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Synthesis and β–adrenergic activity of atypical β-adrenergic phenylethanolaminotetralin stereoisomers†

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Summary — A series of substituted phenylethanolaminotetralins were synthesized as pure stereoisomers and their ability to stimulate atypical β-adrenoceptors selectively was evaluated. The compounds *in vitro* relative potencies were assessed using the atypical β response of inhibition of rat proximal colon motility and the typical β1 (increase in guinea-pig right atrial frequency) and β2 (guinea-pig tracheal relaxation and rat uterus motility inhibition) responses. Compound **42** (SR 58611A) was found to be the most potent and selective.

phenylethanolaminotetralins / SR 58611A / β-atypical adrenergic activity / chiral separation

Introduction

The existence of atypical β-adrenoceptors (*ie*, not β1 or β2) mediating a variety of functions in different tissues and organ systems, such as adipose tissue, intestine, heart and the central nervous system, is becoming widely accepted [1–7]. Arch *et al* [1] have reported that, unlike currently available β-adrenergic agonists, racemic phenylethanolamines with 2 stereogenic carbon atoms selectively stimulated lipolysis in brown adipocytes, with weaker effects on atria (β1) and trachea (β2), indicating that the receptor that mediates lipolysis is neither β1 nor β2 but 'atypical' [5]. *In vitro* inhibition of colonic motility through stimulation of atypical β-adrenoceptors by optically pure phenylethanolaminotetralins (PEATs) has also been reported [6, 8]. In these studies, the PEAT agonist effects were found to be resistant to the β-adrenergic unselective antagonists propranolol and alprenolol, and insensitive to the β2 and β1 selective antagonists ICI 118 551 and CGP 20 712. We have recently demonstrated [9] that alprenolol acts as a competitive antagonist at atypical β-adrenoceptors of rat colon and adipose tissue with the same affinity (pA_2,7), suggesting high functional homology of the atypical β-adrenoceptors in both tissues.

Although atypical β-adrenoceptors have been described in a variety of peripheral tissues, there is also evidence for their existence in the brain, where they might be potential targets for psychotropic agents, as suggested by the peculiar antidepressant profile of **42** (SR 58611A) [10]. This compound appears to have no cardiovascular [11] or antidiuretic [12] effects *in vivo*, unlike typical β-adrenoceptor agonists.

The synthesis of several PEAT analogues through incorporation of a number of substituents into various positions on the aromatic ring of the phenylethanolamino group and on the aromatic ring of the tetralin moiety, revealed the beneficial effects of a *meta*-chloro substitution on the former and a 7-oxygenated substituent on the latter [13].

The aim of this report is to provide an account of the chemistry used to synthesize the stereoisomers of this new class of compounds and an evaluation of the influence of the C-2' carbon atom of the tetralin moiety on the activity of the PEAT derivatives,

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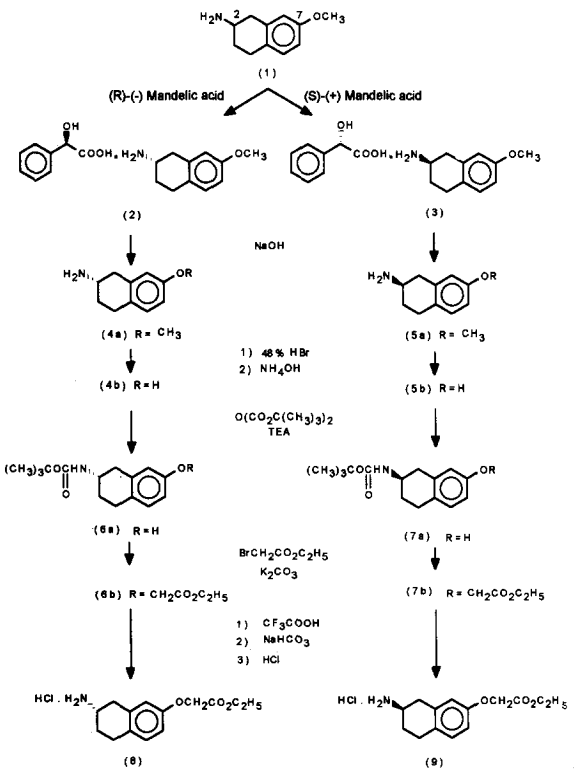
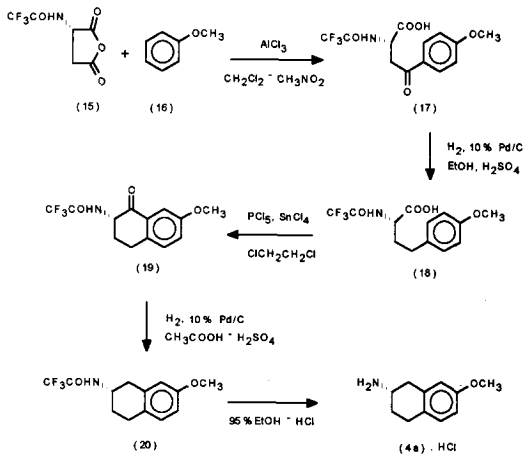
since it is known that the (*R*)-configuration of the C-2 benzylic carbon is essential for β-adrenergic activity [14].

Chemistry

Scheme 1 depicts the resolution with (*R*)-(–) and (*S*)-(+)-mandelic acid of racemic 2-amino-7-methoxytetralin **1** prepared from commercially available 2-keto-7-methoxytetralin by a published procedure [15] to give the corresponding diastereoisomeric salts **2** and **3**, from which the 2 enantiomers **4a** and **5a** were obtained. The corresponding phenol derivatives **4b** and **5b** were prepared by acid hydrolysis from the methoxy precursors and the absolute configuration was determined by comparison with known optical rotation values [16]. Finally, the enantiomers **8** and **9** of 2-amino-7-ethoxycarbonylmethoxytetralin were synthesized as outlined in scheme 1. The synthesis of *S*-(–)-2-amino-7-methoxytetralin **4a** was also carried out stereoselectively, as shown in scheme 2. After reacting anisole **16** with (*S*)-*N*-(trifluoroacetyl) aspartic anhydride **15** [17] under Friedel–Crafts conditions, the methoxyarylketone **17** was obtained and submitted

