.8	5	-7.09 ± 0.18	1.49 ± 0.05	5
Lisuride	-6.07	-7.23 ± 0.22	15.8 ± 2.2	12	1.16	-6.51 ± 0.16 <sup>b</sup>	73.1 ± 8.0	5	No response	No response	5	No response	No response	5
Rilmenidine	IC <sub>50</sub> > -3	100 μM	20.0 ± 3.9	5	No response	No response	No response	5	ND	ND	ND	ND	ND	ND
Epedrine	IC <sub>50</sub> > -3	100 μM	18.5 ± 2.9	9	-4.67 ± 0.17	19.1 ± 4.0	7	ND	ND	ND	ND	ND	ND	ND
Atipamezole	-4.68	100 μM	18.1 ± 4.8	5	No response	No response	5	ND	ND	ND	ND	ND	ND	ND
Midodrine	No binding	100 μM	16.9 ± 2.0	10	100 μM	20.4 ± 8.0	6	ND	ND	ND	ND	ND	ND	ND
BHT933	No binding	100 μM	16.6 ± 2.1	6	ND	ND	6	ND	ND	ND	ND	ND	ND	ND
BHT920	-3.73	100 μM	16.3 ± 4.5	6	-5.31 ± 0.04	12.2 ± 3.6	6	ND	ND	ND	ND	ND	ND	ND
Salmeterol	-5.13	10 μM	15.2 ± 4.0	5	ND	ND	5	ND	ND	ND	ND	ND	ND	ND
BRL44408	-4.68	100 μM	13.7 ± 6.5	5	100 μM	47.0 ± 7.0	6	ND	ND	ND	ND	ND	ND	ND
Xylometazoline	-5.16	100 μM	13.6 ± 3.3	8	-6.71 ± 0.16 <sup>b</sup>	66.2 ± 6.0	6	No response	No response	5	-8.04 ± 0.18 <sup>c</sup>	13.5 ± 1.8% <sup>a</sup>	5	
Chloroethylclonidine	-5.35	100 μM	10.2 ± 2.0	5	No response	No response	6	ND	ND	ND	ND	ND	ND	ND
RWJ52353	IC <sub>50</sub> > -3	10 μM	8.1 ± 3.1	5	ND	ND	5	ND	ND	ND	ND	ND	ND	ND
BMY7378	-6.23	100 μM	8.0 ± 2.1	9	No response	No response	6	ND	ND	ND	ND	ND	ND	ND
Sunepitron	-4.65	100 μM	8.2 ± 1.3	9	?-6.36 ± 0.29	7.8 ± 2.8	7	ND	ND	ND	ND	ND	ND	ND
Dihydroergotamine	-6.92	10 μM	6.4 ± 2.1	5	-7.85 ± 0.13 <sup>b</sup>	50.1 ± 4.8	6	ND	ND	ND	ND	ND	ND	ND
PF3774076	-4.74	10 μM	5.2 ± 1.7	5	No response	No response	6	ND	ND	ND	ND	ND	ND	ND
ARC239	-8.15	100 μM	4.8 ± 1.7	5	No response	No response	5	ND	ND	ND	ND	ND	ND	ND
3-MPPI	-8.59	100 μM	4.6 ± 1.9	7	No response	No response	5	ND	ND	ND	ND	ND	ND	ND
2-MPMDQ	-7.37	100 μM	3.1 ± 1.5	5	No response	No response	5	ND	ND	ND	ND	ND	ND	ND
Dobutamine	-5.44	100 μM	3.0 ± 1.1	8	100 μM	56.3 ± 11.3	6	No response	No response	5	-5.70 ± 0.07	1.24 ± 0.04	5	
Tetrohydrozoline	-5.22	100 μM	2.9 ± 1.4	5	No response	No response	6	ND	ND	ND	ND	ND	ND	ND

(Continues)

TABLE 3 (Continued)

CHO- $\alpha$ 1B	<sup>3</sup> H-prazosin binding	Intracellular calcium release		Log efficacy ratio	ERK1/2-phosphorylation			cAMP accumulation			cAMP accumulation (in presence of forskolin)			
		Log EC <sub>50</sub>	% ionomycin		n	K <sub>D</sub> /EC <sub>50</sub>	Log EC <sub>50</sub>	% PDBu	n	Log EC <sub>50</sub>	% forskolin	n	Log EC <sub>50</sub>	Fold increase
Labetolol	-5.91	100 $\mu$ M	2.5 $\pm$ 1.2	7		-5.42 $\pm$ 0.14 <sup>b</sup>	22.9 $\pm$ 6.3	6	No response	5	No response	5	No response	5
CGP 12177	IC <sub>50</sub> > -4	No response	No response	5		-5.98 $\pm$ 0.12 <sup>b</sup>	38.1 $\pm$ 3.8	6	ND	ND	ND	ND	ND	ND
Eforaxan	-4.27	No response	No response	5		100 $\mu$ M	11.9 $\pm$ 2.4	7	ND	ND	ND	ND	ND	ND
T-CG 1000	-5.18	No response	No response	6		ND	ND	7	ND	ND	ND	ND	ND	ND
2-PMDQ	-6.95	No response	No response	5		No response	No response	7	ND	ND	ND	ND	ND	ND
Amitraz	IC <sub>50</sub> > -4	No response	No response	5		ND	ND	5	ND	ND	ND	ND	ND	ND
Methylidopa	No binding	No response	No response	5		ND	ND	5	ND	ND	ND	ND	ND	ND

Note: Values represent mean  $\pm$  SEM of *n* separate experiments. The ligands are arranged in order of intrinsic efficacy ratio as determined from the calcium response (EC<sub>50</sub>) and binding (K<sub>D</sub>). Abbreviation: ND, not determined.

<sup>a</sup>These compounds had a bi-phasic response. Log EC<sub>50</sub> and % PDBu given for initial stimulatory part of response.

<sup>b</sup>These compounds stimulate ERK1/2-phosphorylation in parent CHO cells, see Supplementary data Table S1, Figure S1.

<sup>c</sup>Xylometazoline caused a decrease in forskolin-stimulated cAMP accumulation. The data given are log IC<sub>50</sub> and % inhibition of forskolin-stimulated cAMP.

Figure S1). Oxymetazoline and xylometazoline responses had >10-fold higher potency in the CHO- $\alpha$ 1A cells, suggesting these responses may be  $\alpha$ 1A-receptor mediated. All other ERK1/2-phosphorylation responses to these six ligands are similar in parent cells, CHO- $\alpha$ 1A, CHO- $\alpha$ 1B, and CHO- $\alpha$ 1D cells and are likely non- $\alpha$ 1-receptor mediated. Oxymetazoline and xylometazoline both resulted in a decrease in cAMP accumulation in the presence of forskolin in the parent cells and CHO- $\alpha$ 1D cells and for xylometazoline (less efficacious than oxymetazoline) in the CHO- $\alpha$ 1B. This cAMP inhibition was also not  $\alpha$ 1-mediated. The stimulatory cAMP responses to oxymetazoline and xylometazoline in CHO- $\alpha$ 1A, and the stimulatory response to oxymetazoline in the CHO- $\alpha$ 1B cells are therefore likely  $\alpha$ -adrenoceptor mediated. These six compounds are not included in the calcium mobilization versus ERK1/2-phosphorylation correlation plots in Figure 6A-C.

