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Benzamides Derived from 1,2-Diaminocyclopropane as Novel Ligands for Human D_2 and D_3 Dopamine Receptors

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Abstract—Benzamides (**3a**–**f**) derived from 4-amino-5-chloro-2-methoxybenzoic acid and either *cis* or *trans* 1,2-diaminocyclopropane were synthesised and were evaluated in binding assays employing, bovine striatal D_2 receptors, recombinant human hD_2 and hD_3 receptors expressed in CHO cells and rat, cortical 5-HT_3 and striatal 5-HT_4 receptors. The *cis* and *trans* isomers of the derivatives were isolated and characterised. The results demonstrated the superiority of the *cis* conformers over the *trans* conformers in dopamine receptor binding assays (K_i $hD_2 = 13.4$ and 6.9 nM and K_i $hD_3 = 17.7$ and 4.5 nM for the *cis*-**3b** and *cis*-**3f** compounds, respectively; K_i $hD_2 = 816$ and >1000 nM and K_i $hD_3 = 469$ and >1000 nM for the corresponding *trans*-**3b** and *trans*-**3f** compounds respectively). The *cis* compounds are folded: the benzamide group and the basic nitrogen atom were in a *syn* relationship. Compound **3f** can be superimposed with a conformation of the tropane derivative, BRL 25594, having the benzyl group in an axial position to give a suitable fit, indicating that both compounds may have a common binding site in the dopamine receptor.

Introduction

The generic benzamide family has provided a number of potent compounds possessing serotoninergic and dopaminergic activity.[1,2] The parent compound of this family is metoclopramide,[3,4] a gastro-kinetic and anti-emetic drug, which is characterised by its weak affinity and lack of selectivity for D_2, 5-HT_3 and 5-HT_4 receptors.[5] The weak affinity and lack of selectivity of metoclopramide for these receptors can be imputed to the large number of permissible conformers of the basic chain. To date, several potent and selective metoclopramide derivatives for these three receptors have been described. These structures are characterised by the 4-amino-5-chloro-2-methoxy benzamide moiety and a basic nitrogen in a rigid amino framework. Thus, zacopride,[6] with a quinuclidine ring and (**1**) BRL 24682,[7] a derivative of *N*-methylnortropane, are potent 5-HT_3 receptor antagonists and 5-HT_4 receptor agonists, while (**2**) BRL 25594,[8] a potent D_2 receptor antagonist, has a benzyl residue attached to the tropyl nitrogen. The influence of the structure of the basic framework on the potency and selectivity has been pointed out by several other compounds of this class.[9–12] Thus, hydrophobic parameters, the orientation of the nitrogen lone-pair and the relative positions of the aromatic benzamide and the heterocycle seem to be crucial parameters for recognition by dopaminergic[13] and serotoninergic receptors.[14] In order to improve the understanding of the influence of conformational parameters on the recognition of benzamide derivatives by serotoninergic and dopaminergic receptors, a cyclopropane ring was introduced in the ethyl amino chain of metoclopramide: the obtained conformationally restricted *cis* and *trans* stereoisomers are producing different orientations of the aromatic ring and the basic nitrogen atom. We present here the results concerning the conformationally constrained benzamides **3a**–**f** (Scheme 1).

Chemistry

1,2-Diaminocyclopropane derivatives were prepared from *trans* and *cis* 1,2-cyclopropanediacids which were synthesised according to a previously-described process.[15] Briefly, *cis* and *trans* 1,2-cyclopropanedicarboxylic acids were prepared by the condensation of methyl acrylate and ethyl chloroacetate which provided