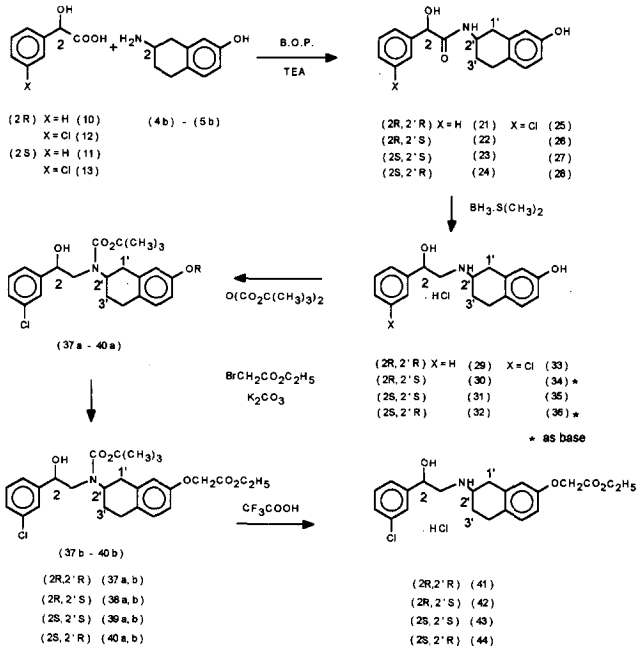


Scheme 1.



Scheme 2.

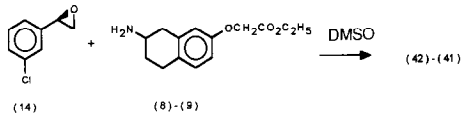
without purification to reductive deoxygenation to give **18**. Cyclization under Friedel–Crafts conditions yielded (*S*)-2-trifluoroacetamido-7-methoxytetralone **19**, which upon reductive deoxygenation, gave **20**. Hydrolysis of the amide gave (*S*)-2-amino-7-methoxytetralin **4a**. The final compounds were prepared as outlined in scheme 3. The enantiomers **4b** and **5b** of 2-amino-7-hydroxytetralin were reacted with commercially available enantiomers of mandelic acid **10** and **11** and with the enantiomers of 3-chloromandelic



Scheme 3.

acid **12** and **13** [18] to give the corresponding amides **21–24** and **25–28**.

Reduction of the amides with borane dimethylsulfide complex gave the phenylethanolaminotetralins **29–32** and the chlorophenylethanolaminotetralins **33–36**. Compounds **33–36** were treated with di-*tert*-butyl-dicarbonate to obtain the Boc-amino-protected derivatives **37a–40a**. Subsequent alkylation with ethyl bromoacetate gave **37b–40b**. The Boc-protecting groups were removed by acid treatment to give the chlorophenyl derivatives **41–44**. Compounds **41** and **42** were also prepared by an alternative method, by reacting (*R*)-3-chlorostyrene oxide **14** [19] with enantiomers **8** and **9** as shown in scheme 4. The physical data for the final products are summarized in table I.



Scheme 4.

Analytical chemistry

The optical purities of the amines **4a** and **5a** were checked by chiral HPLC, using a Chiralcel OD column. The enantiomers of the amine itself were not resolved by the column selected, but satisfactory separation (α = 1.1) was obtained after a pre-column amidation with benzoic acid. The enantiomeric excess was found to be more than 98%. The optical purities of the 3-chloromandelic acids **12** and **13** were also checked by chiral HPLC on the Chiralcel OD column after derivatization as methyl esters. The selectivity factor α was 1.21 and the enantiomeric excess was not less than 97.5%.

The synthetic pathways depicted in schemes 1 and 3 cannot cause inversion of the C-2' carbon atom, but there is the possibility of epimerization of the benzylic chiral center of the mandelic acids. The ^{13}C-NMR spectra for the final PEATs were run to look for possible epimerization (table I). The chemical shifts of the stereogenic carbon atoms were not significantly different in the diastereoisomeric pair (2*R*, 2'*R*)-(2*R*, 2'*S*). Nevertheless, the chemical shifts of the

Table I. Physical data of phenylethanolaminotetralins (PEATs).

Compounds	*mp (°C)*	$[\alpha]_D^{20}$	*Formula*[a]	*^{13}C-NMR (δ(TMS), ppm)*[b]						*Stereoisomeric purity*[c]
				C(1)	C(2)	C(1')	C(2')	C(3')	C(4')	
Phenol compounds										
29(2*R*, 2'*R*)	178–180	+44°[d]	$C_{18}H_{21}NO_2$·HCl	51.3	68.6	31.5	54.0	25.3	26.4	–
30(2*R*, 2'*S*)	205–207	−109°[d]	$C_{18}H_{21}NO_2$·HCl	51.0	68.6	31.2	54.0	25.9	26.4	–
31(2*S*, 2'*S*)	179–180	−41.5°[d]	$C_{18}H_{21}NO_2$·HCl	51.3	68.6	31.5	54.0	25.3	26.4	–
32(2*S*, 2'*R*)	200–202	+105°[d]	$C_{18}H_{21}NO_2$·HCl	50.9	68.6	31.1	53.9	26.0	26.5	–
Chlorophenol compounds										
33(2*R*, 2'*R*)	203–205	+36.4°[e]	$C_{18}H_{20}ClNO_2$·HCl	50.9	68.0	31.5	54.1	25.3	26.4	–
34(2*R*, 2'*S*)	185–187	−78.5°[f]	$C_{18}H_{20}ClNO_2$	50.5	68.0	31.1	54.0	25.9	26.4	–
35(2*S*, 2'*S*)	203–205	−35.8°[e]	$C_{18}H_{20}ClNO_2$·HCl	50.9	68.0	31.5	54.1	25.3	26.4	–
36(2*S*, 2'*R*)	186–188	+76°[f]	$C_{18}H_{20}ClNO_2$	50.5	68.0	31.1	54.0	25.9	26.4	–
Chlorophenoxy compounds										
41(2*R*, 2'*R*)	164–166	+32.6°[e]	$C_{22}H_{26}ClNO_4$·HCl	50.9	68.0	31.5	53.9	25.1	26.4	99.4
42(2*R*, 2'*S*)	155–157	−87.4°[f]	$C_{22}H_{26}ClNO_4$·HCl	51.0	67.8	31.2	53.7	25.5	26.4	99.8
43(2*S*, 2'*S*)	164–166	−34.3°[e]	$C_{22}H_{26}ClNO_4$·HCl	51.0	67.9	31.5	53.9	25.1	26.4	>99.9

