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Original article

Structure–activity relationship of dopaminergic halogenated 1-benzyl-tetrahydroisoquinoline derivatives

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ABSTRACT

Two series of halogenated 1-benzyl-7-chloro-6-hydroxy-tetrahydroisoquinolines were prepared to explore the influence of each series on the affinity for dopamine receptors. All the compounds displayed a high affinity for D_1-like and/or D_2-like dopamine receptors in striatal membranes, although they were unable to inhibit [^{3}H]-dopamine uptake in striatal synaptosomes. The halogen placed on the benzylic ring in 1-benzyl-THIQs, compounds of the series **1**, 2′-bromobenzyl derivatives with K_i values into the nanomolar range, and the series **2**, 2′,4′-dichlorobenzyl-THIQ homologues, proves to be an important factor to modulate affinity at dopamine receptor.

1. Introduction

Dopamine-mediated neurotransmission plays an important role in several psychiatric and neurological disorders which affect several million people worldwide. Researchers have focused on various approaches towards modulating dopaminergic activity via the dopamine receptors (DR) as a potential means of treating schizophrenia and Parkinson's diseases. The consequences of an activation or blockade of DR are wide-ranging, and a perturbation of dopamine neurotransmission may result in profound neurological, psychiatric, or physiological signs and symptoms. For these reasons, much research has focused on the discovery of novel dopaminergic ligands as potential drug candidates [1]. DR can be classified into two pharmacological families (D_1 and D_2-like) that are encoded by at least five genes. Which receptor(s) needs to be activated to obtain therapeutic effects in Parkinson's disease remains controversial [2]. The D_2-like DR shows high affinities for the drugs (antagonists) used in the treatment of schizophrenia (antipsychotics) and those (agonists) utilised to treat Parkinson's disease [3].

Substituted isoquinolines (IQ) represent a class of natural and synthetic compounds that have drawn considerable attention because of their significant and powerful biological activities, including the inhibition of cellular proliferation, and their anti-tumour, antibiotic and anticonvulsant properties. A large number of IQ alkaloids have been obtained from various plants species, and have been evaluated for their ability to inhibit the dopamine transporter and to display affinities at D_1 and D_2-like DR binding sites in rat brain tissue [4]. The neurotransmitter dopamine (3,4-dihydroxyphenethylamine) is involved in the regulation of several functions, including locomotor activity, emotion, cognition and neuroendocrine secretion. Tetrahydroisoquinolines (THIQs), the most numerous naturally occurring alkaloids, include 1-benzyl-THIQs and aporphines, both of which have structural similarities to dopamine and can interact with DR [5].

Previous results of works done in our group suggest that some natural and synthetic 1-benzyl-THIQs alkaloids are able to bind to

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DR [6–8]. We described the enantioselective syntheses of pairs of dopaminergic (1*S*)- and (1*R*)-benzyl-THIQs using (*R*)- and (*S*)-phenylglycinol as the chiral source. We observed that in these 1-benzyl-THIQs series, their (1*S*)-enantiomers were 5–15 times more effective at the D_1-like DR and D_2-like DR than the (1*R*)-enantiomers [9]. Conversely, we described the preparation in a 'one-pot' sequence of 1-cyclohexylmethyl 7,8-dioxygenated-THIQs, which was substituted and unsubstituted in the C ring by the application of the photo-Fries transposition, followed by a tandem reduction–cyclisation and further reduction. Indeed, we accomplished a regioselective hydrogenation of the benzyl ring in the THIQ system for the first time. All the synthesised 1-cyclohexylmethyl THIQs were able to displace the D_2-like DR radioligand from its specific binding sites in rat striatal membranes, while the *N*-methylated derivatives also showed affinity for the D_1-like DR [10].

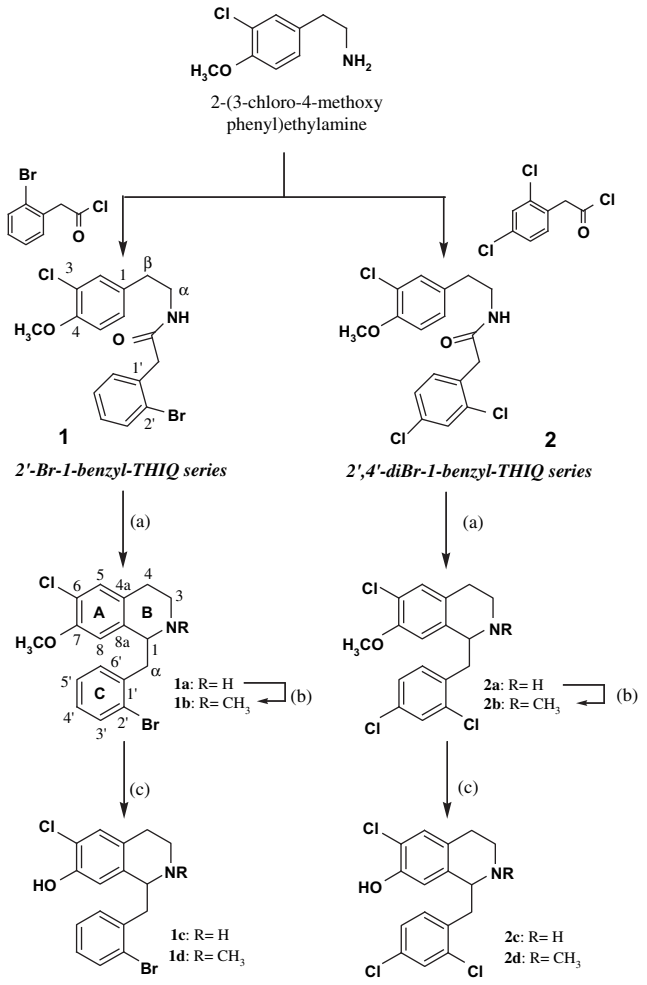
For the purpose of comparing the binding affinities of 1-substituted-THIQs for DR, and as a part of our research into the synthesis of new dopaminergic THIQ derivatives, we prepared two series of halogenated 1-benzyl-tetrahydroisoquinolines which support constant structural factors 6-chloro and 7-oxygenated substitutions, as well as a basic secondary (*N*H) or tertiary (*N*CH_3) amine.