### 3.6 | Correlation plots

In order to examine for any evidence of bias signaling, the log EC<sub>50</sub> values for calcium mobilization were correlated with those for ERK1/2-phosphorylation (Figure 6A-C). This suggests little evidence for biased signaling between these two responses at any of the  $\alpha$ 1-adrenoceptor subtypes. To examine for potential calcium-cAMP-bias, a similar plot was constructed for calcium versus cAMP accumulation. Here, data are plotted for the augmented cAMP accumulation in the presence of forskolin as this has more ligands with measurable agonist responses and the only method by which  $\alpha$ 1D-cAMP responses could be measured. Although this 5-h assay has more potential for ligand degradation (especially of the catecholamines), in a similar study of  $\beta$ -adrenoceptor cAMP accumulation, potency (measured at 10, 30 min, and 5 h) for both catecholamines and synthetic ligands remained the same, suggesting little loss of response due to ligand degradation.<sup>5</sup> There was also little evidence for calcium versus cAMP accumulation bias at the  $\alpha$ 1A-adrenoceptor. There was however some scatter from the line of best fit for the  $\alpha$ 1B-adrenoceptor, suggesting some potential biased signaling. For example  $\alpha$ -methylnorepinephrine and naphazoline had similar calcium responses (log EC<sub>50</sub> -8.10 and -8.03, respectively) but rather different cAMP accumulation responses (log EC<sub>50</sub> -5.56 and -8.26, respectively).

Finally returning to a major aim of the study - to look for any evidence of intrinsic efficacy selectivity - the efficacy ratios for calcium release were compared for  $\alpha$ 1A and  $\alpha$ 1B (Figure 6g) and  $\alpha$ 1A and  $\alpha$ 1D (Figure 6h). Here, dobutamine was the ligand furthest from the line of best fit suggesting it has some  $\alpha$ 1D-selective efficacy relative to that seen at the  $\alpha$ 1A or  $\alpha$ 1B-adrenoceptors.

## 4 | DISCUSSION

This study compared the binding affinity and functional responses of 62 compounds at the human  $\alpha$ 1A,  $\alpha$ 1B,  $\alpha$ 1D-adrenoceptors.  $\alpha$ 1A and  $\alpha$ 1B-adrenoceptors are present in human heart.<sup>13</sup> Although



**TABLE 4** Log  $K_D$  values from  $^3\text{H}$ -prazosin whole-cell binding (from Table 1), log  $\text{EC}_{50}$  values and % ionomycin maximal responses obtained from intracellular calcium mobilization and intrinsic efficacy ratios ( $K_D/\text{EC}_{50 \text{ calcium}}$ ) obtained from CHO- $\alpha 1\text{D}$  cells. The log  $\text{EC}_{50}$  values obtained from ERK1/2-phosphorylation (and % PDBu responses), and those obtained from cAMP accumulation (and % forskolin maximum response), and cAMP accumulation in the presence of forskolin (with fold increase of that response) are also given

