Eur J Med Chem (1994) 29, 55–60

Short communication

Synthesis of some 1-(3-hydroxy-4-methoxybenzyl)-2-alkyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinolines and their binding affinities to DA receptor subtypes

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(Received 4 March 1993; accepted 1 July 1993)

Summary — 1-(3-Hydroxy-4-methoxybenzyl)-2-alkyl-6,7-methylenedioxytetrahydroisoquinolines (**3a–c**), derived from the D_1 selective antagonist *S*-bulbocapnine by cleavage of the bond between the 2 aromatic moieties, have been synthesized and their *in vitro* affinity towards D_1 and D_2 receptors evaluated. Of the compounds tested, the 2-methyl derivative **3a** while showing poor affinity towards D_1 receptors was able to inhibit the D_2 radioligand ^{3}H-raclopride binding by 60% at 10^{-5} M. Conformational analysis allows reasonable explanations of the loss of D_1-affinity of **3a** with respect to the model.

substituted 1-(3-hydroxy-4-methoxybenzyl)-6,7-methylenedioxytetrahydroisoquinoline / D_1 and D_2 receptor binding / bulbocapnine conformational analysis

Introduction

Several biochemical and pharmacological studies have led to the identification of different subpopulations of dopamine (DA) receptors in the mammalian central nervous system (CNS) [1]. Of these receptors, the D_1 are located postsynaptically and are positively linked to adenylate cyclase as its second messenger while the D_2 receptors are located both pre- and postsynaptically and are either not coupled or negatively coupled to adenylate cyclase [2–4]. While research has mainly been concentrated on the function of D_2 receptors in the CNS, little is known about the functional significance of D_1 receptors.

Only recently, studies on the D_1 selective antagonist SCH 23390 **1** [5] and *S*-bulbocapnine **2** [6, 7] evidenced a possible role of D_1 receptors in the pathophysiology of some mental diseases [8, 9]. Thus, SCH 23390 has been suggested as an useful drug in the treatment of schizophrenia and mania. It has been found to possess antipsychotic activity [10] without inducing extrapyramidal side-effects such as Parkinsonism and possible dyskinesia, which are induced by the D_2 antagonists currently used as neuroleptics [11, 12].

These considerations together with the evidence that bulbocapnine is quite a selective blocker of DA-sensitive adenylate cyclase prompted us to synthesize and test new tetrahydrobenzyl isoquinolines (**3a–c**) for D_1 and D_2 receptor affinity. They can either be regarded as open derivatives of *S*-bulbocapnine, or as ring-contracted analogs of SCH 23390.

Chemistry

The synthesis of compounds **3a–c** is depicted in scheme 1. The key intermediate **4** [13] was prepared by condensing 3-benzyloxy-4-methoxy-phenylacetic acid [14, 15] and 3,4-methylenedioxy-β-phenethylamine [10] in refluxing xylene with removal of water. Cyclization of the resulting amide **4** in boiling toluene with $POCl_3$ gave the hydrochloride of **5** in 77% yield. The free base was reacted with the requisite alkyl iodide to give excellent yields of the quaternary salts **6a–c** which were then reduced with sodium boro-

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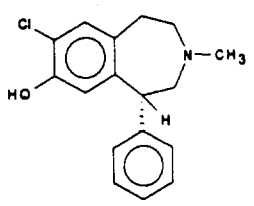

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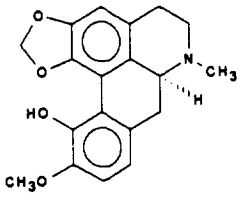

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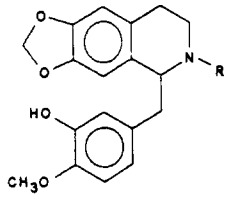

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hydride to the corresponding tetrahydroisoquinolines **7a–c**. Catalytic hydrogenation of the latter over palladium or $PdCl_2$ on carbon led to the requisite **3a–c** in satisfactory yield.

