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Synthesis of a Selective Alpha-2A Adrenoceptor Antagonist, BRL 48962, and Its Characterization at Cloned Human Alpha-Adrenoceptors

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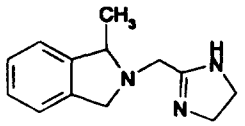
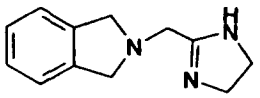
Abstract—The chiral synthesis of the potent and selective alpha-2A antagonist, BRL 48962, is described. Evaluation of BRL 48962 at cloned human alpha-adrenoceptors indicates that this antagonist has a selectivity in the order of 30-fold for the alpha-2A subtype.

Introduction

The racemic 2,3-dihydroisoindolylmethylimidazoline, BRL 44408, is a potent and selective alpha-2A adrenoceptor antagonist.[1] Evaluation of the individual enantiomers of BRL 44408 indicates that the *R*-(–)-enantiomer, BRL 48962,[2] has greater affinity and selectivity for the alpha-2A versus alpha-2B adrenoceptor than the *S*-(+)-enantiomer, BRL 48553.[3] However, these results on BRL 48962 were obtained in tissues from different species and with mixed populations of receptors.



Racemate; BRL 44408
R-enantiomer; BRL 48962
S-enantiomer; BRL 48553



BRL 43520

Alpha-adrenoceptors are classified[4] on a pharmacological basis into alpha-1 and alpha-2 adrenoceptors. Both subtypes are heterogeneous and several independent systems of nomenclature have emerged to define this heterogeneity. The cloning of genes encoding three alpha-1 subtypes (alpha-1A, alpha-1B and alpha-1D)[5] and three alpha-2 subtypes (alpha-2A,[6] alpha-2B and alpha-2C) has helped to clarify the situation and it is now generally accepted that the pharmacology of these cloned receptors relates to those of the subtypes defined by classical pharmacology. The human homologues for all six alpha-adrenoceptor subtypes have been cloned. We are now in a position to report the synthesis and evaluation of BRL 48962, an alpha-2A selective antagonist, in a homogenous receptor population from a single species. Such selective alpha-2 adrenoceptor antagonists may have potential therapeutic utility in several disease states, including diabetes and depression.[7]

Chemistry

We have previously described[8] the synthesis of racemic 1-alkyl-2,3-dihydroisoindoles from 2-formamidino-2,3-dihydroisoindoles using the methodology developed by Meyers *et al.*[9] The subsequent development of chiral auxiliaries for the stereoselective α-alkylation of cyclic secondary amines[10] provided a proven and versatile method for the preparation of the single enantiomers of BRL 44408. The synthesis of the *R*-enantiomer, BRL 48962, from 2,3-dihydroisoindole[11] is shown (Scheme 1).

