Eur J Med Chem (1996) 31, 231–239

Serotoninergic properties of new conformationally restricted benzamides

D Yang, B Brémont, S Shen, S Kefi, M Langlois*

BIOCIS-CNRS (URA 1843), Faculté de pharmacie, 5, rue JB-Clément, 92296, Châtenay-Malabry Cedex, France

(Received 18 October 1995; accepted 23 November 1995)

Summary — A new series of benzamides derived from metoclopramide have been synthesized, in which the vicinal carbon of the basic nitrogen atom of the ethyl chain is situated on the C_3, C_4, C_5 and C_6 rings. The diamino derivatives were prepared through Strecker's reaction from the corresponding ketones except for the cyclopropyl derivatives where 1-ethoxy-1-trimethylsiloxy cyclopropane was used as the starting material. The benzamides were prepared using the mixed anhydride method. They were tested in binding assays for D_2, 5-HT_3 and 5-HT_4 receptors. The results show a marked increase in the selectivity and potency of these derivatives for 5-HT_3 receptors with regard to metoclopramide (compound **1d**: 5-HT_3 K_i = 9.03 nM; 5-HT_4 K_i > 5000; D_2 K_i > 5000). The influences of steric hindrance and hydrophobic properties on the affinity of benzamide derivatives for 5-HT_3 receptors were also emphasized by these data. The X-ray crystal structure of compound **1d** was compared with that of the minimal energy conformer of BRL 24682, a reference 5-HT_3 receptor antagonist benzamide, determined using the Random Search program. Superimposition of the two structures showed a suitable fit between the pharmacophore groups previously determined to be important for 5-HT_3 receptor antagonists. On the other hand, the hydrophobic parts of the basic moieties had different spatial occupancies.

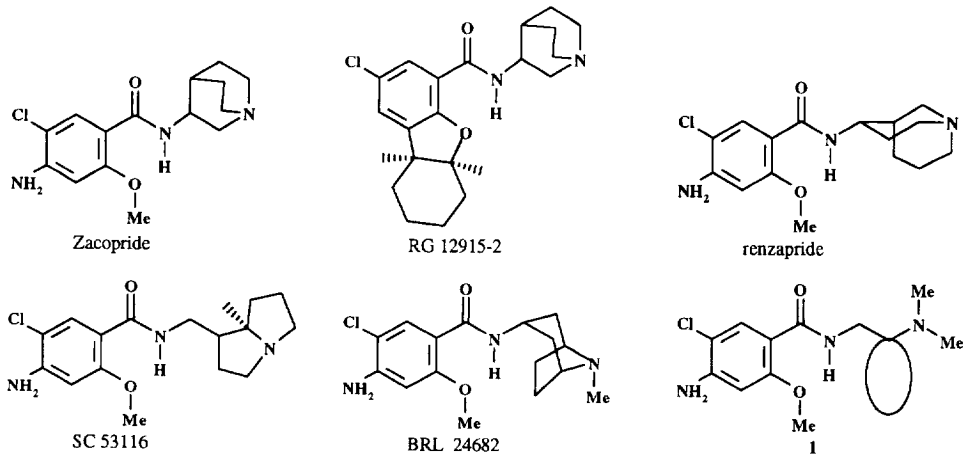
5-HT_3 receptor antagonist / benzamide / Strecker reaction / 5-HT_4 receptor / D_2 receptor

Introduction

In the past decade there have been significant advances in the understanding of the physiology and the role of 5-HT_3 receptors [1, 2]. They are ligand-gated-cation channels [3] similar to NMDA and nicotinic receptors for which the neurotransmitter binding sites are thought to be located on the extracellular part of the α-subunit. 5-HT_3 receptors differ from other serotonin receptor subtypes which are G-protein-coupled receptors [2]. 5-HT_3 receptors are present within the central and peripheral nervous systems and there is considerable interest in the development of specific antagonists because of the numerous pharmacological properties of such compounds which make them very attractive for the treatment of a number of gastrointestinal [1] and brain disorders [4–6]. However, so far 5-HT_3 receptor antagonists have only been used clinically to prevent the emesis induced by oncolytic drugs such as cisplatin; several compounds are on the market [7] or in clinical trials for this purpose. These compounds are derived from various chemical families and several structural analyses have been published. These agree upon the existence of three structural elements in the binding of the antagonists to the receptor site: an aromatic moiety, a carbonyl function or a bioisosteric group, and a basic nitrogen atom [8–11].

A number of 5-HT_3 receptor antagonists, such as zacopride and RG 12915-2, are members of the benzamide family and possess a quinuclidine ring, a basic framework which fits particularly well into the 5-HT_3 receptor binding site [12]. However, benzamide molecules can possess potent affinity for other receptors such as D_2, D_3, and 5-HT_4 receptors and it has been shown that the potency and the selectivity of benzamides is dependent upon the structure of the basic moiety. Thus, extended *N*-benzyl nortropane derivatives, such as tropapride and BRL 25594, provide potent D_2 receptor antagonists [13], whereas the basic rigid folded framework of renzapride, zacopride and SC 53116 leads to compounds with good affinity for 5-HT_3 and/or 5-HT_4 receptors [14–16]. It seemed to us that the selectivity and potency for one receptor or another was related to a particular spatial position of the basic nitrogen with regard to the benzamide moiety. Thus, the weak affinity and lack of selectivity of metoclopramide, the parent compound of the benzamide family, for dopaminergic and serotoninergic receptors can be explained by the large number of permissible conformers due to the flexibility of the amino chain.

*Correspondence and reprints.



Recently [14], we reported a relatively constrained benzamide which had a higher affinity for 5-HT_4 receptors than for 5-HT_3 receptors, demonstrating the value of designing conformationally restricted amino moieties to gain selectivity. We present here new structural data on constrained structures designed to increase potency and selectivity for the various receptors discussed above. Metoclopramide derivatives **1** (table I), in which the vicinal carbon atom of the basic nitrogen atom of the ethyl chain was introduced into a ring, were synthesized and evaluated in 5-HT_3, 5-HT_4 and D_2 receptor binding assays. The tetrasubstitution of the carbon atom brought about a marked steric hindrance and decreased the number of minimal energy conformers. According to previous results obtained with the benzamide series, such a structural modification should give compounds with no dopaminergic properties but with high potency and selectivity for the 5-HT_3 and 5-HT_4 receptors.

