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Synthesis and bovine β_3-adrenergic agonistic activities of a novel series of aryloxypropanolamines

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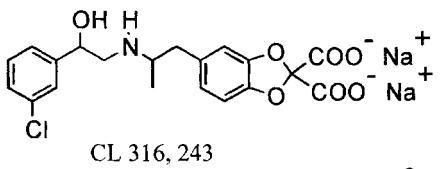
We synthesized a novel series of 21 aryloxypropanolamine compounds characterized by *N*-alkyl, aralkyl, and aryl substituents. The compounds showed potent β_3-adrenergic agonistic activities in Chinese hamster ovary cells expressing the bovine β_3-adrenoceptors with K_{act} and K_i values of 4.2 $\pm$ 3.0 nM and 459 $\pm$ 169 nM respectively, for the ligand with the best compromise between potency and affinity. Structure-activity relationships are discussed.

1. Introduction

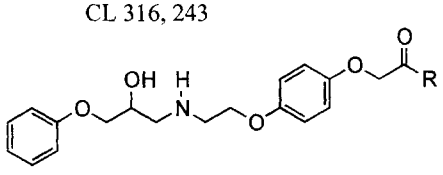
Noradrenaline released from sympathetic nerve endings stimulates two different types of receptors at the cell surface, α and β-adrenoceptors. The three β-adrenoceptor subtypes are coupled to Gs protein causing increased cAMP and protein-kinase A activation [1]. Increased heart rate is the primary consequence of β_1-adrenoceptor stimulation, while broncho dilation and smooth muscle relaxation typically result from β_2-adrenoceptors. The β_3-adrenoceptor is predominantly localized in fat tissues, it is most abundantly expressed in brown adipose tissue where it regulates noradrenaline-induced changes in energy expenditure and thermogenesis [2]. It is also expressed in white adipose tissue where it is involved in lipolysis. Promising results after treating obese animals allowed the evaluation of compounds as anti-obesity and anti-diabetic agents. Thus, the search for specific β_3-adrenergic agonists may also be beneficial in the veterinarian treatment of cattle, for which large mass gains have to be balanced against an upper limit of acceptable fat concentration. We report the synthesis and the biological activity of a novel series of β_3-agonists fit for use in the field of veterinarian medicine and animal husbandry, aimed at increasing overall lean body mass.

Major support for the third subtype of β-adrenoceptors came from a series of potent β_1 and β_2 blocking agents with aryloxypropanolamine structure, also potent β_3-agonists [3, 4]. Among these compounds, ICI 201651 (or its metabolic precursor ICI D 7114) has been extensively studied in animals. It has been shown to be a high potent agonist the bovine β_3-adrenoceptor with also potent β_1 and β_2-adrenoceptor blocking effect [5]. More precisely it has been suggested that the presence of a long and bulky amine substituent moiety of ligand promoted a good β_3 selectivity. In addition, it was demonstrated that either mono or dicarboxylic groups improve the β_3 selectivity by decreasing the β_1 and β_2 binding affinity [6]. In other respects, Bloom *et al.* observed that the *N*-(1,3-benzodioxole) group vastly increased antihyperglycemia, anti-obesity properties, and β_3 selectivity of a series of arylethanolamine derivatives, as exemplified by the CL 316,243 compound [7, 10].

For these reasons we synthesized a new series of aryloxypropanolamines substituted on the terminal nitrogen either by a 1,3-benzodioxole moiety or by various alkyl, aralkyl, and aryl substituents. We then examined their pharmacological properties on the bovine β_3-adrenoceptor, in terms of binding affinities and adenylyl cyclase activation.



CL 316, 243



R : $NHCH_2CH_2OMe$ (ICI D 7114 - *(S)* - isomer)
R : OH (ICI 201651)

2. Investigations, results and discussion

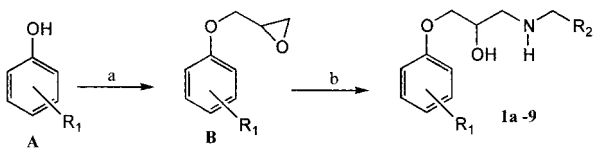
2.1. Chemistry

The synthetic routes to the target compounds **1a** to **9** are illustrated in Scheme 1, their properties are listed in Table 1. The key compounds in the preparation of a series of aryloxypropanolamines were the readily available epoxides and the requisite amines.

The starting material for compounds **1e, 3c** and **5a** was 3-hydroxyphenol. It was preferable to first protect one phenolic function with benzyl chloride before the epichlorohydrin alkylation process (Scheme 2) [11]. Surprisingly, treating **10** with sodium hydride and epichlorohydrine in DMF led to the alkylation of the free phenolic group and to the deprotection of the other one, in a single step. The intermediate epoxide **11** prepared by this method was treated with amine and used without further purification up to the final step of the synthesis.

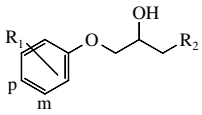
Similarly, treating 3-nitrophenol with sodium hydride and epichlorohydrin in dry DMF at 60 °C during 1 h gave the expected epoxide which was opened with isopropylamine

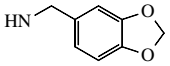
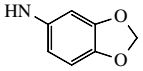
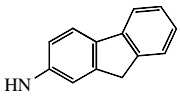
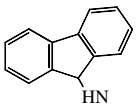
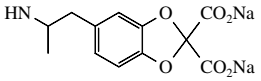
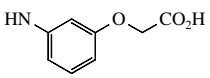
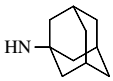
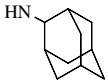
Scheme 1



Reagents : (a) NaH, DMF, epichlorohydrin ; (b) R_2NH_2, DMF, 80°C.