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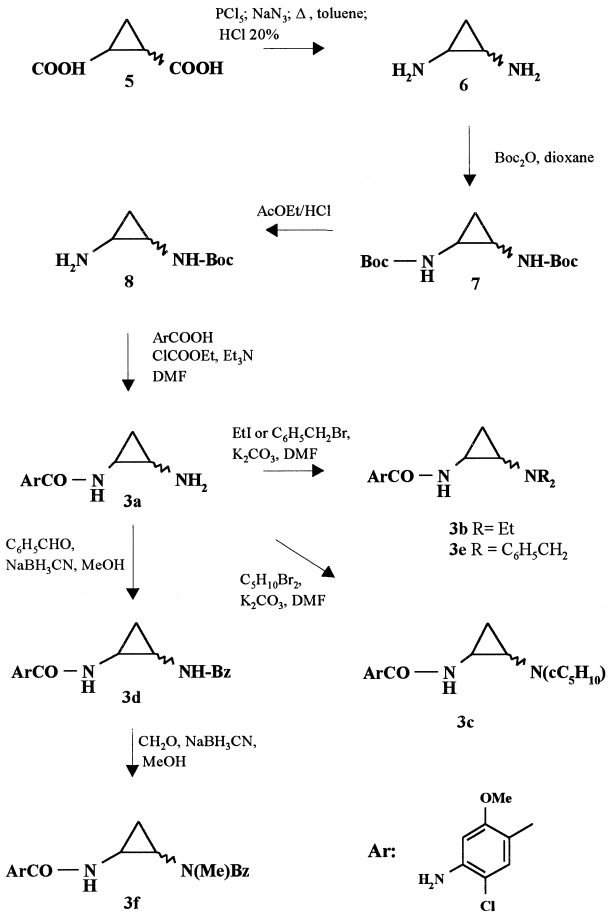


Scheme 1.

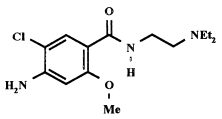
a mixture of *cis* and *trans* cyclopropane esters. The *cis* and *trans* acids **5** were separated during the work up by the hydrolysis of the esters by KOH in methanol. *Cis* and *trans* 1,2-cyclopropanediamine **6** were obtained from the acids **5** using the classical conditions of the Curtius reaction (Scheme 1).[16–18] *t*-Butyloxycarboxylic anhydride was condensed with the amines **6** to give the diprotected compounds *cis* and *trans* **7** which were treated with anhydrous HCl in AcOEt to yield the monoamino derivatives, *cis* and *trans* **8**, respectively. They were condensed with 4-amino-5-chloro-2-methoxybenzoic acid using the mixed anhydride method in DMF and de-protected according to the previous process into the *cis* and *trans* amino derivatives **3a**. These compounds were the essential intermediates to prepare the different *N*,*N*-disubstituted benzamides **3b–f**. *Cis* and *trans* **3a** were condensed with EtI in the presence of K_2CO_3 in DMF to give the *cis* and *trans* diethyl-*N*-substituted derivatives **3b**, the strict structural analogues of metoclopramide. The piperidino analogues **3c**, which, with the *cis* stereoisomers, could mimic the putative active conformer of ML 10302[19] (2-(1-piperidinyl)ethyl 4-amino-5-chloro-2-methoxybenzoate), a 5-HT_4 agonist, were synthesised from **3a** by the double condensation of 1,5 dibromopentane in the presence of K_2CO_3 in MeCN. Successive reactions of the reductive amination of compounds **3a** with benzaldehyde and CH_2O,[20] respectively, gave compounds **3f** that were structurally related by benzyl substitution to the highly potent D_2/D_3 receptor antagonists, clebopride, BRL 25594 and 4-amino-*N*[2-(*N*-benzyl-*N*-methylamino)-2-ethyl]-5-chloro-2-methoxy benzamide **4**, a *N*-benzylated metoclopramide-like compound.

Biological Methods

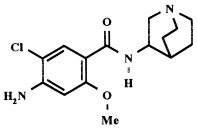
The affinities of the different compounds were evaluated at bovine striatal D_2 receptors, human D_2 and D_3 receptors and rat brain 5-HT_3 and 5-HT_4 receptors. The binding assay for bovine D_2 receptors was performed using [^{3}H]spiperone.[21] Human D_2 and D_3 receptor-containing membranes were prepared from CHO cells expressing one or other of the receptors according to the methods described by Sokoloff[22] and competition experiments were carried out with [^{125}I]iodosulpride. 5-HT_3 receptors affinities were determined with [^{3}H]BRL 43694 in rat posterior cortex membranes and affinities for 5-HT_4 receptors were determined by using rat striatal membranes and [^{3}H]GR-113808 according to conditions previously reported by us.[19]