Chemistry

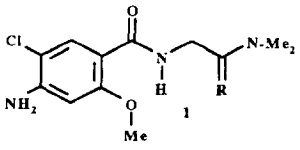
The benzamides **1a–h** (table I) were essentially prepared by condensation of the amine derivatives **4** and 4-amino-5-chloro-2-methybenzoic acid using the mixed anhydride method in THF [17]. The amino derivatives **4a,c–h** were synthesized according to the method described in scheme 1. The ketones **2a,c–e,g,h** were commercially available and tetrahydrofuran-3-one **2f** was synthesized by the cyclization of 1,2,4-trihydroxybutane [18] with *para*-toluenesulfonic acid in tetrahydrofuran-3-ol followed by oxidation with Jones's reagent. The amino nitriles **3a,c–h** were prepared by Strecker's reaction [19] of the ketones with potassium cyanide and dimethylamine hydrochloride in water at room temperature to give the corresponding nitriles in good yield.

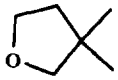
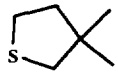
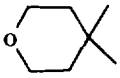
With the exception of **3g**, reduction by $LiAlH_4$ or BH_3-THF failed and led to hydrogenolysis of the C–CN bond [20] to the corresponding dimethylamino derivatives. Only the use of a $LiAlH_4/H_2SO_4$/THF mixture [21] gave the diamino derivatives **4** in good yield. A modification of the classical Strecker reaction was used for the synthesis of the cyclopropane derivative **4b** (scheme 2). 1-Ethoxy-1-trimethylsiloxy cyclopropane was synthesized according to a previously reported method [22] and reacted with KCN and 1-methylbenzylamine to give the cyano derivative **5**, which was then hydrolyzed with sulfuric acid in methylene chloride to give the corresponding amide. It was debenzylated in acetic acid with hydrogen under atmospheric pressure in the presence of 20% Pd/C to the amino amide **6** [32]. This compound was dimethylated according to the Borch reduction procedure to give compound **7** and reduced by $LiAlH_4$ to give the diamine **4b**. Condensation of the amines **4a–h** with 4-amino-5-chloro-2-methoxybenzoic acid gave the corresponding amides **1a–h** reported in table I.

Biological results and discussion

The affinity of compounds **1** for 5-HT_3 receptors in the central nervous system was determined by inhibition of the specific binding of [^{3}H]BRL 43694 [23] (granisetron) to rat posterior cortex membranes using seven to eleven different concentrations according to conditions previously reported for [^{3}H]zacopride binding [24]. The affinities for 5-HT_4 and D_2 receptors were evaluated in binding assays by competition for the binding of the radioligands [^{3}H]GR-113808, a recently described potent 5-HT_4 receptor antagonist [25], and [^{3}H]spiperone, a D_2 receptor antagonist [26] to binding sites in rat and bovine striatum respectively. The results are expressed as K_i values and are reported in table II.

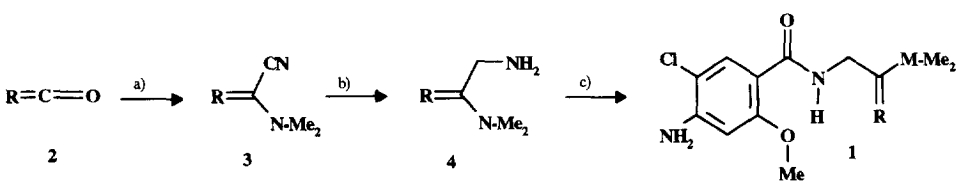
Table I. Physical properties of the benzamides **1**.



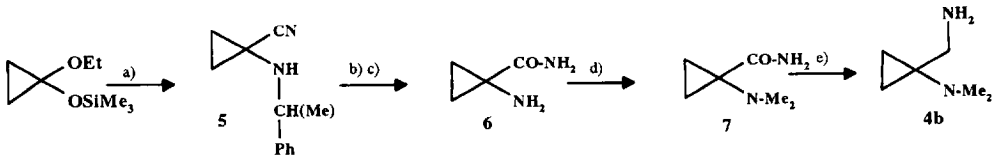
Compound	*R=C*	*Mp (°C)*	*Yield (%)*[a]	*Crystallization solvent*[b]	*Anal found*[c]
1a		149	33	AcOEt/PE	$C_{14}H_{22}N_3O_2Cl$
1b		137	29	AcOEt/EtOH	$C_{14}H_{20}N_3O_2Cl{\cdot}HCl$[d]
1c		152	66	AcOEt/PE	$C_{15}H_{22}N_3O_2Cl$
1d		120	88	AcOEt/EtOH	$C_{16}H_{24}N_3O_2Cl$
1e		223	65	AcOEt/PE	$C_{17}H_{26}N_3O_2Cl$
1f		161	58	AcOEt/PE	$C_{15}H_{22}N_3O_3Cl$
1g		104	62	AcOEt	$C_{15}H_{22}N_3O_2ClS$
1h		225	43	AcOEt/MeOH	$C_{16}H_{24}N_3O_3Cl{\cdot}HCl$[d]

[a]Yield of the condensation reaction between the amine and 4-amino-2-methoxy benzoic acid; [b]PE: petroleum ether; [c]results within ±0.4% of the theoretical values; [d]analyzed as the hydrochloride salt.

The newly synthesized compounds were compared to metoclopramide and to the corresponding dimethyl derivative **1i** (R = H_2). BRL 24682, a potent 5-HT_3 receptor antagonist with marked 5-HT_4 receptor agonist properties [27], was also selected as a reference compound for pharmacological and structural comparison. The data reported for metoclopramide showed its moderate activity and weak selectivity for the three receptors considered. On the other hand, the potency and relative selectivity of BRL 24682 for 5-HT_3 receptors was clearly emphasized. The weak activity of the dimethyl derivative **1i** with regard to metoclopramide is also noteworthy, whereas the introduction of steric constraints (compounds **1a–h**) on the



Scheme 1. Synthetic route to the benzamides **1a,c–h**. R=C: **a**, $C(Me)_2$; **c**, $C(CH_2)_3$; **d**, $C(CH_2)_4$; **e**, $C(CH_2)_5$; **f**, $C(CH_2OCH_2CH_2)$; **g**, $C(CH_2SCH_2CH_2)$; **h**, $C((CH_2)_2O(CH_2)_2)$. a) KCN/HN (Me)z·HCl; b) $LiAlH_4/H_2SO_4$/THF; c) ClCOOEt, THF, EtzN, rt.