Table 1: Structure and chemical data of aryloxypropanolamines 1a–9



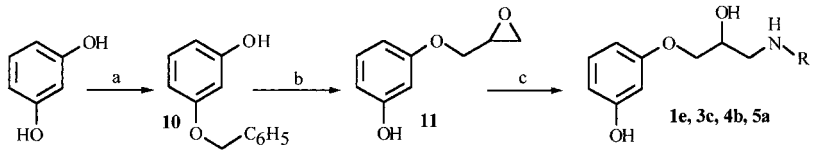
Compd.[a]	R_1	R_2	Method	Yield (%)[b]	Crystn solvent	M.p. (°C)[c]
1a	H		B	67	MeOH	102–103
1b	p-OMe		B	80	MeOH	107–108
1c	m-Cl		A	62	MeOH	128–129
1d	p-Cl		B	74	EtOH	98–99
1e	m-OH		A	62	MeCN	230–231[d]
1f	m-NO_2		A	47	EtOH	129–130
1g	m-CN		A	55	MeOH	138–139
1h	m-CF_3		A	39	EtOH	121–122
2a	H		B	45	EtOAc/cyclohex. (2/8)	80–81
2b	p-OMe		B	65	Et_2O/cyclohex.	80–81
3a	H		B	55	EtOAc	138–139
3b	p-OMe		B	64	EtOAc/cyclohex. (2/8)	119–120
3c	m-OH		A	42	EtOH/Et_2O	94–95
4a	H		B	63	MeCN	96–97[e]
5a	m-OH		A	60	EtOAc/MeOH (95/5)[f]	184–185[g]
5b	m-NH_2	$HNCH(CH_3)_2$	A	66	EtOAc/MeOH (99/1)[f]	hygroscopic
5c	H		B	82	MeCN	83–84
6	H		B	45	H_2O/EtOH (2/8)	>300
7	H		B	39	MeCN	138–139
8	H		B	79	MeOH	230–231[e]
9	H		B	82	EtOH/Et_2O	62–63

[a] All described compounds were fully characterized including spectroscopic and elemental analysis. [b] Yield is based on the epoxide. [c] Melting points of compounds were determined with [d] maleate salt and [e] hydrochloride salt. [f] Purification by column chromatography on silica gel using a mixture of solvents. [g] Melting points of compounds were determined with oxalate salt.

at 70 °C overnight to afford compound **12** which was then reduced to the amino derivative **5b** by H_2, Pd/C (10%) under 1 atm. during 12 h (Scheme 3).

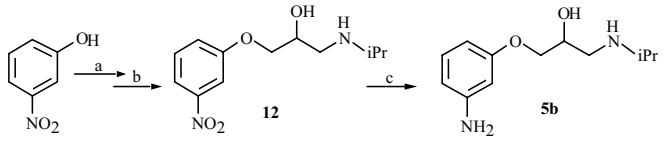
The synthesis of the desired disodium benzodioxole dicarboxylate **6** from the known 1,2-epoxy-3-phenoxypropane and the synthetic amine **16** is outlined in Scheme 4. Compound **13** was obtained by reacting 3,4-dimethoxyphenyl carboxaldehyde with nitroethane to afford the corresponding nitrostyrene derivative, the ethylenic function of which was reduced by $NaBH_4$ [12, 13]. Demethylation of the ether functions of **13** was carried out with boron tribromide in dichloromethane to give the phenolic derivative **14** which was then reacted with diethyl dibromomalonate and anhydrous potassium carbonate in acetone giving ben-

Scheme 2



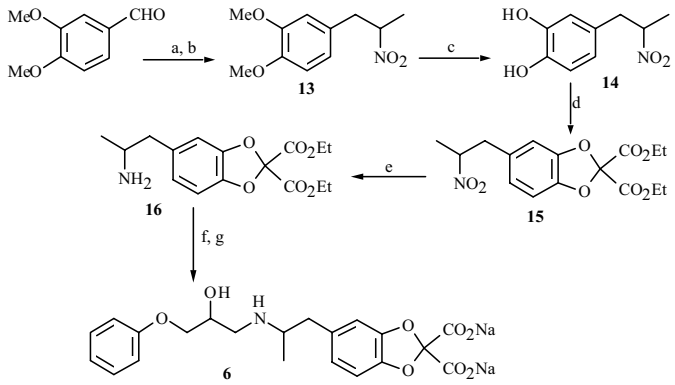
Reagents : (a) Cl-CH2-C6H5, K2CO3, CH3COCH3; (b) NaH, DMF, epichlorohydrin; (c) RNH 2 , DMF, 70°C.