Biological Results and Discussion

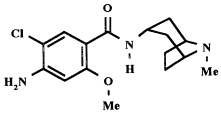
The data from the binding assays are reported in Table 1 where their affinity values for bovine D2, hD_2, hD_3, and rat 5-HT_3 and 5-HT_4 receptors are compared to those of the reference compounds: metoclopramide, clebopride,



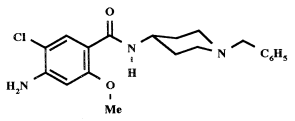
Metoclopramide



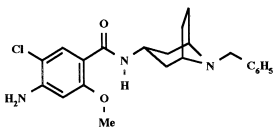
Zacopride



(1) BRL 24682



Clebopride



(2) BRL 25594

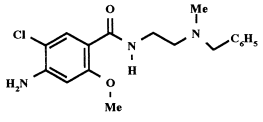


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Table 1. Binding profiles of the benzamides **3a–e** for bovine D_2, human D_2 and D_3 rat 5-HT_3 and rat 5-HT_4 receptors

Compound	Receptor affinity (K_i, nM)[e]				
	Bovine D_2[a]	Human D_2[b]	Human D_3[b]	Rat 5-HT_3[c]	Rat 5-HT_4[d]
Metoclopramide	140 ± 6.5	21 ± 1	27 ± 3	443 ± 45	970
Clebopride	11.9 ± 3.8	0.48 ± 0.03	1.45 ± 0.06	> 1000	104 ± 58
BRL 25594	0.28 ± 0.04	0.05 ± 0.02	0.23 ± 0.02	> 1000	233 ± 60
4	24.3 ± 6.7	1.4 ± 0.05	4.6 ± 0.7	> 1000	867 ± 71
cis-**3a**	I[f]	NT[g]	NT	NT	> 1000
trans-**3a**	I	I	I	NT	> 1000
cis-**3b**	34.8 ± 1.6	13.4 ± 0.3	17.7 ± 2.8	> 1000	567 ± 29
trans-**3b**	2400	816 ± 52	> 1000	> 1000	488 ± 32
cis-**3c**	250 ± 19	NT	NT	> 1000	> 1000
trans-**3c**	> 1000	NT	NT	> 1000	> 1000
cis-**3d**	232 ± 20	112 ± 6.9	89.5 ± 7.1	I	> 1000
trans-**3d**	355 ± 22	118 ± 14	> 1000	I	> 1000
cis-**3f**	31.3 ± 2.3	6.9 ± 0.5	4.5 ± 0.8	I	> 1000
trans-**3f**	952 ± 22	469 ± 6	> 1000	> 1000	> 1000
trans-**3e**	> 1000	> 1000	> 1000	I	> 1000

[a][^{3}H] Spiperone was used as the radioligand and the binding assays were carried out using bovine striatal membranes (25 °C, 30 min); nonspecific binding was determined with butaclamol (10 μM).
[b][^{125}I] Iodosulpride was used as the radioligand and the binding assays were carried out using membranes from CHO cells expressing either the human D_2 (short) receptor (hD_2) or the human D_3 receptor (hD_3) (30 min, 30°C), nonspecific binding was determined in the presence of raclopride (10 μM).
[c][^{3}H]-BRL-43694 was used as he radioligand ant the binding assays were carried out using rat posterior cortex membranes (30 min, 26 °C); nonspecific binding was determined with GR 38032F (10 μM).
[d][^{3}H]-GR113808 was used as the radioligand in rat striatal membranes and nonspecific binding was determined with the 5-HT_4 receptor agonist, ML 10302 (10 μM).
[e]K_i ± standard error of the mean (SEM) values were determined from the Cheng–Prussof equation.
[f]I = inactive up to 10–5 M.
[g]NT = not tested.