[a] All compounds were analysed for CHN and found to be within ± 0.4% of the theoretical values; [b] in DMSO-d_6 at 313K; trifluoroacetic acid-d_1 (40 µl/ml) was added to **34** and **36**; [c] determined by supercritical fluid chromatography; [d] *c* = 2%, EtOH / H_2O 1:1; [e] *c* = 1%, MeOH; [f] *c* = 0.5%, MeOH.

carbon atoms 1' and 3', both on the tetrahydronaphthalene ring, are sensitive to the relative stereochemistry. In the 3 series of compounds in table I, the 1' carbon atom of the (2*R*, 2'*R*) isomer resonates 0.3 ppm downfield of the corresponding carbon atom of the (2*R*, 2'*S*) isomer. There is an inverse relationship between the chemical shift of the 3' carbon atom and the relative stereochemistry. The limit of detection for the diastereoisomeric impurities by ^{13}C-NMR was 3%, and no impurities greater than this were found.

For the more pharmacologically interesting compounds **41–44**, each possible stereoisomeric impurity was determined by chiral chromatography. The same HPLC column (Chiralcel OD) used for chromatography of the starting material was first used for this separation, but only supercritical fluid chromatography satisfactorily separated the 4 stereoisomers, giving a 0.1% limit of quantification of each stereoisomer in each pure compound **41–44**.

Pharmacological results

The *in vitro* abilities of all the compounds to inhibit rat proximal colon spontaneous motility (effect essentially mediated by atypical β-adrenoceptors), to relax guinea-pig trachea and inhibit rat uterus motility (both β2 effects) or to induce chronotropic effects on guinea-pig right atrium (β1 effects) were assayed and the data are reported in table II. The 2*R*,2'*R* and 2*R*,2'*S* isomers and the reference compounds (isoprenaline, adrenaline, noradrenaline) reduced rat colon motility potently and dose dependently, unlike the 2*S*,2'*S* and 2*S*,2'*R* isomers. Compounds **41** and **42** were found to be the most potent, with EC_{50} close to that of (*R*)-isoprenaline (0.5 nM). However, only compound **42** was highly selective, being 143 times more active on rat colon than on rat uterus; **42** was inactive on trachea up to 10^{-5} M. In guinea-pig trachea, the PEATs **29**, **33** and **41** (IC_{50}, 233–480 nM) had intrinsic activities of 0.6, 0.7, which are lower than those of the reference compounds, which behaved as full agonists. It is worth noting that PEATs, unlike the reference compounds, were all inactive on guinea-pig right atrium up to 3×10^{-5} M. The abilities of compound **42** (1000 and 10 000 nM) to antagonize (*R*)-isoprenaline chronotropic effects on right atrium and of (±)-salbutamol on trachea relaxation were tested. The data (0–17 % inhibition) do not support an antagonist action of this compound at β1, β2 receptors. Exposure of the rat colon to the unselective β-adrenergic antagonist (±)-propranolol at a concentration about 100 times greater than its equilibrium dissociation constant at β1 or β2 adrenoceptors (10^{-7} M, 30-min incubation) strongly inhibited (~10 fold) the (*R*)-isoprenaline effect but slightly reduced (2–3 fold) or left unaltered the agonist response of **29**, **33**, **34**, **41**, **42** (table III). This suggests that these compounds, unlike (*R*)-isoprenaline, reduce colon motility essentially through activation of atypical β adrenoceptors. Details of the isolated organ preparations and testing procedures are given in the experimental protocols.

Discussion and conclusions

This study shows that the PEATs are potent inhibitors of rat proximal colon motility through activation of atypical β adrenoceptors and that (2*R*,2'*S*) and (2*R*,2'*R*) isomers are more potent than their enantiomers (2*S*,2'*R*) and (2*S*,2'*S*). Selectivity for the uterus *versus* the colon is greater for (2*R*,2'*S*) than for (2*R*,2'*R*) isomers. This indicates that the configuration *S* of carbon 2' of the tetralin moiety is important for the selectivity of PEAT derivatives. PEATs behaved as partial agonists on β2 adrenoceptors of guinea-pig trachea and were full agonists on both atypical β adrenoceptors of the rat colon and β2 adrenoceptors of the rat uterus.

In conclusion, we have reported a novel class of phenylethanolamine adrenergic agonists, of which compound **42** appears to be the most potent and selective for rat atypical β-adrenoceptors [20]. Unlike the other atypical β-adrenoceptor agonists, such as ICI D7114 [21], compound **42** had no agonist or antagonist properties on the β1 adrenergic receptors of guinea-pig atrium or β2 adrenergic receptors of trachea. *In vivo* studies have shown a good bioavailability of **42** and have confirmed its colonic selectivity in conscious rats [10] and dogs [22, 23]; indeed **42** reduced colonic motility at doses with only mild or no cardiovascular effects. These observations and others from *in vivo* studies of lipolysis and thermogenesis [9, 24] impelled us to select **42** (SR 58611A) for clinical development.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 510 apparatus and are uncorrected. IR spectra were recorded on film with a Perkin-Elmer 881 IR spectrophotometer. ^{1}H-NMR spectra were obtained for $(CD_3)_2SO$ solutions ($(CH_3)_4Si$ as a internal standard) on a Brucker WP 80 spectrometer or, where indicated, on a Brucker AC 200 F spectrometer. ^{13}C-NMR were obtained on a Brucker AC 200 F spectrometer. Optical rotations were obtained on a Perkin-Elmer 141 Polarimeter at 20°C. Purification by gravity column chromatography on Merck silica-gel 60, 70–230 mesh and by flash chromatography on 230–400 mesh were carried out using the slurry method for column packing. Elemental analyses were within ± 0.4% of the theoretical values. IR and ^{1}H-NMR of enantiomers and /or diastereoisomers are only reported once when not significally different.