CHO- $\alpha 1\text{D}$	3H-prazosin binding		Intracellular calcium release		Log efficacy ratio		ERK1/2-phosphorylation		cAMP		cAMP (in presence of forskolin)		
	Log $K_D$	Log $\text{EC}_{50}$	% Ionomycin	n	$K_D/\text{EC}_{50}$	n	Log $\text{EC}_{50}$	% PDBu	Log $\text{EC}_{50}$	n	Log $\text{EC}_{50}$	Fold increase	n
R-phenylephrine	-4.65	-7.20 $\pm$ 0.10	27.8 $\pm$ 4.4	7	2.55	7	-6.17 $\pm$ 0.13	15.8 $\pm$ 2.9	No response	5	-6.77 $\pm$ 0.14	1.21 $\pm$ 0.04	5
Adrenaline	-5.31	-7.74 $\pm$ 0.10	30.3 $\pm$ 2.3	12	2.43	12	-6.74 $\pm$ 0.18	32.2 $\pm$ 4.0	No response	10	-6.06 $\pm$ 0.15	1.26 $\pm$ 0.04	10
Noradrenaline	-5.48	-7.77 $\pm$ 0.07	26.6 $\pm$ 3.6	5	2.29	5	-6.56 $\pm$ 0.19	30.0 $\pm$ 5.3	No response	5	-6.17 $\pm$ 0.15	1.20 $\pm$ 0.06	5
DOBUTAMINE	-5.36	-7.62 $\pm$ 0.15	10.6 $\pm$ 1.8	13	2.26	7	-6.05 $\pm$ 0.14	26.2 $\pm$ 7.5	No response	5	-6.51 $\pm$ 0.05	1.16 $\pm$ 0.06	5
octopamine	$\text{IC}_{50} > -3$	-5.20 $\pm$ 0.15	22.3 $\pm$ 4.1	9	>2.20	5	-4.73 $\pm$ 0.11	14.5 $\pm$ 5.9	No response	5	-5.74 $\pm$ 0.23	1.17 $\pm$ 0.06	5
$\alpha$ -MethylInorepinephrine	-4.84	-7.02 $\pm$ 0.10	29.9 $\pm$ 3.6	8	2.18	8	-5.74 $\pm$ 0.11	27.7 $\pm$ 4.9	No response	5	-6.05 $\pm$ 0.12	1.23 $\pm$ 0.06	5
Metaraminol	-4.25	-6.12 $\pm$ 0.13	23.1 $\pm$ 3.8	11	1.87	11	-5.57 $\pm$ 0.19	20.5 $\pm$ 3.0	No response	5	-6.49 $\pm$ 0.07	1.19 $\pm$ 0.04	5
Synephrine	$\text{IC}_{50} > -3.5$	-5.28 $\pm$ 0.14	16.3 $\pm$ 2.9	7	>1.78	11	-5.19 $\pm$ 0.14	14.5 $\pm$ 2.8	No response	4	No response		4
Dopamine	-4.09	-5.78 $\pm$ 0.09	23.5 $\pm$ 1.8	6	1.69	7	-5.13 $\pm$ 0.08	17.7 $\pm$ 4.3	No response	5	-5.34 $\pm$ 0.12	1.22 $\pm$ 0.08	5
Methoxamine	-3.82	-5.38 $\pm$ 0.11	22.6 $\pm$ 4.7	5	1.56	6	-5.08 $\pm$ 0.07	14.2 $\pm$ 3.9	No response	5	-4.98 $\pm$ 0.25	1.21 $\pm$ 0.04	5
Etilefrine	-4.45	-5.87 $\pm$ 0.16	13.7 $\pm$ 1.8	9	1.42	9	-5.82 $\pm$ 0.17	16.8 $\pm$ 2.0	No response	5	-6.12 $\pm$ 0.10	1.18 $\pm$ 0.04	5
A61603	-3.92	-5.29 $\pm$ 0.12	12.0 $\pm$ 3.6	8	1.37	9	100 $\mu\text{M}$	11.7 $\pm$ 2.9	No response	5	No response		5
Cirazoline	-5.53	-6.90 $\pm$ 0.09	17.3 $\pm$ 3.8	7	1.37	12	-5.42 $\pm$ 0.20	36.5 $\pm$ 3.2	No response	5	No response		5
Moxonidine	-3.96	-5.21 $\pm$ 0.11	16.6 $\pm$ 2.9	5	1.25	8	-4.83 $\pm$ 0.19	16.4 $\pm$ 1.6	No response	5	No response		5
Naphazoline	-5.69	-6.93 $\pm$ 0.12	11.8 $\pm$ 2.2	8	1.24	6	-4.86 $\pm$ 0.15	33.0 $\pm$ 4.7	No response	5	No response		5
ST-91	-5.24	-6.34 $\pm$ 0.15	6.3 $\pm$ 1.6	8	1.10	12	-5.23 $\pm$ 0.21	21.1 $\pm$ 4.4	No response	4	No response		4
Allyphenyline	-5.85	-6.50 $\pm$ 0.21	12.3 $\pm$ 1.4	5	0.65	12	-5.16 $\pm$ 0.13	29.1 $\pm$ 4.7	No response	5	No response		5
Guanfacine	-4.93	-5.46 $\pm$ 0.15	28.6 $\pm$ 2.0	5	0.53	7	-5.16 $\pm$ 0.14	16.1 $\pm$ 4.0	No response	5	-6.64 $\pm$ 0.21	1.20 $\pm$ 0.7	5
Oxymethazoline	-5.28	-5.56 $\pm$ 0.22	7.6 $\pm$ 1.0	10	0.28	12	-7.26 $\pm$ 0.13 <sup>b</sup>	48.6 $\pm$ 5.4	No response	5	<sup>s</sup> -8.24 $\pm$ 0.12	*28.7 $\pm$ 2.9%	8
Buspirone	-5.90	-5.89 $\pm$ 0.22	9.0 $\pm$ 1.5	8	-0.01	10	-5.59 $\pm$ 0.18	11.3 $\pm$ 2.5	No response	5	-5.44 $\pm$ 0.25	1.25 $\pm$ 0.06	5
Guanabenz	-6.02	100 $\mu\text{M}$	20.2 $\pm$ 5.1	6		7	100 $\mu\text{M}$	20.8 $\pm$ 6.6	No response	4	No response		4
Xylometazoline	-5.23	100 $\mu\text{M}$	18.6 $\pm$ 2.0	7		11	-6.92 $\pm$ 0.09 <sup>a</sup>	70.8 $\pm$ 7.8	No response	5	-7.53 $\pm$ 0.22 <sup>b</sup>	*26.5 $\pm$ 1.1%	5
Dexmedetomidine	-5.91	100 $\mu\text{M}$	13.1 $\pm$ 1.7	6		5	100 $\mu\text{M}$	19.7 $\pm$ 3.3	No response	5	No response		5
Rilmenidine	-4.73	100 $\mu\text{M}$	12.0 $\pm$ 0.9	5		5	No response		ND	5	ND		
Medetomidine	-5.67	100 $\mu\text{M}$	11.5 $\pm$ 2.3	7		5	-5.14 $\pm$ 0.20	22.6 $\pm$ 5.1	ND	5	ND		
Labetalol	-6.12	100 $\mu\text{M}$	8.1 $\pm$ 1.6	6		7	100 $\mu\text{M}$	36.5 $\pm$ 5.2 <sup>a</sup>	ND	7	ND		
Clonidine	-5.59	100 $\mu\text{M}$	8.0 $\pm$ 0.5	6		7	100 $\mu\text{M}$	6.1 $\pm$ 1.3	ND	7	ND		
Idazoxan	-5.23	100 $\mu\text{M}$	5.7 $\pm$ 0.7	5		5	100 $\mu\text{M}$	2.2 $\pm$ 1.3	ND	5	ND		

(Continues)



TABLE 4 (Continued)

CHO- $\alpha$ 1D	3H-prazosin binding		Intracellular calcium release		Log efficacy ratio $K_D/EC_{50}$	ERK1/2- phosphorylation		cAMP		cAMP (in presence of forskolin)		
	Log $K_D$	Log $EC_{50}$	% Ionomycin	n		Log $EC_{50}$	% PDBu	n	Log $EC_{50}$	n	Log $EC_{50}$	Fold increase
BRL 44408	-5.06	100 $\mu$ M	4.5 $\pm$ 2.1	6		100 $\mu$ M	47.4 $\pm$ 5.7	6	ND	ND	ND	5
Fenoterol	-4.35	100 $\mu$ M	4.4 $\pm$ 1.8	6		-5.40 $\pm$ 0.17	7.6 $\pm$ 1.4	9	No response	No response	No response	5
BMY7378	-8.60 site 1	100 $\mu$ M	4.2 $\pm$ 1.0	6		100 $\mu$ M	3.0 $\pm$ 1.5	5	ND	ND	ND	5
Detomidine	-6.01	100 $\mu$ M	3.8 $\pm$ 0.8	5		100 $\mu$ M	12.3 $\pm$ 2.4	6	ND	ND	ND	5
Xylazine	-4.56	100 $\mu$ M	3.8 $\pm$ 0.8	5		No response		5	ND	ND	ND	5
Isoprenaline	-3.96	100 $\mu$ M	3.6 $\pm$ 1.2	10		-5.20 $\pm$ 0.21	8.5 $\pm$ 1.7	9	No response	No response	No response	5
Eforaxan	-4.97	100 $\mu$ M	2.8 $\pm$ 0.8	5		100 $\mu$ M	2.7 $\pm$ 1.5	5	ND	ND	ND	5
Ephedrine	-3.57	100 $\mu$ M	2.6 $\pm$ 1.3	5		100 $\mu$ M	3.3 $\pm$ 2.1	5	ND	ND	ND	5
Tizanidine	-5.41	100 $\mu$ M	2.5 $\pm$ 0.8	6		No response		5	ND	ND	ND	5
Sunepitirion	-5.33	100 $\mu$ M	2.5 $\pm$ 0.5	5		100 $\mu$ M	2.4 $\pm$ 1.1	6	ND	ND	ND	5
Tetrahydrozoline	-5.34	100 $\mu$ M	2.3 $\pm$ 1.1	5		No response		5	ND	ND	ND	5
Lisuride	-6.93	10 $\mu$ M	2.1 $\pm$ 0.6	5		-6.34 $\pm$ 0.11 <sup>a</sup>	36.0 $\pm$ 5.1	6	ND	ND	ND	5
Amitraz	-5.08	No response		5		No response		5	ND	ND	ND	5
ARC 239	-8.74 site 1	No response		5		No response		5	ND	ND	ND	5
Atipamezole	-5.33	No response		5		10 $\mu$ M	5.3 $\pm$ 4.6	5	ND	ND	ND	5
BHT920	-4.49	No response		5		No response		5	ND	ND	ND	5
BHT933	-3.70	No response		5		No response		5	ND	ND	ND	5
Brimonidine	-5.27	No response		6		No response		5	ND	ND	ND	5
CGP 12177	-4.20	No response		5		-5.60 $\pm$ 0.18 <sup>a</sup>	25.8 $\pm$ 3.2	7	ND	ND	ND	5
Chloroethylclonidine	-5.50	No response		5		No response		5	ND	ND	ND	5
Dihydroergotamine	-7.19	No response		6		-8.41 $\pm$ 0.11 <sup>a</sup>	32.9 $\pm$ 3.5	6	ND	ND	ND	5
Formoterol	-5.15	No response		5		No response		5	ND	ND	ND	5
Methyldopa	IC <sub>50</sub> > -4	No response		5		No response		5	ND	ND	ND	5
Midodrine	IC <sub>50</sub> > -3	No response		5		No response		5	ND	ND	ND	5
2-MPMDQ	-9.01 site 1	No response		5		No response		5	ND	ND	ND	5
3-MIPPI	-9.76 site 1	No response		5		No response		5	ND	ND	ND	5
2-PMDDQ	-8.42 site 1	No response		5		No response		5	ND	ND	ND	5
Para-amino-clonidine	-5.39	No response		5		No response		5	ND	ND	ND	5
PF3774076	-5.24	No response		5		No response		5	ND	ND	ND	5
RWJ52353	-4.30	No response		6		No response		5	ND	ND	ND	5