BRL 24682 and compound **4**. Metoclopramide was characterised by its moderate affinity for bovine D_2 and rat 5-HT_3 and 5-HT_4 receptors, while values for hD_2 and hD_3 receptors were higher. Clebopride, BRL 25594 and **4** were particularly potent at human hD_2 and hD_3 receptors. These data support the well-known, favourable role of *N*-benzyl substitution on the basic nitrogen atom for improving affinity for dopamine receptors. However, these compounds did not have differential selectivity for hD_2 and hD_3 receptors.

The data obtained for the cyclopropane derivatives demonstrated that affinity for the dopaminergic receptors is dependent on substitution on the nitrogen atom, **3a**, **3d**, **3e**, **3c** were inactive. Compounds **3b** and **3f**, the strict analogues of metoclopramide and **4**, respectively, showed clearly that the *trans* isomers were weakly active or inactive at the different receptors tested, while the corresponding *cis* compounds had nanomolar affinity for dopamine receptors, indicating that these receptors prefer the folded conformation of the amino chain. These results are in contrast to those obtained for the reference compounds, clebopride and BRL 25594 (**2**) and structurally-related dopaminergic antagonists,[13] which possess an extended shape. In particular, for the nortropane derivatives, the *exo* derivatives have been shown to be superior to the *endo* derivatives.[23]

Comparison of the molecular structure of *cis*-**3f** and BRL 25594 (**2**) showed a large structural difference between the two molecules when **2** was represented in an extended conformation with the benzyl group in the equatorial position. However, structural similarity was seen when the benzyl group adopted the axial position. This point was confirmed by conformational analysis of **2** and *cis*-**3f** performed with SYBYL software 6.3. It was carried out using the Molecular Dynamic program and the selected minimal energy conformers were minimized. The structural parameters of the minimum energy conformers selected for **2** were similar to those reported previously for the X-ray structure analysis of benzamides derived from tropane.[24] The carbonyl function was orientated in the anti-parallel position to the axial bond of the basic nitrogen atom and the benzamide ring was maintained co-planar to the carbonyl group by an intramolecular hydrogen bond. The energy difference between the benzyl axial and equatorial conformers of **2** was small ($\Delta E = 0.31$ kcal/mol), demonstrating that the axial conformer can be considered as a putative active conformer in the binding site of D_2 and D_3 receptors. The geometrical parameters of the selected minimum energy conformer of compound *cis* **3f** were similar to the axial conformer of **2**. Thus, the distances between the oxygen and the basic nitrogen atoms of compounds **2** and *cis* **3f** were 5.49 and 5.10 Å, respectively. These two compounds were fitted (Fig. 1) with regard to the carbonyl group and the basic nitrogen associated with the lone pair, which were considered as the binding points in the receptor site. The RMS value (0.184) showed that the compounds were well superimposed and indicated that the new *cis* cyclopropane benzamides could bind to the same site as the nortropane derivatives. These data indicate that the benzamide pharmacophore of the nortropane and

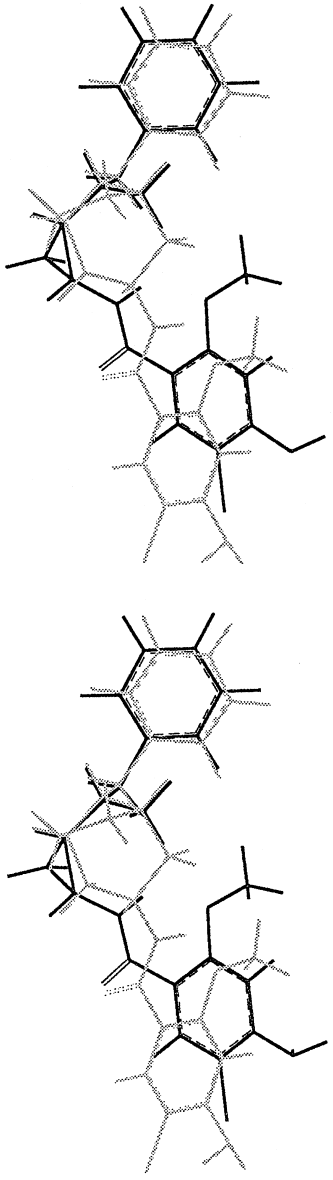

Figure 1. Stereoview of the superimposition of BRL 25594 (**2**) with the benzyl group in the axial position and compound *cis*-**3f** with regard to the oxygen atom of the carbonyl function, the nitrogen basic atom and the lone pair (RMS = 0.184).

piperidine derivatives has a folded shape rather than the extended shape which hitherto was thought to be essential. Examination of structures from the X-ray crystallography analysis of benzamides derived from *N*-benzyl pyrrolidine[25,26] is consistent with the existence of such a folded pharmacophore.