The structures of the resulting 1-benzyl-THIQs were determined on the basis of their NMR spectral data and mass spectrometry analysis. All the synthesised compounds were tested for their ability to displace the selective radioligands of D_1 and D_2-like DR from their specific binding sites in striatal membranes in order to establish their structure–activity relationships (SARs) as dopaminergic agents.

2. Results and discussion

In the present work, we have studied the effect of the attachment of a chlorine or bromine atom to an aromatic C-ring in a series of 1-benzyl-6-chloro-7-hydroxy-THIQ derivatives. In previous works [10–13], we determined both the importance of the absolute configuration of the carbon at position 1 and the role of certain structural requirements for improving the affinity for the D_1 and D_2-like DR families. Thus, it has been postulated that the substitution into the A-ring for a hydroxyl (OH) and a halogen could lead to molecules which selectively bind at least one of the two aforementioned groups of receptors [12,13]. Previously, we have observed that 1-substituted-THIQ derivatives with a benzyl moiety at position 1 showed an affinity for D_1 and D_2 receptors [10,14]. Now, we decided to study the influence of the halogenation of this benzyl group by maintaining the chlorine and OH groups at positions C-6 and C-7 in the THIQ A-ring, respectively.

In this way, we prepared two series of compounds (Scheme 1): 2′-bromobenzyl-THIQs (series **1**: **1a**–**1d**) and 2′,4′-dichlorobenzyl-THIQs (series **2**: **2a**–**2d**) with an identical substitution in the A-ring (6-chloro, 7-hydroxy) and the nitrogen as a secondary or a tertiary amine (*N*H and *N*Me). The structures of the resulting eight 1-substituted-THIQs were determined on the basis of their NMR spectral data and mass spectrometry analysis.



Scheme 1. Synthesis of halogenated 1-benzyl-THIQ analogues (a) $POCl_3$-P_2O_5/toluene; $NaBH_4$/MeOH; (b) HCHO/$NaBH_4$; (c) BBr_3 – 3 h.

2.1. Chemistry

The synthesis of 1-halogenated benzyl-7-chloro-6-hydroxy-THIQ derivatives has been accomplished as an outline in Scheme 1, starting from β-(3-chloro-4-methoxyphenyl) ethylamine, and the 2-bromobenzyl and 2,4-dichlorobenzyl acid chlorides for series **1** and **2**, respectively. Firstly, that previously prepared by standard methods 2-(3-chloro-4-methoxy)ethylamine (Scheme 1) was treated with 2-bromobenzyl or 2,4-dichlorobenzyl acid chloride under Schotten–Baumann conditions and afforded the two amide derivatives with good yields: *N*-(3-chloro-4-methoxyphenethyl)-2-(2′-bromophenyl)acetamide (**1**) and *N*-(3-chloro-4-methoxy-phenethyl)-2-(2′,4′-dichlorophenyl)acetamide (**2**). Afterwards, these *N*-phenylethylamides (**1** and **2**) were converted into the corresponding 1-substituted-THIQs using the Bischler–Napieralski cyclodehydration reaction for which it was necessary to use a mixture of $POCl_3$ and P_2O_5 in dry toluene [15–17], followed by $NaBH_4$ reduction. Subsequently, these BTHIQ were treated with formaldehyde and formic acid, followed by $NaBH_4$ reduction, to afford the *N*-methyl derivatives. Finally, the *O*-demethylation of all the synthesised isoquinolines was performed by adding of 4 equivalents of BBr_3 reagent for 3 h at room temperature to obtain the resulting phenolic tertiary amines **1d** and **2d**.

2.2. Structure–activity relationship

All the synthesised compounds (see Scheme 1) were assayed *in vitro* for their ability to displace the selective ligands of D_1 and D_2 DR from their respective specific binding sites in the striatal membranes. All the compounds were unable to inhibit [^{3}H]-dopamine uptake in striatal synaptosomes. Many of these compounds were able to displace both ^{3}H-SCH 23390 and ^{3}H-raclopride from their specific binding sites in rat striatum at micromolar (μM) or nanomolar (nM) concentrations. The binding affinities for D_1 and D_2 DR are summarised in Table 1, and these

Table 1
Dissociation constants (K_i and pK_i) and selectivity of different compounds at the D_1-like and D_2-like dopaminergic receptors.

