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(–)-5-Methyl-8-hydroxy-(di-*n*-propylamino)tetralin: A new 5-HT_{1A} receptor antagonist

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Abstract – (±)-5-Me-8-OH-DPAT **4** was synthesized by a new synthetic pathway recently described by us. The (+)- and (–)-enantiomers **4** were prepared from the primary amine **8** by crystallisation of the (+)- and (–)-mandelic acid salts. The enantiomers reacted with propyl iodide and were demethylated by 48% HBr to the (+)- and (–)-**4** compounds. These compounds had good affinity for 5-HT_{1A} receptors (K_i = 32.9 ± 0.8 and 45.6 ± 2 nM, respectively) but lacked enantioselectivity. In contrast to 8-OH-DPAT, but similar to WAY 100635 and (+)-WAY 100135, the addition of GTP-γS did not decrease the affinity of these compounds for 5-HT_{1A} receptors, suggesting a partial agonist or antagonist profile. Adenylyl cyclase assays with rat hippocampal membranes showed that (–)-**4** was totally inactive as an agonist over a wide concentration range in contrast to (+)-**4** which was a partial agonist. (–)-**4** (1 and 10 μM) shifted the concentration–effect curve for the inhibition by 8-OH-DPAT of forskolin-stimulated cyclic AMP production to the right (pA_2 = 7.6), demonstrating a competitive interaction between the two drugs. © Elsevier, Paris

serotonin / 5-HT_{1A} receptor antagonist / tetralin / adenylyl cyclase activity

1. Introduction

5-HT_{1A} receptors have received considerable interest over the last decade because several agonists for this receptor have been shown to possess anxiolytic and antidepressant properties in man [1]. Moreover, 5-HT_{1A} receptors play an important role in the central nervous system (CNS) in a number of behaviours such as impulsivity, sexual behaviour and food intake [2]. 5-HT_{1A} receptors are located both presynaptically on the soma and dendrites of the raphe nuclei and postsynaptically in the projection areas [3]. 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) reduce serotoninergic function by inhibiting the firing of 5-HT-containing neurones in the raphe nuclei and, consequently, decreasing extracellular 5-HT concentration in both the raphe nuclei and the projection areas [4]. However, many 5-HT_{1A} receptor ligands act as full agonists at the somatodentritic receptors while being partial agonists or antagonists at the postsynaptic receptors [5]. Thus, it is not clear whether the pharmacological properties observed are due to a pre-synaptic agonist action, a post-synaptic antagonist action or a combination of both [6]. The development of selective and 'silent' 5-HT_{1A} receptor antagonists (i.e. with no agonist properties) is an essential step for the understanding of the physiological role of these receptors and the elucidation of the exact mechanism of the pharmacological effects of the recently developed 5-HT_{1A} receptor ligands.

In the past, several compounds such as BMY 7378 (8-[2-4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5]-decane-7,9-dione dihydrochloride) [7], NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phtalimido)-butyl]butyl]-piperazine) [8] and SDZ 216-525 (methyl 4-(4-[4-(1,1,3-trioxo-2H-1,2-benzisothiazol-2-yl)-butyl]-1-piperazinyl)-1H-indole-2-carboxylate) [9] have been demonstrated to possess antagonist-like activity in a number of pharmacological assays

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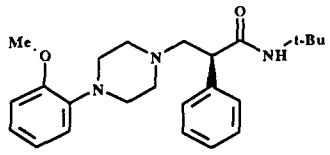
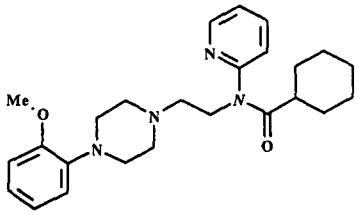
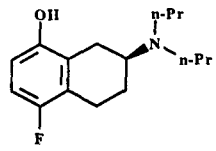


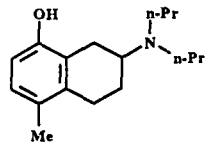
(S)-(+)-WAY 100135 (1)



WAY 100635 (2)



(S)-UH-301 (3)



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measuring postsynaptic 5-HT_{1A} receptor activation. However, these compounds have also shown agonist-like activity when studied at the somatodentritic 5-HT_{1A} autoreceptor. More recently, several compounds, (*S*)-(+) WAY 100135 [10], WAY 100635 [11] and (*S*)-(–)-UH 301 [12] were described as the first selective and silent 5-HT_{1A} receptor antagonists. WAY 100135 and WAY 100635 are members of the phenylpiperazine family which has provided many agonists or partial agonists for this class of receptor and (*S*)-(–)-UH301 is an 5-fluorine derivative of 8-OH-DPAT, the well-known 5-HT_{1A} receptor agonist. To date, only WAY 100635 seems to fulfil the criteria for selectivity and lack of intrinsic activity in models of pre- and postsynaptic 5-HT_{1A} receptor function [13]. Recent data have demonstrated partial 5-HT_{1A} receptor agonist activity at somatodentritic receptors for (*S*)-(+) WAY 100135 [14] and (*S*)-(–)-UH 301 possesses D_2 receptor agonist activity [12], limiting its use, particularly in vivo.