Scheme 3



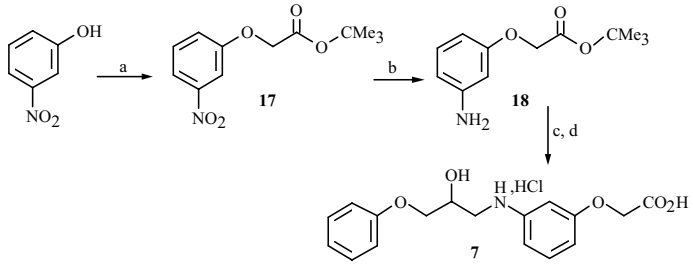
Reagents: (a) NaH, DMF, epichlorohydrin; (b) RNH_2, DMF, 70 °C; (c) H_2, Pd/C (10%), EtOH, 1 atm

Scheme 4



Reagents: (a) $EtNO_2$, $MeCO_2NH_4$, $MeCO_2H$; (b) $NaBH_4$, MeOH; (c) BBr_3, CH_2Cl_2, 0 °C; (d) $Br_2C(CO_2Et)_2$, K_2CO_3, acetone; (e) H_2/Raney Ni, EtOH; 20 atm.; (f) 1,2-epoxy-3-phenoxy propane, 70 °C; (g) 1 N NaOH, CH_3CN, 25 °C

Scheme 5



Reagents: (a) $BrCH_2CO_2CMe_3$, K_2CO_3, acetone; (b) H_2, Pd/C (10%), EtOH, 1 atm.; (c) 1,2-epoxy-3-phenoxypropane, 70%; (d) 6 N HCl, 80 °C

zodioxole-2,2-dicarboxylic acid diethyl ester **15** [11]. Reduction of the NO_2 group of **15** by Raney nickel afforded amine **16** which was first reacted with epoxide **B** (Scheme 1, R_1=H). Ester hydrolysis was then carried out with 1 N NaOH in CH_3CN to give the final product **6**.

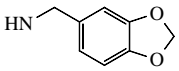
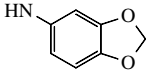
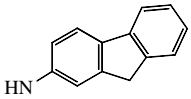
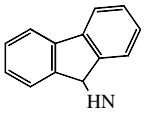
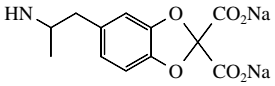
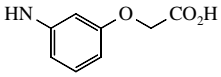
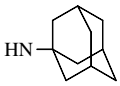
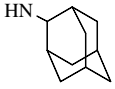
Furthermore, treating 3-nitrophenol with *tert*-butyl bromoacetate and anhydrous potassium carbonate in acetone yielded **17** which led to the required amine **18** by hydrogenation in the presence of Pd-C/10% (Scheme 5). Amine **18** was then reacted with epoxide **B** (R_1=H) as described previously, to give an intermediate ester, the *tert*-butyl ester function of which was hydrolysed by 6N HCl to afford compound 7.

2.2. Biological results and discussion

The compounds were classified in two groups with regards to their agonistic or antagonistic activity so as to define the pharmacological properties of the studied ligands (Table 2). The stimulation of adenylyl cyclase was measured by the determination of cAMP accumulation in CHO-K1 cells expressing bovine β_3-adrenoceptors.

16 out of 21 derivatives were qualified as bovine β_3-adrenoceptor agonists, 2 as antagonists and 3 were inactive. For the agonist class, intrinsic activity (IA) values were calculated for each ligand relative to the maximal cAMP accumulation obtained with (−)-isoproterenol. 9 ligands displayed full agonistic activity with an IA superior to 0.8. 7 ligands were further examined in terms of binding and potency to determine their inhibition constant (K_i) and their adenylate cyclase activation constant (K_{act}). The data obtained allowed to select 5 ligands e.g. **1a**, **4a**, **6**, **8** and **9**, displaying high affinity and/or potency for bovine β_3-adrenoceptors. The ligand with the best compromise between potency and affinity was **1a**, with K_{act} and K_i values of 4.2 ± 3.0 nM and 459 ± 169 nM, respectively. Thus, compound **1a,** containing a benzodioxole moiety, like the potent and selective β_3 agonist CL 316,243, compares favorably with this reference, showing K_{act} and K_i values of 68 nM and 14,000 nM, respectively, determined on CHO-K_1 cells expressing human β_3-adrenoceptors. It should be noted that, as far as we know, CL 316,243 has not been evaluated on bovine β_3-adrenoceptors yet.

Table 2: Biological activity on the bovine β_3-adrenoceptor

			Agonist			Antagonist
Compd.	R1	R2	IA[a]	Ki(μM)[b]	Kact (nM)[c]	% inhib[d]
1a	H		0.93 ± 0.01	0.459 ± 0.169	4.2 ± 3.0	–
1b	p-OMe		–			32 ± 20
1c	m-Cl		0.43 ± 0.08			–
1d	p-Cl		–			88 ± 12
1e	m-OH		inactive			inactive
1f	m-NO_2		0.21 ± 0.005			–
1g	m-CN		0.32 ± 0.01			–
1h	m-CF_3		inactive			inactive
2a	H		0.61 ± 0.04			–
2b	p-OMe		0.22 ± 0.10			–
3a	H		0.74 ± 0.21	6.7 ± 1.6	17,000 ± 5,000	–
3b	p-OMe		inactive			inactive
3c	m-OH		0.81 ± 0.01	2.27	1880	–
4a	H		0.97 ± 0.03	3.8 ± 1.9	270 ± 30	–
4b	m-OH		nd			nd
5a	m-OH		0.86 ± 0.05			–
5b	m-NH_2	$HNCH(CH_3)_2$	0.79 ± 0.05			–
5c	H		1.05 ± 0.17			–
6	H		0.93 ± 0.13	0.224 ± 0.134	15.6 ± 15.0	–
7	H		1.09 ± 0.13		3,400 ± 2,400	–
8	H		1.00 ± 0.14	1.75 ± 0.5	11.5 ± 4.5	–
9	H		1.07 ± 0.07	11 ± 8	29.2 ± 8.0	–
CL 316,243			1.6 [e]	14	68	

All the values are means ± SEM of at least 2 or 3 independent experiments performed in duplicate. [a] IA: intrinsic activity measured relatively to the maximal cyclase stimulation obtained for (−)-isoproterenol 10^{-4} M. [b] Ki: inhibition constant determined in the presence of ^{125}Iodocyano pindolol (according to Cheng and Prusoff) [14]. [c] Kact: activation constant defined as the concentration of ligand necessary to obtain 50% of the maximal effect. [d] % inhib: for antagonistic class, the inhibition activity of each ligand was calculated relative to their capacity to inhibit (−)-isoproterenol effect. [e] Values obtained from CHO-K1 cells expressing human β-adrenoceptors [15].