Compound	Specific-D_1 ligand [^{3}H]-SCH23390		Specific-D_2 ligand [^{3}H]-raclopride		D_1/D_2
	K_i (μM)	pK_i	K_i (μM)	pK_i	
1a	1.528 ± 0.304	5.83 ± 0.08	0.245 ± 0.050	6.63 ± 0.09[c]	6.2
1b	1.319 ± 0.145	5.89 ± 0.05	1.336 ± 0.296	5.90 ± 0.09[e]	1.0
1c	0.110 ± 0.042	7.06 ± 0.18[f]	**0.046 ± 0.012**	7.39 ± 0.14[f]	2.4
1d	**0.065 ± 0.011**	7.21 ± 0.07[f]	**0.018 ± 0.004**	7.76 ± 0.08[b,d,f]	3.6
2a	11.323 ± 4.164	5.01 ± 0.17	3.301 ± 0.797	5.51 ± 0.11[a]	3.4
2b	15.113 ± 2.860	4.84 ± 0.08	6.132 ± 1.381	5.23 ± 0.10	2.5
2c	**0.183 ± 0.014**	6.74 ± 0.03[f]	**0.107 ± 0.027**	7.00 ± 0.11[f]	1.7
2d	0.241 ± 0.054	6.71 ± 0.14[f]	0.269 ± 0.097	6.64 ± 0.19[f]	0.9

The results are expressed as mean ± SEM from 3 to 6 experiments.
ANOVA, post Newmann–Keuls Multiple comparison test:
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$ vs. D_1-like dopaminergic receptor.
[d] $p < 0.05$.
[e] $p < 0.001$ vs. corresponding –NH derivatives (compounds **b** vs. **a**, and **d** vs. **c**).
[f] $p < 0.001$ vs. corresponding $-OCH_3$ derivatives (compounds **c** vs. **a**, and **d** vs. **b**).

results have illustrated some general trends of the structure–activity relationship:

1. In general, all the tested 1-benzyl-6-chloro-7-methoxy-THIQ (**1a**,**b** and **2a**,**b**) showed a lower affinity for D_1 and D_2 DR than their corresponding 7-hydroxy homologues (**1c**,**d** and **2c**,**d**) (about 70-fold). The higher affinity of compounds with free phenol OH has been previously described in several isoquinolines [10,14]. The 1-substituted THIQs synthesised the presence of a hydroxyl (OH) group at position C-7, and their ability to displace the selective ligands of the D_1 and D_2 dopamine receptors from their specific binding sites in the striatal membranes was positively influenced, while the replacement with a methoxyl (OMe) group at position C-7 did not favour dopaminergic activity.
2. Both the **1** (2′-bromobenzyl-THIQs) and **2** (2′,4′-dichlorobenzyl-THIQs) compounds series showed a high affinity for dopaminergic receptors. Surprisingly however, the different type of halogen at the benzyl level did not appear to be important for the selective improvement of the affinities for the D_1 or D_2 receptors. In addition, the secondary amine (*N*H) showed an affinity similar to that of its corresponding tertiary amine (*N*Me) homologues.
3. However, the absence of selectivity that these compounds displayed did not prevent them from behaving like molecules with a high affinity for dopaminergic receptors, and actually showed some K_i values in the nanomolar range (**1d**: 18 nM). We observed that the compounds of series **1** (2′-bromobenzyl derivatives) showed an affinity that was 10 times higher than those of their corresponding 2′,4′-dichlorobenzyl-THIQ homologues (series **2**) since the latter exhibited less potency to displace the ligands of D_1 and/or D_2 DR from their specific binding sites. It is likely that the larger size and atomic number of the bromine atom could justify the better affinity for the D_1 and D_2 receptors (Table 1 and Fig. 1).

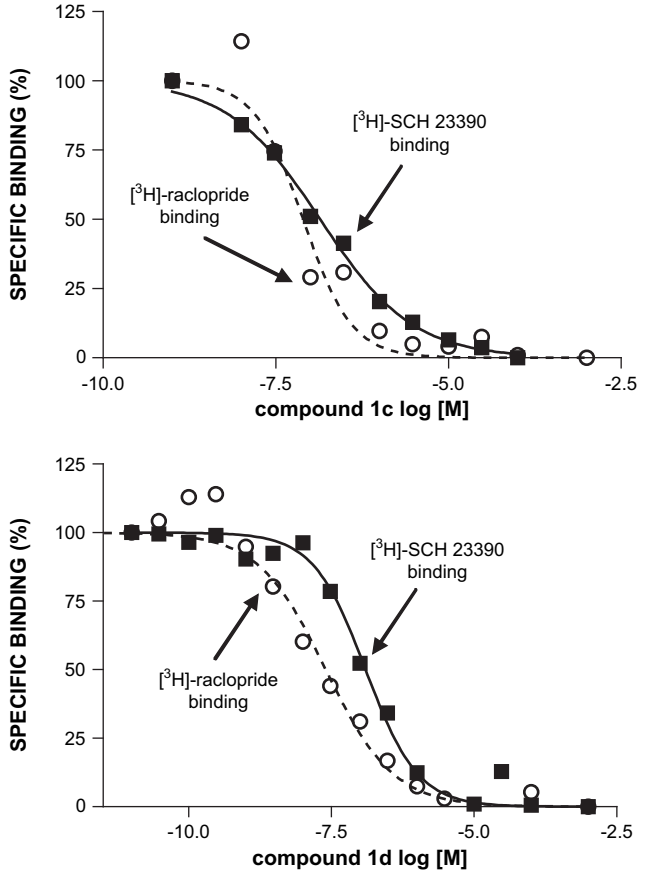


Fig. 1. Displacement of specific binding of [^{3}H]-SCH23390 (D_1-like DR specific ligand) and [^{3}H]-raclopride (D_2-like DR specific ligand) by compounds **1c** and **1d**. The results are expressed as mean ± SEM from 3–6 experiments.