However, (*S*)-(–)-UH 301 is an interesting example as the change in the pharmacological profile can be directly related to the introduction of a fluorine atom in the partial agonist structure of (*S*)-8-OH-DPAT. Modifications of the electrostatic potential properties of both molecules could explain the differences in their orientation in the binding site [15] observed using molecular modelling and, consequently, the change in the pharmacological profile.

We present, herein, additional information on the influence of a structural modification of 8-OH-DPAT to obtain an antagonist derivative by introducing a small hydrophobic group such as a methyl and the first pharmacological data on (–)-5-methyl-8-hydroxy-(di-*n*-propylamino)tetralin demonstrating the antagonist pharmacological profile of this new compound for 5-HT_{1A} receptors.

2. Chemistry

In previous studies [16] concerning the mapping of the binding site of 5-HT_{1A} receptor ligands, we highlighted using the CoMFA method, the contribution of steric parameters to affinity values of the ligands for the 5-HT_{1A} receptor. The existence of a hydrophobic pocket near the area interacting with the aromatic moiety was demonstrated and this result was supported by data obtained with several derivatives of 8-OH-DPAT. In particular, preliminary results obtained from the binding assays with racemic (±)-5-methyl-8-hydroxy-(di-*n*-propylamino)tetralin indicated a putative antagonist profile. Consequently, it was then essential to prepare the enantiomers of this compound and to study their pharmacological profiles.

(+)- and (–)-**4** were prepared according to the pathway reported by us [16, 17] for the synthesis of 8-OH-DPAT and described in *figure 1*. The key reaction of the synthetic route was the Curtius degradation of the tetralin carboxylic acid derivative **7**. This was synthesized from the succinic acid **5** by cyclisation of the corresponding anhydride through the Friedel–Craft reaction. The ketone function of compound **6** was reduced catalytically in an AcOH medium. **7** was transformed into the amine **8** by a classical Curtius reaction through degradation of the acylazide intermediate in isocyanate and acid hydrolysis. This route was more efficient than that using the reaction of diphenylphosphoryl azide which produced a large number of additional compounds. The (+)- and (–)-**8** enantiomers were prepared from **8** by recrystallization of the mandelic acid salts with a moderate yield. Primary amines (+)- and (–)-**8** were alkylated with propyl iodide in acetonitrile and the corresponding derivatives were reacted with 48% HBr under reflux to give (+)- and (–)-**4** ($[\alpha]_D^{20}$ = +66 and –64, respectively).

3. Biochemistry

The affinities of the compounds (±)-, (+)- and (–)-**4** for 5-HT_{1A} receptors were determined by studying their competition for [^{3}H]-8-OH-DPAT binding sites in rat hippocampal membranes according to previously reported methods [18]. Preliminary evaluation of the pharmacological profiles of these compounds was carried out by examining the influence of GTP-γS on the affinity values. It is well established that

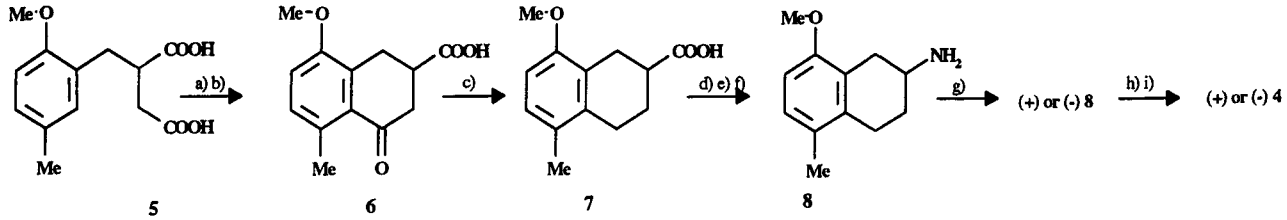

Figure 1. (a) $(CH_3CO)_2O$, reflux; (b) $AlCl_3$, nitrobenzene; (c) Pd/C 10%, AcOH, r.t.; (d) Et_3N, ClCOOEt, NaN_3, acetone, H_2O; (e) toluene, 80 °C; (f) 20% HCl, 80–90 °C; (g) (*S*)-(+)-mandelic acid or (*R*)-(–)-mandelic acid, EtOH; (h) *n*-C_3H_7I, K_2CO_3, acetonitrile or DMF; (i) AcOH, 48% HBr, 140 °C.

agonist affinity for the receptor binding site is decreased by the addition of GTP [19] or analogues such as GTP-γS, while antagonist affinity is little affected. However, for the accurate determination of the pharmacological profile, it was necessary to test the effect of the compounds on receptor function. For this purpose, the compounds were evaluated on 5-HT_{1A} receptors negatively coupled to adenylyl cyclase by using the forskolin-stimulated adenylyl cyclase assay [20] in rat hippocampal membranes. This assay is based on the conversion by the enzyme of [^{32}P]ATP to [^{32}P]cAMP which is then measured using sequential chromatography [21]. 8-OH-DPAT, the reference agonist molecule, produces a concentration-dependent decrease in adenylyl cyclase activity and this concentration–effect curve for the inhibition of adenyl cyclase activity will be shifted to the right in the presence of an antagonist.