Table II. Configuration-dependent action of phenylethanolaminotetralins (PEATs) and reference adrenoceptor agonists on rat proximal colon and other isolated preparations.*

Compounds	*β-atypical receptor rat proximal colon IC_{50}*	*β2-receptor*			*β1-receptor*	*Selectivity uterus vs colon*
		Rat uterus IC_{50}	*Guinea-pig trachea IC_{50}*	*IA*	*Guinea-pig atrium EC_{50}*	
Isoprenaline (*R*)	0.5 (0.4–0.6)	0.2 (0.17–0.25)	1.2 (0.96–1.47)	1	4.8 (3.6–6.0)	0.4
Isoprenaline (*S*)	28 (22–35)	5.2 (4.4–6.1)	17 (14–22)	0.9	97 (72–129)	0.2
Noradrenaline (*R*)	10 (8–14)	90 (75–109)	18 (14–23)	1	41 (25–67)	9
Adrenaline (*R*)	15 (11–21)	1.6 (1.4–1.8)	5.2 (4.3–6.3)	1	78 (61–99)	0.1
29 (2*R*, 2'*R*)	30 (22–41)	29 (23–37)	450 (170–1160)	0.6	>30 000	1
30 (2*R*, 2'*S*)	611 (453–824)	947 (715–1250)	>10 000	ne	>30 000	1.5
31 (2*S*, 2'*S*)	>30 000	9400 (7600–11 600)	nd	–	>30 000	ne
32 (2*S*, 2'*R*)	12 541 (8328–18 884)	8300 (6500–10 600)	nd	–	>30 000	0.7
33 (2*R*, 2'*R*)	7 (6–9)	28 (21–36)	480 (260–880)	0.6	>30 000	4
34 (2*R*, 2'*S*)	36 (26–48)	340 (260–460)	>10 000	ne	>30 000	9
35 (2*S*, 2'*S*)	27 069 (21 818–33 583)	9400 (7800–11 200)	nd	–	>30 000	0.3
36 (2*S*, 2'*R*)	>30 000	~15 000	nd	–	>30 000	ne
41 (2*R*, 2'*R*)	2.7 (2.0–3.5)	66 (45–96)	233 (82–664)	0.7	>30 000	24
42 (2*R*, 2'*S*)	3.5 (2.6–4.7)	499 (372–672)	>10 000	ne	>30 000	143
43 (2*S*, 2'*S*)	11 107 (9697–12 721)	~25 000	>10 000	ne	>30 000	2
44 (2*S*, 2'*R*)	2351 (1712–3228)	18 400 (15 000–22 000)	>10 000	ne	>30 000	7.8

*IC_{50} and EC_{50}: concentration (nM) producing half-maximal effect; in parentheses: the 95% confidence limits; IA: intrinsic activity (isoprenaline = 1); ne: not evaluable; nd: not determined; the maximal dose tested for PEATs was 3 x 10^{-5} M. In rat uterus and colon and in guinea-pig atrium the active compounds behaved as full agonists.

(R)-(+)-2-Amino-7-hydroxytetralin ***5b***

To a solution of **1** (50 g, 0.282 mol) in absolute ethanol (550 ml), was added (*S*)-(+)-mandelic acid (43 g, 0.282 mol) in absolute ethanol (550 ml). After 1 night at room temperature, the precipitate was filtered and crystallized twice from absolute ethanol to give 34.2 g **3**. mp 190–192°C. $[\alpha]_D^{20}$ +90° (*c* 0.5, MeOH). ^{1}H-NMR (200 MHz): δ 1.6–1.9 (1H, m), 2.0–2.2 (1H, m), 2.6–3.2 (4H, m), 3.3–3.5 (1H, m), 3.75 (3H, s), 4.6 (1H, s), 6.7–7.5 (8H, m).

The salt was taken up in water (300 ml), made alkaline with 1 N sodium hydroxide solution and the tetralin extracted with ethyl acetate. The organic phase was washed with water and evaporated to dryness under vacuum to give 19.8 g **5a**. The residue was taken up with 48% hydrobromic acid (260 ml) and then stirred at reflux for 3 h. The solution was evaporated to dryness under reduced pressure, taken up with water (70 ml), made alkaline with 5 N ammonium hydroxide solution, cooled overnight and filtered to give 15.5 g (30%) **5b**. mp 143–144°C. $[\alpha]_D^{20}$ +91° (*c* 0.5, MeOH). IR (KBr) 3485, 3328, 3280, 1604, 1461, 1305 cm^{-1}. ^{1}H-NMR: δ 1–3.5 (7H, m), 6.5–7 (3H, m). Anal $C_{10}H_{13}NO{\cdot}H_2O$ (C, H, N, H_2O). The hydrochloride of **5b** has $[\alpha]_D^{20}$ +68.56° (*c* 0.5, MeOH); lit [16]: $[\alpha]_D^{20}$ +71.7° (*c* 1, MeOH).

Table III. Inhibition by propranolol of effects of PEATs IC_{50} on rat proximal colon.*

Compounds	*Inhibition of spontaneous colonic motility IC_{50} (nM)*	
	Control	*Propranolol*
(*R*)-isoprenaline	0.5 (0.4–0.6)	5.1 (4.2–6.3)
29	30 (22–41)	87 (67–113)
33	7 (6–9)	7 (5.6–9)
34	36 (26–48)	53 (43–65)
41	2.7 (2.0–3.5)	7 (6–9)
42	3.5 (2.6–4.7)	3.4 (2.5–4.5)

*Propranolol (100 nM) was added to the bath 30 min before the agonist; IC_{50}: concentration (nM) producing 50% maximal effect; in parentheses: the 95% confidence limits.

*(S)-(–)-2-Amino-7-hydroxytetralin **4b***
The mother liquors obtained from **3** and its first crystallization were combined and evaporated to dryness. The residue was taken up with water (300 ml), the suspension was made alkaline with 1 N sodium hydroxide solution, extracted with ethyl acetate and concentrated to dryness under vacuum to give 2-amino-7-methoxy tetralin (26.7 g, 0.150 mol). The solid was taken up with absolute ethanol (250 ml) and treated with a solution of (*R*)-(–)-mandelic acid (23 g, 0.15 mol) in absolute ethanol (250 ml). After 1 night at room temperature, a precipitate was filtered and crystallized from ethanol to give 34.2 g of the pure salt **2.** mp 189–191°C. $[\alpha]_D^{20}$ –92.3° (*c* 0.5, MeOH).

