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Laboratory note

Synthesis and β–adrenoceptor agonist properties of (±)–1–(3′,4′-dihydroxyphenoxy)–3–(3″,4″–dimethoxyphenyl) ethylamino–2–propanol hydrochloride, (±)–RO363.HCl, and the (2*S*)–(–)–isomer

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Abstract – The synthesis of (±)-1-(3′,4′-dihydroxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol hydrochloride, (±)-RO363.HCl, and the (2*S*)-(-)-isomer is described for the first time. The binding affinities for (±)-RO363.HCl, (2*S*)-(-)-RO363.HCl and a number of well known β-adrenoceptor agonists for transfected human β_1-, β_2- and β_3-adrenoceptors expressed in Chinese hamster ovary cells have been determined and compared with the functional potencies in rat atria (β_1) and trachea (β_2). The results indicate that both (±)-RO363 and (2*S*)-(-)-RO363 are selective for the human and rat β_1-adrenoceptors. The (2*S*)-(-)-isomer of RO363, as expected, has a higher binding affinity for the human and functional potency for rat β-adrenoceptor subtypes than the racemate. However, in contrast to the catecholamines and formoterol, the functional potency of the racemic mixture and its (-)-enantiomer are not significantly different from their binding affinity, suggesting that they are examples of partial agonists with sufficient intrinsic activity to produce full agonist responses. © Elsevier, Paris

(±)-RO363 / (2S)-(-)-RO363 / β1-adrenoceptor agonist / chiral synthesis / CHO cells

1. Introduction

The first pharmacological reports on (±)-1-(3′,4′-dihydroxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol ((±)-RO363, *figure 1*), a synthetic catecholamine, appeared in 1978 [1] and it has been described as a potent, selective β_1-adrenoceptor agonist in the guinea-pig, rat, rabbit and cat [1–3]. More recently, work carried out by Molenaar et al. [4, 5] suggests that (2*S*)-(-)-RO363 is a potent partial agonist for both human β_1- and β_3-adrenoceptors. In addition, they report that (2*S*)-(-)-RO363 is able to activate a novel human atrial β-adrenoceptor, which they have designated as a putative β_4-adrenoceptor [4, 5].

The original synthesis of (±)-RO363, and the subsequent resolution of the (2*S*)-(-)- and (2*R*)-(+)-enantiomers, was carried out by D. Iakovidis as part of his doctoral studies [6] and has never been formally reported. In light of the renewed interest in RO363 as a research tool, we describe here the synthesis of (±)-RO363 (**7**, *figure 2*) using a shorter (5 steps) and simpler procedure than the original 9 step process [6]. We also report the chiral synthesis of (2*S*)-(-)-RO363 (**11**, *figure 3*). Both (±)-RO363 **7** and (2*S*)-(-)-RO363 **11** have been prepared as the hydrochloride salts.

The binding affinity of (±)-RO363 **7** and (2*S*)-(-)-RO363 **11** for human β_1-, β_2- and β_3-adrenoceptors transfected and expressed in Chinese hamster ovary (CHO) cells and their functional potency in rat atria (β_1) and trachea (β_2) have been determined and compared to

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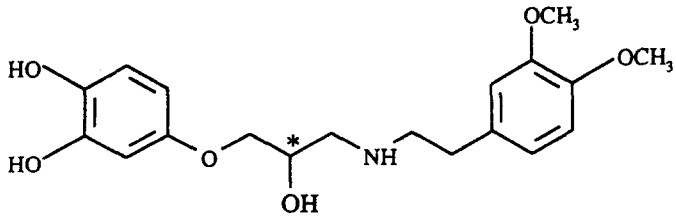
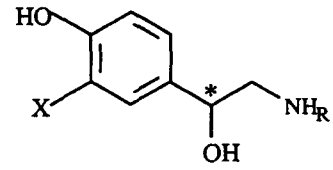
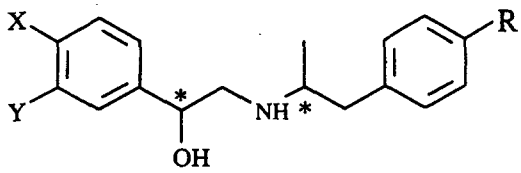
Abbreviations: 1-(3′,4′-dihydroxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol, RO363; Chinese hamster ovary, CHO; m-chloroperbenzoic acid, MCBA; 1-(1H-2-cyano-3-iodoindol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol, iodocyanopindolol, ICYP; sodium-4-[2-[2-hydroxy-2-(3-chlorophenyl) ethylamino]propyl]phenoxyacetate, BRL37344; 2-(t-butylamino)-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol, salbutamol; N-[2-hydroxy-5-[1-hydroxy-2[[2-(p-methoxyphenyl)-2-propyl]amino]-ethyl]phenyl]formamide, formoterol; 1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol, isoprenaline; Kd, dissociation constant; Bmax, maximal density of binding sites.



(a)



(b) X = OH, R = H
(c) X = OH, R = iPr
(d) X = CH_2OH, R = tBu



(e) X = OH, Y = NHCHO, R = OMe
(f) X = H, Y = Cl, R = OCH_2CO_2H

Figure 1. Structures of (a) RO363, (b) noradrenaline, (c) isoprenaline, (d) salbutamol, (e) formoterol and (f) BRL37344. The asterisks denote a chiral centre.

other well known β-adrenoceptor agonists. In addition, we have examined the functional potency of (-)-isoprenaline and (2*S*)-(-)-RO363 in guinea-pig atria and trachea.