Concerning the structure activity relationship, the presence of a bulky amine substituent appears to be favorable for the β_3-adrenergic activity, correlating with our hypothesis. However, it is interesting to note that aralkyl derivatives (**1a**, **4a**) are more active than their *N*-aryl analogs (**2a**, **3a**, **7**). *N*-adamantyl derivatives **8** and **9**, which can be derived respectively from *N-tert*-butyl and *N*-isopropyl derivatives, are equipotent to *N*-aralkyl compounds. It should be noted that compound **6** is more active than CL 316,243 (containing the same *N*-terminal moiety) but less active than the simple benzodioxole derivative **1a**.

The presence of a substituent on the aryloxy part seems to play an important role in the agonistic/antagonistic ratio. The *para* substituent of **1b** and **1d** seems to promote the antagonistic activity, whereas a *meta* substituent decreases or abolishes (**1e**) the agonistic activity. This could be related to a potential hydrogen bond between one of the two oxygen atoms of the benzodioxole ring and the *m*-OH group, leading to a more stacked conformation.

In conclusion, our research has shed some light in the structural requirement for β_3-adrenergic activity and has led to a series of potent β_3-adrenoceptor agonists characterized by an aryloxypropanolamine skeleton [16]. More detailed biological studies are necessary to determine the pharmacological profile of these compounds, particularly for **1a** which possesses the best ratio between potency and affinity against β_3-adrenoceptors.

3. Experimental

3.1. Apparatus

Melting points were determined with Gallenkamp capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 200.13 MHz (Bruker AC 200 F.T.). Chemical shifts are reported in parts per million (δ) downfield from an internal standard of tetramethylsilane (Me_4Si) for $CDCl_3$, D_2O and Me_2SO-d_6. IR spectra were recorded on a PYE unicam SP3-100 (Philips) spectrophotometer. Microanalyses agreed to within ± 0.4% of the calculated values, unless otherwise noted.

3.2. Chemistry

3.2.1. 3-Benzyloxyphenol (**10**)

Resorcinol (36.6 g, 0.33 mol) and 42 g (0.33 mol) of benzylchloride were refluxed overnight with stirring in 250 ml of dry acetone containing 46.6 g of K_2CO_3. The mixture was cooled, filtered, and evaporated. The residue

was diluted with 500 ml of water and extracted with Et_2O. The organic layer was washed with 10% aq. NaOH, and extracted with Et_2O. The red oil obtained was distilled with a "Kugelrohr distillation apparatus" yielding 18 g of 10, b_{15} 240–245 °C (lit. [7, 9] b_{11} 202–210 °C).

3.2.2. 1,2-Epoxy-3-(3-hydroxyphenoxy)propane (**11**)

A solution of 3.3 g (16.5 mmol) of **10** in 50 ml of DMF was treated with 0.8 g (16.5 mmol) of sodium hydride, and the mixture was stirred for 15 min. After addition of 13.75 ml (16.5 mmol) of epichlorohydrin, the mixture was stirred at 60 °C for 1 h to complete alkylation. The excess reagent and solvent were evaporated under reduced pressure, and the residue was partitioned between ethyl acetate and water. The organic layer gave the epoxide, which was used without further purification.

3.2.3. N-Isopropyl-3-(3-nitrophenoxy)-2-hydroxypropylamine (**12**)

A solution of 3 g (21.6 mmol) of 3-nitrophenol in 500 ml of dry DMF was treated with 1.05 g (21.6 mmol) of sodium hydride, and the mixture was stirred for 30 min. After addition of 18 ml (21.6 mmol) of epichlorohydrin, the mixture was stirred at 60 °C for 1 h to complete alkylation. The excess reagent and solvent were evaporated in vacuo, and the residue was partitioned between ethyl acetate and water to give the crude epoxide, which was heated at 70 °C overnight with an excess of isopropylamine. The mixture was evaporated in vacuo and the residue was washed with CH_3CN to provide the corresponding amino-alcohol **12** as a yellow solid.

3.2.4. 2-Nitro-1-(3,4-dimethoxyphenyl)propane (**13**)

To a solution of 14.3 g (86 mmol) of 3,4-dimethoxyphenylcarboxaldehyde in 200 ml of AcOH containing 4 g of NH_4OAc was added 10 g (90 mmol) of nitroethane and the mixture was refluxed overnight. After cooling the excess reagent and solvent were evaporated in vacuo. The residue was chromatographed on silica gel eluting with EtOAc/cyclohexane (20/80) to afford the intermediate nitrostyrene (m.p. 73–74 °C). To a solution of the latter (33.60 mmol) in 75 ml of methanol was added 2.54 g (67.3 mmol) of $NaBH_4$ under stirring at 5–10 °C. After allowing the reaction to proceed for another 1 h at room temperature, the reaction mixture was concentrated, excess of $NaBH_4$ decomposed with acetic acid and extracted with CH_2Cl_2. The organic layer gave the required compound **13** as an oil, yield 70%. ^{1}H NMR ($CDCl_3$) d 1.38 (d, 3 H, J = 7.2 Hz), 2.72 (dd, 1 H, J = 16.0, 8.0 Hz), 3.09 (dd, 1 H, J = 15.0, 8.0 Hz), 3.64 (s, 6 H), 4.49 (m, 1 H), 6.42–6.60 (m, 3 H, Ar–H).