From compound **4a** (19.8 g), 15.6 g (30%) of compound **4b** was obtained according to the above described method for **5b.** mp 142–144 °C. $[\alpha]_D^{20}$ –93° (*c* 0.5, MeOH). Anal $C_{10}H_{13}NO \cdot H_2O$ (C, H, N, H_2O).

*(R)-(+)-N-t-Butyloxycarbonyl-N-2-amino-7-hydroxytetralin **7a***
To a solution of **5b** (9.14 g, 0.050 mol) and triethylamine (39 ml, 0.280 mol) in anhydrous *N,N*-dimethylformamide (100 ml), was added di-*t*-butyldicarbonate (13.6 g, 0.062 mol). The mixture was stirred for 3 h at room temperature, then water (500 ml) was added and the mixture extracted with ethyl ether (200 ml x 3). The organic layer was washed with water and dried over sodium sulfate. The solvent was evaporated and the residue purified by flash-chromatography (eluent: cyclohexane/ethyl acetate 7:3) to give 12.7 g (96%) **7a** as a vitreous solid. $[\alpha]_D^{20}$ +69.3° (*c* 1, MeOH). IR (KBr) 3352, 1680, 1510, 1258, 1166 cm^{-1}. ^{1}H-NMR: δ 1–3.7 (8H, m), 1.45 (9H, s), 6.25–6.75 (3H, m), 6.6 (1H, bs, exchange by D_2O), 8.8 (1H,s exchange by D_2O).

*(S)-(–)-N-t-Butyloxycarbonyl-N-2-amino-7-hydroxytetralin **6a***
Following the above procedure for compound **7a** compound **6a** was obtained as a vitreous solid. $[\alpha]_D^{20}$ –62.4° (*c* 1, MeOH).

*(R)-(+)-2-Amino-7-ethoxycarbonylmethoxytetralin hydrochloride **9***
To a solution of **7a** (10.8 g, 0.041 mol) in acetone (400 ml) were added ethyl bromoacetate (13.6 ml, 0,123 mol) and anhydrous potassium carbonate (17 g, 0.123 mol). The mixture was then stirred at reflux for 6 h and after cooling the solid was filtered off and washed with acetone. The filtrate was evaporated to dryness under vacuum. The residue was treated with ethyl ether (200 ml), washed with water (50 ml x 3) and then dried over sodium sulfate. After evaporation of the solvent, 10.3 g (72%) **7b** were obtained. Mp 112–114°C. $[\alpha]_D^{20}$ +57.97° (*c* 1, MeOH). IR (KBr) 3388, 1747, 1708, 1529 cm^{-1}. ^{1}H-NMR: δ 1–3.7 (7H, m), 1.25 (3H, t, *J* = 7 Hz), 1.4 (9H, s), 4.1 (2H, q, *J* = 7 Hz), 4.6 (2H, s), 6.4–7 (3H, m), 6.6 (1H, bs exchange by D_2O). Anal $C_{19}H_{27}NO_5$ (C, H, N).

The product **7b** (3.5 g, 0.01 mol) was dissolved in dichloromethane (40 ml) and treated at 0–5°C, with a solution of trifluoroacetic acid (7.7 ml, 0,1 mol) in dichloromethane (40 ml). The mixture was stirred at room temperature for 4 h. The organic layer was washed with saturated sodium hydrogen carbonate solution, water and dried over sodium sulfate. After evaporation of the solvent the residue was dissolved in ethyl acetate (10 ml), treated with a 6 N solution of gaseous hydrochloric acid in ethanol and 1.1 g (38.5%) of the solid hydrochloride **9** was filtered off. Mp 169–171°C. $[\alpha]_D^{20}$ +51.14° (*c* 1, MeOH). IR (KBr) 3300–2400, 1745, 1502, 1216 cm^{-1}. ^{1}H-NMR: δ 1.15 (3H, t, *J* = 7Hz), 1.30–3.75 (7H, m), 4.0 (2H, q, *J* = 7 Hz), 4.60 (2H, s), 6.5–7 (3H, m) 8–8.5 (1H, bs, exchange by D_2O). Anal $C_{14}H_{20}ClNO_3$ (C, H, N).

*(S)-(–)-2-Amino-7-ethoxycarbonylmethoxytetralin hydrochloride **8***
Compound **8** was prepared *via* **6b** following the same procedure as for compound **9**. Mp 148–150°C. $[\alpha]_D^{20}$ –47.1 (*c* 1, MeOH). Anal $C_{14}H_{20}ClNO_3$ (C, H, N). Data for (*S*)-(–)-*N*-*t*-butyloxycarbonyl-*N*-2-amino-7-ethoxycarbonylmethoxytetralin **6b**: mp 109–111°C. $[\alpha]_D^{20}$ –53.4 (*c* 1, MeOH). Anal $C_{19}H_{27}NO_5$ (C, H, N).

*(S)-4-(4-Methoxyphenyl)-4-oxo-2-trifluoroacetylaminobutanoic acid **17***
To a suspension of aluminium chloride (29 g, 0.21 mol) in dichloromethane (260 ml) and nitromethane (48 ml) at 0–5°C under stirring, were added anisole **16** (22 g, 0.2 mol) and *N*-(trifluoroacetyl)-L-aspartic anhydride **15** (22 g, 0,1 mol). Stirring was maintained for 1 h at 0–5°C and for 2 d at room temperature. The reaction mixture was poured into ice (1 kg) and extracted with dichloromethane (200 ml x 3). The combined organic layers were washed with 1 N hydrochloric acid solution, water and then extracted with 1 N sodium hydrogen carbonate solution (350 ml x 3). The aqueous alkaline fractions were acidified with 1 N hydrochloric acid solution and extracted with dichloromethane (300 ml x 3). The organic phase was dried (sodium sulfate), filtered and concentrated *in vacuo* to give 29 g (90%) of crude **17**. This was used without further purification.