Scheme 2. Synthetic route to the diamino derivative **4b**. a) KCN, $NH_2CH(Me)Ph$; b) CH_2Cl_2, H_2SO_4, 0 °C; c) H_2, Pd/C 20%, AcOH; d) CH_2O, $NaCNBH_3$, MeCN; e) $LiAlH_4$, THF.

Table II. Binding profiles of the benzamides **1** for 5-HT_3, 5-HT_4 and D_2 receptors.

Compound	*K_i (nM)*		
	(5-HT_3)[a]	*(5-HT_4)*[b]	*(D_2)*[c]
1a	41 ± 2.6	> 1000	1830 ± 270
1b	263 ± 42	NT[d]	> 5000
1c	12.1 ± 1.8	> 5000	1400 ± 280
1d	9.03 ± 0.3	> 5000	1950 ± 260
1e	61.3 ± 3.3	> 1000	Inactive
1f	71.9 ± 4.4	NT[d]	2050 ± 150
1g	173 ± 35	> 1000	920 ± 20
1h	431 ± 39	NT[d]	2200 ± 180
1i	978 ± 36	> 1000	947 ± 35
Metoclopramide	443 ± 58	973 ± 63	285 ± 25
BRL 24682	0.28 ± 0.0.4	48 ± 5 6	> 1000

[a][^{3}H]BRL-43694 was used as the radioligand and the binding assays were carried out using rat posterior cortex membranes (30 min, –25 °C); non-specific binding was determined with GR 38032F (10 μM); [b][^{3}H]GR-113808 was used as the radioligand and the binding assays were carried out using rat striatal membranes (30 min; 25 °C); non-specific binding was determined with the 5-HT_4 receptor agonist ML 10302 [28]; [c][^{3}H]spiperone was used as the radioligand and the binding assays were carried out using bovine striatal membranes (25 °C, 30 min); non-specific binding was determined with butaclamol (10 μM). Each assay was done in triplicate and inhibition curves were analyzed by a computer-assisted curve-fitting program (ALLFIT). K_i values were determined from the Cheng–Prussof equation. [d]NT= not tested.

α carbon of the ethyl chain caused a clear increase in the potency and selectivity for 5-HT_3 receptors. The affinity of these compounds depended upon the ring size and was particularly marked with the C_5 and C_4 rings (compounds **1c,d**) which were the most potent derivatives (K_i = 12.1 and 9.03 nM respectively). The presence of an oxygen or sulfur heteroatom in the ring had an unfavorable effect and gave compounds **1f–h** with moderate levels of activity.

Comparison of the different values reported in table II with those for compound **1i** shows that only the affinity for the 5-HT_3 receptor was increased while the lack of affinity for 5-HT_4 and D_2 receptors was maintained. These results clearly demonstrate the role of the restricted conformation of the basic amino framework in the benzamide series in increasing the affinity and selectivity for the 5-HT_3 receptors. In the past, the structure of several 5-HT_3 receptor antagonists, such as renzapride, zacopride and BRL 24682, have highlighted this aspect [12]. However, it has often been reported that these derivatives are not selective and possess fairly good affinity for 5-HT_4 receptors, related to their gastro-kinetic properties [28].

The lack of affinity of compounds **1c,d** for 5-HT_4 receptors reported here should give new structural information on the recognition parameters for both receptors. For this purpose, a structural analysis of compound **1d** was performed by X-ray crystallography with the crystal as a hydrochloride salt and the

search for the minimum energy conformer was carried out using the Random Search program of Sybyl 6.03 (Sybyl, Saint Louis, MO). The crystal conformation is shown in figure 1 and we observed, as for all the orthopramides reported to date, the planar arrangement of the amide function and the ortho-methoxy group in a virtual six-membered ring [13] due to an intramolecular hydrogen bond between the NH amidic atom and the oxygen atom of the methoxy group. The crystal structure was similar to that of the absolute minimum energy conformer calculated by the Random Search program. The distance between the basic nitrogen atom and the oxygen of the carbonyl function was 4.77 Å, shorter than that calculated (5.41 Å) for the minimum energy conformer of BRL 24682. However, a suitable fit (RMS = 0.33) between both compounds was obtained by superimposition (fig 2) of the aromatic carbons, the carbonyl function and the basic nitrogen atom, the essential groups for binding of 5-HT_3 receptor antagonists to the receptor site, and explains the relatively good affinity of compound **1d** for 5-HT_3 receptors. On the other hand, the hydrophobic parts of the ethylene bridge of the tropane and cyclopentane rings did not have a good fit, suggesting differences in binding to secondary sites within the receptor. This structural difference could explain the lack of affinity of **1d** for the 5-HT_4 receptor and its marked selectivity for the 5-HT_3 receptor.

In conclusion, the compounds and their relative affinities for 5-HT_3, 5-HT_4 and D_2 receptors reported here have shown, as has been demonstrated previously, the importance of steric constraints of the basic amino framework of benzamide derived from 4-amino-5-chloro-2-methoxybenzoic acid for recognition by the 5-HT_3 receptor. The affinities depended upon the nature and size of the ring introduced into the basic chain, confirming our previous results [17] on the influence of hydrophobic properties and steric hindrance of the basic moiety on binding to the 5-HT_3 receptor. In addition, these findings suggest that 1-aminomethyl-1-dimethylaminocyclopentane could

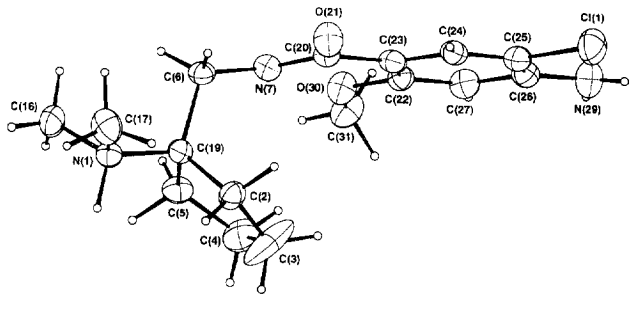


Fig 1. An Ortep drawing of compound **1d**.