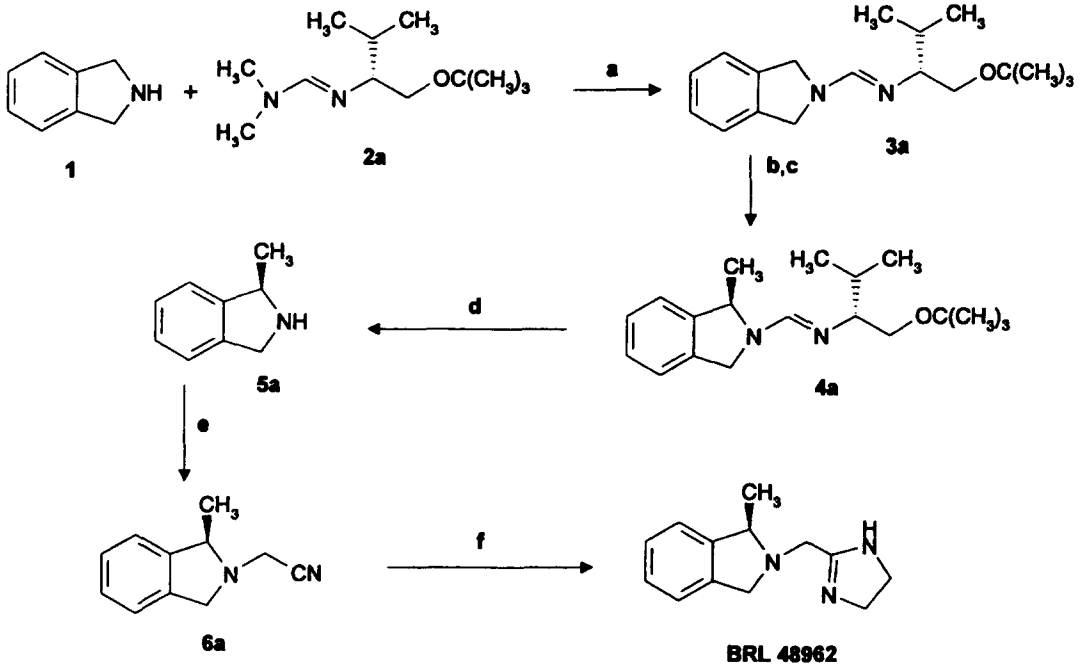
Thus reaction of 2,3-dihydroisoindole (**1**) with the chiral formamidine[12] (**2a**), derived from *R*-valinol, under formamidine exchange conditions gave the intermediate (**3a**) in quantitative yield. Stereoselective deprotonation of this material, with *s*-BuLi in THF at –78 °C followed by quenching at –100 °C with iodomethane, gave the *R*-1-methyl derivative (**4a**) in 71% yield after vacuum distillation. The removal of the chiral auxiliary with hydrazine:acetic acid:ethanol and purification by chromatography gave 27% of 2,3-dihydro-1-methylisoindole (**5a**) as an oil. Compound **5a** was converted via a two-step process,[8] with an overall yield of 22%, into BRL 48962, a white crystalline solid, mp 126 °C. Optical purity was assessed by chiral HPLC as 97% *e.e.* The *S*-enantiomer, BRL 48553, was prepared in a similar manner using the formamidine

derived from *S*-valinol. X-Ray single-crystal analysis[13] of the 1-*S*-camphor-10-sulfonic acid salt of BRL 48553 confirmed it to be the *S*-enantiomer, thereby establishing the methyl group in BRL 48962 to be in the *R*-configuration.

Results and Discussion

The mRNA for the alpha-2A adrenoceptor has been shown to be widely distributed in rat tissues[14] and is present in human brain, kidney, aorta, pancreas, lung, platelets and prostate.[15,16] The alpha-2A adrenoceptor subtype corresponds to the prazosin insensitive alpha-2 adrenoceptor characterised in almost all functional assays.

In a preliminary communication[3] we reported a comparison of BRL 44408 with its enantiomers using human platelets and rat brain cortex binding assays (Table 1). The human platelet alpha-2 adrenoceptor is of the alpha-2A subtype, whereas rat brain cortex is a mixture of alpha-2A and -2B.[17] These binding data suggest that the (–)-enantiomer, BRL 48962, has a greater affinity than the (+)-enantiomer, BRL 48553, for the alpha-2A subtype and that BRL 48962 has greater selectivity against the alpha-2B subtype than BRL 48553. On this basis it would be expected that the (–)-enantiomer should have a greater alpha-2A affinity than the racemate; unfortunately the variability of the experimental results has not allowed such a distinction to be made. Both BRL 44408 and BRL 48962 are antagonists at the alpha-2A adrenoceptor as shown (Table 1) by their ability to antagonise alpha-2 adrenoceptor-mediated inhibition of lipolysis in human adipocytes which are known to possess alpha-2A adrenoceptors.[18] Studies[3] on the alpha-1 adrenoceptor agonist activity of these compounds indicates that the weak alpha-1 agonism associated with BRL 44408 resides predominantly with the (+)-enantiomer (Table 1). The normethyl analogue,



Scheme 1.*
a. $(NH_4)_2SO_4$, toluene, heat; b. s-BuLi, THF, –78 °C; c. CH_3I, THF, –100 °C;
d. N_2H_4, AcOH, heat; e. $ClCH_2CN$, K_2CO_3, heat; f. $(CH_2NH_2)_2$, CS_2, 110°C.

*BRL 48553 was synthesized using intermediates, 2b to 6b, of opposite stereochemistry as described in the experimental.