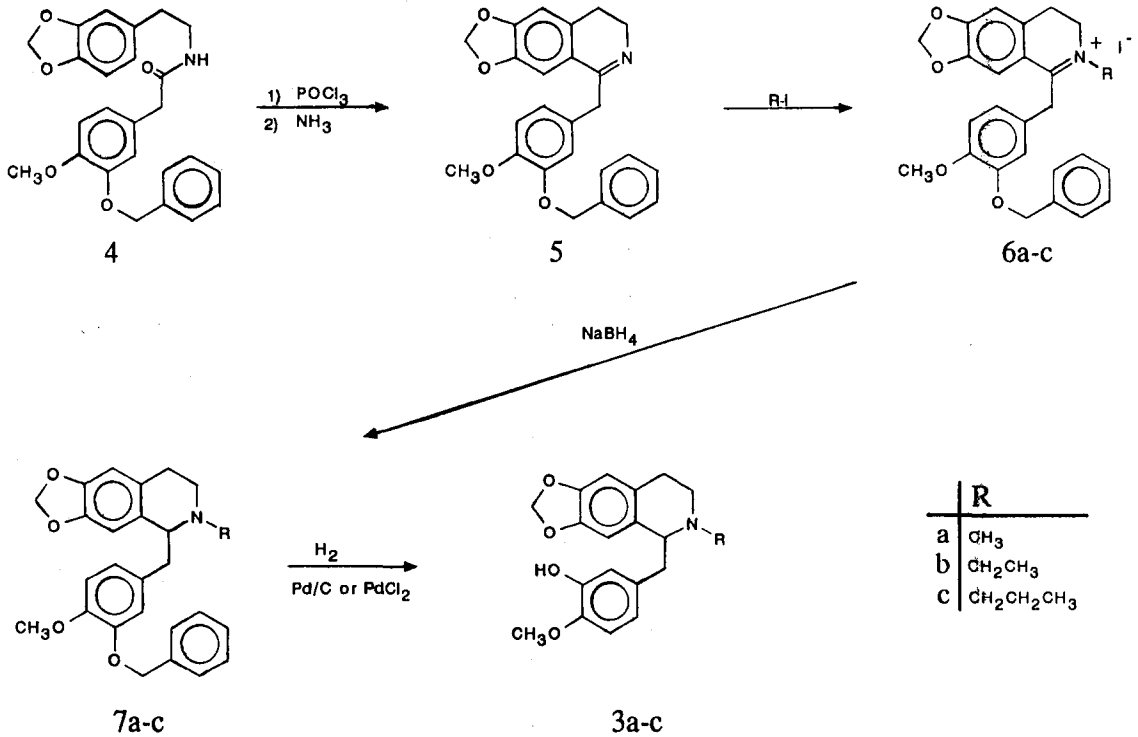
Receptor binding

The potency and selectivity of *N*-substituted tetrahydroisoquinoline derivatives **3a–c** were evaluated by measuring their *in vitro* affinity for the D_1 and D_2 receptors in a competition binding assay, using membrane preparations from rat caudate nucleus. ^{3}H-SCH 23390 [17] and ^{3}H-raclopride [18] were used as selective ligands for D_1 and D_2 dopamine receptors, respectively.

Molecular modelling

A conformational analysis of **3a** and bulbocapnine was performed using the MM2 force field developed by Allinger and included in the Macromodel [19] molecular software package. The dihedral angles involved in the side chain and in the tetrahydroisoquinolinic moiety were allowed to rotate with 30° increments. With bulbocapnine the global minimum at 36.69 kcal/mol corresponded to a neat planar structure in which the 2 aromatic rings formed an angle of 28°.

The global minimum of **3a** (**A-3a**) at 32.01 kcal/mol showed the benzyl group oriented towards the isoquinolinic nitrogen and did not permit superimposition with bulbocapnine (fig 1).



Scheme 1.