3. Conclusions

The halogen placed on the benzylic ring in 1-benzyl-THIQs proves to be an important factor to modulate affinity at DR. Compounds of the series **1**, 2′-bromobenzyl derivatives with K_i values into the nanomolar range, and the series **2**, 2′,4′-dichlorobenzyl-THIQ homologues, proves to be an important factor to modulate affinity at dopamine receptor. The compounds of series **1** showed an affinity that was 10 times higher than those of their corresponding series **2** homologues to displace the ligands of D_1 and/or D_2 DR from their specific binding sites.

4. Experimental section

4.1. General instrumentation

Melting points were taken on a Cambridge microscope instrument coupled with a Reichert-Jung. EI and FAB mass spectra were recorded on a VG Auto Spec Fisons spectrometer instruments (Fisons, Manchester, United Kingdom). ^{1}H NMR and ^{13}C NMR spectra were recorded with $CDCl_3$ as a solvent on a Bruker AC-300, AC-400 or AC-500. Multiplicities of ^{13}C NMR resonances were assigned by DEPT experiments. NOE DIFF irradiations, COSY, HMQC, HSQC and HMBC correlations were recorded at 400 MHz and 500 MHz (Bruker AC-400 or AC-500). All the reactions were monitored by analytical TLC with silica gel 60 F_{254} (Merck 5554). Residues were purified by silica gel 60 (40–63 μm, Merck 9385) column chromatography. Solvents and reagents used and purchased from commercial sources. The cited yields were of purified material. The HCl salts of the synthesised compounds were prepared from the corresponding base with 5% HCl in MeOH.

4.2. General procedure for the synthesis of amides *1* and *2*

2-(3-Chloro-4-methoxyphenyl) ethylamine was obtained by standard methods [8]. Amides **1** and **2** were prepared under Schotten–Baumann conditions by condensing 2-(3-chloro-4-methoxyphenyl)ethylamine with the appropriate benzoyl chloride.

4.2.1. 2-(2′-Bromophenyl)-N-(3-chloro-4-methoxyphenylethyl)acetamide (**1**)

A mixture of 2-(2-bromophenyl)acetic acid (1 g, 4.65 mmol) and $SOCl_2$ (0.4 mL, 5.58 mmol) in anhydrous CH_2Cl_2 was refluxed for 3 h. Then, the solvent was removed to obtain the 2-(2-bromophenyl)acetyl chloride as a pale yellow oil, which was used in the next step without further purification. Then, an amount of 2-bromophenylacetyl chloride (0.6 mL, 4.03 mmol) was added dropwise at 0 °C to a solution of the β-(3-chloro-4-methoxyphenyl)ethylamine (500 mg, 2.69 mmol) in CH_2Cl_2 (20 mL) and 5% aqueous NaOH (4.5 mL), and was stirred at room temperature for 3 h. Next, 2.5% aqueous HCl was added, and the organic solution was washed with brine (2 × 10 mL) and H_2O (2 × 10 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was purified via silica gel column chromatography (hexane/EtOAc, 50:50) to afford 753 mg of the amide **1** (1.97 mmol, 73.2%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (dd, J = 7.80, 1.1 Hz, 1H, H-3′), 7.27–7.16 (m, 3H, H-6′, H-5′, H-4′), 7.08 (d, J = 2.2 Hz, 1H, H-2), 6.93 (dd, J = 8.4, 2.2 Hz, 1H, H-6), 6.78 (d, J = 8.4 Hz, 1H, H-5), 3.94 (s, 3H, OCH_3-4), 3.72 (s, 2H, CH_2CO), 3.37 (td, J = 6.9, 6.0 Hz, 2H, H-α), 2.83 (t, J = 6.9 Hz, 2H, H-β); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6 (CO), 153.6 (C-4), 134.5 (C-1′), 133.8, 133.1, 131.5, 130.4, 129.2, 128.1, 127.6, 124.9 (C-2′), 122.3 (C-3), 112.3 (CH-5), 56.2 (OCH_3), 43.9 (CH_2-α), 40.7 (CH_2), 34.9 (CH_2-β); FAB-MS m/z 383 $[M]^+$, 381, 368, 168; HREIMS m/z 381.0139 ($C_{17}H_{17}BrClNO_2$, calc 381.0131).