2. Chemistry

The synthetic routes used to prepare (±)-RO363.HCl **7** and (2*S*)-(-)-RO363.HCl **11** are outlined in *figures 2* and *3*. Commercially available 3,4-dibenzyloxybenzaldehyde **1** was oxidized with m-chloroperbenzoic acid (MCBA) [7] to produce the corresponding formate **2**, which after mild hydrolysis produced the desired phenol **3**.

The phenol **3** reacted with epichlorohydrin under alkaline conditions to produce the epoxide **4** *(figure 2)*. The ring opening addition reaction of the epoxide **4** with the amine **5**, followed by the conversion of the free base to the hydrochloride salt gave the protected form of the final compound **6**. The benzyl protecting groups were removed by hydrogenation to give (±)-RO363.HCl **7**. The assigned structure of (±)-RO363.HCl was fully confirmed by MS and ^{1}H-NMR spectra and elemental analysis.

To synthesize the (2*S*)-(-)-isomer of RO363 **11**, epichlorohydrin was replaced with (2*S*)-(+)-glycidyl-3-nitrobenzenesulfonate **8** *(figure 3)* to produce the (2*S*)-(+)-epoxide **9**. The optical purity of (2*S*)-(+)-glycidyl-3-nitrobenzenesulfonate **8** is > 99% and is commonly used in the homochiral synthesis of β-adrenoceptor agonists and antagonists [8]. The optical purity of the protected benzyl derivatives was confirmed to be > 99% by chiral HPLC. The assigned structure of (2*S*)-(-)-RO363.HCl **11** was fully confirmed by MS and ^{1}H-NMR spectra and elemental analysis.

3. Pharmacology

The binding affinities for transfected human β_1-, β_2- and β_3-adrenoceptors expressed in CHO cells were determined for the endogenous catecholamine (-)-noradrenaline, the non-selective agonist (-)-isoprenaline, the β_1-selective agonist (±)-RO363 **7** and the isomer

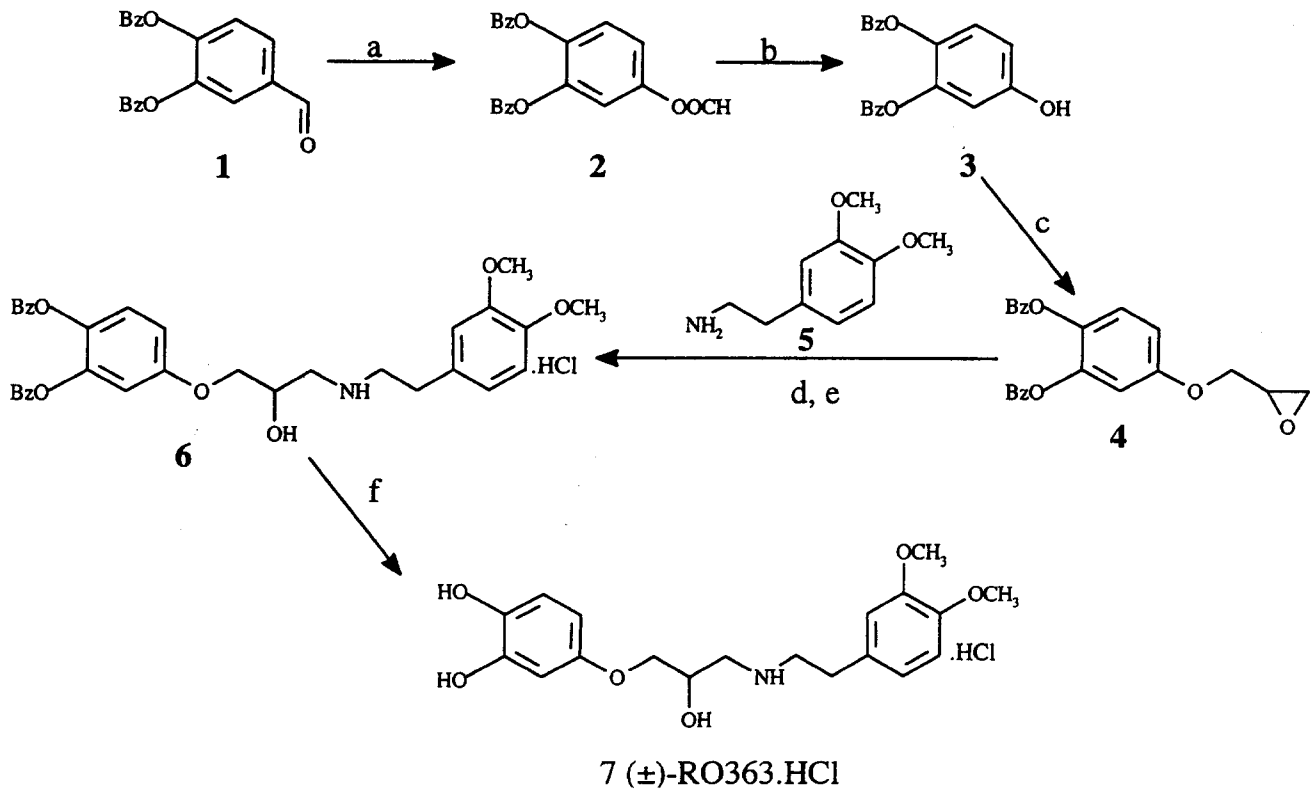


Figure 2. Synthesis of (±)-RO363.HCl **7**. Reagents: (a) MCBA, CH_2Cl_2; (b) KOH, MeOH; (c) epichlorohydrin, KOH, EtOH; (d) EtOH; (e) EtOH, ethereal HCl; (f) H_2, Pd/C.