Table 1. Binding affinity and functional activity in isolated tissues

	Alpha-2 binding affinity		Alpha-2 antagonist activity	Alpha-1 agonist activity
Compound	Ki nM*		pA_2	pD_2*
	Human platelets	Rat brain cortex	Human adipose tissue	Rabbit aorta
BRL 44408	1.7 ± 1.2 (3)	61 ± 3.6 (3)	7.6 (1)	4.6 (4)
BRL 48553	33 ± 11.4 (3)	50.2 (2)	-	4.8 (2)
BRL 48962	2.1 ± 1.8 (3)	58.7 (2)	7.8 ± 0.1 (3)	< 4.0 (2)
BRL 43250	5.0 (2)	13.1 (1)	-	4.4 (2)
Rauwolscine	1.6 ± 0.2 (3)	0.7 ± 0.2 (3)	-	-

*Mean ± SEM (number of observations shown in parentheses)

Table 2. Binding data at cloned human alpha-adrenoceptors

Compound	Human alpha-adrenoceptor in CHO cells K_i (nM) Mean (SEM)					
	Alpha-2A	Alpha-2B	Alpha-2C	Alpha-1A	Alpha-1B	Alpha-1D
Yohimbine	1.60 (0.23)	7.20 (1.8)	1.12 (0.1)	1000 (410)	334 (10)	289 (21)
SK&F 86466	9.40 (0.3)	15.8 (3.4)	19.8 (2.6)	449 (73)	485 (34)	126 (4.8)
Prazosin	2100 (700)	365 (48)	95.3 (1.8)	0.57 (0.12)	0.28 (0.03)	0.29 (0.02)
BRL 44408	109 (23)	1800 (450)	700 (200)	354 (250)	9000 (2060)	4200 (700)
BRL 48962	29.6 (3.2)	1300 (560)	830 (250)	1900(909)	2700 (283)	1800 (140)

BRL 43250, has only a slight bias for the alpha-2A receptor and possesses a lower affinity for this receptor than BRL 48962. This suggests that the 1-methyl substituent in the *R*-configuration is important for the observed alpha-2A adrenoceptor affinity and selectivity.

The binding data for BRL 48962 at cloned human alpha-adrenoceptors are compared with those of BRL 44408, the selective alpha-1 adrenoceptor antagonist, prazosin, and two structurally dissimilar alpha-2 adrenoceptor antagonists, SK & F 86466 and yohimbine (Table 2). The concentration response curves for the binding of BRL 48962 at these human alpha-adrenoceptor subtypes are shown in Figure 1. BRL 48962 has good affinity for the alpha-2A subtype, but significantly lower affinity for all other subtypes. Interestingly, the affinities of BRL 44408 and BRL 48962 were lower in CHO cells transfected with the alpha-2A adrenoceptor than in human platelets. However, the binding affinities of BRL 44408 in COS cells transfected with the alpha-2A adrenoceptor and in the HT-29 cell line, which expresses the alpha-2A adrenoceptor (K_i = 12.4, unpublished data, and K_i = 5.4 nM,[19] respectively), are more in line with the data in human platelets. For the present comparison, it is important to have all the data generated from alpha-adrenoceptors expressed in the same cell line. Although there are several ligands that distinguish between alpha-2 and alpha-1 adrenoceptors, there are few that are selective for individual subtypes of the alpha-2 adrenoceptor. Oxymetazoline[20] is the only other compound to exhibit comparable radioligand binding selectivity for the alpha-2A adrenoceptor over other alpha-2 adrenoceptor subtypes. However, oxymetazoline has a relatively high affinity for the alpha-1A adrenoceptor[21] and partial agonist activity at both alpha-1 and alpha-2 adrenoceptors, which severely limit its utility as a tool for receptor characterisation.