4.2.2. 2-(2′,4′-Dichlorophenyl)-N-(3-chloro-4-methoxyphenylethyl)acetamide (**2**)

The title compound was prepared according to the procedure for **1**, using 2-(2,4-dichlorophenyl)acetyl chloride (0.6 mL, 4.03 mmol) and β-(3-chloro-4-methoxyphenyl) ethylamine (500 mg, 2.69 mmol). The residue was purified via silica gel column chromatography (hexane/EtOAc, 50:50) to afford 682 mg of the amide **2** (1.83 mmol, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, J = 1.1 Hz, 1H, H-3′), 7.22 (m, 2H, H-5′, H-6′), 7.10 (d, J = 2.2 Hz, 1H, H-2), 6.92 (dd, J = 8.4, 2.2 Hz, 1H, H-6), 6.79 (d, J = 8.4 Hz, 1H, H-5), 3.88 (s, 3H, OCH_3-4), 3.60 (s, 2H, CH_2CO), 3.43 (td, J = 6.7, 6.0 Hz, 2H, H-α), 2.68 (t, J = 6.7 Hz, 2H, H-β); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3 (CO), 154.0 (C-4), 135.3 (C-2′), 134.4 (C-1′), 132.8 (C-6′), 132.0 (CH-4′), 131.8 (C-1), 130.8 (CH-2), 130.0 (CH-3′), 128.3 (CH-6), 128.1 (CH-5′), 122.7 (C-3), 112.5 (CH-5), 56.6 (OCH_3), 41.2 (CH_2), 41.0 (CH_2-α), 34.7 (CH_2-β); FAB-MS m/z 372 $[M+H]^+$, 168; HREIMS m/z 372.0312 ($C_{17}H_{17}Cl_3NO_2$, calc 372.0325).

4.3. General procedure for Bischler–Napieralski cyclisation

4.3.1. 1-(2′-Bromobenzyl)-6-chloro-7-methoxy-1,2,3,4-tetrahydroisoquinoline (**1a**)

To a 250 mL three-neck round-bottom flask under N_2, the corresponding amide **1** (500 mg, 1.31 mmol) was added in dry toluene (20 mL), and was treated with P_2O_5 (3.7 g, 13.1 mmol), which was added in portions and followed by the dropwise addition of $POCl_3$ (1.2 mL, 13.1 mmol). The mixture was stirred and refluxed under N_2 for 6–8 h, and then cooled to room temperature. Toluene was concentrated under reduced pressure and the reaction mixture was slowly poured into a mixture of crushed ice. The solid residue was triturated with 10% aqueous NaOH to afford a suspension (pH ≈ 8–9), then extracted with CH_2Cl_2 (3 × 15 mL). The combined CH_2Cl_2 extracts were dried over Na_2SO_4 and the solvent evaporated *in vacuo* to afford reddish oil. The residue was dissolved in MeOH (20 mL), and was then cooled to −78 °C and treated with $NaBH_4$ (76 mg, 2 mmol). The reaction mixture was stirred for 2 h. Water (15 mL) was added and volatiles were evaporated under reduced pressure. The aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography CH_2Cl_2/MeOH/NH_3 (100:2:0.5) to furnish 164 mg of **1a** (0.50 mmol, 34%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (dd, 1H, J = 8.0, 0.9 Hz, H-3′), 7.30–7.16 (m, 3H, H-6′, H-5′, H-4′), 7.11 (s, 1H, H-5), 6.73 (s, 1H, H-8), 4.28 (dd, 1H, J = 9.7, 4.0 Hz, H-1), 3.81 (s, 3H, OCH_3), 3.34 (dd, 1H, J = 13.6, 4.0 Hz, H-αa), 3.25–3.19 (m, 1H, H-3a), 3.04–2.76 (m, 2H, H-αb, H-3b), 2.74–2.71 (m, 2H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.7 (C-7), 138.4 (C-1′), 137.9 (C-8a), 133.5 (CH-3′), 132.4 (CH-6′), 130.8 (CH-5), 128.8 (C-4′), 128.4 (C-4a), 127.9 (C-5′), 124.8 (C-2′), 120.2 (C-6), 110.7 (CH-8), 56.5 (OCH_3), 55.4 (CH-1), 43.5 (CH_2-3), 40.1 (CH_2-α), 29.2 (CH_2-4); FAB-MS m/z 366 $[M+H]^+$, 196; HRFABMS m/z 366.0238 $[M+H]^+$ ($C_{17}H_{18}BrClNO$, calc 366.0260).

4.3.2. 1-(2′,4′-Dichlorobenzyl)-6-chloro-7-methoxy-1,2,3,4-tetrahydroisoquinoline (**2a**)

The title compound was prepared according to the procedure for **1a** using the corresponding amide **2** (500 mg, 1.34 mmol), P_2O_5 (3.8 g, 13.4 mmol) and $POCl_3$ (1.3 mL, 13.4 mmol). The residue obtained as a reddish oil was treated with $NaBH_4$ (76 mg, 2 mmol). The crude product was purified by silica gel column chromatography CH_2Cl_2/MeOH/NH_3 (100:2:0.5) to furnish 193 mg of **2a** (0.51 mmol, 38%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, 1H, J = 1.7 Hz, H-3′), 7.21–7.20 (m, 2H, H-5′, H-6′), 7.12 (s, 1H, H-5), 6.66 (s, 1H, H-8), 4.24 (dd, 1H, J = 9.8, 4.1 Hz, H-1), 3.83 (s, 3H, OCH_3), 3.29 (dd, 1H, J = 13.7, 4.1 Hz, H-αa), 3.25–3.19 (m, 1H, H-3a), 3.00–2.94 (m, 2H, H-3b, H-αb), 2.74–2.71 (m, 2H, H-4), 1.7 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9 (C-7), 137.8 (C-1′), 135.4 (C-8a), 134.9 (C-2′), 133.1 (C-6′), 132.6 (CH-4′), 130.6 (CH-5), 129.5 (CH-3′), 128.2 (C-5′), 127.1 (CH-4a), 120.6 (C-6), 110.2 (CH-8), 56.1 (OCH_3), 54.9 (CH-1), 40.1 (CH_2-3), 39.6 (CH_2-α), 28.6 (CH_2-4); FAB-MS m/z 356 $[M+H]^+$, 196; HRFABMS m/z 356.0321 $[M+H]^+$ ($C_{17}H_{17}Cl_3NO$, calc 356.0375).