4. Results and discussion

The affinity values of (±)-, (+)- and (–)-**4** for 5-HT_{1A} receptors in the presence or the absence of GTP-γS are reported in *table I*. 8-OH-DPAT, (+)-WAY 100135 and WAY 100635 were used as the reference compounds. We only observed a weak enantioselectivity of the 5-HT_{1A} receptors for the enantiomers **4** (K_i = 32.9 and 45 nM for the (+)- and (–)-enantiomers, respectively) and they can therefore be considered to be equipotent. This result contrasted with that reported for UH 301, another tetralin derivative, where the (*R*)-enantiomer was 8-fold more potent than the (*S*)-derivative [22]. The addition of GTP-γS brought about a large decrease in the affinity of 8-OH-DPAT for 5-HT_{1A} receptors while the affinities of the antagonists (+)-WAY 100135, WAY 100635 and the compounds (±)-, (+)- and (–)-**4** were relatively unaffected. These data confirmed the preliminary results

Table I. Effect of GTP-γS on the affinities of (±)-, (+)-, (–)-**4**, 8-OH-DPAT, (+)-WAY 100135 and WAY 100635 for 5-HT_{1A} receptors.

Compound	K_i[a]	K_i (+ 3 x 10^{-5} M GTP-γ-S)[b]	K_i (+ 3 x 10^{-5} M GTP-γ-S)/K_i
(±)-**4**	49.0 ± 1.5	35.5 ± 1.7	0.7
(+)-**4**	32.9 ± 0.8	25.7 ± 1.5	0.8
(–)-**4**	45.8 ± 2.0	42.6 ± 1.0	0.9
8-OH-DPAT	0.7 ± 0.1	16.7 ± 1.5	22.9
(+)-WAY 100135	2.3 ± 0.1	4.0 ± 0.1	1.8
WAY 100635	1.2 ± 0.1	1.9 ± 0.1	1.6

[a]K_i values are expressed in nM ± the standard error of the mean (SEM) and were calculated using the Cheng–Prusoff equation from IC_{50} values obtained from competition curves: the values of the data are at least the results of two determinations done in triplicate. [b]The experimental conditions were identical to those reported in the experimental section but the assays were carried out in the presence of GTP-γS.

with the racemic mixture **4** [16b] and suggested a putative antagonist profile for the (+)- and (–)-enantiomers **4** at 5-HT_{1A} receptors. Consequently, it was considered worthwhile to investigate the effects of these compounds on 5-HT_{1A} receptor function.

The results of the adenylyl cyclase assay are presented in *figure 2*. 8-OH-DPAT inhibited forskolin-stimulated adenylyl cyclase activity over a concentration range of 10^{-9}–10^{-6} M with a maximum inhibition of total cAMP production of 25% at 3 μM (*figure 2A*). (–)-**4** did not significantly affect forskolin-stimulated adenylyl cyclase activity over a range of 10^{-9}–10^{-3} M, while the (+)-enantiomer exhibited a clear partial agonist profile, reducing forskolin-stimulated cyclic AMP production by approximately 15% at 10 μM (*figure 2D*). The partial agonist profile of (+)-**4** was confirmed using WAY 100635 (0.2 μM) which inhibited the reduction in cyclic AMP synthesis produced by (+)-**4** (*figure 2D*). 10 μM (±)-**4** induced (*figure 2A*) a shift to the right of the concentration–effect curve for the inhibition by 8-OH-DPAT of forskolin-stimulated adenylyl cyclase activity, suggesting an antagonist profile for the (–)-**4** compound. This was confirmed by the shift to the right of the inhibiton curve for 8-OH-DPAT observed in the presence of 1 and 10 μM (–)-**4** (*figure 2B,C*) which demonstrated a competitive interaction between these two drugs for the same site. These results demonstrated that (–)-**4** is a postsynaptic antagonist at 5-HT_{1A} receptors in rat hippocampus with a pA_2 value of 7.6. A recent preliminary report [23] from our laboratories demonstrated that (–)-**4**, in microdialysis experiments in rats, had no effect on the basal release of serotonin but produced a presynaptic dose-dependent reduction of 8-OH-DPAT-induced inhibition of serotonin release in the ventral hippocampus, confirming its interest as a potential silent 5-HT_{1A} receptor antagonist. Moreover, (–)-**4** had only a weak affinity for rat striatal D_2 receptors in binding assays using [^{3}H] spiperone (K_i = 570 and 3500 nM for the (+)- and (–)-**4** compounds, respectively), an advantage over (*S*)-UH 301 which was more potent (K_i = 400 nM) at this receptor in binding assays [12].

The (–)-compound has similarity to (*S*)-UH 301 undoubtedly the (*S*)-configuration as it has been demonstrated with a large number of 2-amino tetralins that this configuration is always related to the (–)-sign of the optical rotation [24]. With regard to (*S*)-8-OH-DPAT, which is a partial agonist for 5-HT_{1A} receptors, it can be supposed that the antagonist profiles of (*S*)-UH 301 and (–)-**4** only depend upon the structural variation on the 5 position of the tetralin moiety. However, it is evident that the influences of the fluorine atom and the methyl group on the binding site of the 5-HT_{1A} receptor are very different. It has been reported [15] that the electron-withdrawing effect of the fluorine atom in (*S*)-UH 301 could be implicated in the modification of the pharmacological profile with regard to that of 8-OH-DPAT. In contrast, only steric parameters can be implicated to explain the antagonist profile of (–)-**4**. Therefore it is likely that the methyl group does not greatly modify the recognition properties of this compound by the 5-HT_{1A} receptor site, but it probably hampers the rearrangement of the molecule in the binding site during the G-protein coupling process which is essential for the agonist to activate the receptor. Recently [25], we showed a similar effect in the 5-HT_4 receptor field where a potent antagonist was designed by introducing a methyl group into the structure of the agonist. The influence of the steric hindrance of the methyl group in the binding site is regioselective as it has been demonstrated that the presence of methyl groups on the 1 or 3 position of 8-OH-DPAT only gave compounds with a partial agonist profile [26].