(2*S*)-(-)-RO363 **11**, the β_2-selective agonists (±)-salbutamol and (±)-formoterol and the β_3-selective agonist (±)-BRL37344 (the structures of all agonists are given in *figure 1*). The functional potencies of all seven agonists were also determined in rat spontaneously beating atria (where responses are due to β_1-adrenoceptor stimulation) and rat trachea previously contracted with 1 μM carbachol (where responses are due to β_2-adrenoceptor stimulation). The pharmacological results are listed in *table I*. In addition, we have examined the functional potency of (2*S*)-(-)-RO363 **11** and (-)-isoprenaline for guinea-pig spontaneously beating atria and guinea-pig trachea previously contracted with 300 nM carbachol.

4. Results and discussion

4.1. Binding at human β-adrenoceptors

The dissociation constants (K_d) and maximal density of binding sites (B_{max}) of (-)-[^{125}I]-iodocyanopindolol (ICYP) for the transfected human β_1-, β_2- and β_3-adrenoceptor subtypes were determined by saturation binding experiments. For human β_1-adrenoceptors the K_d was 4.99 ± 0.48 pM and the B_{max} was 7 127 ± fmol mg protein^{-1}; for the β_2-adrenoceptor the K_d was 8.00 ± 1.10 pM and the B_{max} was 3 914 ± 583 fmol mg protein^{-1}; and for the β_3-adrenoceptor the K_d was 313 ± 93.8 pM and the B_{max} was 2 325 ± 322 fmol mg protein^{-1}. For all cell lines, Scatchard analysis gave a straight line ($n_H \approx 1.0$) consistent with the presence of a single β-adrenoceptor subtype.

The binding affinities for the seven compounds were determined for the human β_1-, β_2- and β_3-adrenoceptors in competition with the radioligand ICYP. As expected all compounds displayed monophasic displacement curves ($n_H \approx 1.0$) and of the compounds tested, (2*S*)-(-)-RO363 **11** had the highest binding affinity for human β_1-adrenoceptors (pK_i = 8.71; *table I*), while (±)-formoterol displayed the highest affinity for human β_2-adrenoceptors (pK_i = 6.98, *table I*) and (±)-BRL37344 had the highest binding affinity for human β_3-