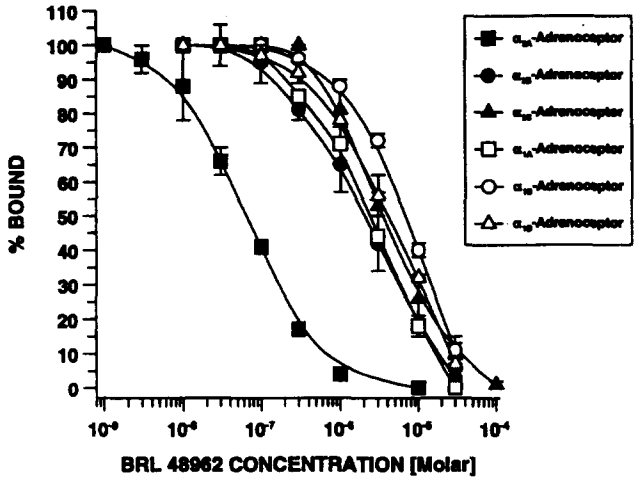


Figure 1. BRL 48962 displacement of ^{3}H rauwolscine (alpha-2) and ^{3}H prazosin (alpha-1) binding to adrenergic subtypes in transfected CHO-cells.*

*Each curve represents the mean (± SEM) of 3–5 experiments.

Conclusion

An effective chiral synthesis of BRL 48962, the *R*-enantiomer of BRL 44408, has been designed. BRL 48962 has been shown to bind to the human alpha-2A adrenoceptor with good affinity and selectivity compared with other alpha-adrenoceptor subtypes. Therefore, BRL 48962 may be useful as a pharmacological tool to characterise this receptor subtype and in the treatment of diseases in which attenuation of adrenergic activity at the alpha-2A adrenoceptor is beneficial.

Experimental

Chemistry

Melting points were determined using a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Joel JNM GX270 spectrometer using TMS as internal standard. Optical rotations and mass spectra were carried out on a Perkin–Elmer 241 polarimeter and VG 70F mass spectrometer, respectively. Microanalyses were determined in a Carlo–Erber 1106 elemental analyser, the results were within ± 0.4% of the theoretical values. Chiral HPLC was performed with a LKB 2150 isocratic HPLC pump and an LDC 5m 3100 UV detector using an LKB Enantiopac column. X-Ray crystallographic data were measured on a Nicolet R3m diffractometer with Cu-K_α radiation (graphite monochromator) using ω-scans.

(R)-2-[[[1-[(1,1-Dimethylethoxy)methyl]-2-methylpropyl]imino]-methyl]-2,3-dihydro-1H-isoindole (3a)

A solution of 2,3-dihydro-1*H*-isoindole (5.38 g, 45.1 mmol), (*R*)-*N'*-(*t*-butoxy-2-amino-3-methylbutyl)-*N,N*-dimethylformamidine (11.65 g, 54.3 mmol) and ammonium sulphate (catalytic) in dry toluene (30 mL), under

nitrogen, was stirred at reflux for 18 h. The solution was cooled and evaporated to yield the crude product as a brown liquid. Distillation (Kugelrohr) gave **1** as a colourless oil, yield 13.0 g (100%), bp 160 °C/0.2 mm Hg. ^{1}H NMR δ ($CDCl_3$): 0.89 (6H, *d*, 2 × CH_3), 1.17 (9H, *s*, 3 × CH_3), 1.84 (1H, *m*, CH), 2.71 (1H, *m*, CH), 3.20 (1H, *d* of *d*, $OC\underline{H}_AH_B$), 3.54 (1H, *d* of *d*, $OCH_A\underline{H}_B$), 4.70 (4H, *s*, 2 × CH_2), 7.29 (4H, *m*, Ar-H), 7.62 (1H, *s*, N=CH); $[\alpha]_D^{25}$ +2.0° (*c* 1.1; THF).

(R,R)-2-[[[1-[(1,1-Dimethylethoxy)methyl]-2-methylpropyl]imino]methyl]-2,3-dihydro-1-methyl-1H-isoindole (4a)