*(S)-(+)-4-(4-Methoxyphenyl)-2-trifluoroacetylaminobutanoic acid **18***
To a solution of **17** (29 g, 0.09 mol) in a mixture of ethanol (200 ml) and concentrated sulfuric acid (1.4 ml), was added 10% palladium on activated carbon (2.3 g) and the mixture was hydrogenated at room temperature and atmospheric pressure until hydrogen uptake ceased. The catalyst was filtered off and solid sodium hydrogen carbonate (14.3 g) was added. The solvent was removed under reduced pressure and the residue

was treated with water (500 ml) and acidified with 2 N hydrochloric acid solution. The precipitate was then filtered and dried to give 18 g (65%) **18**. mp 120–122°C. $[\alpha]_D^{20}$ +8.5° (*c* 0.5, MeOH). IR (KBr) 3305, 3200–2500, 1707 cm^{-1}. ^{1}H-NMR: δ 1.75–2.75 (4H, m), 3.73 (3H, s), 4–4.25 (1H, m), 6.5–7.25 (4H, m), 10.5 (1H, d, *J* = 8 Hz). Anal $C_{13}H_{14}F_3NO_4$ (C, H, N).

(S)-(−)-1-Oxo-2-trifluoroacetylamino-7-methoxy-1,2,3,4-tetrahydronaphthalene ***19***

To a stirred solution of **18** (34 g, 0.11 mol) in 1,2-dichloroethane (830 ml) at 0–5°C, phosphorus pentachloride (20 g, 0.096 mol) was added portionwise. The mixture was stirred at 0–5°C for 2 h, then tin(IV) chloride (34 ml, 0.29 mol) was added. After stirring for 15 min at 0–5°C and then 2 h at room temperature the reaction mixture was poured into ice (1kg). The organic layer was separated and the aqueous phase extracted with 1,2-dichloroethane (250 ml). The organic layers were combined, treated with water, dried (sodium sulfate) and concentrated *in vacuo* to give crude **19** (26.3 g). Crystallization from isopropanol gave 23 g (72%) of pure **19**. Mp 164–166°C. $[\alpha]_D^{20}$ −87.4° (*c* 0.5, MeOH). IR (KBr) 3310, 3200–2880, 1730, 1700 cm^{-1}. ^{1}H-NMR: δ 2–3.5 (4H, m), 3.75 (3H, s), 4.75 (1H, m), 7–7.5 (3H, m). Anal $C_{13}H_{12}F_3NO_3$ (C, H, N).

(S)-(−)-2-Trifluoroacetylamino-7-methoxy-1,2,3,4-tetrahydronaphthalene **20**

To a solution of **19** (25.5 g, 0.09 mol) in a mixture of acetic acid (450 ml) and concentrated sulfuric acid (12 ml), 10% palladium on activated carbon (2.6 g) was added and the mixture was hydrogenated at room temperature and atmospheric pressure until hydrogen uptake ceased (2 h). After filtration, a solution of sodium acetate (68 g, 0.83 mol) in water (150 ml) was added and the mixture was concentrated *in vacuo* to dryness. The residue was treated with water (300 ml) and the solid was filtered, washed with water, 10% sodium hydrogen carbonate solution and water again to give 14.6 g (59%) **20**. Mp 113–118 °C. $[\alpha]_D^{20}$ −90.8° (*c* 0.5, MeOH). IR (KBr) 3340, 1710, 1200 cm^{-1}. ^{1}H-NMR: δ 1.5–3.5 (6H, m), 3.73 (3H, s), 4.0 (1H, m), 6.5–7 (3H, m), 9.75 (1H, m, exchange by D_2O). Anal $C_{13}H_{14}F_3NO_2$ (C, H, N).

(S)-(−)-2-Amino-7-methoxytetralin hydrochloride **4a**

A solution of **20** (5.5 g, 0.02 mol) in a mixture of 95% ethanol (95 ml) and concentrated hydrochloric acid (32.5 ml) was heated under reflux for 3 h. The solvent was removed under reduced pressure and the residue taken up with acetone and filtered to give crude **4a** as the hydrochloride. Crystallization from ethanol gave 2.15 g (50%) of pure **4a** as white crystals. Mp 203–205°C. $[\alpha]_D^{20}$ −66.1° (*c* 0.5, MeOH). ^{1}H-NMR: δ 1.5–3.5 (6H, m), 3.7 (3H, s), 6.5–7 (3H, m), 8.3–8.6 (3H, bs exchange by D_2O). Anal $C_{11}H_{16}ClNO$ (C, H, N).

General method for the preparation of compounds ***21–28****. Preparation of N-[(2S)-7-hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(R)-3-chloromandelamide* ***26***

A solution of **12** (3.6 g, 0.019 mol), **4b**, (3.4 g, 0,019 mol), benzotriazol-1-yloxy-tris (dimethylamino) phosphonium hexafluorophosphate (BOP reagent) (8.5 g, 0.019 mol), triethylamine (2.7 ml, 0.019 mol) in anhydrous dichloromethane (60 ml) was stirred for 5 h at room temperature. Ethyl acetate (400 ml) was added and the reaction mixture washed with a saturated solution of sodium hydrogen carbonate, water, 1 N hydrogen chloride solution, water and then dried (sodium sulfate). After filtration the solvent was evaporated and the oil purified by flash-chromatography (eluent: ethyl acetate/cyclohexane 7:3) to give 4 g (63.4%) **26** as a thick oil which after treatment with petroleum ether gave a vitreous solid. $[\alpha]_D^{20}$ −95° (*c* 1, MeOH). IR (KBr) 3600–2900, 1655–1529 cm^{-1}. ^{1}H-NMR: δ 1.4–3.15 (6H, m), 3.7–4.2 (1H, m), 5.0 (1H, bs), 6.25 (1H, bs, exchange by D_2O), 6.4–6.6 (2H, m), 6.75–7.0 (1H, m), 7.2–7.6 (5H, m), 7.7–8.1 (1H, bd, *J* = 8 Hz, exchange by D_2O), 9.0 (1H, bs, exchange by D_2O).

N-[(2R)-7-Hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(R)-3-chloromandelamide ***25***. The product **25** was a white solid. Mp 144–147°C. $[\alpha]_D^{20}$ +35.1° (*c* 1, MeOH). Anal $C_{18}H_{18}ClNO_3$ (C, H, N).