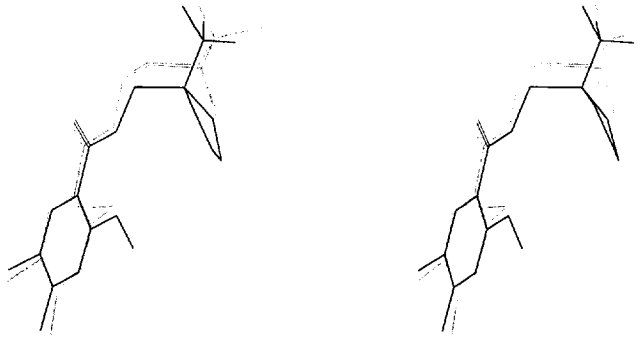


Fig 2. Stereoview of the superimposition of the X-ray crystal structure of **1d** and the minimal energy conformer of BRL 24682 with regard to the aromatic carbon atoms, the carbonyl function and the basic nitrogen atom.

be used as a new building block for the design of novel 5-HT_3 receptor ligands. Finally, these newly synthesized benzamides possessed marked selectivity, lacking affinity for the 5-HT_4 receptor. Experiments are now in progress to examine the potential use of compounds such as **1d** as selective 5-HT_3 receptor antagonists.

Experimental protocols

Chemistry

Melting points were determined on a Mettler FP 61 melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Brucker AC 200 at 200 and 500 MHz respectively. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as an internal standard, and signals are quoted as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), br s (broad singlet), or m (multiplet). Elemental analyses were performed at the CNRS's analytical services in Châtenay-Malabry and were within ±0.4% of the theoretical values unless otherwise noted.

Materials

THF was distilled from sodium/benzophenone. Column chromatography was performed on Merck silica gel 60 (70/230 mesh). Thin-layer chromatography was done on silica gel 60F-254 (0.26 mm thickness) plates. 4-Amino-5-chloro-2-methoxybenzoic acid, acetone, cyclopentanone, 3-tetrahydrothiophenone, tetrahydro-4*H*-pyran-4-one and ethyl chloroformate were purchased from Aldrich (Strasbourg, France).

*General procedure for preparation of nitriles **3a,c–h**. 1-(Dimethylamino)cyclopentanecarbonitrile **3d***

A solution of KCN (6.5 g, 0.1 mol) in 50 mL H_2O was added over 10 min to a stirred, cooled suspension of $HNMe_2$·HCl (8.15 g, 0.1 mol) and cyclopentanone (8.4 g, 0.1 mol). The mixture was stirred overnight at room temperature and extracted with Et_2O. The organic layer was washed with H_2O,

dried over $MgSO_4$, and then evaporated under reduced pressure to give **3d** (12.0 g, 87%) as an oil used without further purification. ^1H NMR ($CDCl_3$) δ: 2.27 (s, 6H, CH_3 x 2), 2.1–2.1 (m, 2H), 1.8–1.6 (m, 6H); ^{13}C NMR ($CDCl_3$) δ: 119.1 (CN), 68.9 (C_1), 41.5 (CH_3 x 2), 37.9 (C_2 and C_5), 23.0 (C_3 and C_4).

2-Dimethylamino-2-methylpropionitrile ***3a***. This compound was synthesized from acetone (3.48 g, 0.06 mol) according to the method described for product **3d**. Yield: 5.6 g (91%) as an oil. ^1H NMR ($CDCl_3$) δ: 1.95 (s, 6H, NMe_2), 1.09 (s, 6H, CMe_2); ^{13}C NMR ($CDCl_3$) δ: 118.8 (CN), 56.5 (C_2), 40.1 (NMe_2); 26.1 (Me).

1-(Dimethylamino)cyclobutanecarbonitrile ***3c***. This compound was synthesized from cyclobutanone (2.1 g, 0.03 mol) according to the method described for product **3d**. Yield: 3.6 g (85%) as an oil. ^1H NMR ($CDCl_3$) δ: 2.0–1.5 (m, 6H, cyclobutyl), 1.8 (s, 6H, CH_3 x 2); ^{13}C NMR ($CDCl_3$) δ: 118.8 (CN), 60.8 (C_1), 38.3 (CH_3 x 2), 32.1 (C_2 and C_4), 13.0 (C_3).

1-(Dimethylamino)cyclohexanecarbonitrile ***3e***. This compound was synthesized from cyclohexanone (19.6 g, 0.2 mol) according to the method described for product **3d**. Yield: 26 g (85%) after recrystallization from petroleum ether; mp: 35 °C. ^1H NMR ($CDCl_3$) δ: 2.22 (s, 6H, CH_3 x 2), 2.1 (m, 2H), 1.7–1.0 (m, 8H); ^{13}C NMR ($CDCl_3$) δ: 118.6 (CN), 62.2 (C_1), 39.6 (CH_3 x 2), 34.3 (C_2 and C_6), 24.6 (C_4), 22.1 (C_3 and C_5).

3-Cyano-3-dimethylamino-tetrahydrofurane ***3f***. This compound was synthesized from 3-tetrahydrofuranone (4.4 g, 0.051 mol) according to the method described for product **3d**. Yield: 6.3 g (88%). ^1H NMR ($CDCl_3$) δ: 4.11 (d, J = 8.7 Hz, 1H, H_{2a}), 4.0–3.9 (m, 2H, H_5), 3.59 (d, J = 8.7 Hz, 1H, H_{2b}), 2.3–2.2 (m, 1H, H_{4a}), 2.3 (s, 6H, NMe_2), 2.2–2.0 (m, 1H, H_{4b}); ^{13}C NMR ($CDCl_3$) δ: 117.8 (CN), 76.3, 68.1 and 67.4 (C_2, C_3 and C_5), 41.8 (CH_3 x 2), 38.0 (C_4).

3-Cyano-3-dimethylamino-tetrahydrothiophene ***3g***. This compound was synthesized from 3-tetrahydrothiophenone (4.6 g, 0.045 mol) according to the method described for product **3d**. Yield: 6.7 g (95%). ^1H NMR ($CDCl_3$) δ: 2.9–2.6 (m, 4H, H_2-H_5), 2.2 (m, 1H, H_{4a}), 2.0 (s, 6H, NMe_2), 1.8 (m, 1H, H_{4b}), ^{13}C NMR ($CDCl_3$) δ: 116.9 (CN), 71.7 (C_3), 42.2 (CH_3 x 2), 40.4 (C_2), 40.0 (C_5), 27.8 (C_4).