To a stirred solution of **1** (7.0 g, 24.3 mmol) in dry THF (200 mL) under nitrogen at –78 °C, was added dropwise 1.3 M *s*-butyllithium in *n*-hexane (20.5 mL, 26.7 mmol). The brown solution was cooled to –100 °C, then treated dropwise with iodomethane (1.86 mL, 29.9 mmol). After warming to –40 °C over 0.5 h, the yellow solution was quenched with water (20 mL) and the product extracted with CH_2Cl_2. The extract was washed with water (3 ×), dried, and evaporated to yield the crude product as a brown liquid. Distillation (Kugelrohr) gave **3** as a pale-red oil, yield 5.22 g (71%), bp 180–90 °C/0.2 mm Hg. ^{1}H NMR δ ($CDCl_3$): 0.89 (3H, *d*, CH_3), 0.91 (3H, *d*, CH_3), 1.16 (9H, *s*, 3 × CH_3), 1.51 (3H, *d*, CH_3), 1.87 (1H, *m*, CH), 2.75 (1H, *q*, CH), 3.23 (1H, *t*, $OC\underline{H}_AH_B$), 3.57 (1H, *d* of *d*, $OCH_A\underline{H}_B$), 4.69 (2H, *s*, CH_2), 4.98 (1H, *m*, CH), 7.24 (4H, *m*, Ar-H), 7.63 (1H, *s*, N = CH); $[\alpha]_D^{25}$ –17.0 ° (*c* 0.46; THF).

(R)-2,3-Dihydro-1-methyl-1H-isoindole (5a)

A solution of **3** (4.12 g, 13.6 mmol), hydrazine hydrate (2.0 mL, 41.1 mmol) and acetic acid (2.3 mL, 41.1 mmol) in 60% (v/v) aqueous ethanol (30 mL) was stirred under nitrogen at 55 °C for 4 h. The reaction was cooled, evaporated and partitioned between EtOAc and saturated $NaHCO_3$. The organic layer was washed with water and dried. Evaporation yielded a red oil which was chromatographed on SiO_2, eluting with EtOAc:Et_3N (95:5) to give **5** as a colourless liquid, yield 0.50 g (27%). ^{1}H NMR δ ($CDCl_3$): 1.40 (3H, *d*, CH_3), 2.18 (1H, *s*, exchanges with D_2O, NH), 4.21 (2H, *s*, CH_2), 4.44 (1H, *q*, CH), 7.29 (4H, *s*, Ar-H).

(R)-1,3-Dihydro-1-methyl-2H-isoindole-2-acetonitrile (6a)

A mixture of **5** (0.50 g, 3.75 mmol), chloroacetonitrile (0.36 mL, 5.69 mmol) and potassium carbonate (0.29 g, 2.10 mmol) in acetone (20 mL) was stirred under nitrogen at reflux temperature for 5 h. The reaction was cooled, evaporated and partitioned between CH_2Cl_2 and 1 M Na_2CO_3 solution. The organic layer was washed with water and dried. Evaporation gave a red oil which was chromatographed on SiO_2, eluting with *n*-hexane: EtOAc (85:15) to yield **4** as a colourless oil, yield 0.33 g (51%). ^{1}H NMR δ ($CDCl_3$): 1.42 (3H, *d*, CH_3), 3.85 (2H, *q*, CH_2), 4.07 (2H, *m*, CH_2), 4.23 (1H, *d*, CH), 7.21 (4H, *m*, Ar-H). $[\alpha]_D^{25}$ –42.9° (*c* 0.67; THF). HPLC, 87% *e.e.*

(R)-2-[(4,5-Dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole (BRL 48962)

A mixture of **7** (0.40 g, 2.32 mmol), 1,2-diaminoethane (0.17 mL, 2.54 mmol) and carbon disulfide (catalytic) was heated under nitrogen at 110 °C for 1 h. The mixture was cooled and partitioned between CH_2Cl_2 and water. The organic layer was washed with water and dried. Evaporation gave a red crystalline solid which was chromatographed on SiO_2, eluting EtOAc:Et_3N:MeOH (8:1:1) to afford **9** as a white crystalline solid, yield 0.22 g (44%), mp 126 °C (dec). ^{1}H NMR δ ($CDCl_3$): 1.42 (3H, *d*, CH_3), 3.55 (2H, *q*, CH_2), 3.63 (4H, *s*, CH_2CH_2), 3.72 (IH, *d* of *d*, $C\underline{H}_AH_B$), 3.91 (1H, *m*, CH), 4.21 (1H, *d* of *d*, $CH_A\underline{H}_B$), 5.00 (1H, *s*, exchanges with D_2O, NH), 7.19 (4H, *m*, Ar-H); $[\alpha]_D^{25}$ –76.2° (*c* 0.38; THF); HPLC, 97% *e.e.*; MS m/z 213 ($M-H_2^+$); Analysis: $C_{13}H_{17}N_3$ (CH, N).