In conclusion, (–)-**4** exhibits interesting properties for 5-HT_{1A} receptors and is a serious candidate as a new, selective, 5-HT_{1A} receptor antagonist. Its effects on behaviour, food intake, hypothermia and the potentiation of the effect of 5-HT uptake inhibitors are under investigation and will be published elsewhere. Moreover, these results confirm and highlight the interest in discrete steric modifications of agonist structures to design an antagonist molecule for a G-protein-coupled receptor.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Mettler FP 61 or a Kofler-Reichert apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 or an AM 300 spectrometer with tetramethylsilane as the internal standard. Chemical shifts are reported in parts per million (ppm) in δ units. ^{1}H NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quadriplet), m (multiplet) and br (broad). Coupling constants (*J*) are given in Hertz. Optical rotations were obtained on a Polartronic E polarimeter. Elemental analyses were performed at the CNRS analysis service in Vernaison (France) or at the Microanalysis Service in the Châtenay-Malabry Faculty (France). Each compound gave an elemental analysis within 0.45% of the theoretical values. Diethyl acetylsuccinate, 5-methylsalicylic acid, (*S*)-(+)-mandelic acid and (*R*)-(–)-mandelic acid were purchased from Aldrich-Chimie (Strasbourg). Methyl 2-methoxy-5-methylbenzoate was synthesized by esterification of the corresponding salicylic acid [27]. 2-Methoxy-5-methylbenzyl alcohol was prepared by the reduction of the corresponding benzoate with $AlLiH_4$ according to the process already described [28].

5.1.1. 2-Methoxy-5-methylbenzyl chloride

A solution of 2-methoxy-5-methylbenzyl alcohol (13.76 g, 90.52 mmol) and pyridine (8.76 mL; 18.63 mmol) in anhydrous toluene (160 mL) was cooled to 0 °C in an ice bath.