N-[(2S)-7-Hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(S)-3-chloromandelamide ***27***. The product **27** was a white solid. Mp 145–146°C. $[\alpha]_D^{20}$ −34.2° (*c* 1, MeOH). Anal $C_{18}H_{18}ClNO_3$ (C, H, N).

N-[(2R)-7-Hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(S)-3-chloromandelamide ***28***. The product **28** was a vitreous solid. $[\alpha]_D^{20}$ +92.5° (*c* 1, MeOH).

N-[(2R)-7-Hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(R)-mandelamide ***21***. The product **21** was a white solid. Mp 145–l47°C. $[\alpha]_D^{20}$ +26.4° (*c* 0.5, MeOH). IR (KBr) 3400, 3300 (b), 1642, 1630, 1612, 1545, 1231 cm^{-1}. ^{1}H-NMR: δ 1.4-3.15 (6H, m), 3.7–4.2 (1H, m), 4.88 (1H, d, *J* = 4 Hz), 6.0 (1H, d, *J* = 4 Hz, exchange by D_2O), 6.25–7 (3H, m), 7–7.5 (5H, b), 7.75 (1H, bd, *J* = 8 Hz, exchange by D_2O), 8.9 (1H, bs, exchange by D_2O). Anal $C_{18}H_{19}NO_3$ (C, H, N).

N-[(2S)-7-Hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(R)-mandelamide ***22***. The product **22** was a white solid. Mp 159–160°C. $[\alpha]_D^{20}$ −113° (*c* 0.5, MeOH). Anal $C_{18}H_{19}NO_3$ (C, H, N).

N-[(2S)-7-Hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(S)-mandelamide ***23***. The product **23** was a white solid. Mp 147–148°C. $[\alpha]_D^{20}$ −27.5° (*c* 0.5, MeOH). Anal $C_{18}H_{19}NO_3$ (C, H, N).

N-[(2R)-7-Hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(R)-mandelamide ***24***. The product **24** was a white solid. Mp 159–160°C. $[\alpha]_D^{20}$ +110° (*c* 0.5, MeOH). Anal $C_{18}H_{19}NO_3$ (C, H, N).

General method for the preparation of compounds ***29-36****. Preparation of N-[(2S)-7-hydroxy-1,2,3,4-tetrahydronaphth-2-yl]-(2R)-2-(3-chlorophenyl)-2-hydroxy ethanamine* ***34***

A solution of **26** (2.5 g, 0.0075 mol) in anhydrous tetrahydrofuran was maintained at reflux under a nitrogen atmosphere, and a solution of 10 M of borane-methylsulfide complex (2.3 ml, 0.023 mol) in tetrahydrofuran (15 ml) was added. After 4 h at reflux, the reaction mixture was cooled to room temperature, treated with methanol (25 ml) and left for 30 min at room temperature and then for 30 min at reflux. The solvent was removed *in vacuo* and the residue was crystallized twice from isopropanol to give 1.6 g (67%) **34**. Mp 185–187°C. IR (KBr) 3290, 3190, 2800–2500, 1348, 1118 cm^{-1}. ^{1}H-NMR: δ 1.2–3.02 (9H, m), 4.5–4.8 (1H, m), 6.4–6.6 (2H, m), 6.7–7 (1H, m), 7.1–7.5 (5H, m).

General method for the preparation of compounds ***41–44****. Preparation of N-[(2S)-7-ethoxycarbonylmethoxy-1,2,3,4-tetrahydronaphth-2-yl]-(2R)-2-(3-chlorophenyl)-2-hydroxy-ethanamine hydrochloride* ***42***

To a stirred solution of **34** (1.8 g, 0.0056 mol) and triethylamine (4 ml) in *N,N*-dimethylformamide (12 ml), was added

di-*t*-butyldicarbonate (1,36 g, 0.0062 mol). The reaction mixture was stirred for 3 h at room temperature. After addition of water (50 ml) the mixture was extracted with ethyl acetate (70 ml x 3). The organic phase was washed with water, dried and concentrated *in vacuo* to dryness to give **38a** (2.3 g) as an oily product. ^{1}H-NMR: δ 1.0-4.1 (9H, m), 1.4 (9H, s), 4.8 (1H, bs, C*H*OH), 5.5 (1H, m, CHO*H*), 6.3–7.5 (7H, m), 8.8 (1H, s, exchange by D_2O). Compound **38a** was dissolved in acetone (90 ml) and treated with ethyl bromoacetate (1.9 ml, 0.017 mol) in the presence of anhydrous potassium carbonate (2.34 g, 0.017 mol). The mixture was then stirred at reflux for 6 h. After cooling the precipitate was filtered off and washed with acetone. The filtrate was evaporated to dryness *in vacuo*. The residue **38b** [^{1}H-NMR: δ 1.0–4.2 (9H, m), 1.25 (3H, t, J = 7 Hz), 1.4 (9H, s), 4.15 (2H, q, J = 7 Hz), 4.3 (2H, s), 4.35 (1H, bs), 5.5 (1H, m), 6.5–7.5 (7H, m)] was dissolved in dichloromethane (20 ml), cooled to 0–5°C and treated with a solution of trifluoroacetic acid (4.97 g, 0.043 mol) in dichloromethane (10 ml). The mixture was stirred at room temperature for 4h, and then neutralized with a sodium hydrogen carbonate solution. The organic phase was washed with water, dried (sodium sulfate), filtered, concentrated *in vacuo* to dryness and purified by chromatography (eluent: ethyl acetate) to give the free base of **42**. The base was dissolved in ethanol and acidified with a 6 N solution of gaseous hydrochloric acid in ethanol and the solid obtained was crystallized from ethyl acetate (12 ml) to give 0.44 g (17.8%) of pure **42**. Mp 155–157°C. IR (KBr) 1755, 1612, 1203 cm^{-1}. ^{1}H-NMR (200 MHz): δ 1.21 (3H, t, J = 7 Hz), 1.9 (1H, m), 2.4 (1H, m), 2.6–3.4 (6H, m), 3.5 (1H, m), 4.18 (2H, q, J = 7 Hz), 4.70 (2H, s, OCH_2COO), 5.2 (1H, m, C*H*OH), 6.3 (1H, bs, exchange by D_2O, OH), 6.7 (1H, d, J = 3 Hz), 6.73 (1H, dd, J_1 = 3 Hz, J_2 = 8.5 Hz), 7.05 (1H, d, J = 8.5 Hz), 7.35–7.46 (3H, m), 7.52 (1H, sb), 9.31 (2H, bs, exchange by D_2O, N+H_2). Supercritical fluid chromatography rt (see below) 14.7 min; rt of **43**, **44**, **41**: 11.9, 25.7, 38 min.