4.4. General procedure for N-methylation

4.4.1. 1-(2′-Bromobenzyl)-6-chloro-7-methoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline (**1b**)

To a stirred solution of **1a** (500 mg, 1.37 mmol) in MeOH (20 mL), 37% formaldehyde (15 mL) and one drop of formic acid were added. The mixture was refluxed for 1 h, cooled to room temperature, treated with $NaBH_4$ (520 mg, 13.7 mmol), and refluxed for an additional 1 h. The reaction mixture was left to warm up to room temperature, and the solvent was removed under reduced pressure. Water (3 mL) was added to the residue and the aqueous mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product which was further purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH, 100:1:0.3) to afford 437 mg of **1b** (1.23 mmol, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (dd, 1H, J = 7.5, 1.6 Hz, H-3′), 7.16 (dd, 1H, J = 7.5, 1.6 Hz, H-5′), 7.09 (s, 1H, H-5), 7.07 (dd, 1H, J = 7.5 Hz, 1.6 Hz, H-6′), 6.97 (dd, 1H, J = 7.5 Hz, 1.6 Hz, H-4′), 5.91 (s, 1H, H-8), 3.92 (m, 1H, H-1), 3.49 (s, 3H, OCH_3), 3.35–3.30 (m, 2H, H-3a, H-αa), 2.93–2.80 (m, 3H, H-4a, H-3b, H-αb), 2.64–2.59 (m, 1H, H-4b), 2.54 (s, 3H, NCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.8 (C-7), 138.8 (C-1′), 136.2 (C-8a), 132.7 (CH-3′ and CH-6′), 130.0 (CH-5), 127.9 (CH-4′), 127.1 (CH-5′), 125.1 (C-2′), 120.1 (C-6), 111.8 (CH-8), 62.3 (CH-1), 55.6 (OCH_3), 45.5 (CH_2-3), 42.4 (NCH_3), 40.5 (CH_2-α), 24.4 (CH_2-4); FAB-MS m/z 380 $[M+H]^+$, 210; HRFABMS m/z 380.0318 $[M+H]^+$ ($C_{18}H_{20}BrClNO$, calc 380.0417).

4.4.2. 1-(2′,4′-Dichlorobenzyl)-6-chloro-7-methoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline (**2b**)

The title compound was prepared according to the procedure for **1b** using the corresponding secondary amine **2a** (500 mg, 1.41 mmol), 37% formaldehyde (15 mL) and $NaBH_4$ (535 mg, 14.1 mmol). The crude product was purified by silica gel column

chromatography (CH_2Cl_2/MeOH/NH_4OH, 100:1:0.3) to afford 469 mg of **2b** (1.27 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, 1H, J = 2.1 Hz, H-3′), 7.13 (dd, 1H, J = 8.2 Hz, 2.1 Hz, H-5′), 6.96 (d, 1H, J = 8.2 Hz, H-6′), 7.09 (s, 1H, H-5), 6.04 (s, 1H, H-8), 3.91–3.82 (m, 1H, H-1), 3.59 (s, 3H, OCH_3), 3.27–3.20 (m, 2H, H-3a, H-αa), 2.91–2.77 (m, 3H, H-3b, H-αb, H-4a), 2.61–2.56 (m, 1H, H-4b), 2.49 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.2 (C-7), 136.2 (C-1′), 135.9 (C-8a), 135.0 (C-2′), 133.2 (CH-6′), 132.6 (C-4′), 130.1 (CH-5), 129.1 (CH-3′), 127.1 (C-4a), 126.7 (CH-5′), 120.4 (C-6), 111.6 (CH-8), 62.5 (CH-1), 55.7 (OCH_3), 45.7 (CH_2-3), 42.4 (NCH_3), 37.7 (CH_2-α), 24.2 (CH_2-4); FAB-MS m/z 370 $[M+H]^+$, 210; HRFABMS m/z 370.0520 $[M+H]^+$ ($C_{18}H_{19}Cl_3NO$, calc 370.0532).