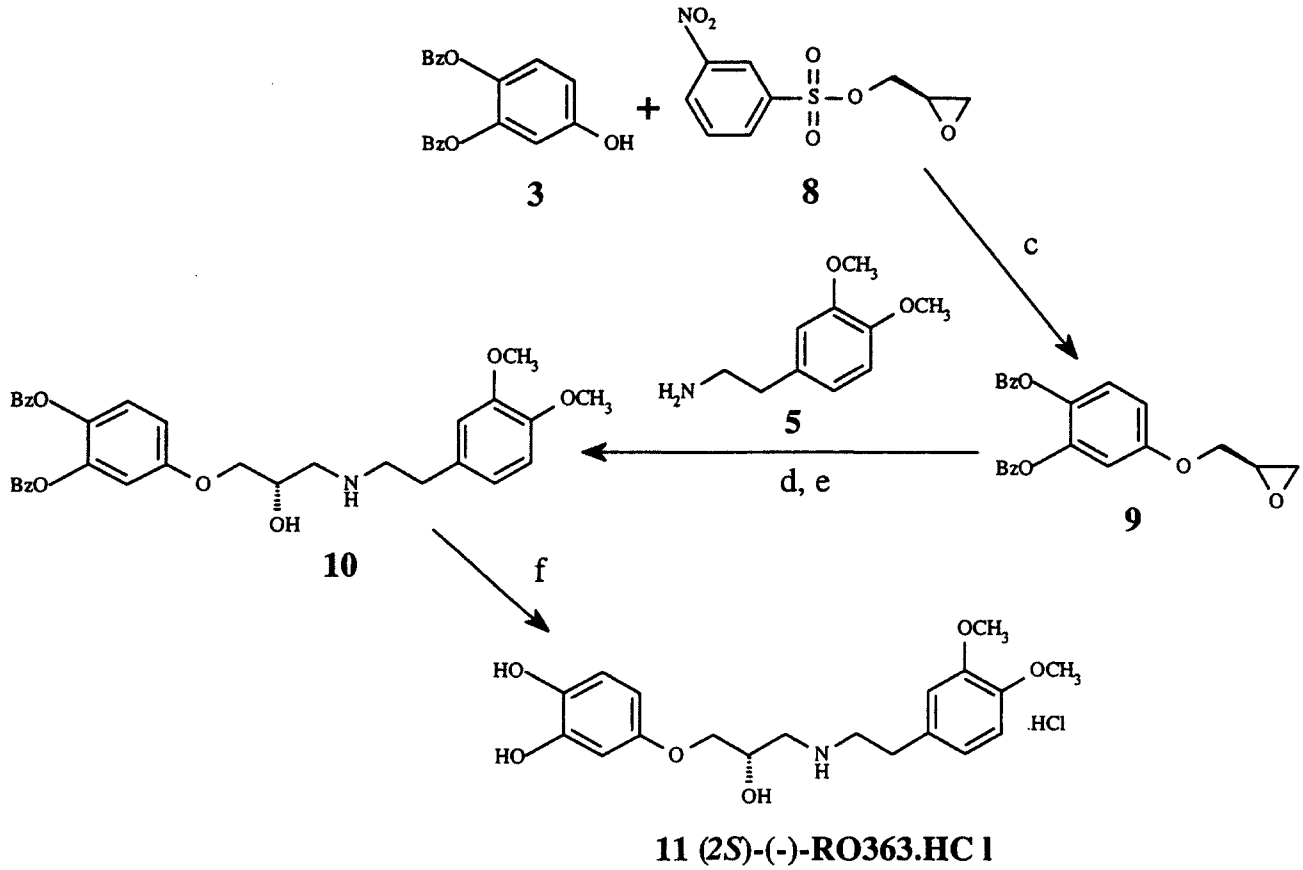


Figure 3. Chiral synthesis of (*2S*)-(-)-RO363.HCl **11**. Reagents: (a) MCBA, CH_2Cl_2; (b) KOH, MeOH; (c) DMF, NaH; (d) EtOH; (e) EtOH, ethereal HCl; (f) H_2, Pd/C.

adrenoceptors (pK_i = 5.84, *table I*). Overall, the results are consistent with the reported selectivities of these three agonists. The relative affinity order for human β_1-adrenoceptors was (*2S*)-(-)-RO363 **11** > (±)-RO363 **7** > (-)-isoprenaline > (-)-noradrenaline > (±)-formoterol > (±)-salbutamol > (±)-BRL37344; for human β_2-adrenoceptors it was (±)-formoterol > (*2S*)-(-)-RO363 **11** > (±)-RO363 **7** > (-)-isoprenaline = (±)-salbutamol > (±)-

Table I. Agonist binding affinity for human β_1-, β_2- and β_3-adrenoceptors and functional potency at rat β_1- and β_2-adrenoceptors.

Compound	Human binding affinity			Rat functional potency	
	β_1 pK_i	β_2 pK_i	β_3 pK_i	atria (β_1) pD_2	trachea (β_2) pD_2
(-)-isoprenaline	6.89 ± 0.06	6.24 ± 0.07	5.06 ± 0.07	8.53 ± 0.16	7.05 ± 0.05
(-)-noradrenaline	6.02 ± 0.11	4.41 ± 0.15	4.64 ± 0.05	7.25 ± 0.33	5.55 ± 0.29
(±)-RO363	8.22 ± 0.03	6.63 ± 0.09	4.23 ± 0.17	8.23 ± 0.10	6.56 ± 0.08
(*2S*)-(-)-RO363	8.71 ± 0.13	6.87 ± 0.04	4.48 ± 0.12	8.88 ± 0.16	6.59 ± 0.11
(±)-formoterol	5.97 ± 0.15	6.98 ± 0.25	4.71 ± 0.14	7.10 ± 0.07	7.70 ± 0.25
(±)-salbutamol	5.86 ± 0.25	6.24 ± 0.11	4.20 ± 0.08	5.70 ± 0.01	6.45 ± 0.20
(±)-BRL37344	5.76 ± 0.04	6.10 ± 0.03	5.84 ± 0.05	5.84 ± 0.12	ND[a]

[a]Value not determined since (±)-BRL37344 produced variable responses in this tissue.

BRL37344 > (-)-noradrenaline; and for β_3-adrenoceptors was (±)-BRL37344 > (-)-isoprenaline > (±)-formoterol > (-)-noradrenaline > (2*S*)-(-)-RO363 **11** > (±)-RO363 **7** ≥ (±)-salbutamol *(table I)*.

4.2. Functional studies in rat tissues

The potency of the agonists for stimulating chronotropic effects in isolated rat atria (β_1-mediated), and in relaxing tracheal chain preparations previously contracted with 1 μM carbachol (β_2-mediated), were determined *(table I)*. (2*S*)-(-)-RO363 **11**, (-)-isoprenaline and (±)-RO363 **7** were the most potent compounds for rat β_1-adrenoceptors, whereas (-)-isoprenaline and the β_2-selective agonist (±)-formoterol were the most potent at rat β_2-adrenoceptors. (±)-BRL37344 produced variable responses in the rat trachea making estimations of the functional potency (pD_2) difficult. In these preparations, (±)-RO363 **7** and (2*S*)-(-)-RO363 **11** were more β_1-selective than (-)-isoprenaline (≈ 1.5-fold and ≈ 6.5-fold, respectively).

These studies were conducted in the absence of neuronal and extra neuronal uptake inhibitors which might explain the relatively low potency of (-)-noradrenaline. However, the literature is unclear on this issue [9, 10] and suggests that the presence of neuronal and extraneuronal uptake inhibitors has little or no effect on the potency of catecholamines in rat atrial preparations [9, 10]. This mechanism is not important for (-)-isoprenaline, the other ethanolamine agonists studied, and phenoxypropanolamines like (±)-RO363 **7** [11].

The relative order of potency of the seven agonists at rat β_1-adrenoceptors was (2*S*)-(-)-RO363 **11** > (-)-isoprenaline > (±)-RO363 **7** > (-)-noradrenaline > (±)-formoterol > (±)-BRL37344 > (±)-salbutamol; at β_2-adrenoceptors the order was (±)-formoterol > (-)-isoprenaline > (2*S*)-(-)-RO363 **11** ≥ (±)-RO363 **7** > (±)-salbutamol > (-)-noradrenaline *(table I)*.