(Continues)

TABLE 4 (Continued)

CHO- $\alpha$ 1D	3H-prazosin binding		Intracellular calcium release		Log efficacy ratio		ERK1/2- phosphorylation		cAMP		cAMP (in presence of forskolin)		
	Log $K_D$	Log $EC_{50}$	% Ionomycin	n	$K_D/EC_{50}$	Log $EC_{50}$	% PDBu	n	Log $EC_{50}$	n	Log $EC_{50}$	Fold increase	n
Salbutamol	-3.87	No response	No response	5		100 $\mu$ M	4.0 $\pm$ 2.5	5	ND	5	ND	ND	ND
Salmeterol	-5.77	No response	No response	5		No response		5	ND	5	ND	ND	ND
T-CG 1000	-5.91	No response	No response	5		10 $\mu$ M	16.3 $\pm$ 5.9	6	ND	6	ND	ND	ND
UK14304	-5.36	No response	No response	5		No response		5	ND	5	ND	ND	ND
Ziprasidone	-7.20	No response	No response	5		10 $\mu$ M	4.6 $\pm$ 2.7	5	ND	5	ND	ND	ND

Note: Values represent mean  $\pm$  SEM of *n* separate experiments. The ligands are arranged in order of intrinsic efficacy ratio as determined from the calcium response ( $EC_{50}$ ) and binding ( $K_D$ ).

Abbreviation: ND, not determined.

<sup>a</sup>These compounds stimulate ERK1/2-phosphorylation in parent CHO cells, see Supplementary data Table S1, Figure S1.

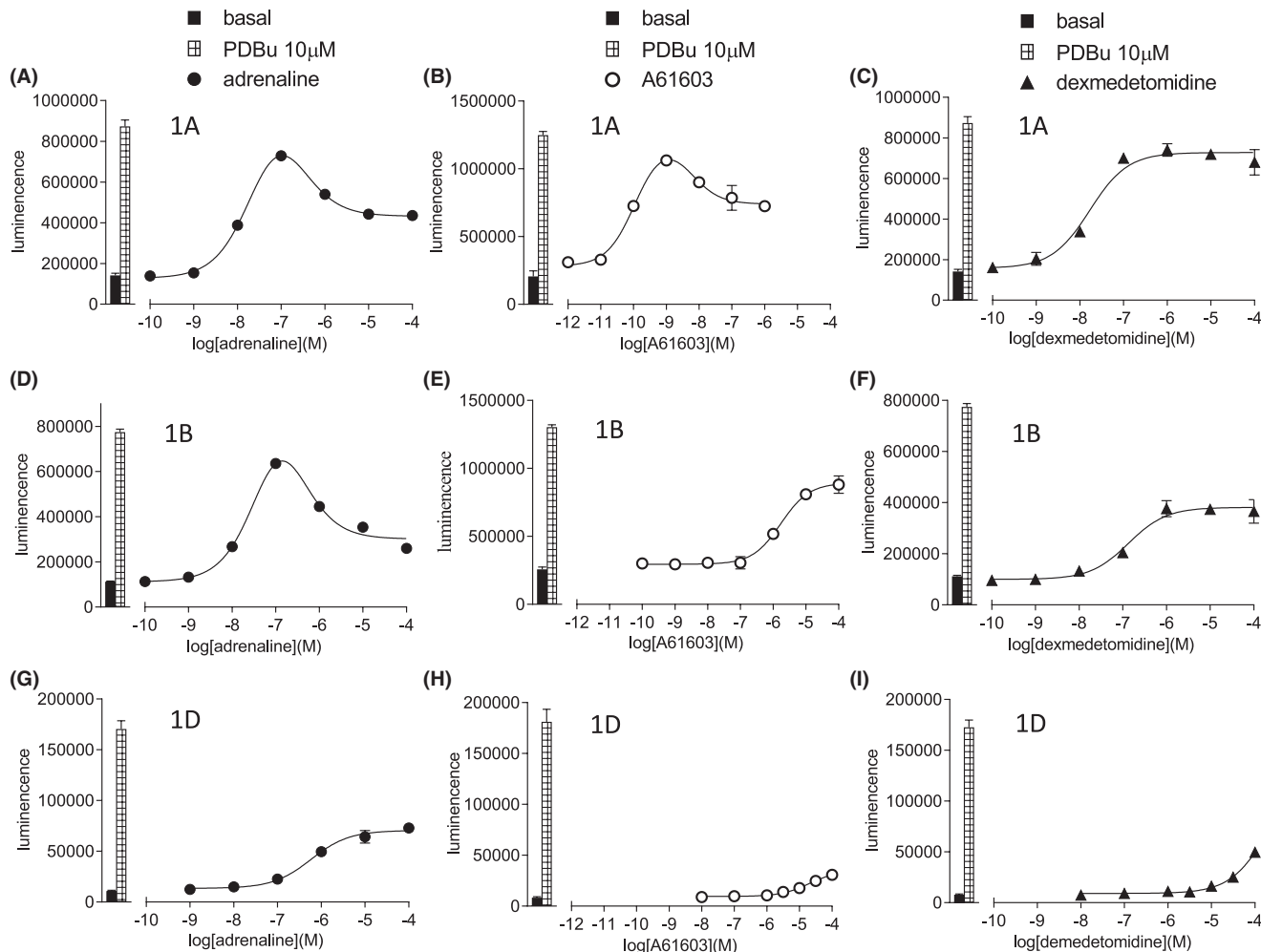
<sup>b</sup>Oxymetazoline and xylometazoline cause a decrease in forskolin-stimulated cAMP accumulation. The data given are log  $IC_{50}$  and % inhibition of forskolin-stimulated cAMP as both compounds caused a decrease in cAMP accumulation.