Compounds **3a**–**e** had no affinity for 5-HT_3 receptors and these results further demonstrate the unfavourable influence of the hydrophobic substituent on the basic nitrogen atom for binding to this receptor. The compounds were inactive or had moderate affinity for 5-HT_4 receptors and these data agree with a number of previous studies on the structure–activity relationships benzamides.[14]

Conclusion

The relative affinities for bovine D_2, cloned hD_2, hD_3 and rat 5-HT_3 and rat 5-HT_4 receptors of the ligands reported here underline the importance of steric constraints of the basic nitrogen framework of benzamides derived from 4-amino-5-chloro-2-methoxybenzoic acid for recognition by these receptors. *cis*-1,2-Diamino cyclopropane derivatives are novel benzamides with nanomolar affinity for hD_2 and hD_3 receptors. Structural comparison of these compounds with the reference compound, BRL 25594, demonstrated the propensity of tropane and piperidine benzamides to adopt a folded conformation, where the benzyl group is in the axial position, in the receptor binding site. *cis*-1,2-Diamino cyclopropane constitutes a new basic moiety of the benzamide family which recognises both hD_2 and hD_3 receptors with similar potency.

Experimental

General

Melting points were determined on a Kofler 7841 and were uncorrected. Analytical samples were homogeneous by TLC and afforded spectroscopic results consistent with the assigned structures. ^{1}H NMR spectra were obtained using either a Bruker ARX-400 or AC-200 spectrometer (residual $CHCl_3$ ($\delta\varepsilon_H$ 7.26) as internal standard). ^{13}C NMR spectra were recorded in the same solvent stated for the ^{1}H spectra. Elemental analyses were performed on a Perkin–Elmer 240 analyser. TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light. Preparative chromatography was performed under pressure with SDS Chromagel Silica 60, 35–70 mesh. All solvents and reagents were reagent grade unless otherwise noted.

4-Amino-*N*-[2-(*N*-benzyl-*N*-methylamino)ethyl]-5-chloro-2-methoxy benzamide (4). To a stirred suspension of 4-amino-5-chloro-2-methoxybenzoic acid (1 g, 4.96 mmol) and triethylamine (0.52 g, 5.2 mmol) in DMF (100 mL) 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. *N*-benzyl-*N*-(methyl)ethylene diamine (0.82 g, 5 mmol) was added dropwise and the mixture was stirred overnight. The majority of the solvent was removed in vacuo and the residue was partitioned between EtOAc and an aqueous K_2CO_3 (10%) solution. The organic layers were dried over $MgSO_4$. The organic solvent was evaporated to give a solid which was recrystallised in EtOAc/diisopropyl ether mixture to give **4**: 1.33 g (yield: 64%), mp 130 °C. ^{1}H NMR ($CDCl_3$) δ 8.2 (m, 1H, NH), 8.1 (s, 1H, *H*CCCl), 7.3 (m, 5H, H_{ar}), 6.3 (s, 1H, *H*CCOCH$_3$), 4.4 (s, 2H, NH_2), 3.9 (s, 3H, OCH_3), 3.6 (m, 4H, C*H*$_2$C$_6$H$_5$ and NHC*H*$_2$), 2.6 (t, J = 6 Hz, 2H, CONHCH$_2$C*H*$_2$), 2.3 (s, 3H, NCH_3). ^{13}C NMR ($CDCl_3$) δ 164 (CONH), 158 ($C_{ar}OCH_3$), 147 ($C_{ar}NH_2$), 139 ($C_{ar}CH_2$), 133 (C_{ar}HCCl), 129–127 (C_{ar}), 113 ($C_{ar}Cl$), 111 (C_{ar}CONH), 98 ($C_{ar}HCOCH_3$), 62 (NCH$_2$CH$_2$NH), 56 (OCH_3), 55 (NCH$_2$C$_6$H$_5$), 42 (NCH_3), 37 (NCH$_2$CH$_2$NH). Anal.