4.5. General procedure for O-demethylation

4.5.1. 1-(2′-Bromobenzyl)-6-chloro-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (**1c**)

A solution of the appropriate isoquinoline **1a** (260 mg, 0.71 mmol) in dry CH_2Cl_2 (10 mL) was cooled to −78 °C. To this stirring solution, BBr_3 (0.28 mL, 2.8 mmol) was added dropwise. After 15 min, the reaction mixture was left to warm up to room temperature and stirred for 3 h. The reaction was terminated by the addition of MeOH (5 mL), dropwise and the mixture was stirred for another 30 min. The solvent was concentrated to dryness. The residue was dissolved in EtOAc (2 mL) and made alkaline with 37% aqueous NH_4OH to pH ≈ 11, and was subsequently neutralised with 1 M HCl to pH ≈ 7–8. The aqueous layer was then extracted with the EtOAc (3 × 10 mL). The combined EtOAc extracts were dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH, 100:6:0.5) to afford 185 mg of **1c** (0.53 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (dd, 1H, J = 7.6, 1.2 Hz, H-3′), 7.27 (s, 1H, H-5), 7.22–7.02 (m, 3H, H-6′, H-5′, H-4′), 6.91 (s, 1H, H-8), 4.29 (dd, 1H, J = 9.7, 4.0 Hz, H-1), 3.25–3.04 (m, 2H, H-3a, H-αa), 3.02–2.76 (m, 2H, H-3b, H-αb), 2.79–2.75 (m, 2H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.4 (C-7), 139.3 (C-1′), 136.2 (C-8a), 132.4 (CH-3′), 131.1 (CH-6′), 130.7 (C-4a), 130.4 (CH-5), 128.1 (CH-4′), 127.6 (CH-5′), 124.4 (C-2′), 117.2 (C-6), 114.1 (CH-8), 57.6 (CH-1), 42.1 (CH_2-3), 39.4 (CH_2-α), 27.9 (CH_2-4); FAB-MS m/z 353 $[M+H]^+$, 183.

4.5.2. 1-(2′-Bromobenzyl)-6-chloro-7-hydroxy-N-methyl-1,2,3,4-tetrahydroisoquinoline (**1d**)

The title compound was prepared according to the procedure for **1c** using the corresponding compound **1b** (260 mg, 0.73 mmol) and BBr_3 (0.29 mL, 2.9 mmol). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH, 100:3:0.5) to afford 190 mg of **1d** (0.56 mmol, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (dd, 1H, J = 8.0, 0.8 Hz, H-3′), 7.30–7.16 (m, 2H, H-6′, H-5′, H-4′), 7.27 (s, 1H, H-5), 6.92 (s, 1H, H-8), 4.30–4.02 (m, 1H, H-1), 3.35–3.30 (m, 2H, H-3a, H-αa), 2.93–2.80 (m, 3H, H-4a, H-3b, H-αb), 2.64–2.59 (m, 1H, H-4b), 2.53 (s, 3H, NCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.3 (C-7), 139.6 (C-1′), 135.3 (C-8a), 132.6 (C-3′), 131.4 (C-4a), 131.1(C-6′), 130.1 (C-5), 128.2 (C-4′), 127.6 (C-5′), 124.4 (C-2′), 119.9 (C-6), 115.8 (C-8), 64.8 (C-1), 47.5 (CH_2-3), 39.1 (CH_2-α), 26.5 (CH_2-4); FAB-MS m/z 367 $[M+1]^+$, 183.

4.5.3. 1-(2′,4′-Dichlorobenzyl)-6-chloro-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (**2c**)

The title compound was prepared according to the procedure for **1c** using the corresponding isoquinoline **2a** (260 mg, 0.69 mmol) and BBr_3 (0.27 mL, 2.7 mmol). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH, 100:6:0.5) to afford 182 mg of **2c** (0.5 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, 1H, J = 2.1 Hz, H-3′), 7.31 (dd, 1H, J = 8.2, 2.1 Hz, H-5′), 7.25 (d, 1H, J = 8.2 Hz, H-6′), 7.18 (s, 1H, H-5), 6.45 (s, 1H, H-8), 4.01–3.96 (m, 1H, H-1), 3.35–3.25 (m, 2H, H-3), 3.09–2.89 (m, 2H, H-α), 2.79–2.75 (m, 2H, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.4 (C-7), 138.1 (C-1′), 136.0 (C-8a), 134.0 (C-2′), 132.4 (C-6′), 132.0 (C-4′), 130.5 (CH-4a), 130.2 (CH-5), 130.1 (CH-3′), 126.8 (CH-5′), 118.7 (C-6), 114. 1 (CH-8), 61.6 (CH-1), 42.7 (CH_2-3), 37.1 (CH_2-α), 26.8 (CH_2-4); FAB-MS m/z 343 $[M+1]^+$, 183, 148.

4.5.4. 1-(2′,4′-Dichlorobenzyl)-6-chloro-7-hydroxy-N-methyl-1,2,3,4-tetrahydroisoquinoline (**2d**)

The title compound was prepared according to the procedure for **1c** using the corresponding isoquinoline **2b** (260 mg, 0.70 mmol) and BBr_3 (0.28 mL, 2.8 mmol). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH, 100:3:0.5) to afford 198 mg of **2d** (0.56 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, 1H, J = 1.7 Hz, H-3′), 7.31–7.20 (m, 2H, H-5′, H-6′), 7.18 (s, 1H, H-5), 6.92 (s, 1H, H-8), 4.27 (dd, 1H, J = 9.8, 4.0 Hz, H-1), 3.20–2.81 (m, 4H, H-3, H-α), 2.77–2.73 (m, 2H, H-4), 2.46 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.8 (C-7), 139.9 (C-1′), 135.3 (C-8a), 134.1 (C-2′), 132.9 (C-6′), 132.8 (C-4′), 131.5 (CH-4a), 130.3 (CH-3′), 130.1 (CH-5), 126.8 (CH-5′), 118.3 (C-6), 115.8 (CH-8), 64.1 (CH-1), 48.3 (CH_2-3), 43.6 (NCH_3), 36.5 (CH_2-α), 26.5 (CH_2-4); FAB-MS m/z 357 $[M+1]^+$, 196.