3.2.5. 2-Nitro-1-(3,4-dihydroxyphenyl)propane (**14**)

To an ice cold solution of 7 g (31.10 mmol) of **13** in 200 ml of CH_2Cl_2 was added dropwise 8.8 ml of boron tribromide. The mixture was stirred at 0 °C to 5 °C for 15 min, then at room temperature for 30 min, quenched with H_2O and stirred for 20 min. The CH_2Cl_2 layer was separated, washed with H_2O, dried and evaporated to afford 73% of **14**, which was used for the next step without purification.

3.2.6. Diethyl-5-(2-nitropropyl)benzodioxol-2,2-dicarboxylate (**15**)

A mixture of 3 g (15.2 mmol) of catechol **14**, 4.9 g (15.2 mmol) of diethyl dibromomalonate, 8 g of anh. K_2CO_3 and 120 ml of acetone was stirred overnight at room temperature. The mixture was filtered, washed with acetone and the combined filtrates were evaporated to give a yellow oil which was purified by CC on silica gel eluting with EtOAc/cyclohexane (50/50). The pure fractions were combined and evaporated to give 87% of **15** as a colorless oil. ^{1}H NMR ($CDCl_3$) d 1.32 (t, 6 H, J = 7.07 Hz), 1.53 (d, 3 H, J = 6.67 Hz), 2.93 (dd, 1 H, J = 14.17, 6.57 Hz), 3.25 (dd, 1 H, J = 14.15, 7.62 Hz), 4.36 (q, 4 H, J = 7.14 Hz), 4.71 (m, 1 H), 6.70 (dd, 1 H Ar, J = 8.10, 1.55 Hz), 6.76 (d, 1 H Ar, J = 1.57 Hz), 6.86 (d, 1 H Ar, J = 8.0 Hz).

3.2.7. Diethyl-5-(2-aminopropyl)benzodioxol-2,2-dicarboxylate (**16**)

To a solution of 3.54 g (10 mmol) of 15 in 20 ml of ethanol was added 2 g of Raney nickel and the mixture was hydrogenated overnight at 20 atm. The catalyst was filtered off and the solvent evaporated in vacuo to give the crude amine **16** which was used for the next step without further purification.

3.2.8. tert-Butyl-3-nitrophenoxyacetate (**17**)

A mixture of 3 g (21.56 mmol) of 3-nitrophenol, 4.3 g (22 mmol) of tert-butyl bromoacetate, 3.2 g of anh. K_2CO_3 and 50 ml of acetone was stirred overnight at room temperature. The mixture was filtered, washed with acetone and the combined filtrates were evaporated to a yellow oil which was purified by CC on silica gel, eluting with EtOAc/cyclohexane (50/50) to give 5 g (92%) of **17** as a colorless oil.

3.2.9. tert-Butyl-3-aminophenoxyacetate (**18**)

To a solution of 4.8 g (18.95 mmol) of **17** in ethanol was added 2 g of Pd/C (10%) and the mixture was hydrogenated overnight at 1 atm. The catalyst was filtered off and the solvent evaporated in vacuo to yield 3.38 g (80%) of the required amine **18** which was chromatographed on silica gel column eluting with EtOAc/cyclohexane (50/50).

3.2.10. General synthetic procedure for aryloxypropanolamines **1a** *to* **9**

Aryloxypropanolamines were prepared from epoxides and amines intermediates using method A or B. The preparation of **1e** is illustrative of method A and that of **6** of method B (see Table 1).

3.2.10.1. [3-(m-Hydroxyphenyl)oxy-2-hydroxypropyl]amino methyl-1,3-benzodioxole (**1e**) (Method A)

To a solution of epoxide **11** in DMF was added 1 equiv. of piperonylamine and the mixture was heated overnight at 80 °C. After cooling, the solvent was removed in vacuo, and the oily residue was chromatographed on silica gel using a mixture of solvents and recrystallized as indicated in Table 1.

3.2.10.2. N-Isopropyl-3-(3-aminophenoxy)-2-hydroxypropylamine (**5b**)

To a solution of 0.4 g (1.57 mmol) of **12** in 30 ml of methanol was added 0.05 g of Pd/C (10%) and the mixture was hydrogenated for 12 h at 1 atm. The catalyst was filtered off and the solvent evaporated in vacuo yielding the expected amine which was chromatographed on a silica gel eluting with EtOAc/MeOH (99/1).

3.2.10.3. 5-[2-(3-Phenoxy-2-hydroxypropylamino)propyl]-1,3-benzo dioxole-2,2-dicarboxylic acid, disodium salt (**6**) (Method B)

A mixture of 0.19 g (1.24 mmol) of 1,2-epoxy-3-phenoxypropane and 0.4 g (1.24 mmol) of amine **16** was heated at 70 °C overnight. After cooling, the product was chromatographed on silica gel eluting with EtOAc/MeOH (95/5) to afford a hygroscopic solid which was washed with small portions of EtOH to furnish 0.41 g (70%) of benzodioxole-2,2-diethylester. 0.4 g (0.85 mmol) of this diethylester, 1.7 ml of 1 N sodium hydroxide and 20 ml of CH_3CN were stirred for 48 h at room temperature. The solvents were evaporated and the residue was washed with small portions of EtOH, CH_3CN and MeOH and then crystallized from H_2O/EtOH (2/8) to yield 0.25 g (64%) of the disodium salt **6**.