4-Cyano-4-dimethylamino-tetrahydro-4H-pyrane ***3h***. This compound was synthesized from tetrahydro-4H-pyran-4-one (1.0 g, 0.01 mol) according to the method described for product **3d**. Yield: 1.46 g (95%). ^1H NMR ($CDCl_3$) δ: 3.80 (m, 2H, H_{2eq} and H_{6eq}), 3.42 (m, 2H, H_{2ax} and H_{6ax}), 2.14 (s, 6H, CH_3 x 2), 1.84 (m, 2H, H_{3eq} and H_{5eq}), 1.51 (m, 2H, H_{3ax} and H_{5ax}); ^{13}C NMR ($CDCl_3$) δ: 117 4 (CN), 63.9 (C_2 and C_6), 60 5 (C_4), 38.9 (CH_3 x 2), 34.5 (C_3 and C_5).

General procedure for preparation of diamines ***4a,c–h***. *1-Aminomethyl-1-dimethylamino-cyclopentane* ***4d***

To a stirred suspension of $LiAlH_4$ (3.04 g, 0.08 mol) in 80 mL dry THF, cooled to 0 °C under N_2, was added dropwise a solution of H_2SO_4 (2.12 mL, 0.04 mol) in dry THF (8 mL). The mixture was stirred for 1 h and then allowed to rest overnight at room temperature. To this suspension, a solution of **3d** (3.45 g, 0.025 mol) in 20 mL THF was added dropwise at 0 °C. The mixture was warmed at 40–50 °C for 1 h, and then cooled and hydrolyzed with H_2O. The mixture was filtered and the filtrate was concentrated under reduced pressure. The oily residue was purified by bulb-to-bulb distillation to give **4d** (2.0 g, 56%); bp 70 °C (60 mmHg); ^1H NMR ($CDCl_3$) δ: 2.57 (s, 2H, NCH_2), 2.14 (s, 6H, CH_3 x 2), 2.09 (s, 2H, NH_2), 1.8–1.21 (m, 8H, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ: 69.3 (C_1), 47.7 (CNH_2), 38.7 (CH_3 x 2), 30.1 (C_2 and C_5), 25.2 (C_3 and C_4).

2-Dimethylamino-2-methylpropylamine ***4a***. This product was synthesized from **3a** (4.08 g, 0.04 mol) according to the method described for product **4d**. The crude product was used without further purification: yield: 2.8 g (68%). ^1H NMR ($CDCl_3$) δ: 2.50 (s, 2H, H_1), 2.12 (s, 6H, NMe_2), 2.05 (s, 2H, NH_2), 0.90 (s, 6H, CMe_2); ^{13}C NMR ($CDCl_3$) δ: 56.0 (C_2), 50.0 (C_1), 37.5 (NMe_2), 17.6 (C_3).

1-Aminomethyl-1-dimethylaminocyclobutane ***4c***. This product was synthesized from **3c** (3.35 g, 0.027 mol) according to the method described for product **4d**. Yield: 1.1 g (30%). ^1H NMR ($CDCl_3$) δ: 2.73 (s, 2H, NCH_2), 2.16 (s, 6H, CH_3 x 2), 2.1 (m, 2H), 1.6 (m, 4H), 1.4 (m, 2H, NH_2).

1-Aminomethyl-1-dimethylaminocyclohexane ***4e***. This product was synthesized from **3e** (4.6 g, 0.03 mol) according to the method described for product **4d**. Yield: 3.1 g (66%); bp 50 °C (0.1 mmHg); ^1H NMR ($CDCl_3$) δ: 2.23 (s, 2H, NCH_2), 1.77 (s, 6H, CH_3 x 2), 1.4–0.7 (m, 10H, cyclohexyl); ^{13}C NMR ($CDCl_3$) δ: 57.0 (C_1), 42.0 (CNH_2), 36.8 (CH_3 x 2), 28.0 (C_2 and C_6), 25.6 (C_4), 21.4 (C_3 and C_5).

3-Aminomethyl-3-dimethylamino-tetrahydrofurane ***4f***. This product was synthesized from **3f** (6.3 g, 0.045 mol) according to the method described for product **4d**. The crude product was used without further purification: yield: 3.2 g (49%). ^1H NMR ($CDCl_3$) δ: 3.5–3.3 (m, 2H, H_5), 3.4 (d, J = 9.5 Hz, 1H, H_{2a}), 3.1 (d, J = 9.5 Hz, 1H, H_{2b}), 2.5 (d, J = 13.0 Hz, NCH_a), 2.3 (d, J = 13.0 Hz, NCH_b), 1.8 (s, 6H, NMe_2), 1.6–1.5 (m, 1H, H_{4a}), 1.1–1.0 (m, 1H, H_{4b}), 0.9 (s large, 2H, NH_2); ^{13}C NMR ($CDCl_3$) δ: 73.6, 69.8 and 67.8 (C_2, C_3 and C_5), 46.4 (CNH_2), 39.2 (NMe_2), 29.3 (C_4).

3-Aminomethyl-3-dimethylamino-tetrahydrothiophene ***4g***. To a stirred suspension of $LiAlH_4$ (1.9 g, 0.05 mol) in 50 mL dry THF, cooled to 35 °C, was added dropwise a solution of **3g** (3.1 g, 0.02 mol) in 20 mL THF under N_2. The mixture was diluted with 50 mL Et_2O, and then warmed to room temperature and stirred for 1 h. The resulting mixture was hydrolyzed with a 15% aqueous NaOH solution and filtered. The filtrate was dried over anhydrous $MgSO_4$ and then evaporated in vacuo to give crude **4g** (2.6 g, 82%) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 2.83 (d, J = 11.0 Hz, 1H, H_{2a}), 2.6–2.8 (m, 4H, H_5 and NCH_2), 2.40 (d, J = 11.0 Hz, 1H, H_{2b}), 2.25 (s, 6H, NMe_2), 2.1–1.8 (m, 2H, H_4), 1.35 (s, 2H, NH_2); ^{13}C NMR ($CDCl_3$) δ: 72.3 (C_3), 43.8 (CNH_2), 39.2 (CH_3 x 2), 33.4 (C_2), 32.7 (C_5), 28.8 (C_4).

4-Aminomethyl-4-dimethylamino-tetrahydro-4H-pyrane ***4h***. This product was synthesized from **3h** (1.23 g, 0.008 mol) according to the method described for product **4d**. Yield: 0.86 g (68%). ^1H NMR ($CDCl_3$) δ: 3.76 (m, 2H, H_{2eq} and H_{6eq}), 3.46 (m, 2H, H_{2ax} and H_{6ax}), 2.77 (s, 2H, NCH_2), 2.14 (s, 6H, CH_3 x 2), 1.8–1.5 (m, 4H, H_3 and H_5).