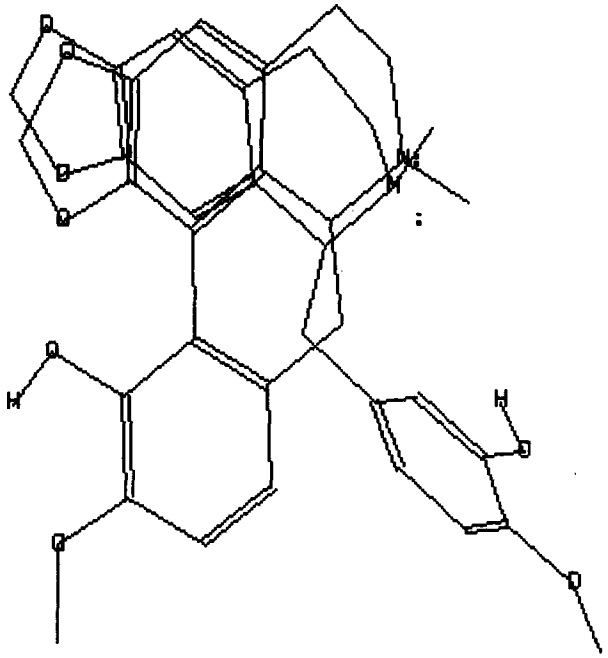


Fig 1. Superimposition of **A-3a** and bulbocapnine.

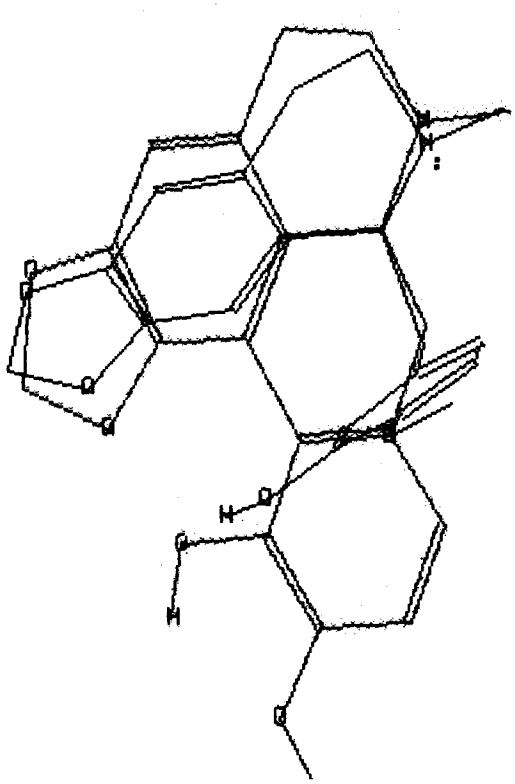


Fig 2. Superimposition of **B-3a** and bulbocapnine.

The conformer in which the benzyl group was oriented in the opposite direction (**B-3a**) was very close to the former (32.77 kcal/mol). In any case no minimum structure of **3a** could superimpose the benzyl group on the bulbocapnine moiety. In fact, even considering the conformers with the desired orientation of the side chain towards the dioxymethylenic moiety, the aromatic rings assumed different bond angles values, that were never close to the 28° angle shown in bulbocapnine (fig 2).

It is interesting to note that both in bulbocapnine and **3a**, the distance (> 4 Å) between the phenolic hydrogen and the methylenedioxy oxygen did not allow the formation of an intramolecular hydrogen bond.

Results and discussion

As illustrated in figure 3, bulbocapnine displaced ^{3}H-SCH 23390 binding in a concentration-dependent manner. This finding suggests that in the rat CNS this compound binds with higher affinity to D_1 than to D_2 receptors.

In fact, as shown in figure 4, bulbocapnine was much less potent in displacing ^{3}H-raclopride from D_2 sites than ^{3}H-SCH 23390 was from D_1 sites (fig 3). On the contrary, the tetrahydroisoquinoline analog of bulbocapnine, **3a**, was able to maximally inhibit (– 78%) ^{3}H-SCH 23390 binding at 10^{-4} M, thus evidencing a significant decrease in D_1 receptor affinity with respect to the reference compound.

Substitution of the nitrogen with alkyl groups larger than methyl, as in **3b**, **3c**, led to compounds with lower affinity for the D_1 receptor (inhibition at 10^{-4} M being 58 and 32%, respectively) (fig 3). Moreover, **3a** was the most potent compound at inhibiting ^{3}H-raclopride binding at D_2 sites. In fact, at 10^{-5} M **3a** displaced ^{3}H-raclopride binding by 60%, while the homologous **3b** and **3c** at the same concentration showed only ≈ 20% displacement; by increasing the concentration to 10^{-4} M, the binding inhibition was 93, 77 and 83%, respectively. By assuming that interaction of bulbocapnine with D_1 receptors involves fitting of the 2 aromatic rings on properly oriented flat surfaces, the poor affinity of **3a** could reside in the exclusion of its 3-hydroxy-4-methoxy phenyl group from fitting on the flat area of the receptor. On the contrary, the comparable affinity of **3a** and bulbocapnine towards D_2 receptors could be explained by a possible prominent role of the isoquinoline moiety in the interaction with these receptors.