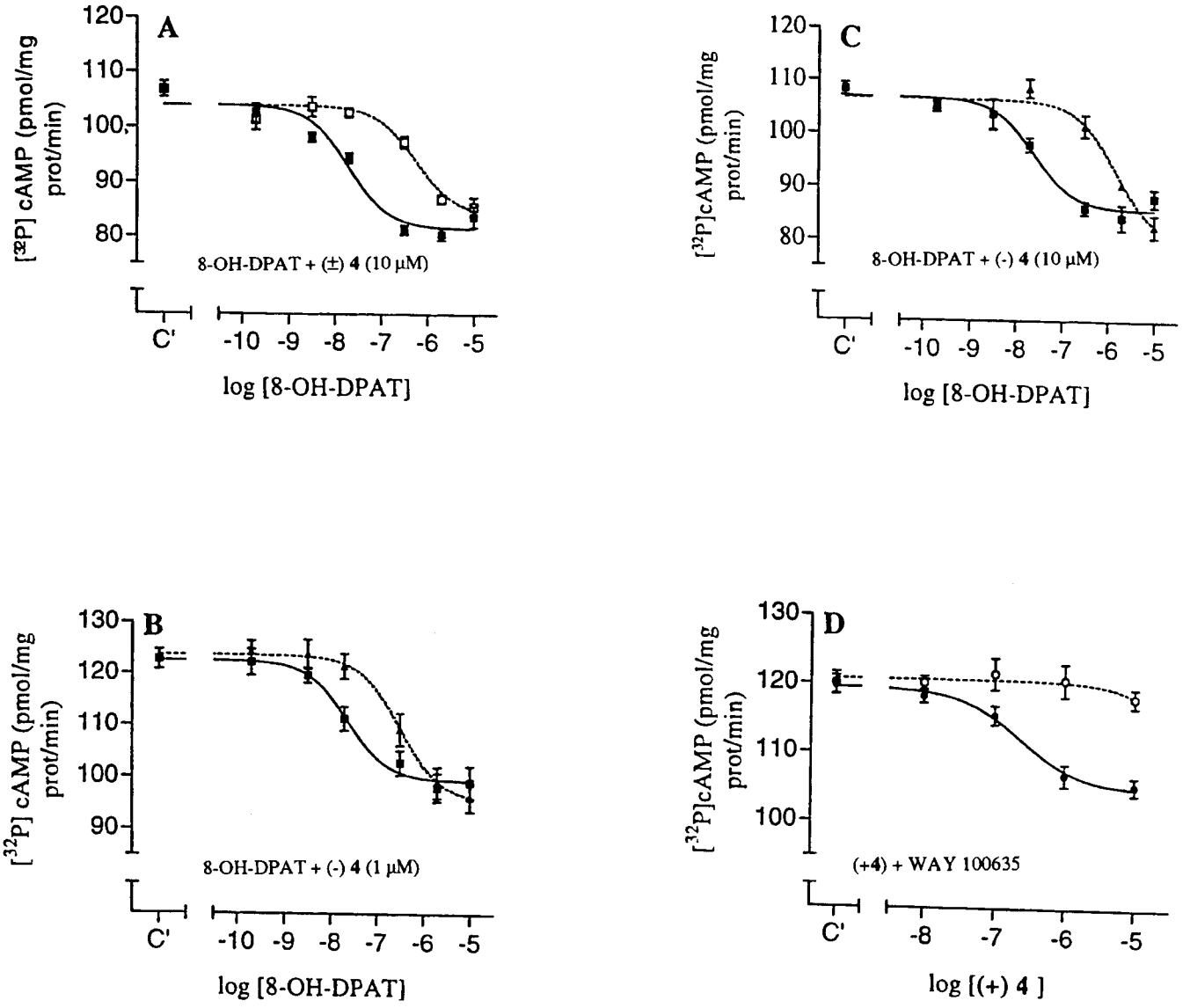

Figure 2. Concentration–effect curves of 8-OH-DPAT (A, B, C) and (+)-**4** (D) on forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes; (A) 8-OH-DPAT alone (■) or in the presence of 10 μM of (±)-**4** (□), B 8-OH-DPAT alone (■) or in the presence of 1 μM of (–)-**4** (▲), (C) 8-OH-DPAT alone (■) or in the presence of 10 μM of (–)-**4** (▲). (D) (+)-**4** (●) alone or in the presence of 0.2 μM of WAY 100635 (○). Adenylyl cyclase activity is expressed as pmol [^{32}P]cyclic AMP synthesized per mg protein per minute at 30 °C. Each point is the mean ± SEM of triplicate determinations in 2–3 separate experiments. C' on obscissa represents the control data without agonist.

Thionyl chloride (15.85 mL, 217.86 mmol, 2.4 equiv.) was added slowly while stirring vigorously. The reaction mixture was stirred at room temperature overnight and then poured into ice. The solution was stirred for one hour. The organic layer was separated and washed twice with water and then with a saturated aqueous solution of $NaHCO_3$ and brine. After drying over $MgSO_4$, the solvent was evaporated to give 13.57 g of the crude chloride, which was purified by distillation under reduced pressure at ca. 12 mmHg (82%). B.p. 112–114 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.2 (s, 1H), 7.1 (d, J = 8.3 Hz, 1H), 6.8 (d, J = 8.3 Hz, 1H), 4.7 (s, 2H), 3.9 (s, 3H), 2.3 (s, 3H); ^{13}C NMR (200 MHz, $CDCl_3$) δ: 155.2, 131.1, 130.4, 129.7, 125.4, 110.7, 55.5, 41.6, 20.3.

5.1.2. (2-Methoxy-5-methylbenzyl)succinic acid **5**

To a solution of diethyl acetylsuccinate (21 mL, 104 mmol) in 100 mL of dry DMF was added in small portions at 0 °C, a

suspension of 60% sodium hydride in oil (4.94 g, 124 mmol) under an inert atmosphere. The solution was stirred until no more gaseous emissions were observed. 2-methoxy-5-methylbenzyl chloride (17.9 g, 104 mmol) dissolved in 50 mL of dry DMF was added dropwise and the reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo and the residue taken up with ether and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give a residue which was hydrolysed by boiling in a mixture of 299 mL of 2 N NaOH and 50 mL of dioxane for 20 h. The cold alkaline solution was washed with ether and acidification with concentrated HCl produced the (arylmethyl)succinic acid which was extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and the solvent evaporated to give 17.35 g (67%) of the pure compound **5**. M.p. 117 °C. ^{1}H NMR (200 MHz, CD_3OD) δ: 7.1–6.8 (m, 3H), 3.8 (s, 3H), 3.2–2.3 (m, 5H), 2.3 (s, 3H); ^{13}C NMR (200 MHz, CD_3OD) δ: 178.8, 176.1, 157.0, 132.1, 130.6, 129.6, 127.6, 111.5, 55.9, 42.9, 36.1, 33.5, 20.8. Anal. (C, H) $C_{13}H_{16}O_5$.

5.1.3. 3-Carboxy-5-methoxy-8-methyl-1-tetralone ***6***

The (arylmethyl)succinic acid **5** (10 g, 39.68 mmol) was converted to the corresponding anhydride by refluxing for 2 h with acetic anhydride (80 g). The solution was removed in vacuo to give a brown oil which was the crude anhydride and this was used without further purification. It was dissolved in 80 mL of nitrobenzene and added slowly to a cooled solution (0 °C) of $AlCl_3$ (16 g, 3 equiv.) in 80 mL of nitrobenzene. The reaction mixture was stirred for 10 min and a solution of 60 g of ice with 60 mL of concentrated HCl was added. After standing overnight, the nitrobenzene was removed by steam distillation. The cooled solution was extracted with ethyl acetate, the organic layer was dried over $MgSO_4$ and the solvent evaporated. The crude product was purified by chromatography (silica gel, CH_2Cl_2–MeOH, 95:5) to give 7 g of the pure tetralone **6** (75%). M.p. 144 °C. ^{1}H NMR (200 MHz, CD_3OD) δ: 6.9 (m, 2H), 3.7 (s, 3H), 3.2–2.4 (m, 5H), 2.3 (s, 3H); ^{13}C NMR (200 MHz, CD_3OD) δ: 200.2, 176.6, 156.1, 133.1, 132.8, 131.7, 131.6, 115.5, 56.1, 42.9, 39.2, 26.8, 22.7. Anal. (C, H) $C_{13}H_{14}O_4$.