1-(Dimethylamino)cyclopropanecarboxamide ***7***

To a stirred solution of **6** [30] (400 mg, 4 mmol) and 37% aqueous formaldehyde (2 mL, 25 mmol) in 15 mL CH_3CN was added $NaCNBH_3$ (500 mg, 8 mmol). The reaction mixture was stirred for 15 min and then AcOH was added dropwise until the solution tested neutral on wet pH paper. Stirring was continued

for an additional 2 h. The solvent was evaporated under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with an aqueous 5 N NaOH solution. The organic layer was dried over $MgSO_4$ and evaporated to give crude **7** (400 mg, 87%). ^1H NMR ($CDCl_3$) δ: 7.25 (s broad, 1H, H_{amide}), 6.60 (s broad, 1H, H_{amide}), 1.89 (s, 6H, NMe_2), 0.8 (m, 2H cyclopropyl), 0.6 (m, 2H cyclopropyl); ^{13}C NMR ($CDCl_3$) δ: 177.5 (CO), 47.1 (C_1), 41.6 (CH_3 x 2), 11.9 (C_2, C_3).

1-Aminomethyl-1-dimethylaminocyclopropane ***4b***
To a stirred suspension of $LiAlH_4$ (0.27 g, 7 mmol) in 20 mL dry THF, cooled to 0 °C, was added dropwise a solution of **7** (0.40 g, 3.1 mmol) in 10 mL THF under N_2 and the mixture was then refluxed for 2 h. The reaction mixture was allowed to cool and the excess $LiAlH_4$ was quenched with a 15% aqueous NaOH solution. The resulting mixture was filtered and the filtrate was dried over anhydrous $MgSO_4$. This solution of **4b** was used for the synthesis of **1b** without further purification.

General procedure for preparation of benzamides ***1a–h****. 4-Amino-5-chloro-N-[1-(dimethylamino)-1-cyclopentyl]methyl-2-methoxybenzamide* ***1d***
To a stirred suspension of 4-amino-5-chloro-2-methoxybenzoic acid (1.0 g, 5 mmol) and triethylamine (0.51 g, 5 mmol) in 50 mL THF cooled to 0 °C, was added dropwise ethyl chloroformate (0.54 g, 5 mmol) under N_2. The mixture was warmed to room temperature and stirred for 1 h. A solution of 1-aminomethyl-1-dimethylamino-cyclopentane **4d** (0.68 g, 4.7 mmol) was added dropwise and the reaction mixture was stirred overnight. The majority of the solvent was removed in vacuo and the residue was partitioned between AcOEt and an aqueous K_2CO_3 (10%) solution. The organic layers were dried over $MgSO_4$, concentrated and recrystallized from AcOEt/petroleum ether to give 1.35 g (88%) **1d**: mp 120 °C; ^1H NMR ($CDCl_3$) δ: 8.25 (s broad, 1H, CONH), 7.95 (s, 1H, H_{ar}), 6.23 (s, 1H, H_{ar}), 4.73 (s, 2H, NH_2), 3.71 (s, 3H, OCH_3), 3.34 (d, J = 4.7 Hz, 2H, NCH_2), 2.17 (s, 6H, CH_3 x 2), 1.7–1.3 (m, 8H, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ: 164.9 (C=O), 157.6 (C_2), 147.1 (C_4), 132.4 (C_6), 112.2, 111.1 (C_1 and C_5), 97.9 (C_3), 68.4 ($C_{1'}$-cyclopentyl), 55.9 ($C_{methoxy}$) 45-4 (NCH_2) 39-3 (CH_3 x 2), 31.3 ($C_{2'}$ and $C_{5'}$), 25.3 ($C_{3'}$ and $C_{4'}$); Anal $C_{16}H_{24}N_3O_2Cl$ (C, H, N).

4-Amino-5-chloro-N-[2-dimethylamino-2-methylpropyl]-2-methoxybenzamide ***1a***. This compound was prepared according to the method described for **1d** from **4a** (2.8 g, 0.02 mol). The crude product was purified by flash chromatography and then recrystallized from AcOEt/petroleum ether to give 2.4 g (33%) of compound **1a** as a white solid: mp 149 °C; ^1H NMR ($CDCl_3$) δ: 8.15 (s broad, 1H, CONH), 7.90 (s, 1H, H_{ar}), 6.12 (s, 1H, H_{ar}), 4.35 (s, 2H, NH_2), 3.66 (s, 3H, OCH_3), 3.16 (d, J = 4.5 Hz, 2H, NCH_2), 2.06 (s, 6H, NMe_2), 0.86 (s, 6H, CMe_2); Anal $C_{14}H_{22}N_3O_2Cl$ (C, H, N, Cl).

4-Amino-5-chloro-N-[1-(dimethylamino)-1-cyclopropyl]-methyl-2-methoxybenzamide hydrochloride ***1b***. This compound was prepared according to a method analogous to that described for compound **1d**. From the solution of crude 1-aminomethyl-1-dimethylaminocyclopropane **4b** was obtained 0.30 g (29% from 3.1 mmol of **7**) of compound **1b**. The hydrochloride salt was prepared and recrystallized from EtOH/AcOEt. mp ~ 137 °C; ^1H NMR ($CDCl_3$) δ: 8.03 (s, 1H, H_{ar}), 7.79 (s broad, 1H, CONH), 6.23 (s, H_{ar}); 4.34 (s, 2H, NH_2), 3.83 (s, OCH_3), 3.47 (d, J = 5.5 Hz, 2H, NCH_2), 2.37 (s, 6H, NMe_2), 0.6–0.5 (m, 4H, $H_{cyclopropyl}$); Anal $C_{14}H_{20}N_3O_2Cl$•HCl (C, H, N, Cl).

4-Amino-5-chloro-N-[1-(dimethylamino)-1-cyclobutyl]methyl-2-methoxybenzamide ***1c***. This product was synthesized according to the method described for **1d** from **4c** (1.0 g, 7.8 mmol). The crude product was purified by recrystallization of the free amine from AcOEt/petroleum ether to yield 1.6 g (66%) of **1c**: mp 152 °C; ^1H NMR ($CDCl_3$) δ: 8.04 (s broad, 1H, CONH), 8.02 (s, 1H, H_{ar}), 6.23 (s, 1H, H_{ar}), 4.45 (s, 2H, NH_2), 3.77 (s, 3H, OCH_3), 3.56 (d, J = 5.0 Hz, 2H, NCH_2), 2.19 (s, 6H, CH_3 x 2), 2.1 (m, 2H), 1.7 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 164.7 (C=O), 157.3 (C_2), 147.7 (C_4), 132.4 (C_6), 112.0, 111.0 (C_1, C_5), 97.6 (C_3), 62.4 ($C_{1'}$), 55.7 ($C_{methoxy}$), 42.2 (NCH_2), 37.6 (CH_3 x 2), 26.5 ($C_{2'}$ and $C_{4'}$), 12.6 ($C_{3'}$). Anal $C_{15}H_{22}N_3O_2Cl$ (C, H, N, Cl).