The functional potency of (2*S*)-(-)-RO363 **11** for the rat β_1-adrenoceptor was 4.5-fold greater than the potency of the racemic compound, whereas both the racemate and the (2*S*)-(-)-isomer had similar rat β_2-adrenoceptor potencies. The agonist efficacy of both (±)-RO363 **7** and (2*S*)-(-)-RO363 **11** were equivalent to (-)-isoprenaline in the rat atria (β_1-mediated) and the concentration-response curves of the three compounds were parallel *(figure 4a)*, ie., both (±)-RO363 **7** and (2*S*)-(-)-RO363 **11** produce an equivalent response to a full agonist on rat β_1-adrenoceptors. However, in the rat trachea (β_2-mediated) (±)-RO363 **7** and (2*S*)-(-)-RO363 **11** were only able to achieve 35 ± 6% and 34 ± 7% respectively of the maximum agonist effect obtained with (-)-isoprenaline.

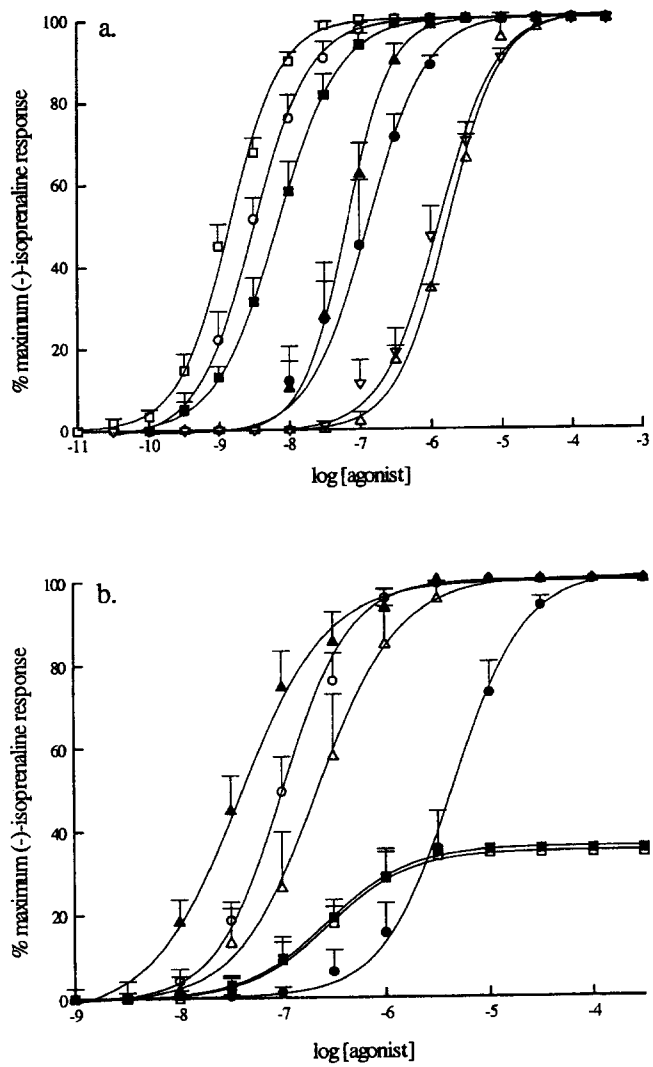


Figure 4. Concentration-response curves for: **a.** chronotropic effects on isolated rat atria; and **b.** relaxation of rat tracheal chain previously contracted with 1 μM carbachol by (-)-isoprenaline (○), (-)-noradrenaline (●), (2*S*)-(-)-RO363 (□), (±)-RO363 (■), (±)-salbutamol (Δ), (±)-formoterol (▲) and (±)-BRL 37344 (▽). Values given represent mean ± s.e.m. of 4–9 individual experiments.

The slopes of the (±)-RO363 **7** and (2*S*)-(-)-RO363 **11** concentration-response curves were different to that obtained with (-)-isoprenaline *(figure 4b)*, ie., both (±)-RO363 **7** and (2*S*)-(-)-RO363 **11** were acting as partial agonists on rat β_2-adrenoceptors.

4.3. Functional studies in guinea-pig tissues

In the present studies, the rat tracheal chain model displayed relatively low sensitivity to β_2-adrenoceptor mediated relaxation, which resulted in the non-selective β-adrenoceptor agonist (-)-isoprenaline displaying ≈ 30-fold β_1-selectivity. We therefore repeated our functional studies for (-)-isoprenaline and (2*S*)-(-)-RO363 **11** in guinea-pig atrial and tracheal preparations. In guinea-pig tissues, both (-)-isoprenaline and (2*S*)-(-)-RO363 **11** had similar potencies and efficacies for β_1-mediated chronotropic effects in atrial preparations (pD_2 values = 8.27 ± 0.08 and 8.30 ± 0.07, respectively). In guinea-pig trachea, precontracted with 300 nM carbachol, (-)-isoprenaline was ≈ 2.3-fold more potent than (2*S*)-(-)-RO363 **11** at stimulating β_2-mediated relaxation (pD_2 values = 7.23 ± 0.04 and 6.86 ± 0.09, respectively). Thus in guinea-pig tissue (-)-isoprenaline had a β_1-selectivity of ≈ 10 compared with ≈ 28 for (2*S*)-(-)-RO363 **11**, moreover, (2*S*)-(-)-RO363 **11** was only able to achieve 70 ± 3% of the maximum agonist effect obtained with (-)-isoprenaline and behaved as a partial agonist at β_2-adrenoceptors.