5.1.4. 2-Carboxy-8-methoxy-5-methyltetralin ***7***

The ketone **6** (8.3 g, 35.5 mmol) was reduced with H_2 under atmospheric pressure, using 10% palladium on activated carbon as the catalyst (800 mg) in 160 mL of acetic acid, at room temperature for 26 h. The catalyst was filtered off and the solution was concentrated under reduced pressure. The residue was purified by recrystallization in ethanol to give 5.9 g of the pure compound **7** (75%). M.p. 171 °C. ^{1}H NMR (200 MHz, CD_3OD) δ: 6.85 (d, J = 8.0 Hz, 1H), 6.5 (d, J = 8.0 Hz, 1H), 3.7 (s, 3H), 3.0 (m, 1H), 2.8–2.4 (m, 4H), 2.15 (m, 1H), 2.1 (s, 3H), 1.8 (m, 1H); ^{13}C NMR (200 MHz, CD_3OD) δ: 178.4, 155.1, 134.9, 127.6, 126.8, 123.4, 106.5, 54.8, 38.8, 26.1, 25.7, 25.1, 18.3. Anal. (C, H) $C_{13}H_{16}O_3$.

*5.1.5. (±)-2-Amino-8-methoxy-5-methyltetralin hydrochloride (±)-***8***•HCl*

Triethylamine (0.37 mL, 2.63 mmol) in acetone (6 mL) was added dropwise under cooling to a solution of the 2-carboxytetralin **7** (0.5 g, 2.27 mmol) in acetone (6 mL) and water (0.5 mL). While maintaining the temperature at 0 °C, ethyl chloroformate (0.27 mL, 2.87 mmol) in acetone (2 mL) was slowly added. The mixture was stirred for 30 min at 0 °C and then a solution of sodium azide (0.18 g, 2.87 mmol) in water (0.8 mL) was added. The mixture was stirred at 0 °C for 1 h and was then poured into 25 g of ice-water and extracted twice with ether. The ether extracts were washed with water, dried over Na_2SO_4, and evaporated under vacuum without heating to give the azide of the carboxylic acid **7** as a solid. The azide was dissolved in dry toluene (4 mL), and the solution was heated in an oil bath at 80 °C until no more nitrogen was evolved. Removal of the toluene under vacuum afforded 0.45 g of the corresponding isocyanate as an oil which was used without purification. A mixture of the isocyanate and 20% hydrochloric acid (4 mL) was stirred at 90–100 °C for 3 h and then allowed to stand at room temperature overnight. The reaction mixture was diluted with water and extracted with ether. The water phase was made alkaline with solid Na_2CO_3 and extracted with ether. The combined organic extracts were washed with water and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave 0.35 g (81%) of the amine which was converted to its hydrochloride salt (±)-**8**•HCl with a 4 N hydrochloric ether solution. M.p. > 300 °C. ^{1}H NMR (200 MHz, CD_3OD) δ: 6.85 (d, J = 8.3 Hz, 1H), 6.55 (d, J = 8.3 Hz, 1H), 3.6 (s, 3H), 3.3 (m, 1H), 3.1–3.0 (m, 1H), 2.7–2.55 (m, 2H), 2.4 (m, 1H), 2.2–2.0 (m, 1H), 2.0 (s, 3H), 1.8–1.6 (m, 1H); ^{13}C NMR (200 MHz, CD_3OD) δ: 157.0, 135.3, 129.4, 129.2, 121.9, 108.6, 55.9, 49.0, 29.3, 28.2, 26.3, 19.1. Anal. (C, H, N, Cl) $C_{12}H_{18}ClNO$.

*5.1.6. Resolution of (±)-***8**

To a solution of (±)-**8** (8.5 g, 44.44 mmol) in 200 mL of absolute ethanol was added (*S*)-(+)-mandelic acid ($[\alpha]_D^{20}$ + 155, *c* 2.8, H_2O) (6.76 g, 44.44 mmol) in 200 mL of absolute ethanol. The mixture was boiled under stirring and after one night at room temperature, the precipitate was filtered. The progress of the resolution was monitored by measuring changes in the optical rotation of the free amine released from the corresponding diastereoisomeric salt. Four recrystallizations from absolute ethanol gave 2.96 g (19%) of the resolved amine (+)-**8**-(+)-mandelate. M.p. 218–220 °C, $[\alpha]_D^{20}$ +86 (*c* 0.5, MeOH). It was released with a 2 N NaOH solution into dichloromethane. The organic phase was dried over $MgSO_4$ and evaporated to give 1.56 g (95%) of the free amine (+)-**8**: $[\alpha]_D^{20}$ +62 (*c* 0.5, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.95 (d, J = 8.36 Hz, 1H), 6.61 (d, J = 8.36 Hz, 1H), 3.79 (s, 3H), 3.18–3.0 (m, 2H), 2.86–2.53 (m, 2H), 2.36–2.23 (m, 1H), 2.17 (s, 3H), 2.1–1.95 (m, 1H), 1.61–1.52 (m, 1H), 1.48 (br s, 2H).