3.2.10.4. 3-[(3-Phenoxy-2-hydroxypropyl)amino]phenoxyacetic acid (**7**)

A mixture of 0.34 g (2.24 mmol) of 1,2-epoxy-3-phenoxypropane and 0.5 g (2.24 mmol) of amine **18** was heated at 70 °C overnight. After cooling, the product was washed with EtOH and the precipitate was filtered off to give 0.6 g of the tert-butylester. 0.6 g of this ester and 15 ml of 6 N hydrochloride were stirred overnight at 80 °C. The solvent was evaporated in vacuo and the residue was washed with EtOH, CH_3CN and then recrystallized from CH_3CN to give 0.36 g of the hydrochloride salt **7**.

3.2.10.5. ^{1}H NMR data of final compounds **1a–9**

1a (free base in Me_2SO-d_6) : δ ppm = 2.59 (m, 2 H), 3.62 (s, 2 H), 3.90 (m, 3 H), 5.95 (s, 2 H), 6.78 (m, 2 H Ar), 6.90 (m, 4 H Ar), 7.26 (dt, 2 H Ar, J = 7.73, 1.02 Hz).

1b (free base in Me_2SO-d_6) : δ ppm = 2.52 (m, 2 H), 3.61 (s, 2 H), 3.68 (s, 3 H), 3.83 (m, 3 H), 4.88 (b, NH), 5.95 (s, 2 H), 6.75 (dd, 1 H Ar, J = 8.0, 1.38 Hz), 6.81 (d, 1 H Ar, J = 8.0 Hz), 6.83 (s, 4 H), 6.89 (d, 1 H Ar, J = 1.1 Hz).

1c (maleate in Me_2SO-d_6) : δ ppm = 2.90 (dd, 1 H, J = 13.56, 9.40 Hz), 3.07 (dd, 1 H, J = 12.59, 2.94 Hz), 3.97 (d, 2 H, J = 5.07 Hz), 4.10 (s, 2 H), 4.12 (m, 1 H), 6.01 (s, 2 H), 6.04 (s, 2 H), 6.90 (dd, 1 H Ar, J = 8.31, 2.14 Hz), 7.00 (m, 4 H Ar), 7.09 (s, 1 H Ar), 7.31 (t, 1 H Ar, J = 8.42 Hz).

1d (maleate in Me_2SO-d_6) : δ ppm = 2.90 (dd, 1 H, J = 12.63, 9.20 Hz), 3.08 (dd, 1 H, J = 12.67, 3.18 Hz), 3.93 (d, 2 H, J = 5.18 Hz), 4.10 (s, 2 H), 4.12 (m, 2 H), 6.03 (s, 2 H), 6.04 (s, 2 H), 6.95 (m, 4 H Ar), 7.09 (s, 1 H), 7.34 (d, 2 H, J = 8.99 Hz).

1e (free base in Me_2SO-d_6) : δ ppm = 2.85 (dd, 1 H, J = 12.47, 8.89 Hz), 3.04 (dd, 1 H, J = 12.63, 3.02 Hz), 3.93 (d, 2 H, J = 5.18 Hz), 4.07 (s, 2 H), 4.23 (m, 1 H), 5.85 (m, 1 H Ar), 6.03 (s, 2 H), 6.53 (m, 2 H Ar), 6.92 (d, 1 H Ar, J = 8.0 Hz), 7.02 (dd, 1 H Ar, J = 8.0, 1.58 Hz), 7.19 (m, 2 H Ar).

1f (free base in Me_2SO-d_6) : δ ppm = 2.58 (t, 2 H, J = 5.64 Hz), 3.62 (s, 2 H), 3.89 (m, 1 H), 3.97 (dd, 1 H, J = 15.59, 5.98 Hz), 4.11 (dd, 1 H, J = 15.14, 4.0 Hz), 4.99 (b, 1 H), 5.94 (s, 2 H), 6.78 (m, 2 H), 6.89 (s, 1 H), 7.39 (ddd, 1 H, J = 8.29, 2.47, 0.95 Hz), 7.55 (dd, 1 H, J = 6.93, 1.05 Hz).

1g (free base in Me_2SO-d_6) : δ ppm = 2.55 (m, 2 H), 3.61 (s, 2 H), 3.89–4.05 (m, 3 H), 4.97 (s, 1 H), 5.95 (s, 2 H), 6.72–6.78 (m, 2 H), 6.88 (s, 1 H), 7.24–7.50 (m, 4 H).

1h (free base in Me_2SO-d_6) 2.58 (m, 2 H), 3.61 (s, 2 H), 3.90–4.05 (m, 3 H), 4.97 (s 1 H), 5.94 (s, 2 H), 6.72–6.78 (m, 2 H), 6.89 (s, 1 H), 7.20–7.27 (m, 3 H), 7.49 (t, 1 H, J = 7.69 Hz).