5. Conclusion

(2*S*)-(-)-RO363 **11** is unusual in that, in spite of its phenoxypropanolamine structure, it possesses high intrinsic efficacy with approximately 66-fold greater affinity for transfected human β_1-adrenoceptors than (-)-isoprenaline and approximately 2-fold greater potency at stimulating chronotropic responses in rat atria (β_1-mediated) than (-)-isoprenaline. Interestingly the phenoxypropanolamine agonists ((±)-RO363 **7** and its (2S)-(-)-enantiomer **11**), in contrast to (-)-isoprenaline, (-)-noradrenaline and formoterol display a functional

the rat compared to the guinea-pig, so that while 10 nM RO363 gives a full atrial response in the rat, it produces virtually no tracheal response. Traditionally, for compounds of the phenoxypropanolamine class, the (2*S*)-isomer is more potent than the (2*R*)-isomer. For RO363, this is confirmed by our pharmacological results where the (2*S*)-(-)-isomer **11** acts as a potent selective β_1-adrenoceptor agonist at the rat β_1-adrenoceptor, with a pD_2 of 8.88 (cf the racemate pD_2 of 8.23) and a selectivity ratio β_1/β_2 of 195 (cf the racemate of 47). (2*S*)-(-)-RO363 **11** also binds selectively, with high affinity, to the human β_1-adrenoceptor with a selectivity ratio β_1/β_2 of 69 and β_1/β_3 of 17 000. By contrast, other β_1-adrenoceptor agonists reported in the literature [13–15] lack the β_1-potency of (2*S*)-(-)-RO363 **11,** and most are either partial β_1-adrenoceptor agonists with low intrinsic efficacy (eg. xamoterol [13, 14]) or possess no appreciable selectivity (eg. dobutamine [12, 13, 15]).

6. Experimental protocols

6.1. Chemistry

6.1.1. General

Melting points were determined using a manual Gallenkamp electrothermal apparatus (range 0–400 °C) in glass capillary tubes and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT/IR 1600 spectrometer. ^{1}H-NMR spectra were recorded on a Varian EM 360 spectrometer. Chemical ionisation (C.I., methane gas) mass spectra were recorded on either a Finnigan GCQ™ or a Finnigan 4000 series GC/MS mass spectrometer. All spectra were consistent with the assigned structures. Optical rotation values were obtained using a Bellingham

6.1.3. 3,4-dibenzyloxyphenol **3**

MCBA (5.06 g, 29 mmol) was added in small portions, over 30 min, to a stirred solution of 3,4-dibenzyloxybenzaldehyde (6.36 g, 20 mmol) in anhydrous CH_2Cl_2 (100 mL) at 0 °C. The resulting mixture was stirred overnight, by which time no aldehyde proton could be detected by ^{1}H-NMR. The solids were dissolved by the addition of ether and the organic layer was washed three times with 10% $NaHCO_3$ solution, once with brine and then dried over anhydrous Na_2SO_4. Evaporation of the solvent furnished the crude formate ester **2** which was hydrolysed without further purification. Yield (crude) = 5.81 g, 87%; MS m/e 335 (M + 1); ^{1}H-NMR ($CDCl_3$) δ 5.28 (4H, s), 6.35–7.18 (3H, m), 7.54 (10H, s), 8.26 (1H, s).

The formate **2** was dissolved in the minimum amount of MeOH and a slight molar excess of KOH was then added while stirring under nitrogen. Half an hour later, water was added, followed by dilute aqueous HCl to bring the pH of the mixture to 6 and the solution was extracted with ether. The ethereal solution was washed with 10% aqueous $NaHCO_3$ solution, then washed with water and dried over anhydrous $MgSO_4$. The phenol was recovered after evaporation of the solvent and small traces of 3,4-dibenzyloxybenzoic acid were removed by column chromatography (silica, eluent CH_2Cl_2). The protected phenol **3** was then recrystallized from CCl_4. Yield (overall) = 4.84 g, 79%; m.p. = 113.6–114.4 °C (Lit. m.p. = 110.5–111.5 °C [16], Lit. m.p. = 101 °C [17], Lit. m.p. = 106–108 °C [18]); MS m/e 307 (M + 1); ^{1}H-NMR ($CDCl_3$) δ 5.08 (1H, s), 5.23 (4H, s), 6.31–7.07 (3H, m), 7.49 (10H, s).

6.1.4. (±)-1-(3′4′-Dibenzyloxyphenoxy)-2,3-epoxypropane **4**

3,4-Dibenzyloxyphenol **3** (3.06 g, 10 mmol) in EtOH (30 mL) was treated with aqueous KOH (0.62 g, 11 mmol, in 5 mL of water) and epichlorohydrin (2.78 g, 30 mmol) and stirred overnight. The reaction mixture was evaporated to dryness under reduced pressure and extracted with ethyl acetate. The extract was washed with water, then with 5% aqueous NaOH, again with water and then dried over anhydrous Na_2SO_4. The filtered solution was evaporated under reduced pressure and used without further purification. The epoxide **4** was homogenous by TLC (silica, eluent CH_2Cl_2). Yield (crude) = 3.12 g, 86%; MS m/e 363 (M + 1); ^{1}H NMR ($CDCl_3$) δ 2.68–3.02 (2H, q), 3.20–3.47 (1H, m), 3.78–4.42 (2H, m), 5.03 (2H, s), 5.10 (2H, s), 6.28–7.02 (3H, m), 7.37 (10H, s).