The free amine was isolated from the combined mother liquors from the two first recrystallizations and treated with an equimolar amount of (*R*)-(–)-mandelic acid ($[\alpha]_D^{23}$ –153, *c* 2.8, H_2O) in absolute ethanol. This yielded, after four recrystallizations done as above, 2.00 g (13%) of (–)-**8**-(–)-mandelate. This salt was taken up in a 2 N NaOH solution and extracted with dichloromethane to give 1.04 g (93%) of the pure amine (–)-**8**: $[\alpha]_D^{20}$ –68 (*c* 0.5, MeOH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.7 (d, J = 8.0 Hz, 1H), 6.52 (d, J = 8.0 Hz, 1H), 3.7 (s, 3H), 3.1–2.92 (m, 2H), 2.78–2.44 (m, 2H), 2.28–2.15 (m, 1H), 2.08 (s, 3H), 1.97–1.84 (m, 1H), 1.73 (br s, 2H), 1.58–1.3 (m, 1H).

*5.1.7. (±)-8-Hydroxy-5-methyl-di-n-propylaminotetralin hydrobromide (±)-***4**•*HBr*

A suspension of (±)-**8**•HCl (1.14 g, 5 mmol) and *n*-propyliodide (1 mL, 10 mmol, 1.2 equiv.) in 20 mL of CH_3CN was stirred with anhydrous K_2CO_3 (2.07 g, 30 mmol, 3 equiv.) for 4 days at room temperature. Ether was added and the precipitate was eliminated by filtration. The filtrate was concentrated and the crude product was purified by chromatography (alumina, Et_2O) to give 0.64 g (47%) of (±)-8-methoxy-5-methyl-di-*n*-propylaminotetralin. It was dissolved in 10 mL of a 48% HBr solution and stirred at 130 °C for 2 h under nitrogen. The hydrobromic acid was evaporated and the residue

recrystallized from EtOH/Et_2O to give 0.21 g (43%) of the pure hydrobromide (±)-**4**•HBr. M.p. 236 °C. ^1H NMR (200 MHz, CD_3OD) δ: 6.8 (d, J = 8.1 Hz, 1H), 6.4 (d, J = 8.1 Hz, 1H), 3.55–3.45 (m, 1H), 3.2–2.45 (m, 8H), 2.25–2.2 (m, 1H), 1.95 (s, 3H), 1.8–1.6 (m, 5H), 0.9 (t, J = 7.3 Hz, 6H); ^{13}C NMR (200 MHz, CD_3OD) δ: 155.0, 135.3, 129.2, 127.6, 120.5, 112.8, 61.85, 53.9, 53.7, 27.35, 25.5, 24.9, 19.65 (2C), 18.0, 11.4 (2C). Anal. (C, H, N, Br) $C_{17}H_{28}BrNO + 0.16\ H_2O$.

5.1.8. (+)-8-Hydroxy-5-methyl-di-n-propylaminotetralin hydrochloride (+)-4•HCl

A solution of *n*-propyliodide (1 mL, 10.34 mmol, 1.2 equiv.) and (+)-**8** (0.9 g, 4.7 mmol) in 20 mL of CH_3CN and 20 mL of dry DMF was stirred with anhydrous K_2CO_3 (1.9 g, 14 mmol, 3 equiv.) at 80 °C for 24 h. The solvents were removed and the crude product purified by chromatography (silica gel, CH_2Cl_2–MeOH, 98:2 then Et_2O) to give 0.8 g (62%) of (+)-**8**-methoxy-5-methyl-di-*n*-propylaminotetralin as a solid. It was dissolved in AcOH (15 mL) and 7.5 mL of 48% HBr solution was added. The reaction mixture was stirred at 140 °C for 4 h and, after cooling, poured into a cooled solution of saturated Na_2CO_3 (380 mL). The free amine was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and evaporated. The brown solid obtained was dissolved in EtOH and a 4 N hydrochloric ether solution was added to give after fitration 0.6 g of a solid which was the pure chlorhydrate (+)-**4**•HCl (69%). M.p. 214–216 °C; $[\alpha]_D^{20}$ +66 (*c* 0.5, MeOH). ^1H NMR (200 MHz, CD_3OD) δ: 6.7 (d, J = 8.1 Hz, 1H), 6.4 (d, J = 8.1 Hz, 1H), 3.65–3.45 (m, 1H), 3.5–2.25 (m, 8H), 2.3–2.15 (m, 1H), 2.0 (s, 3H), 1.9–1.55 (m, 5H), 0.95 (t, J = 7.3 Hz, 6H); ^{13}C NMR (200 MHz, CD_3OD) δ: 154.3, 135.2, 129.25, 127.5, 120.4, 112.8, 61.8, 53.9, 53.9, 27.35, 25.5, 24.8, 19.6 (2C), 18.0, 11.3 (2C). Anal. (C, H, N) $C_{17}H_{28}ClNO•H_2O$.

5.1.9. (–)-8-Hydroxy-5-methyl-di-n-propylaminotetralin hydrochloride (–)-4•HCl

Compound (–)-**4**•HCl (0.6 g, 41%) was prepared from (–)-**8** (0.94 g, 4.91 mmol) following the same procedure as for compound (+)-**4**•HCl. M.p. 215–216 °C, $[\alpha]_D^{20}$ –64 (*c* 0.5, MeOH). ^1H NMR (200 MHz, CD_3OD) δ: 6.741 (d, J = 8.12 Hz, 1H), 6.45 (d, J = 8.12 Hz, 1H), 3.64–3.5 (m, 1H), 3.2–2.51 (m, 8H), 2.28–2.2 (m, 1H), 2.05 (s, 3H), 1.84–1.66 (m, 5H), 0.95 (t, J = 7.3 Hz, 6H). Anal. (C, H, N) $C_{17}H_{28}ClNO•H_2O$.