4-Amino-5-chloro-N-[1-(dimethylamino)-1-cyclohexyl]methyl-2-methoxybenzamide hydrochloride ***1e***. This compound was prepared according to the method described for compound **1d** from **4e** (1.4 g, 9 mmol). The hydrochloride salt was prepared and recrystallized from EtOH/AcOEt to give 2.2 g (65%) of **1e** as a white solid: mp 223 °C; ^1H NMR ($CD_3OD/CDCl_3$ 10%) δ: 7.87 (s, 1H, H_{ar}), 6.60 (s, 1H, H_{ar}), 3.99 (s, 3H, OCH_3), 3.95 (s, 2H, NCH_2), 2.96 (s, 6H, CH_3 x 2), 2.1–1.4 (m, 10H, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ: 164.6 (C=O), 157.6 (C_2), 147.0 (C_4), 132.4 (C_6), 112.2, 111.0, 97.9 (C_3), 57.4 ($C_{1'}$), 55.8 ($C_{methoxy}$), 41.6 (NCH_2), 37.4 (CH_3 x 2), 28.9 ($C_{2'}$ and $C_{6'}$), 25.9 ($C_{4'}$), 22.1 ($C_{3'}$ and $C_{5'}$). Anal $C_{17}H_{26}N_3O_2Cl$•HCl (C, N, H, Cl).

(R,S) 4-Amino-5-chloro-N-[3-dimethylamino-3-tetrahydrofuranyl]methyl-2-methoxybenzamide ***1f***. This compound was synthesized according to the method described for product **1d**. From the amine **4f** (1.4 g, 0.01 mol), and 4-amino-5-chloro-2-methoxybenzoic acid (2.1 g, 0.011 mol) was obtained 1.9 g (58%) of **1f** after purification by recrystallization from AcOEt/petroleum ether: mp 161 °C; ^1H NMR ($CDCl_3$) δ: 8.25 (s broad, 1H, CONH), 8.01 (s, 1H, H_{ar}) 6.23 (s, 1H, H_{ar}), 4.43 (s, 2H, NH_2), 3.78 (s, 3H, OCH_3), 3.91–3.45 (m, 6H, H_5, H_2 and NCH_2); 2.25 (s, 6H, NMe_2), 2.1–1.9 (m, 1H, H_{4a}), 1.65–1.6 (m, 1H, H_{4b}); Anal $C_{15}H_{22}N_3O_3Cl$ (C, H, N, Cl).

4-Amino-5-chloro-N-[3-dimethylamino-3-tetrahydrothiophenyl]methyl-2-methoxybenzamide ***1g***. This compound was prepared according to the method described for compound **1d**. From the amine **4g** (2.48 g, 0.015 mol) and 4-amino-5-chloro-2-methoxybenzoic acid (3.2 g, 0.016 mol) was obtained 3.2 g (62%) of **1g**, purified by recrystallization from AcOEt: mp 104 °C; ^1H NMR ($CDCl_3$) δ: 8.22 (s broad, 1H, CONH), 7.97 (s, 1H, H_{ar}), 6.27 (s, 1H, H_{ar}), 4.67 (s, 2H, NH_2), 3.76 (s, 3H, OCH_3), 3.70 (m, 1H, NCH_a), 3.36 (m, 1H, NCH_b), 2.88 (d, J = 11.0 Hz, 1H, H_{2a}), 2.8–2.75 (m, 2H, H_5), 2.46 (d, J = 11.0 Hz, 1H, H_{2b}), 2.29 (s, 6H, CH_3 x 2), 2.1–1.8 (m, 2H, H_4); ^{13}C NMR ($CDCl_3$) δ: 164.5 (C=O), 157.3 (C_2), 146.8 (C_4), 132.4 (C_6), 111.8, 110.9, 97.6 (C_3), 71.3 ($C_{3'}$), 55.7 (OCH_3), 41.4 (NCH_2), 39.2 (CH_3 x 2), 34.3 ($C_{2'}$), 33.3 ($C_{5'}$), 28.7 ($C_{4'}$). Anal $C_{15}H_{22}N_3O_2ClS$ (C, H, N: calc 12.22, found 10.84; Cl: calc 10.31, found 9.21; S: calc 9.32, found 8.47).

4-Amino-5-chloro-N-[4-dimethylamino-4-tetrahydro-4H-pyranyl]-methyl-2-methoxybenzamide hydrochloride ***1h***. This compound was prepared according to a method analogous to that described for compound **1d**. From the crude compound **4h** was obtained 0.9 g (43%) of **1h**. The hydrochloride salt was prepared and recrystallized from EtOH/AcOEt as a white solid: mp 225 °C; ^1H ($CDCl_3$) δ: 8.10 (s broad, 1H, CONH), 8.00 (s, 1H, H_{ar}), 6.20 (s, 1H, H_{ar}), 4.40 (s, 2H, NH_2), 3.80 (s, 3H,

OCH_3), 3.8–3.7 (m, 2H, $H_{2'eq}$ and $H_{6'eq}$), 3.59 (d, J = 5.1 Hz, 2H, NCH_2), 3.54 (m, 2H, $H_{2'ax}$ and $H_{6'ax}$), 2.28 (s, 6H, CH_3 x 2), 1.78 (m, 2H, $H_{3'ax}$ and $H_{5'ax}$), 1.41 (m, 2H, $H_{3'eq}$ and $H_{5'eq}$). Anal $C_{16}H_{24}N_3O_3Cl$•HCl (C, H, N, Cl).

4-Amino-5-chloro-N-[2-(dimethylamino)ethyl]-2-methoxybenzamide hydrochloride ***1i.*** This compound was synthesized from 2-(dimethylamino)ethylamine (0.88 g, 0.01 mol) according to the method described for product **1d**. The free base was treated with a solution of HCl (3 N) in AcOEt to give 2.3 g (77%) of **1i**. ^1H NMR (CD_3OD) δ: 7.8 (s, 1H, H_{ar}), 6.65 (s, 1H, H_{ar}), 3.8 (s, 3H, OCH_3), 3.6 (t, J = 5.7 Hz, 2H), 3.2 (t, J = 5.7 Hz, 2H), 2.8 (s, 6H, CMe_2). Anal $C_{12}H_{18}N_3O_2Cl$•HCl (C: calc 46.76%; found 46.10%, H, N, Cl: calc 23.01%; found 23.51%).