6.1.5. (±)-1-(3′,4′-Dibenzyloxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol hydrochloride **6**

(±)-1-(3′,4′-Dibenzyloxyphenoxy)-2,3-epoxypropane **4** (2.90 g, 8 mmol) and 3,4-dimethoxyphenylethylamine **5** (1.45 g, 8 mmol) in EtOH (20 mL) were heated to reflux for 6 h while stirring. The reaction mixture was evaporated to dryness and the residue was purified by column chromatography (silica, eluent: CH_2Cl_2/MeOH/NH_4OH 28%, 90:9:1). The fractions containing the desired product were combined, freed from solvents and the residue was dissolved in EtOH and treated with excess ethereal HCl. The precipitated hydrochloride **6** was recrystallized from EtOH/ether. Yield = 3.56 g, 82%; m.p. = 184.5–186.5 °C; MS m/e 546 (M + 1); ^{1}H NMR ($CDCl_3$) δ 1.96–2.16 (4H, m), 2.81–2.94 (2H, m), 3.88–4.02 (2H, m), 3.92 (3H, s), 3.96 (3H, s), 4.73–4.86 (1H, m), 5.19 (4H, s), 6.52–7.06 (6H, m), 7.48 (10H, s).

6.1.6. (±)-1-(3′,4′-Dihydroxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol hydrochloride **7**

(±)-1-(3′,4′-Dibenzyloxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol hydrochloride **6** (3.0 g, 5 mmol) in EtOH (50 mL) was hydrogenated at ambient temperature and pressure over Pd/C 10% (0.1 g) until hydrogen absorption ceased. The reaction mixture was filtered in a dark, dry box, under nitrogen and the solvent was evaporated under vacuum. The residue (**7**) was recrystallized from EtOH/ether, in the dark, under nitrogen and filtered under the same conditions. Yield = 1.75 g, 88%; m.p. = 69.5–71.5 °C; MS m/e 364 (M + 1); ^{1}H NMR ($CDCl_3$) δ 1.92–2.14 (4H, m), 2.80–2.92 (2H, m), 3.86–4.00 (2H, m), 3.92 (3H, s), 3.96 (3H, s), 4.72–4.85 (1H, m), 6.21–7.12 (6H, m). Anal. $[C_{19}H_{26}ClNO_6][H_2O]_{0.5}$, ie. elemental analysis is consistent with the presence of 0.5 mol of water of crystallization, % C calculated 55.81%, found 55.28%; % H calculated 6.61%, found 7.00%; % Cl calculated 8.69%, found 9.09% and % N calculated 3.43%, found 3.56% (Note: the oxalate salt of **8** was free of the water of crystallization [6]).

6.1.7. (2S)-(+)-1-(3′4′-dibenzyloxyphenoxy)-2,3-epoxypropane **9**

Sodium hydride, 60% suspension in mineral oil (0.52 g, 13 mmol), was washed with dry ether to remove the oil and suspended in anhydrous DMF (10 mL). While stirring, 3,4-dibenzyloxyphenol **3** (3.06 g, 10 mmol) in anhydrous DMF (7 mL) was added dropwise under nitrogen. Stirring continued for 30 min. The resulting mixture was cooled to 5 °C with the aid of an icebath and treated with a solution of (2*S*)-(+)-glycidyl-3-

nitrobenzenesulfonate **8** (2.59 g, 10 mmol) in DMF (7 mL). The mixture was stirred for 3 h under nitrogen whereupon it was cautiously treated with distilled water (2 mL). The mixture was diluted with twice its volume of water and extracted twice with 25 mL of ethyl acetate. The combined organic extracts were washed with 5% aqueous NaOH solution, followed by brine, dried over anhydrous Na_2SO_4 and evaporated. The residue was dried overnight under reduced pressure. The epoxide **9** was homogenous by TLC (silica, eluent CH_2Cl_2). Yield (crude) = 2.99 g, 83%; MS m/e 363 (M + 1); ^{1}H-NMR ($CDCl_3$) δ 2.68–3.02 (2H, q), 3.20–3.47 (1H, m), 3.78–4.42 (2H, m), 5.03 (2H, s), 5.10 (2H, s), 6.28–7.02 (3H, m), 7.37 (10H, s).

6.1.8. (2S)-(-)-1-(3′,4′-dibenzyloxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol hydrochloride **10**

(2*S*)-(+)-1-(3′,4′-dibenzyloxyphenoxy)-2,3-epoxypropane **9** (2.90 g, 8 mmol) and 3,4-dimethoxyphenylethylamine **5** (1.45 g, 8 mmol) in EtOH (20 mL) were heated to reflux for 6 h while stirring. The reaction mixture was evaporated to dryness and the residue was purified by column chromatography (silica, eluent: CH_2Cl_2/MeOH/NH_4OH 28%, 90:9:1). The fractions containing the desired product were combined, freed from solvents and the residue was dissolved in EtOH and treated with excess ethereal HCl. The precipitated hydrochloride **10** was recrystallized from EtOH/ether. Yield = 3.40 g, 78%; m.p. = 185.5–188.0 °C; MS m/e 546 (M + 1); ^{1}H-NMR ($CDCl_3$) δ 1.96–2.16 (4H, m), 2.81–2.94 (2H, m), 3.88–4.02 (2H, m), 3.92 (3H, s), 3.96 (3H, s), 4.73–4.86 (1H, m), 5.19 (4H, s), 6.52–7.06 (6H, m), 7.48 (10H, s). $[\alpha]^{20}_D = -7.2°$ (2.05% solution, DMSO:CH_3CN 1:1).