(S)-2-[[[1-[(1,1-Dimethylethoxy)methyl]-2-methylpropyl]imino]methyl]-2,3-dihydro-1H-isoindole (3b)

The title compound was prepared in an analogous manner to **1** using 2,3-dihydro-1*H*-isoindole (3.35 g, 28.1 mmol) and (*S*)-*N'*-(*t*-butoxy-2-amino-3-methylbutyl)-*N,N*-dimethylformamidine (6.70 g, 31.3 mmol). Yield 5.75 g (71%), bp 190–195 °C/0.5 mm Hg. ^{1}H NMR δ ($CDCl_3$): 0.94 (6H, *d*, 2 × CH_3), 1.21 (9H, *s*, 3 × CH_3), 1.84 (1H, *m*, CH), 2.78 (1H, *m*, CH), 3.26 (1H, *t*, $OC\underline{H}_AH_B$), 3.57 (1H, *d* of *d*, $OCH_A\underline{H}_B$), 4.77 (4H, *s*, 2 × CH_3), 7.33 (4H, *s*, Ar-H), 7.67 (1H, *s*, CH=N); $[\alpha]_D^{25}$ – 14.1° (*c* 0.17; THF).

(S,S)-2-[[[1-[(1,1-Dimethylethoxy)methyl]-2-methylpropyl]imino]methyl]-2,3-dihydro-1-methyl-1H-isoindole (4b)

The title compound was prepared in an analogous manner to **3**, using **2** (5.75 g, 19.9 mmol), 1.3 M *s*-butyllithium in *n*-hexane (16.9 mL, 22.0 mmol) and iodomethane (1.37 mL, 22.0 mmol). Yield 4.85 g (80%), bp 180–190 °C/0.1 mm Hg. ^{1}H-NMR δ ($CDCl_3$): 0.82 (3H, *d*, CH_3), 0.83 (3H, *d*, CH_3), 1.09 (9H, *s*, 3 × CH_3), 1.44 (3H, *d*, CH_3), 1.80 (1H, *m*, CH), 2.67 (1H, *m*, CH), 3.15 (IH, *d* of *d*, $OC\underline{H}_AH_B$), 3.48 (1H, *d* of *d*, OCH_AH_B), 4.61 (2H, *s*, CH_2), 4.91 (1H, *q*, CH), 7.16 (4H, *m*, Ar-H), 7.55 (1H, *s*, CH=N); $[\alpha]_D^{25}$ +13.2° (*c* 0.14; THF).

(S)-2,3-Dihydro-1-methyl-1H-isoindol, (5b)

The title compound was prepared in an analogous manner to **5** using **4** (4.50 g, 14.9 mmol), hydrazine hydrate (2.20 mL, 45.3 mmol) and acetic acid (2.57 mL, 45.0 mmol). Yield 0.93 g (47%). ^{1}H NMR δ ($CDCl_3$): 1.45 (3H, *d*, CH_3), 2.90 (1H, *s*, exchanges with D_2O, NH), 4.40 (2H, *m*, CH_2), 4.46 (1H, *q*, CH), 7.22 (4H, *m*, Ar-H); $[\alpha]_D^{25}$ +23.4° (*c* 0.95; THF).

(S)-1,3-Dihydro-1-methyl-2H-isoindole-2-acetonitrile (6b)

The title compound was prepared in an analogous manner to **7** using **6** (0.93 g, 6.98 mmol), chloroacetonitrile (0.49 mL, 7.74 mmol) and potassium carbonate (0.48 g, 3.47 mmol). Yield 0.55 g (46%). ^{1}H NMR δ ($CDCl_3$): 1.44 (3H, *d*, CH_3), 3.85 (2H, *q*, CH_2), 4.13 (3H, *m*, CH_2 and CH), 7.22 (4H, *m*, Ar-H); $[\alpha]_D^{25}$ +40.3° (*c* 0.90; THF); HPLC 92% *e.e.*