$\alpha$ 1A and  $\alpha$ 1D-adrenoceptors are important for vasoconstriction, the role of the  $\alpha$ 1B-adrenoceptor (also present in blood vessels) is less certain.<sup>10,14,36,37</sup> Interestingly, the affinity of adrenaline and noradrenaline was substantially lower for the  $\alpha$ 1B-adrenoceptor than for  $\alpha$ 1A or  $\alpha$ 1D-adrenoceptors. Adrenaline and noradrenaline had high intrinsic efficacy, with adrenaline being marginally higher at each receptor (in keeping with the slightly more potent adrenaline vs. noradrenaline responses observed by [21,25,38]). Phenylephrine (patented 1927, in clinical use since 1934<sup>39</sup>) and noted as potent by others,<sup>25,26</sup> also had very high intrinsic efficacy.

A61603 was the most selective agonist studied, with a calcium release and ERK1/2-phosphorylation potency ( $EC_{50}$  value) in CHO- $\alpha$ 1A cells in the sub-nanomolar range, rather than the micromolar range of the CHO- $\alpha$ 1B and CHO- $\alpha$ 1D cells. This high  $\alpha$ 1A-potency has been previously reported.<sup>11,24-26,40</sup> However to understand more, examination of both affinity and agonist responses is necessary. A61603 has high (>660-fold)  $\alpha$ 1A-adrenoceptor-binding selectivity, and this explains its high selectivity. Other agonist compounds had  $\alpha$ 1A-selective affinity, including PF3774076, oxymetazoline, lisuride, xylometazoline, and dihydroergotamine. No compound had  $\alpha$ 1B-selectivity, and BMY7378 (a known  $\alpha$ 1D-antagonist<sup>31,41</sup>) was the only compound with  $\alpha$ 1D-selective affinity.

Phenylephrine, naphazoline, oxymetazoline, and xylometazoline are present in many non-prescription nasal congestion treatments. They cause  $\alpha$ -agonist-induced vasoconstriction, reducing blood flow in nasal mucosa,<sup>10</sup> although there is still uncertainty about their clinical value.<sup>20,42</sup> Even topical preparations have problems including rebound congestion (first reported by Feinberg and Friedlaender,<sup>43</sup> and is still debated,<sup>44,45</sup>) and predictable systemic  $\alpha$ 1A-adrenoceptor complications for example hypertension and headache.<sup>46,47</sup> The  $\alpha$ 1A-adrenoceptor subtype (rather than  $\alpha$ 1B or  $\alpha$ 1D), along with  $\alpha$ 2A and  $\alpha$ 2B, has the highest mRNA expression in human nasal mucosa and is thought to be the primary target.<sup>21</sup> Phenylephrine (high intrinsic efficacy) and naphazoline (moderate intrinsic efficacy) were both non-selective  $\alpha$ 1-agonists (Tables 1-4), however, both oxymetazoline and xylometazoline had  $\alpha$ 1A-adrenoceptor selective affinity. A degree of  $\alpha$ 1A selective affinity of these two compounds has also been previously reported.<sup>21,25,26,38,48</sup>

Although clear agonist responses were seen with oxymetazoline for both calcium mobilization and ERK1/2-phosphorylation in CHO- $\alpha$ 1A and CHO- $\alpha$ 1B cell lines; in CHO- $\alpha$ 1D cells, ERK1/2-phosphorylation responses were substantially greater than the  $\alpha$ 1D-calcium response raising the possibility of  $\alpha$ 1D-biased-signaling. Examination of other ligands reveals several compounds with substantial ERK1/2-phosphorylation relative to calcium responses in the CHO- $\alpha$ 1D cells (e.g. oxymetazoline, xylometazoline, dihydroergotamine, and lisuride). Studies in untransfected parent CHO cells revealed similar agonist responses (see Supplementary data). Thus the ERK1/2-phosphorylation responses in CHO- $\alpha$ 1D cells (low receptor expression) were not occurring via the transfected receptor and were not due to biased signaling. Indeed, with the exception of oxymetazoline and xylometazoline in CHO- $\alpha$ 1A-cells where the responses were more potent, these agonist responses measured in any of the cell lines are unlikely to be  $\alpha$ 1-adrenoceptor mediated.