2a (free base in Me_2SO-d_6) : δ ppm = 3.22 (dd, 1 H, J = 12.77, 7.02 Hz), 3.37 (dd, 1 H, J = 12.76, 4.21 Hz), 4.05 (m, 2 H), 4.22 (m, 1 H), 5.86 (s, 2 H), 6.11 (dd, 1 H Ar, J = 8.26, 2.27 Hz), 6.32 (d, 1 H Ar, J = 2.25 Hz), 6.66 (d, 1 H Ar, J = 8.26 Hz), 6.96 (m, 3 H Ar), 7.30 (m, 2 H Ar).

2b (free base in Me_2SO-d_6) : δ ppm = 3.21 (dd, 1 H, J = 12.75, 7.07 Hz), 3.35 (dd, 1 H, J = 12.73, 4.22 Hz), 3.78 (s, 3 H), 3.97 (dd, 1 H, J = 9.55, 6.06 Hz), 4.03 (dd, 1 H, J = 11.49, 6.18 Hz), 4.20 (m, 1 H), 5.86 (s, 2 H), 6.11 (dd, 1 H Ar, J = 8.31, 2.34 Hz), 6.31 (d, 1 H Ar, J = 2.30 Hz), 6.66 (d, 1 H Ar, J = 8.28 Hz), 6.85 (s, 4 H Ar).

3a (maleate in Me_2SO-d_6) : δ ppm = 3.17 (dd, 1 H, J = 11.97, 5.21 Hz), 3.31 (dd, 1 H, J = 12.0, 4.9 Hz), 3.74 (s, 2 H), 4.01 (m, 3 H), 6.25 (s, 2 H), 6.68 (dd, 1 H Ar, J = 8.27, 2.06 Hz), 6.88 (m, 4 H Ar), 7.10 (m, 1 H Ar), 7.27 (m, 3 H Ar), 7.43 (d, 1 H Ar, J = 7.16 Hz), 7.58 (m, 2 H Ar).

3b (free base in Me_2SO-d_6) : δ ppm = 3.16 (m, 1 H), 3.30 (m, 1 H), 3.69 (s, 3 H), 3.73 (s, 2 H), 3.90 (m, 3 H), 5.16 (d, 1 H, J = 4.84 Hz), 5.72 (t, 1 H N H, J = 5.79 Hz), 6.66 (dd, 1 H, J = 8.27, 2.01 Hz), 6.89 (m, 5 H Ar), 7.10 (dt, 1 H Ar, J = 7.36, 1.09 Hz), 7.25 (dt, 1 H Ar, J = 7.43, 0.8 Hz), 7.42 (d, 1 H Ar, J = 7.26 Hz), 7.54 (d, 1 H Ar, J = 8.27 Hz), 7.60 (d, 1 H Ar, J = 7.14 Hz).

3c (maleate in Me_2SO-d_6) : δ ppm = 3.58 (m, 2 H), 3.94 (m, 4 H), 4.11 (m, 1 H), 6.17 (s, 2 H), 6.37 (s, 1 H Ar), 6.56 (d, 2 H, J = 7.96 Hz), 6.81 (d, 1 H Ar, J = 7.58 Hz), 6.97–7.23 (m, 4 H Ar), 7.42 (m, 1 H Ar), 7.57 (m, 2 H Ar).

4a (HCl in Me_2SO-d_6) : δ ppm = 3.54 (m, 2 H), 3.85 (m, 3 H), 4.15 (m, 1 H), 5.64 (s, 1 H), 6.75–6.92 (m, 4 H Ar), 7.17–7.25 (m, 2 H), 7.38–7.56 (m, 4 H Ar), 7.96 (m, 2 H Ar), 8.14 (m, 1 H Ar).

5a (free base in Me_2SO-d_6) : δ ppm = 1.09 (d, 6 H, J = 6.25 Hz), 2.83 (m, 3 H), 3.86 (m, 2 H), 4.04 (m, 1 H), 6.35–6.48 (m, 3 H Ar), 7.04 (t, 1 H Ar, J = 8.26 Hz).

5b (free base in Me_2SO-d_6) : δ ppm = 0.96 (d, 6 H, J = 6.19 Hz), 2.65 (m, 2 H), 3.85 (m, 1 H), 3.98 (dd, 1 H, J = 9.79, 6.07 Hz), 4.11 (dd, 1 H, J = 9.83, 4.22 Hz), 7.41 (dd, 1 H Ar, J = 8.16, 2.51 Hz), 7.55 (t, 1 H Ar, J = 8.16 Hz), 7.71 (t, 1 H Ar, J = 2.32 Hz), 7.79 (dd, 1 H Ar, J = 7.21, 1.89 Hz).

5c (HCl in Me_2SO-d_6) : δ ppm = 0.96 (d, 6 H, J = 6.2 Hz), 2.54 (m, 1 H), 2.67 (m, 1 H), 3.87 (m, 3 H), 6.90 (m, 3 H Ar), 7.26 (dd, 2 H Ar, J = 9.05, 7.13 Hz).

6 (disodium salt in D_20) : δ ppm = 0.93 (d, 3/2 H, J = 6.25 Hz), 1.05 (d, 3/2 H, J = 6.43 Hz), 2.53 (m, 5 H), 3.84 (m, 3 H), 6.52–6.93 (m, 6 H Ar), 7.10–7.30 (m, 2 H Ar).

7 (HCl in Me_2SO-d_6) : δ ppm = 3.82 (m, 2 H), 3.96 (m, 4 H), 4.06 (m, 1 H), 4.72 (s, 2 H), 6.88–7.00 (m, 5 H Ar), 7.24–7.45 (m, 4 H Ar).