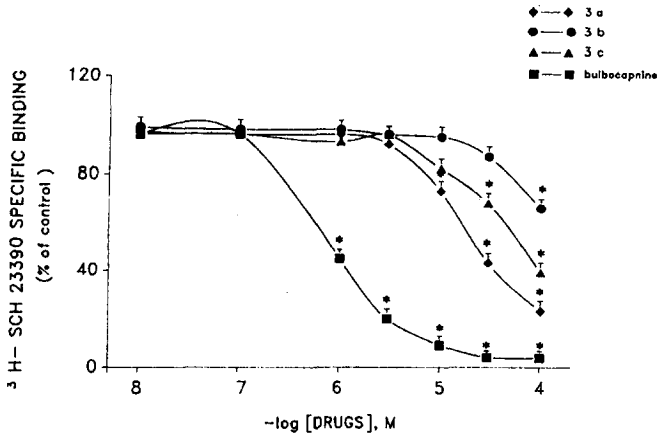


Fig 3. Effect of *N*-substituted tetrahydroisoquinoline derivatives and bulbocapnine on ^{3}H-SCH 23390 binding in membrane preparations of caudate nucleus from rat brain. ^{3}H-SCH 23390 binding was performed as described in the *Experimental protocols* using a concentration of 1 nM of the ligand. Data are mean ± SEM of 5 experiments. $*p < 0.05$ *vs* solvent (ANOVA followed by Sheffe's test).

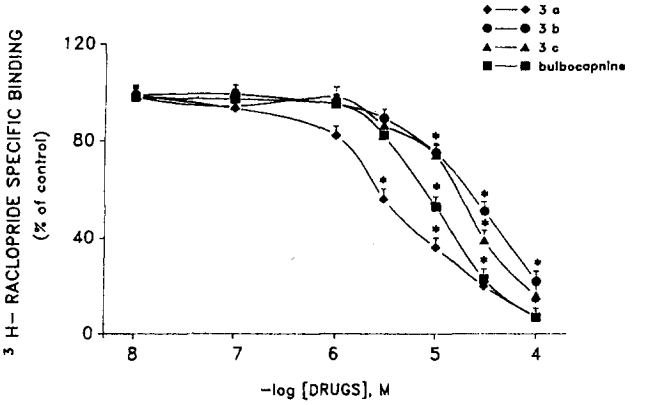


Fig 4. Effect of *N*-substituted tetrahydroisoquinoline derivatives and bulbocapnine on ^{3}H-raclopride binding in membrane preparations of caudate nucleus from rat brain. ^{3}H-Raclopride binding was performed as described in the *Experimental protocols* using a concentration of 2 nM of the ligand. Data are mean ± SEM of 5 experiments. $*p < 0.05$ *vs* solvent (ANOVA followed by Sheffe's test).

Experimental protocols

Chemical section

Melting points were determined on a Kofler apparatus or in open capillaries with a Büchi apparatus and are uncorrected. IR spectra were measured on a Perkin–Elmer model (7.81) infrared spectrometer in nujol mulls or as film. Ultraviolet spectra were determined in 95% ethanol on a Perkin–Elmer Lambda 5 model. Nuclear magnetic resonance spectra were determined on a Varian XL–200 instrument with absorptions recorded in ppm downfield from internal $(Me)_4Si$. Elemental analyses were performed by the Padua University microanalysis laboratory and are within ± 0.4% calculated values. TLC was carried out on 2-mm thick E Merck 60F-254 plates. Flash chromatography was performed with Merck 9385 silica gel. Chemical and spectroscopic data of the new differently substituted analogs synthesized have been reported in tables I and II.

N-(3,4-Methylenedioxyphenethyl)-3-benzyloxy-4-methoxyphenylacetic acid amide ***4***

A slurry of 5.95 g (0.022 mol) of 3-benzyloxy-4-methoxyphenylacetic acid [14, 15], 3.6 g (0.022 mol) and β-(3,4-methylenedioxyphenyl) ethylamine [16] in 7.5 ml xylene was refluxed under N_2 for 18 h with azeotropic removal of water. On cooling, 7.1 g (78.02%) of **4** crystallized as a yellow solid, mp: 130–131°C (lit [13] 131–132°C).