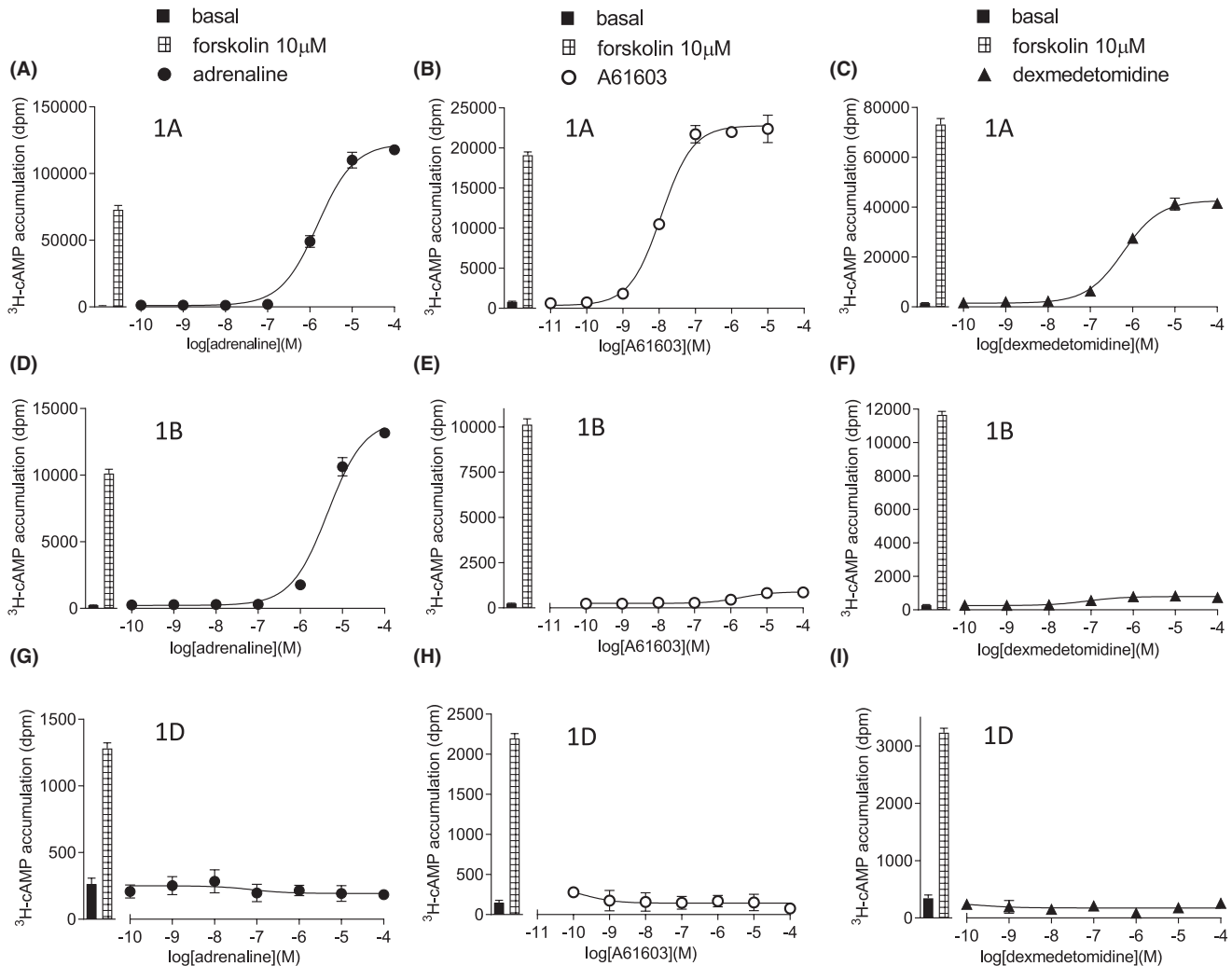


**FIGURE 3** ERK1/2-phosphorylation in CHO- $\alpha$ 1A cells (A-C), CHO- $\alpha$ 1B cells (D-F), or CHO- $\alpha$ 1D cells (G-I) in response to adrenaline (A, D, G), A61603 (B, E, H) or dexmedetomidine (C, F, I). Bars represent basal ERK1/2-phosphorylation and that in response to 10  $\mu$ M PDBu alone. Data points are mean  $\pm$  SEM of triplicate determinations

An “impossible” situation of negative efficacy ratios was seen for 2-PMDQ, ARC239, 2-MPMDQ, and 3-MPPI in CHO- $\alpha$ 1A cells: a higher concentration was required to stimulate agonist responses ( $EC_{50}$ ) than required to occupy the receptors ( $K_D$ ). These compounds had the smallest responses when compared with the ionomycin control. No agonist responses were observed in parent CHO cells, nor in CHO- $\alpha$ 1B or CHO- $\alpha$ 1D cells, suggesting that they are indeed  $\alpha$ 1A-adrenoceptor-mediated responses. A similar “impossible” situation occurs in  $\beta$ 1 and  $\beta$ 3-adrenoceptors, where certain lower efficacy compounds activate a secondary agonist conformation<sup>49–51</sup> involving the extracellular end of transmembrane 4.<sup>52</sup> A “low” affinity state of the  $\alpha$ 1A-adrenoceptor has been previously proposed ( $\alpha$ 1L), initially reported as having a lower prazosin affinity<sup>10</sup> and references therein) but also seen with affinity measurements in functional assays.<sup>11</sup> Further studies are required to determine whether the low potency of these agonists are occurring at a lower affinity  $\alpha$ 1A-secondary agonist conformation, akin to that of the  $\alpha$ 1 and  $\alpha$ 2-adrenoceptors, and whether this has any relationship of this to the “ $\alpha$ 1L”-adrenoceptor.

Overall, there was very close alignment between the calcium mobilization and the ERK1/2-phosphorylation responses (Figure 6a–c), suggesting no biased Gq/calcium versus ERK1/2-signaling in CHO- $\alpha$ 1A, CHO- $\alpha$ 1B, or CHO- $\alpha$ 1D cells. Copik et al.<sup>24</sup> examined  $\alpha$ 1A-adrenoceptor isoprenaline responses in HEK cells in detail and concluded that although isoprenaline induced similar calcium and ERK1/2-phosphorylation responses, isoprenaline did not induce phospholipase C or inositol phosphate responses. They concluded that their calcium response was a non-Gq-coupled event, and thus isoprenaline was an ERK versus Gq-biased ligand. Evans et al.<sup>25</sup> and da Silva et al.<sup>26</sup> report phenylephrine and methoxamine as having ERK versus Gq-calcium bias. In our study, phenylephrine, methoxamine, and isoprenaline have different intrinsic efficacies, but no calcium versus ERK-phosphorylation bias. It is possible that the ERK1/2-phosphorylation in our study could be downstream from the calcium response (as suggested by [22]).

Previous studies suggest that  $\alpha$ 1-adrenoceptors stimulate cAMP.<sup>24,38,53,54</sup> CHO- $\alpha$ 1A and CHO- $\alpha$ 1B agonist cAMP responses were seen with several compounds, although not in the  $\alpha$ 1D cells. In



**FIGURE 4**  $^3$ H-cAMP accumulation in CHO- $\alpha$ 1A cells (A–C), CHO- $\alpha$ 1B cells (D–F), or CHO- $\alpha$ 1D cells (G–I) in response to adrenaline (A, D, G), A61603 (B, E, H), or dexmedetomidine (C, F, I). Bars represent basal  $^3$ H-cAMP accumulation and that in response to 10  $\mu$ M forskolin alone. Data points are mean  $\pm$  SEM, of triplicate determinations