8 (HCl in Me_2SO-d_6) : δ ppm = 1.61 (m, 5 H), 1.91 (m, 6 H), 2.10 (m, 4 H), 2.94 (m, 1 H), 3.12 (m, 1 H), 3.99 (dd, 2 H, J = 5.08 Hz), 4.22 (m, 1 H), 6.90–6.97 (m, 3 H Ar), 7.29 (t, 2 H Ar, J = 8.29 Hz).

9 (HCl in Me_2SO-d_6) : δ ppm = 1.59–1.92 (m, 10 H), 2.41 (m, 4 H), 3.62 (m, 3 H), 4.03 (m, 2 H), 4.56 (m, 1 H), 6.92–6.99 (m, 3 H Ar), 7.29 (t, 2 H Ar, J = 7.31 Hz).

3.3. Pharmacology

3.3.1. cAMP accumulation experiments

For studies of agonist activity, CHO-β_3-adrenoceptors, were grown to preconfluence in six-well dishes to a density of approximately 0.6×10^6 cells/well. After washing with 1 ml Ham's F12 medium buffered with 20 mM Hepes (pH 7.4) supplemented with 1 mM IBMX, cell monolayers were incubated for 30 min at 37 °C in the absence or in the presence of 10^{-4}M (−)-isoproterenol, or 10^{-12} M to 10^{-4} M of ligand. For studies of antagonistic effect, cells were preincubated at 37 °C for 10 min with 100 μM of ligand tested before addition of 10 nM of (−)-isoproterenol and incubation for an another 20 min period. The reaction was stopped by washing once with 1 ml PBS and by immediate addition of 500 μl 1 N sodium hydroxide. After 20 min at 37 °C, dissolved cells were collected, buffered with 1N acetic acid and centrifuged at 3000 × g for 10 min at 4 °C. The total amount of cAMP contained in an aliquot of supernatant was determined using the Amersham kit according to the manufacturers instructions. For eight of the full agonists activation constant (K_{act} : activation constant defined as the concentration of ligand necessary to obtain 50% of the maximal effect) was calculated from dose-response curve experiments.

3.3.2. Binding experiments

Competition analysis was carried out using a saturating concentration of [^{125}I] ICYP (2 nM), and increasing concentrations of the β_3-selective agonists. CHOK1-β_3 were incubated with drugs for 30 min. at 37 °C, before dilution with ice-cold PBS and rapid filtration over Whatmann GF/C glass fibber filters soaked in 0.3% polyethylenimine using a Brandel cell harvester, followed by extensive washing with ice-cold PBS. Radioactivity was assessed by γ-counting. IC_{50} values (concentration of ligand inducing 50% inhibition of [^{125}I] ICYP binding) and K_i (inhibition constant) values were calculated from the Cheng and Prussof equation [14]. K_{act} (activation constant) values, the concentrations of ligand necessary to obtain 50% of the maximal effect were determined from the dose-response adenylyl cyclase activation. K_i and K_{act} parameters were calculated using computerized iterative non-linear regression curve fitting, with the INPLOT-4 program (GraphPad software; ©1987 by H. J. Motulsky).

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References

1 Strosberg, A. D. : Prot. Sci. 1993, 2, 1198.
2 Krief, S.; Lönnqvist, F.; Raimbault, S. Baude, B.; Van Sprosen, A.; Arner, P.; Srosberg, A. D.; Ricquier, D.; Emorine, L. J.: J. Clin. Invest. **91**, 344 (1993)
3 Blin, N.; Camoin, L.; Maigret, B.; Strosberg, A.D.: Mol. Pharmacol. **44**, 1094 (1993)
4 Strosberg, A. D.; Pietri-Rouxel, F.: Trends Pharmacol. Sci. **17**, 373 (1996)
5 Pietri-Rouxel, F.; Lenzen, G.; Kapoor, A.; Drumare, M.-F.; Archimbault, P.; Strosberg, A. D.; St J. Manning, B.: Eur. J. Biochem. **230**, 350 (1995)
6 Sher, P. M.; Fisher, L. G.; Skwish, S.; Michel, I. M.; Seiler, S. M.; Washburn, W. N.; Dickinson, E. J.: Bioorg. Med. Chem. Lett. **7**, 2253 (1997)
7 Bloom, J. D.; Claus, T. H.; Devries, V. G.; Dolan, J. A.; Dutia, M. D. U.S. Patent 5, 106, 867
8 Drugs Fut.: **18**, 541 (1993)
9 Bloom, J. D.; Dutia, M. D.; Johnson, B. D; Wissner, A.; Burns, M. G.; Largis, E. E.; Dolan, J. A.; Claus, T. H.: J. Med. Chem. **35**, 3081 (1992)
10 Drugs Fut. **19**, 23 (1994)
11 Filton, A. O.; Ramage, G. R.: J. Chem. Soc. 4870 (1962)
12 Rastogi, S.; Kansal, V. K.; Bhaduri, A. P.: Indian J. Chem. **22B**, 234 (1983)
13 Bhanu, A. S.; Bhaskar, J. V.; Periasamy, M.: Tetrahedron **48**, 4623 (1992)
14 Cheng, Y. C.; Prusoff, W. H.: Biochem. Pharmacol. **22**, 3099 (1973)
15 Pietri-Rouxel, F.; Strosberg, A. D.: Fund. Clin. Pharmacol. **9**, 211 (1995)
16 Archimbault, Ph.; Leclerc, G.; Strosberg, A. D.; Piétri-Rouxel, F.; PCT/Fr. Patent 97 01963, 1997.

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