*Alternative general method for the preparation of compounds **41** and **42**. Preparation of N-[(2R)-7-ethoxycarbonylmethoxy-1,2,3,4-tetrahydronaphth-2-yl]-(2R)-2-(3-chlorophenyl)-2-hydroxyethanamine hydrochloride **41***

A mixture of **9** as a base (1.29 g, 0.005 mol) and **14** (1.2 g, 0.0077 mol) in dimethylsulfoxide (5 ml) was heated at 80°C over night. After cooling to room temperature, the solution was diluted with water and extracted with dichloromethane (30 ml x 3). The organic phase was dried (sodium sulfate), concentrated *in vacuo* and the product obtained was purified by chromatography (eluent: ethyl acetate). The amine was treated with a 6 N solution of gaseous hydrochloric acid in ethanol and the solid hydrochloride filtered off. Crystallization from ethyl acetate gave 0.75 g (34%) of pure **41**.

Chromatographic methods

The chiral HPLC instrument consisted of a Waters M 590 pump, a Waters U6K injector, a Waters M840 integration system and a Chiralcel OD column (Daicel). The chiral supercritical fluid chromatographic instrument consisted of 2 pumps Varian 2050, an UV detector Varian 2550, a column oven Croco-cil, a Tescom high-pressure valve and a Varian Vista 402 integration system.

*Chiral HPLC of compounds **4a** and **5a***

Preparation of the derivatization reagent (benzoylimidazole). Carbonyldiimidazole (0.5 g) was dissolved in dichloromethane (10 ml). A solution of benzoic acid (0.18 g) in dichloromethane (10 ml) was added and the mixture was stirred for 5 min.

Preparation of the test solution. **4a** or **5a** (5 mg) was added to the derivatization reagent (1 ml); after 10 min the organic layer was washed with 5% sodium carbonate (1 ml), with 1 N hydrochloric acid (1 ml) and with water (1 ml). The organic phase was dried with anhydrous sodium sulfate and evaporated to dryness with nitrogen, and then the residue was dissolved in the mobile phase (2 ml).

HPLC chromatography. The mobile phase was a mixture of hexane and 2-propanol (80:20) at a flow rate of 1 ml/min.

*Chiral HPLC of compounds **12** and **13***

Preparation of test solution. The sample (20 mg) was dissolved in a 10% solution of boron trichloride in methanol (1 ml). After 3 min at 100°C the solution was cooled and dichloromethane (3 ml) and water (1 ml) were added. The organic layer was separated, dried with anhydrous sodium sulfate and evaporated with nitrogen. The residue was dissolved in the mobile phase (1 ml).

HPLC chromatography. The mobile phase was a mixture of hexane and 2-propanol (90:10) at a flow rate of 1 ml/min.

*Chiral supercritical fluid chromatography of compounds **41–44***

The compounds were dissolved in methanol. The eluent was a mixture of CO_2 (75 vol) and 0.3% diethylamine in 2-propanol (25 vol) at a flow rate of 2 ml/min; the column temperature was 32°C, the pressure was 160 atm and the UV detector was set at 280 nm.

Pharmacological methods

In vitro tests were performed as described by Bianchetti and Manara [6]. All the isolated preparations, from rats (Crl: CD®BR Charles River) (250–350 g) of either sex or from male DH IFFA-CREDO guinea-pigs (500–700 g), were set up in appropriate solutions (containing 200 μgml^{-1} ascorbic acid) in a 20 ml organ bath maintained at 37°C and aerated with 95% O_2, 5% CO_2 unless otherwise specified. Concentrations of agonists inducing tracheal relaxation or increasing atrial frequency by 50% over the basal value (EC_{50}), were extrapolated from cumulative log–concentration–response curves [25]. In the other preparations (rat colon and uterus), the EC_{50} (concentrations of agonists reducing spontaneous contractions by 50%) were obtained from log–concentration–response curves built by averaging the individual responses for each preparation. At least 6 different concentration–response curves were constructed.

Rat proximal colon

The first 3 cm segment of the rat proximal colon, starting from the ileo-caecal junction, was mounted longitudinally in Krebs–Ringer solution. Spontaneous motility (rhythmic phasic contractions) was recorded isotonically under a constant load of 1 g, in the presence of 10 μM phentolamine, 0.5 μM desmethylimipramine and 30 μM hydrocortisone hemisuccinate. Quantitative computer analysis was based on a motility index consisting of the area under the pressure waves over a 10-min period. Only 1 agonist concentration was tested on each preparation.

Guinea-pig right atrium

Spontaneously beating right atria of guinea-pigs were set up in Krebs solution maintained at 30°C and preincubated (30 min) with 12 μM phenoxybenzamine before cumulative addition of

the tested compounds with appropriate log–concentration increments (contact time for each dose 2.5 min); responses were expressed as percentages of the maximal response.

Guinea-pig trachea
Guinea-pig tracheal chains were set up in Krebs solution and preincubated (30 min) with 12 μM phenoxybenzamine before the addition of the tested compounds in log–concentration increments; only 1 cumulative concentration–response curve was obtained for each preparation. Drug-induced relaxation was recorded isotonically under a constant load of 0.5 g. The contact time between doses was 5 min or longer, until the effect reached a steady state.

Rat uterus
Uterine horns from unprimed estrus rats were set up in Locke's solution aerated with 100% O_2 and preincubated (30 min) with 12 μM phenoxybenzamine before addition of the drugs. Spontaneous contractions were recorded isotonically under a constant load of 1 g. Only 1 agonist concentration was tested on each preparation and allowed to act for at least 5 min. Activity was expressed as the percentage reduction of the amplitude of contractions from the basal value.

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