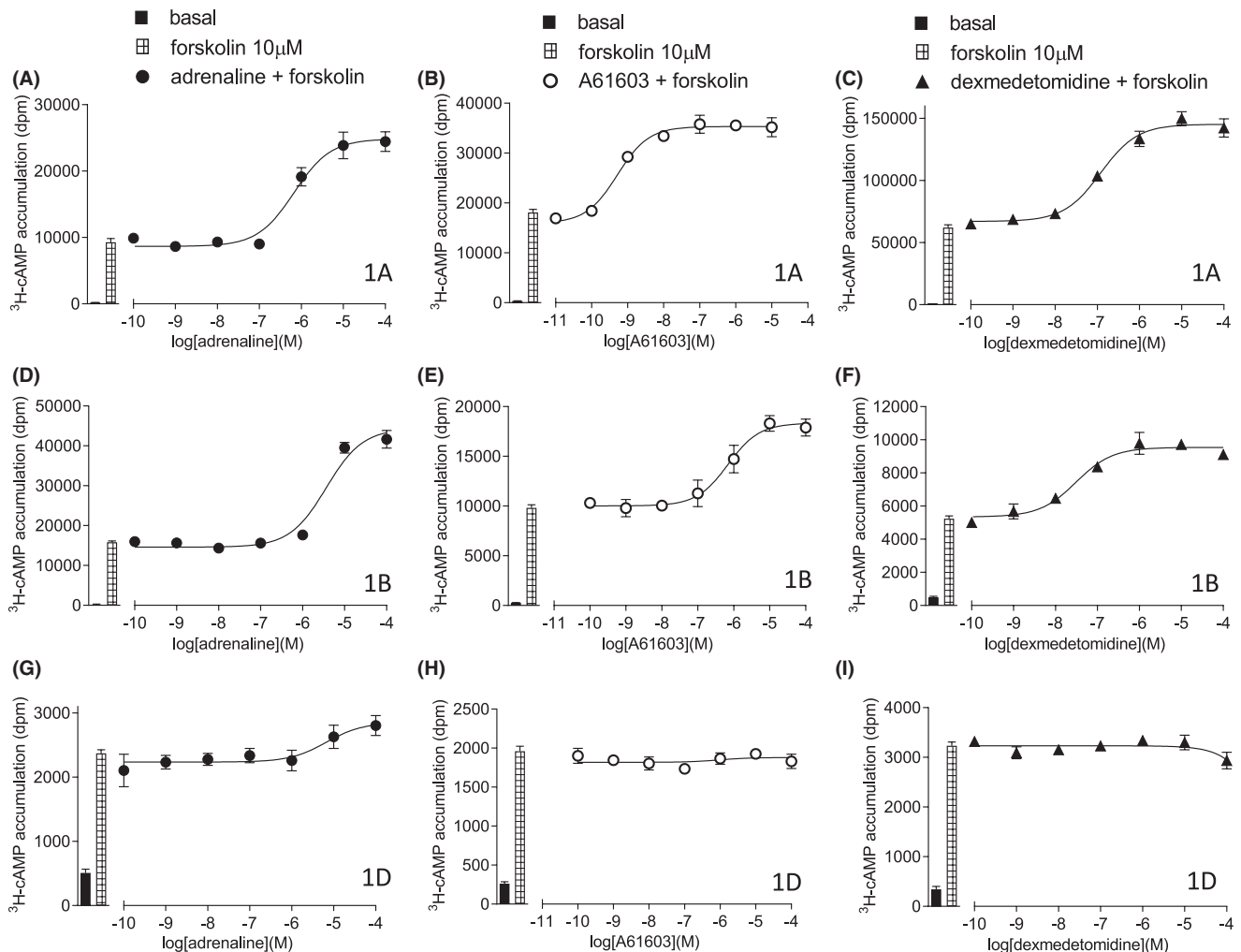
CHO- $\alpha$ 1A cells, cAMP responses required much higher agonist concentrations than that required for calcium release (as in [24–26,54]). This lower potency Gs-coupling is similar to that seen at the adenosine A1 receptor<sup>55</sup> and may represent a lower agonist affinity for the Gs-coupled conformation of the  $\alpha$ 1-adrenoceptors than for the Gq-coupled conformation. This was not always the case for  $\alpha$ 1B- see below.

There was no inhibition of forskolin-stimulated cAMP in CHO- $\alpha$ 1A or CHO- $\alpha$ 1B cells, suggesting no evidence for Gi receptor coupling. In fact, forskolin further increased the cAMP stimulatory responses, in keeping with forskolin-induced enhancement of GPCR-Gs-adenylyl cyclase coupling (proposed by [35] and [34]), and da Silva et al.<sup>26</sup> who were not able to measure an oxymetazoline-cAMP response, but observed an oxymetazoline response in the presence of 1  $\mu$ M forskolin. In CHO- $\alpha$ 1D cells, an inhibitory cAMP response was seen with oxymetazoline and xylometazoline, similar to that seen in parent CHO cells, suggesting that this was not  $\alpha$ 1D-receptor mediated. Thus oxymetazoline and xylometazoline

cause non- $\alpha$ 1-adrenoceptor-mediated responses in CHO cells that decrease cAMP and stimulate significant ERK-phosphorylation, very much in keeping with the CHO Gi-coupled 5HT-1B receptor proposed by da Silva et al.<sup>26</sup> The stimulatory response seen in CHO- $\alpha$ 1A and CHO- $\alpha$ 1B cells is likely receptor-mediated due to the higher level of transfected  $\alpha$ -adrenoceptors in these cell lines.

There was a good correlation between calcium mobilization and cAMP stimulation in CHO- $\alpha$ 1A-cells suggesting little calcium versus cAMP biased signaling. However, the correlation plot for the  $\alpha$ 1B-adrenoceptor shows substantially more scatter with adrenaline, noradrenaline, and  $\alpha$ -methylnorepinephrine having substantially more potent calcium than cAMP responses, whereas naphazoline, dexmedetomidine, medetomidine, allyphenylene, detomidine, guanabenz, and dobutamine had more potent cAMP responses than calcium. There may therefore be some bias signaling with respect to calcium and cAMP pathways via the  $\alpha$ 1B-adrenoceptor.

In  $\alpha$ 1A-cells, six ligands stimulated biphasic ERK1/2-phosphorylation responses: an initial increase in phospho-ERK1/2-phosphorylation



**FIGURE 5**  $^3\text{H}$ -cAMP accumulation in the presence of  $10\ \mu\text{M}$  forskolin in CHO- $\alpha 1\text{A}$  cells (A-C), CHO- $\alpha 1\text{B}$  cells (D-F), or CHO- $\alpha 1\text{D}$  cells (G-I) induced in response to adrenaline (A, D, G), A61603 (B, E, H) or dexmedetomidine (C, F, I). Bars represent basal  $^3\text{H}$ -cAMP accumulation and that in response to  $10\ \mu\text{M}$  forskolin alone. Data points are mean  $\pm$  SEM of triplicate determinations