6.1.9. (2S)-(-)-1-(3′,4′-dihydroxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol hydrochloride **11**

(2*S*)-(-)-1-(3′,4′-dibenzyloxyphenoxy)-3-(3″,4″-dimethoxyphenyl)ethylamino-2-propanol hydrochloride **10** (3.0 g, 5 mmol) in EtOH (50 mL) was hydrogenated at ambient temperature and pressure over Pd/C 10% (0.1 g) until hydrogen absorption ceased. The reaction mixture was filtered in a dark, dry box, under nitrogen and the solvent was evaporated under vacuum. The residue (**11**) was recrystallized from EtOH/ether, in the dark, under nitrogen and filtered under the same conditions. Yield = 1.86 g, 93%; m.p. = 71.0–72.5 °C; MS m/e 364 (M + 1); ^{1}H-NMR ($CDCl_3$) δ 1.92–2.14 (4H, m), 2.80–2.92 (2H, m), 3.86–4.00 (2H, m), 3.92 (3H, s), 3.96 (3H, s), 4.72–4.85 (1H, m), 6.21–7.12 (6H, m). $[\alpha]^{20}_D = -11.6°$ (2.88% solution, MeOH). Anal. $C_{19}H_{26}ClNO_6$ (C, H, N, Cl).

6.1.10 Optical purity of (2S)-(-)-RO363

The immediate precursor of (±)-RO363, the di-O-benzyl derivative, was completely resolved into the R- and S-isomers on a column of Chiracel OD (4 × 250 mm, Daicel Chemical Industries Ltd) using a mobile phase of hexane/isopropanol/diethylamine (60:40:0.1) at a flow rate of 1 mL/min. Detection was by UV absorbance at 283 nm. The R-isomer eluted at 17 min and the S-isomer at 25 min, each isomer producing a similar peak area. The (2*S*)-(-)-RO363 derivative showed a major peak of the S-isomer with a trace of the R-isomer from which we calculate the optical purity of > 99%. (±)-RO363 **7** was partially resolved on the same column with a mobile phase of hexane/isopropanol/ethanol/diethylamine (30:45:25:0.1), the two isomers eluting at 8.9 and 10.2 min. (2*S*)-(-)-RO363 **11** produced a single peak at 10 min, consistent with a high optical purity. Because of the incomplete resolution of the two isomers, an exact figure for the optical purity could not be estimated, however the chromatograms suggest a similar purity to that of the precursor.

6.2. Pharmacology

6.2.1. Drugs and chemicals

(-)-Isoprenaline, (-)-noradrenaline and (±)-propranolol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). (-)-[^{125}I]-ICYP was purchased from Amersham (Buckinghamshire, UK) and (±)-BRL37344.HCl was from Tocris Cookson Ltd. (Bristol, UK). (±)-Formoterol was a gift from Professor J. Angus of the Department of Pharmacology of the University of Melbourne. (±)-Salbutamol and (±)-bupranolol were synthesized within our department by Dr D. Iakovidis. All other chemicals were of reagent grade from BDH Chemicals (Kilsyth, Australia).

6.2.2. Isolated tissue preparations

All studies were performed in agreement with and according to the Prevention of Cruelty to Animals Act (1986), the NH & MRC/CSIRO/AAC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) and with the approval of the Animal Welfare Committee at the Austin and Repatriation Medical Centre. Male and female Sprague-Dawley rats, 200–300 g, and guinea-pigs (400–450 g) were used and killed by a blow to the neck followed by cervical dislocation and/or decapitation.

Studies were carried out on rat isolated atria and tracheal rings as we described previously [19]. All tissues were allowed to equilibrate for 45 min with Krebs Ringer physiological salt solution; the composition of which in mmol L^{-1} was NaCl, 120; KCl, 5.6; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.4; $NaHCO_3$, 25; glucose 11.2 and EGTA, 0.0025. Cumulative concentration-response curves were obtained for agonists in each preparation.

6.2.2.1. Rat isolated spontaneously beating atria

Rat hearts were removed from adult animals and placed in Krebs Ringer salt solution (pH 7.4) aerated with 5% CO_2 in O_2. The atria were dissected free of the ventricles and overlying tissue and placed in a 20 mL bath maintained at 37 °C and connected to an isotonic transducer. A tension of 1 g was applied and chronotropic activity was amplified and recorded on a Grass Polygraph.

the dark. Saturation studies were performed with 0.5–500 pM [^{125}I]-ICYP for the β_1- and β_2-adrenoceptor cell lines and 1–3 000 pM for the β_3-adrenoceptor cell line. Non-specific binding was determined in the presence of 2 μM (±)-propranolol for the β_1- and β_2-adrenoceptor cell lines and 100 μM (±)-bupranolol for the β_3-adrenoceptor cells as described by Blin et al. and Gros et al. [20, 21]. Incubations were terminated by rapid filtration onto GF/C Whatman glass fibre filters presoaked with 0.01% polyethyleneimine and washing with 4 × 5 mL ice-cold phosphate buffer (pH 7.4). Filter-bound radioactivity was counted for 1 min using an LKB Multigamma counter.

6.2.4. Data analysis

6.2.4.1. Functional studies

All concentration-response curves were expressed as a percentage of the maximum response by the agonist and

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