1-(3-Benzyloxy-4-methoxybenzyl)-6,7-methylenedioxy-3,4-dihydroisoquinoline ***5***

A solution of 3 g (0.0075 mol) of the amide **4** and 3 ml $POCl_3$ in 210 ml dry toluene (dissolution at 50°C) was refluxed for

Table I. Chemical data of 3,4-dihydroisoquinolinium **6** and 1,2,3,4-tetrahydroisoquinoline (**7** and **3**) derivatives.

Compd	*Yield (%)*	*Mp (°C) (solvent)*
6a	91	189–192[a] (EtOH)
6b	81.5	170–172 (EtOH)
6c	93.5	196 (EtOH)
7a	92	Oil 92–94 (HCl)[b]
7b	72.1	Oil 164–167 (HCl) (Acetone)
7c	89	Oil 188–190 (HCl) (Acetone)
3a	53.9	123–125 (EtOH)
3b	58	94–96 (IPA)[c]
3c	65	78–80 (IPA/H_2O)

[a]See [13], mp: 197°C (ethanol); [b]after trituration in ether; [c]isopropyl alcohol.

Table II. Spectroscopic data of compounds **6**, **7** and **3**.

Compd	*UV λ max (log ε)*	*IR ν (cm^{-1})*	*^{1}H-NMR (δ, ppm)*[a]
6a	205.5 (4.92); 254.0 (4.27); 281.0 (3.90); 307.0 (3.83); 368.3 (3.94)	1640, 1590	3.29 (t, 2H, C-4 CH_2); 3.67 (s, 3H, N-CH_3); 3.88 (s, 3H, O-CH_3); 4.02 (t, 2H, C-3 CH_2); 4.47 (s, 2H, CH_2-Ph); 5.16 (s, 2H, O-CH_2-Ph); 6.13 (s, 2H, O-CH_2-O); 6.50–7.40 (m, 10H, Ar-H)
6b	205.5 (4.70); 254.0 (4.20); 286.0 (3.87); 304.9 (3.89); 3.63.3 (4.03)	1630, 1590	1.60 (t, 3H, CH_2-CH_3); 3.23 (t, 2H, C-4 CH_2); 3.87 (s, 3H, O-CH_3); 3.96–4.15 (m, 4H, CH_2-*CH_3*- and C-3 CH_2); 4.51 (s, 2H, CH_2-Ph); 5.17 (s, 2H, O-CH_2-Ph); 6.13 (s, 2H, O-CH_2-O); 6.58–7.40 (m, 10H, Ar-H)
6c	205.3 (4.75); 287.0 (3.91); 301.7 (3.92); 364.4 (3.90)	1630, 1590	0.82 (t, 3H, CH_2-CH_2-*CH_3*); 1.49–1.62 (m, 2H, CH_2-*CH_2*-CH_3); 3.24 (t, 2H, C-4 CH_2); 3.88 (s, 3H, O-CH_3); 3.94–4.10 (m, 4H, *CH_2*-CH_2-CH_3 and C-3 CH_2); 4.49 (s, 2H, CH_2-Ph); 5.16 (s, 2H, O-CH_2-Ph); 6.13 (s, 2H, O-CH_2-O); 6.50–7.41 (m, 10H, Ar-H)
7a	205.3 (4.75); sh 229.0 (4.34); 282.7 (3.83)	1610, 1590	2.38 (s, 3H, N-CH_3); 2.40–3.18 (m, 6H, CH_2 x 3); 3.65 (t, 1H, C-1 CH); 3.77 (s, 3H, O-CH_3); 5.01 (s, 2H, O-CH_2-Ph); 5.88 (s, 2H, O-CH_2-O); 6.46–7.45 (m, 10H, Ar-H)
7b	204.2 (4.63); 229.0 (4.11); 282.6 (3.60)	1610, 1590	1.01 (t, 3H, CH_2-*CH_3*); 2.32–3.20 (m, 8H, CH_2 x 4); 3.69 (t, 1H, C-1 CH); 3.87 (s, 3H, O-CH_3); 5.08 (s, 2H, O-CH_2-Ph); 5.84 (s, 2H, O-CH_2-O); 6.10–7.50 (m, 10H, Ar-H)
7c	205.0 (4.67); sh 232.0 (4.08); 286.8 (3.74)	1610, 1590	0.79 (t, 3H, CH_3); 1.38 (m, 2H, CH_2CH_3); 2.40–3.20 (m, 8H, CH_2 x 4); 3.65 (t, 1H, C-1 CH); 3.87 (s, 3H, O-CH_3); 5.09 (s, 2H, O-CH_2-O); 6.15–7.45 (m, 10H, Ar-H)
3a	206.1 (4.74); 234.0 (4.10); 287.8 (3.91)	3400, 1594	2.46 (s, 3H, CH_3); 2.48–3.22 (m, 6H, CH_2 x 3); 3.68 (t, 1H, C-1 CH); 3.84 (s, 3H, O-CH_3); 5.83–5.86 (m, 2H, O-CH_2-O); 6.20–6.80 (m, 5H, Ar-H)
3b	205.8 (4.62); 232.0 (3.98); 288.1 (3.76)	3450, 1590	1.05 (t, 3H, CH_3); 2.40–3.25 (m, 8H, CH_2 x 4); 3.79 (t, 1H, C-1, CH); 3.87 (s, 3H, O-CH_3); 5.83–5.87 (m, 2H, O-CH_2-O); 6.17–6.78 (m, 5H, Ar-H)
3c	206.1 (4.64); 232.5 (4.00); 288.0 (3.80)	3380, 1600	0.79 (t, 3H, CH_3); 1.38–1.49 (m, 2H, CH_2); 2.40–3.30 (m, 10H, CH_2 x 5); 3.70 (t, 1H, C-1 CH); 3.83 (s, 3H, O-CH_3); 5.82–5.86 (m, 2H, O-CH_2-O); 6.10–6.80 (m, 5H, Ar-H); 8.25 (br s, 1H, OH, exchange with D_2O)