X-ray crystallography

A selected crystal was set up on a Nonius CAD4 automatic diffractometer. Unit cell dimensions with estimated standard deviations were obtained from least-square refinements of the setting angles of 25 well-centered reflections. Two standard reflections were monitored periodically, and showed no change during data collection. Computations were performed using Crystals (University of Oxford, UK) adapted on a MicroVax II. The structure was solved by a direct method using the Shelx86 program (University of Göttingen, Germany 1986). All non-hydrogen atoms were isotropically refined because of the small number of reflections. Hydrogen atoms were placed in calculated positions and given an overall isotropic thermal parameter; full matrix least-squares refinements were carried out by minimizing the function $\Sigma w (|F_0| - |F_c|)^2$ where F_0 and F_c are the observed and calculated structure factors. Unit weight was used. Models reached convergence with $R = \Sigma (||F_0| - |F_c||)/\Sigma |F_0|$ and $R_W = [\Sigma w (|F_0| - |F_c|)^2/\Sigma w (F_0)^2]^{1/2}$ having the 0.072 value. Criteria for a satisfactory complete analysis were the ratio of the RMS shift to the standard deviation being less than 0.1 and no significant features in the final difference map.

Compound ***1d***

$C_{16}H_{24}ClN_3O_2$, HCl, MW = 362.6, crystals are parallelepiped, colorless and belong to the monoclinic system, space group Pa. Cell parameters: a = 10.851 Å, b = 7.263 Å, c = 12.273 Å, β = 107.16°, V = 942 Å^3, d = 1.30 g/cm^3. Tables of atomic coordinates, bond distances and angles are available from the author.

Binding assays

Male Sprague–Dawley rats from Janvier laboratory (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 the experiments.

5-HT$_3$ receptor

Membranes were prepared from rat posterior cortex according to the procedure described by Hall and Gozlan [24]. [^{3}H]BRL 43694 (61 Ci/mmol) was purchased from NEN research products. GR 38032F was a generous gift from Glaxo (UK). All other chemicals and reagents were commercially available from Sigma. The binding of 1.2 nM [^{3}H]BRL 43694 (K_D = 1.5 nM, B_{max} = 30 fmol/mg protein for 5-HT$_3$ receptors) was measured using membranes (100 µL aliquots equivalent to 0.95 mg protein) suspended in a final volume of 0.5 mL of 50 mM Hepes, pH 8.4 and incubated at 25 °C for 30 min. Seven to eleven concentrations of each drug were used in triplicate. Non-specific binding was determined by the addition of 10 µM GR 38032F in duplicate. Total binding was defined in quadruplicate.

5-HT$_4$ receptor

Membranes were prepared from rat striatum and olfactori tubercule which were pooled separately and stored at –80 °C. Tissues were thawed at 0 °C and homogenized in 15 volumes of ice-cold 50 mM Hepes (pH 7.4) using a Polytron homogenizer and then centriguged once at 40 000 g at 4 °C for 15 min. The resulting pellet was resuspended in 5 volumes of Hepes to a final concentration of 4–5 mg prot/mL determined by the Bradford method. Membrane aliquots of 2.6 mL were kept frozen at –80 °C until subsequent use.

Binding assays were performed according to the method previously described [25] with modifications. The binding of [^{3}H]GR-113808 (85 Ci/mmol, purchased from Amersham) were measured using membranes (50 µL aliquots equivalent to 0.1–0.2 mg protein) suspended in a final volume of 0.5 mL of 50 mM Hepes (pH 7.4). Seven concentrations of each drug were used and the assay was done in triplicate. The incubation (25 °C, 30 min) was stopped by the addition of 1 mL of cold buffer solution.

Non-specific binding was defined with 10 µM of ML 10302 [31] and represented less than 10% of the total binding.

D$_2$ receptor

Frozen striata of female bovine brains (Cellubio, France) were used in all studies and were stored at –80 °C prior to use. The tissue was dissected and homogenized in 40 volumes of buffer solution (ice-cold Tris, 50 mM, pH 7.4 at 23 °C) with a Polytron. The homogenate was first centrifuged at 3000 g for 5 min. The supernatant was then homogenized in an equal volume of buffer and centrifuged at 48 000 g for 15 min. The pellet was resuspended and centrifuged again at 48 000 g. The same volume of the buffer solution was added, the pellet was homogenized and the solution was incubated for 10 min at 37 °C and centrifuged. The final pellet was resuspended in 5 volumes of cold buffer solution (50 mM Tris, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, pH 7.4 at 23 °C). Binding assays were performed in glass tubes in a final volume of 0.5 mL with 100 µL of membrane aliquots corresponding to 0.5 mg of protein. Seven concentrations of each drug in triplicate were used to inhibit the binding of 0.9 nM [^{3}H]spiperone (32.4 Ci/mmol, NEN Research Products) in the presence of ketanserin (100 nM) to block 5-HT$_2$ receptors and incubated for 30 min at 23 °C. Non-specific binding was determined by the addition of 10 µM butaclamol in duplicate. Total binding was defined in quadruplicate.

Protein estimation

The protein concentrations of bovine striatum and rat posterior cortex were determined by the method of Lowry [29] using bovine serum albumin as the standard. The protein concentration of rat striatum was determined by the method of Bradford [30].

For each assay the bound radioactivity was separated by vacuum filtration through Whatman GF/B glass filters, presoaked in 0.1% poly(ethyleneimine), using a Brandel Cell Harvester. The filters were then washed twice with 5 mL of 50 mM Tris-HCl (pH 8.4) at room temperature and dried. The filters were placed in polyethylene vials to which were added 4 mL of a scintillation cocktail (Beckman, Ready-safe) and, after equilibration, the radioactivity was determined using liquid scintillation spectrometry. The data were analyzed by a computer-assisted curve-fitting program in Lotus 1.2.3 to provide IC_{50}, K_i and r^2 values, K_i values being calculated from the Cheng–Prussof equation.

Acknowledgments

We are grateful to Sanofi Recherche for financial support. We thank E Kidd for assistance in the correction of the manuscript.

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