(S)-2-[(4,5-Dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole (BRL 48553)

The title compound was prepared in an analogous manner to **9** using **8** (0.55 g, 3.19 mmol) and 1,2-diaminoethane (0.23 mL, 3.44 mmol). Yield 0.36 g (52%), mp 134–135 °C (EtOAc). ^{1}H NMR δ ($CDCl_3$): 1.42 (3H, *d*, CH_3), 3.55 (2H, *q*, CH_2), 3.63 (4H, *s*, CH_2CH_2), 3.72 (1H, *d* of *d*, $C\underline{H}_AH_B$), 3.90 (1H, *m*, CH), 4.21 (1H, *d* of *d*, $CH_A\underline{H}_B$), 5.10 (1H, *s*, exchanges with D_2O, NH), 7.20 (4H, *m*, Ar-H); $[\alpha]_D^{25}$ +76.1° (*c* 0.90; THF); HPLC, 98% *e.e.*; MS m/z 213 ($M-H_2^+$); Analysis: $C_{13}H_{17}N_3$ (C, H, N).

Pharmacology

Determination of binding affinity for recombinant alpha-adrenoceptors and tissues

Preparation of platelet and rat cerebral cortex membranes, together with the conditions of the binding assays, have been previously detailed.[1] With respect to assays using recombinant adrenoceptors, the three human alpha-1 adrenoceptors (alpha-1A, alpha-1B, alpha-1D) and alpha-2 adrenoceptors (alpha-2A, alpha-2B, alpha-2C) were cloned and stably expressed in Chinese hamster ovary cells (CHO) as previously described.[22] Cell pellets were homogenized (Brinkman Polytron) with cold TME buffer (10 mM Tris, 2 mM EDTA, pH 7.4). The homogenate was centrifuged at 500 × g for 10 min at 4 °C. The supernatant was collected and centrifuged at 100,000 × *g* for 30 min at 4 °C. The resulting membrane pellet was resuspended in 50 mM Tris buffer, pH 7.4.

To assess binding affinity membranes were incubated with test antagonists at concentrations ranging from 0.1 nM to 100 μM. Assays were initiated by the addition of membrane protein and incubated at 25 °C for 30 (^{3}H rauwolscine) or 45 (^{3}H prazosin) min. 50 mM Tris-EDTA buffer, pH 7.4, was used for ^{3}H rauwolscine and 50 mM Tris-HCl, pH 7.4, was used for ^{3}H prazosin. Radioligand was present at a concentration near its K_d, determined previously in saturation experiments. Non-specific binding was determined using 10 μM phentolamine. K_i values were calculated using the London Software program for non-linear regression. The receptor density and radioligand binding affinity for each of the cloned receptors were as in Table 3.

Table 3.

Receptor	K_d (nM)	B_{max} (pmol mg^{-1} protein)
Alpha-1A	0.23 (^{3}H prazosin)	41
Alpha-1B	0.15 (^{3}H prazosin)	9
Alpha-1D	0.18 (^{3}H prazosin)	10
Alpha-2A	1.0 (^{3}H rauwolscine)	13
Alpha-2B	2.3 (^{3}H rauwolscine)	14
Alpha-2C	0.23 (^{3}H rauwolscine)	27

Determination of alpha-1 adrenoceptor agonist activity

Ring segments of rabbit aorta were mounted in organ baths at 37 °C containing Krebs' medium, D-glucose (11.1 mM), propranolol (2 μM), disodium EDTA (30 μM) and L-ascorbic acid (30 μM). Contractions of this tissue in response to noradrenaline or compound are mediated by post-junctional alpha-1 adrenoceptors.[23]

pA_2 Evaluation of BRL 44408 and BRL 48962 in human adipocyte lipolysis

Human adipocytes were prepared by the collagenase digestion method of Rodbell.[24] Theophylline (1.6 mM) was used to stimulate lipolysis and dose response curves for inhibition of lipolysis by the α_2-adrenoceptor agonist UK 14304 were constructed.[25] Dose–response curves for UK 14304 were competed by four concentrations of antagonist. The resulting Schild plot[26] was used to generate pA_2 values shown in Table 1.

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