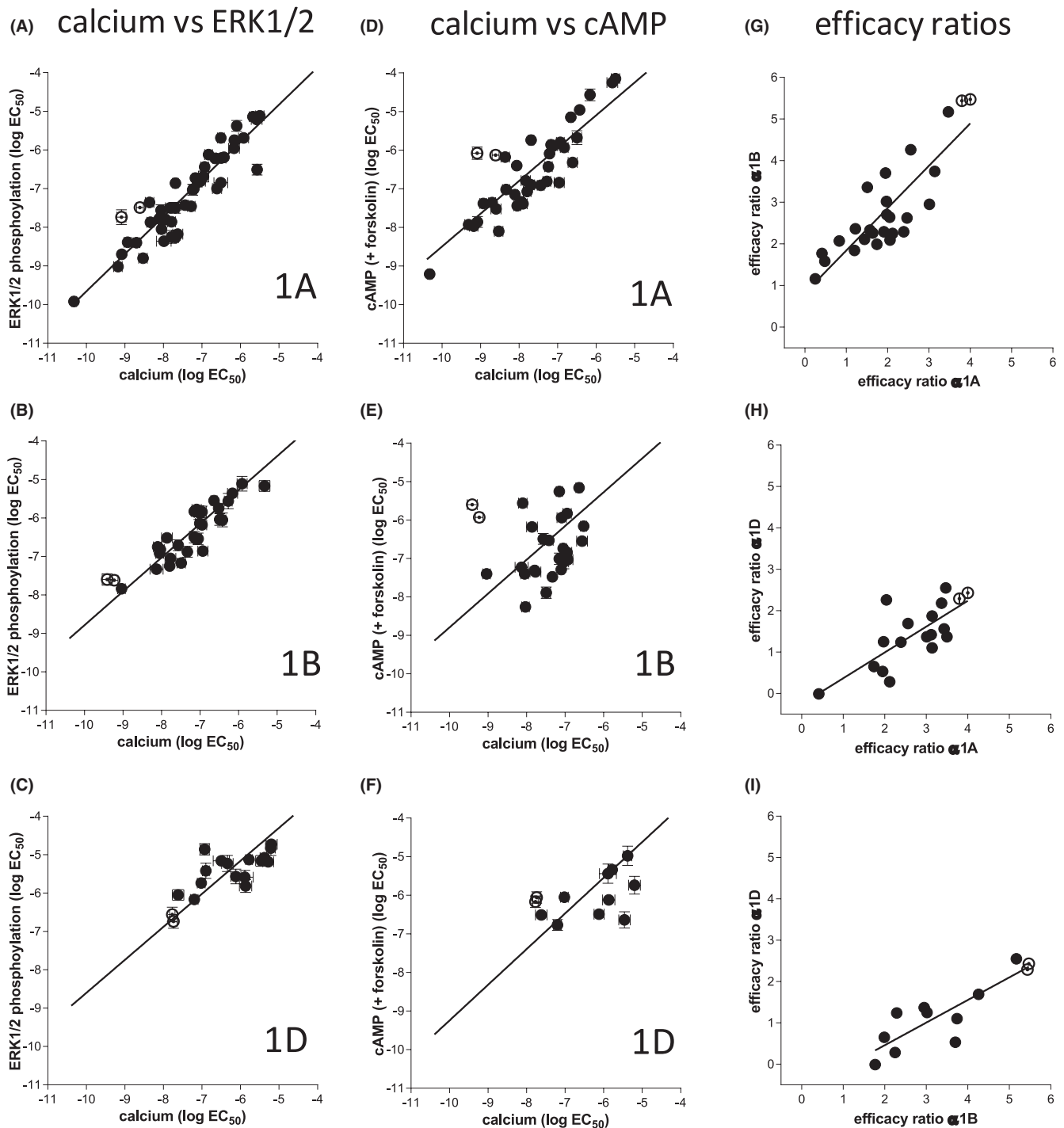
was followed by a decrease at higher agonist concentrations (Figure 3). This appears to be an efficacy driven phenomena because these six ligands had the highest intrinsic efficacy as determined from the calcium release assay. This phenomena was also seen with adrenaline and noradrenaline in CHO- $\alpha 1\text{B}$  cells, but not in CHO- $\alpha 1\text{D}$  cells (lower receptor expression) where all responses were smaller relative to the PDBu response. Interestingly,<sup>22</sup> proposed that  $\alpha 1\text{A}$ -induced cAMP stimulation could have a negative effect on ERK1/2-phosphorylation. Thus the Gs-coupled cAMP stimulation, which only occurs at higher agonist concentrations, could be the explanation for the decrease in ERK1/2-phosphorylation seen at higher agonist concentrations.

Finally, the intrinsic efficacy of ligands was examined. Although direct  $\text{EC}_{50}$  comparisons are not possible across cell lines, the rank order of intrinsic efficacies are either as presented in Tables 2-4 or pictorially from correlation plots (Figure 6). There was a good correlation for the intrinsic efficacy of agonists at these receptors, suggesting little intrinsic activity selectivity.

The ligand with the most selective intrinsic efficacy was dobutamine (ranked 4<sup>th</sup> in the  $\alpha 1\text{D}$  table, and furthest from the line of best fit, Figure 6). Dobutamine stimulated a response with similar affinity, potency, and intrinsic efficacy to that of noradrenaline in the CHO- $\alpha 1\text{D}$  cells, but despite a similar affinity, did not stimulate any measurable calcium or ERK1/2-phosphorylation CHO- $\alpha 1\text{B}$  response and only a mid-table response intrinsic efficacy response in the CHO- $\alpha 1\text{A}$  cells. Dobutamine has previously been shown to have affinity for  $\alpha 1$ -adrenoceptors.<sup>56</sup> However, A61603 apart, given the lack of selectivity of most  $\alpha 1$ -adrenoceptor agonists, there seems plenty of scope to develop both affinity-selective and intrinsic efficacy-selective agonist drugs for the  $\alpha 1$ -adrenoceptors in future.

#### ACKNOWLEDGMENTS

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**FIGURE 6** (A–C) Correlation plots of log EC<sub>50</sub> determined from intracellular calcium mobilization with the EC<sub>50</sub> determined from ERK1/2-phosphorylation in (A) CHO-α1A cells, (B) CHO-α1B cells, and (C) CHO α1D cells. The endogenous hormones adrenaline and noradrenaline are represented by open circles. The line is that of best fit. The data for oxymetazoline, xylometazoline, dihydroergotamine, lisuride, labetalol, and CGP 12177 are not included in these plots as the compounds generated agonist ERK1/2-phosphorylation responses in non-transfected cells and are therefore non-α1-mediated responses. (D–F) Correlation plots of log EC<sub>50</sub> determined from intracellular calcium mobilization with the EC<sub>50</sub> determined from cAMP accumulation in the presence of forskolin in (D) CHO-α1A cells, (E) CHO-α1B cells, and (F) CHO-α1D cells. The endogenous hormones adrenaline and noradrenaline are represented by open circles. The line is that of best fit. (G–I) Plots of efficacy ratio ( $K_D/EC_{50}$ ) for (G) α1A versus α1B, (H) α1A versus α1D, and (I) α1B versus α1D as determined from whole-cell binding affinity measurements and intracellular calcium mobilization. The endogenous hormones adrenaline and noradrenaline are represented by open circles. The line is that of best fit and the slope is not 1 and does not necessarily go through the origin as this represents a function of efficacy (i.e. differences in cell line which include receptor number, receptor-effector coupling etc.). Compounds with the greatest perpendicular distance from the line represent compounds with the greatest degree of selective intrinsic efficacy



## DISCLOSURE

JGB is on the Scientific Advisory Board for CuraSen Therapeutics. The majority of the data in this study predates that appointment.

## AUTHOR CONTRIBUTIONS

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

## DATA AVAILABILITY STATEMENT

Data available on request from the authors: The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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#### SUPPORTING INFORMATION

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

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