5.2. Pharmacological methods

5.2.1. Materials

Tris, EGTA, NaCl, sucrose, $MgSO_4$, 3-isobutyl-1-methylxanthine (IBMX), Forskolin, GTP-γS, 5-HT and 8-OH-DPAT were purchased from Sigma; GTP, ATP, creatine phosphate, creatine kinase and cAMP from Boehringer and [^{3}H]8-OH-DPAT, [^{32}P]α-ATP and [^{3}H]cAMP from Dupont, NEN.

Male Sprague–Dawley rats from Janvier Laboratories (France) were used. Animals were housed at 22 ± 1 °C , with 55% humidity, on a 12 h light–dark cycle with free access to food and water for 4 days before use.

5.2.2. Binding assay-5-HT_{1A} receptors

Rats were decapitated, the brains were rapidly removed and the hippocampi dissected on ice. The tissues were thawed at 0 °C and homogenized in 40 vol. of ice-cold 50 mM Tris-HCl (pH 7.4 at 25 °C) using a Polytron tissue disrupter and then centrifuged twice at 40 000 *g* at 4 °C for 20 min. The supernatant was removed and the final pellet was resuspended in Tris-HCl buffer and incubated at 37 °C for 10 min to eliminate endogenous serotonin. The membranes were again centrifuged twice at 40 000 *g* and the final pellet was resuspended in 10 vol. of Tris-HCl. The protein concentration was determined using the Folin–Lowry method [29] with bovine serum albumin as the standard. Membrane aliquots of 2.6 mL were kept frozen at –80 °C. On the day of the study, the membranes were thawed at 0 °C. The K_i value of a compound for binding to 5-HT_{1A} receptors was determined by incubating 50 µL of the hippocampal membrane suspension (0.25 mg protein/mL) at 25 °C for 30 min with 20 µL of [^{3}H]8-OH-DPAT (specific activity 240 Ci/mmol, final concentration 0.3 nM) and 20 µL of the competing drugs at various concentrations suspended in a final volume of 0.5 mL 50 mM Tris-HCl. Seven concentrations of each drug were used and the assay was done in triplicate. K_i values were also determined in the presence of GTP-γS (30 µM) using the same method. Non-specific binding was determined by the addition of 20 µL of 10 µM 5-HT in duplicate. Total binding was defined in quadruplicate. Bound radioactivity was separated by vacuum filtration through Whatman GF/B glass filters, presoaked in a 0.1% polyethylenimine solution, using a Brandel Cell Harvester, followed by two washes with 5 mL of ice-cold buffer solution. The filters were placed in polyethylene vials to which 8 mL of a scintillation cocktail were added (Beckman, Ready Safe). After equilibration, the radioactivity remaining in the samples was determined using liquid scintillation spectrometry. IC_{50} values were calculated by a computer-assisted curve-fitting program (ALLFIT) based on a one-site model. K_i values were determined using the Cheng–Prusoff equation.

5.2.3. Adenylyl cyclase assays

Rat hippocampi were dissected on ice immediately after death by decapitation and homogenized in 50 volumes (v/w) of 2 mM ice-cold Tris-maleate containing 2 mM ethylene glycol bis β-aminoethyl-ether tetra acetic acid (EGTA) and 0.3 M sucrose, pH 7.2, using an Arthur H. Thomas tissue homogenizer. The homogenate was centrifuged at 500 *g* for 5 min at 4 °C and the supernatant was collected for a further centrifugation at 35 000 *g* for 10 min at 4 °C. The pelleted material was then resuspended in the same volume of the isotonic Tris-maleate buffer, and aliquots (40 µL) were used for the adenylyl cyclase assays [20–30]. Forskolin (10 µM), GTP (10 µM) and NaCl (0.1 M) were added to the assay mixture (25 mM Tris-maleate, 1 mM $MgSO_4$, 0.1 mM IBMX, 0.5 mM ATP plus 1 µCi of [^{32}P]α-ATP, [^{3}H]cAMP (about 10 000 cpm), 20 mM creatine phosphate, 0.2 mg/mL creatine kinase, 1 mM cAMP, pH 7.2) along with various concentrations of 8-OH-DPAT (0.2 nM–10 µM) or (+)-**4** (0.01–10 µM) in the presence or absence of (±)-**4** (10 µM), (–)-**4** (1–10 µM) or WAY 100635 (0.2 µM), (total assay volume 0.1 mL). The assay mixture was incubated for 20 min at 30 °C, and the reaction was stopped by adding 0.1 mL of a mixture containing 50 mM Tris-HCl, 5 mM ATP, 2 mM cAMP and 1% sodium dodecylsulfate, pH 7.4. Newly synthesized [^{32}P] cyclic AMP was extracted finally by ion exchange and adsorption chromatography as described by Salomon [21]. The protein concentration was determined using a Micro BCA protein Assay reagent Kit (Pierce) with bovine serum albumin as the standard. Adenylyl cyclase activity is expressed as pmol [^{32}P]cyclic AMP synthesized per mg of protein per minute at 30 °C.

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