4.6. Pharmacological in vitro assays

4.6.1. Animals

Female Wistar rats (200–220 g), bred in a standard experimental animal room of the Faculty of Pharmacy, were used for [^{3}H]-dopamine uptake assays and radioligand binding experiments. Rats were housed under a 12-h light/dark cycle at 22 °C and 60% humidity. All the protocols complied with European Community guidelines for the use of experimental animals and were approved by the University of Valencia's Ethics Committee.

4.6.2. [^{3}H]-dopamine uptake assay

[^{3}H]-dopamine uptake was studied using a preparation of rat striatal synaptosomes. All the experimental procedures for synaptosomes preparation were carried out at 0–4 °C. The rat striatum was dissected, homogenised in 10 volumes (w/v) of 0.32 M sucrose with an ultraturrax T25 (Janke & Kinkel) (4 s, maximal scale) and centrifuged at 1000g for 10 min. The supernatant was stored and the pellet was resuspended in 10 volumes of 0.32 M sucrose, and was centrifuged again at 1000g for 10 min. The two supernatants were combined and the mixture was centrifuged at 16,000g for 30 min. The resultant pellet was suspended in 10 volumes of ice-cold Krebs medium (pH 7.6) containing (mM): 118 mM NaCl; 4.75 mM KCl; 1.2 mM KH_2PO_4; 1.8 mM $CaCl_2$; 1.2 mM $MgCl_2$; 25 mM $NaHCO_3$; and 11 mM glucose. Aliquots were pre-incubated for 10 min at 37 °C in Krebs buffer containing 10 μM pargyline (to block the metabolism of dopamine by monoamine oxidase), [^{3}H]-dopamine (47 Ci/mmol, Amersham) was added to a final concentration of 0.5 nM, and incubation continued for another 10 min. Compounds were screened at 100 μM. Incubation was terminated by dilution into ice-cold Krebs medium, and the samples were filtered rapidly through fibreglass filters (Schleicher & Schuell Grade 30) using a Brandel cell harvester (model M-24, Biochemical Research and Development Laboratories, Inc.). Filters were washed twice with 3 mL cold Krebs medium and dried. Non-specific [^{3}H]-dopamine uptake was determined in the presence of 10 μM nomifensine (dopamine uptake inhibitor). Filters were placed into a scintillation mixture (Optiphase "Hisafe" 2, Perkin Elmer), and radioactivity was determined by scintillation spectrometry [6,9]. Protein concentrations were determined using the Bradford protein assay (BioRad).

4.6.3. Radioligand binding assays

[^{3}H]-SCH23390 and [^{3}H]-raclopride binding experiments were performed on rat striatal membranes. The rat striatum was homogenised in 10 volumes (w/v) of Tris–HCl buffer (50 mM, pH 7.4 at 22 °C) with an ultraturrax T25 (Janke & Kinkel) (4 s, maximal scale). The homogenate was centrifuged twice at 49,000*g* for 15 min at 4 °C with a resuspension cycle in the same volume of Tris–HCl buffer between each centrifugation. The final pellet was resuspended in Tris-ions buffer containing 120 mM NaCl; 2 mM $CaCl_2$, 5 mM KCl; 1 mM $MgCl_2$; and 0.1% ascorbic acid (pH 7.4). For D_1-like receptor binding assays, membranes (100 μg/mL) were incubated with [^{3}H]-SCH23390 (0.25 nM; 66 Ci/mmol, Amersham, GE Healthcare, UK) and various concentrations of competition compound (10^{-10} M to 10^{-4} M) for 1 h at 23 °C. Non-specific binding was determined in the presence of 30 μM SK&F38393. For D_2-like receptor binding assays, membranes (200 μg/mL) were incubated with [^{3}H]-raclopride (0.5 nM; 62.2 Ci/mmol, Perkin Elmer) and various concentrations of competition compound (10^{-10} M to 10^{-4} M) for 1 h at 23 °C. Non-specific binding was determined in the presence of 50 μM apomorphine (Sigma). In both cases, incubations were stopped by the addition of 3 mL ice-cold Tris-ions buffer followed by a rapid filtration through fibreglass filters (Schleicher & Schuell Grade 30) using a Brandel cell harvester (model M-24, Biochemical Research and Development Laboratories, Inc.). Filters were washed twice with 3 mL cold Tris-ions buffer. After the filters had been dried, radioactivity was counted in 4 mL scintillation liquid (Optiphase "Hisafe" 2, Perkin Elmer). All the compounds were used as hydrochloride salts. Data were analysed by Prim (Graph Pad Software; San Diego, California, U.S.A), and K_i values were determined using the K_D value for [^{3}H]-SCH23390 of 0.36 nM and for [^{3}H]-raclopride of 1.25 nM. Values are expressed as the mean ± SEM of three to six independent determinations performed in duplicate.

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