[a]All spectra were run in $CDCl_3$ except for compound **7a** $(CD_3)_2CO$.

12 min and then rapidly cooled to room temperature. The solvent and excess $POCl_3$ were evaporated *in vacuo*, the residue washed with petroleum ether 3 times and then dissolved in 25 ml ethanol. Upon cooling to 0–5°C, 3 ml concentrated HCl was added followed by 75 ml ether. On standing in a refrigerator for 48–72 h, 2.52 g (77.3%) **5**·HCl crystallized as a yellow solid, mp: 117–120°C. R_f ($CHCl_3$/MeOH, 9:1) 0.71. IR ν cm^{-1}: 1660. UV λ max (log ε): 211.1 (4.35); 245.7 (4.19); 303 (3.71); 363.7 (3.95). ^{1}H-NMR ($CDCl_3$/DMSO-d_6) δ: 2.94 (m, 2H, C-4 CH_2); 3.54 (m, 2H, C-3 CH_2); 3.79 (O-CH_3); (s, 2H, CH_2-Ph); 3.80 (s, 3H, O-CH_3); 4.47 (s, 2H, H_2O); 5.13 (s, 2H, O-CH_2-Ph); 6.17 (s, 2H, O-CH_2-O); 6.85–7.62 (m, 10H, Ar-H); 14.02 (br, s, 1H, N-HCl).

The free base **5** was liberated from its hydrochloride in ethanol with 10% ammonium hydroxide followed by dilution with water and cooling, mp: 87–90°C. R_f ($CHCl_3$/MeOH, 9:1) 0.77. IR ν cm^{-1}: 1600, 1590. UV λ max (log ε): 202 (4.17); 273 (3.62); 320 (3.38). ^{1}H-NMR ($CDCl_3$) δ: 2.60 (t, 2H, C-3 CH_2); 3.65 (t, 2H, C-4 CH_2); 3.84 (s, 3H, O-CH_3); 3.89 (s, 2H, CH_2-Ph); 5.10 (s, 2H, O-CH_2-Ph); 5.96 (s, 2H, O-CH_2-O); 6.61–7.50 (m, 10H, Ar-H).

1-(3-Benzyloxy-4-methoxybenzyl)-2-alkyl-6,7-methylenedioxy-3,4-dihydroisoquinolinium iodide ***6a–c***

General procedure. A slurry of 0.00486 mol dihydroisoquinoline **5** in 25 ml of the appropriate alkyl iodide was refluxed for 3 h. After dissolution, a yellow solid was precipitated, which was collected after cooling.

*1-(3-Benzyloxy-4-methoxybenzyl)-2-alkyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline **7a–c***

General procedure. To a stirred solution of 0.0026 mol alkyl iodide in 18 ml $CHCl_3$/MeOH (2:1), 0.4 g (0.0112 mol) $NaBH_4$ was added in small portions over a 1-h period. Stirring was continued for 0.5 h, the solvent evaporated and the residue diluted with water and extracted with $ChCl_3$. The extract was washed with water, dried (Na_2SO_4) and evaporated to give **7a–c** as oils which were purified by flash chromatography.

*1-(3-Hydroxy-4-methoxybenzyl)-2-alkyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline **3a–c***

General procedure. A mixture of 0.00341 mol of **7** in an appropriate solvent (40 ml methanol for **7a**, 30 ml methanol with a few drops of acetic acid for **7b**, and 100 ml ethanol/ethyl acetate (2:1) for **7c**) was shaken with H_2 (50 psi) at room temperature for a given period (3.5 h for **7a** and 45 h for **7b** and **7c**) in the presence of an appropriate catalyst (2.5 ml 2% aqueous $PdCl_2$, 0.5 g active carbon for **7a** and 0.23 g Pd/C (10% by wt) for **7b** and **7c**). The mixture was filtered and the solution evaporated to give a brown oily residue which was purified from the unreacted product by flash chromatography eluting with a solution of $CHCl_3$/MeOH, 95:5 to give **3a–c** as yellow oils which solidified on standing.

Biochemical section

Receptor binding studies

Tissue preparation. Male Sprague–Dawley rats (Charles River, Como, Italy) weighing 150–200 g were used to obtain brain tissue. Rats were killed by decapitation and striata were rapidly dissected and stored frozen at – 80°C until the binding assay.

Striata were homogenized in 100 vol (w/v) of ice-cold 50 mM Tris–HCl buffer, pH 7.40 (buffer A) and centrifuged at 48 000 *g* for 10 min. The resultant pellet was washed twice by resuspension and recentrifugation in 100 vol of the same buffer. The final pellet was resuspended in buffer A containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ (buffer B).

^{3}H-SCH 23390 binding assay. A modification according to Porceddu *et al* [20] of the method of Billard *et al* [17] was used in these experiments. Radioligand binding was performed in 12 x 75-mm culture tubes at a total assay vol of 0.5 ml. Each tube contained 50 µl competitor dissolved in 1% tartaric acid at various concentrations (from 10^{-8} to 10^{-4} M), 50 µl ^{3}H-SCH 23390 (1 nM), 400 µl tissue homogenate (≈ 0.150 mg tissue per assay) prepared as described above. Non-specific binding was determined in the presence of 10^{-6} M ^{3}H-SCH 23390. Tubes were incubated at 37°C for 60 min and rapidly filtered *in vacuo* through Whatman GF/B filters with 3 rinses of ice-cold 50 mm buffer A. The filters were placed in plastic mini-vials containing 4 ml scintillation fluid (Atomlight, New England Nuclear) and left overnight. Radioactivity was measured in a scintillation spectrophotometer with an efficiency of 40%.

^{3}H-Raclopride binding assay. A modification of the method of Kohler *et al* [18] was used in these experiments. Briefly, incubation tubes contained striatal membrane suspension (equivalent to 150 µg protein and ^{3}H-raclopride (spec act 86.9 Ci/mmol; New England Nuclear) at a concentration of 2.0 nM in a final vol of 0.5 ml buffer B containing 0.01% (w/v) ascorbic acid. Non-specific binding was determined in the presence of 1 µM (+)-butaclamol. Incubations were carried out for 60 min at 23°C. After incubation, samples were filtered and counted as described above. Protein was measured according to Lowry *et al* [21] using bovine serum albumin as a standard.

Data were analyzed using ANOVA followed by Scheffe's test.

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