calcd $C_{18}H_{22}ClN_3O_2$: C, 62.15; H, 6.37; N, 12.08. Found: C, 61.92; H, 6.4. N, 11.92.

(±)-(*trans*)-*N*,*N*′-Bis(*tert*-butyloxycarbonyl)-1,2-cyclopropanediamine (*trans*-7). The amine hydrochloride salt *trans*-**6**[15] (0.72 g, 5 mmol) were treated with 5 N NaOH (10 mL). Water (10 mL), dioxane (20 mL) and then *tert*-butyloxycarboxylic anhydride (2.2 g, 10 mmol) were added at 0 °C. After 30 min, the solution was extracted several times with chloroform. After washing with brine, the organic phase was dried over $MgSO_4$, filtered and evaporated. The residue was washed with petroleum ether to give 0.91 g (yield: 67%) of *trans*-**7**. ^{1}H NMR ($CDCl_3$) δ 5.0 (s, 2H, NH), 3.2 (m, 2H, CH), 1.5 (m, 2H, CH_2), 1.4 (s, 18H, CH_3).

(±)-(*cis*)-*N*,*N*′-Bis(*tert*-butyloxycarbonyl)-1,2-cyclopropanediamine (*cis*-7). Following the previous procedure described for *trans*-**7**, the amine hydrochloride salt *cis*-**6** (1 g, 6.9 mmol) gave 1.5 g (yield: 77%) of *cis*-**7**. ^{1}H NMR ($CDCl_3$) δ 5.0 (s, 2H, NH), 2.9 (m, 2H, CH), 1.8 (m, 1H, CH_{2trans}), 1.4 (s, 18H, CH_3), 1.2 (m, 1H, CH_{2cis}).

(±)-(*trans*)-*N*-*tert*-Butyloxycarbonyl-1,2-cyclopropanediamine (*trans*-8). A solution of *trans*-**7** (0.9 g, 3.3 mmol) in ethyl acetate (20 mL) was cooled to 0 °C and treated with a 2.4 N HCl solution (20 mL) in ethyl acetate. The mixture was allowed to warm to room temperature overnight, then filtered and dried to give 0.55 g (80%) of *trans*-**8**. ^{1}H NMR ($CDCl_3$) δ 6.4 (s, 1H, NH), 3.3 (m, 1H, C*H*NH), 3.0 (m, 1H, C*H*NH_2), 1.5 (m, 2H, CH_2), 1.4 (s, 9H, CH_3).

(±)-(*cis*)-*N*-*tert*-Butyloxycarbonyl-1,2-cyclopropanediamine (*cis*-8). Following the previous procedure, 1.5 g (5.5 mmol) of the compound *cis*-**7** gave 0.9 g (yield: 63%) of *cis*-**8**. ^{1}H NMR ($CDCl_3$) δ 6.4 (s, 1H, NH), 3.3 (m, 1H, C*H*NH), 3.0 (m, 1H, C*H*NH_2), 1.8 (m, 1H, CH_2), 1.4 (s, 18H, CH_3), 1.2 (m, 1H, CH_2).

(±)-(*trans*)-4-Amino-*N*-(2-amino-1-cyclopropyl)-5-chloro-2-methoxybenzamide (*trans*-3a). A solution of triethylamine (0.35 mL, 2.5 mmol) in anhydrous DMF (50 mL) was cooled to 4 °C and treated with 4-amino-5-chloro-2-methoxybenzoic acid (0.5 g, 2.5 mmol) and ethyl chloroformate (0.24 mL, 2.5 mmol). After 30 min at 4 °C, *trans*-**8** (0.5 g, 2.5 mmol) was added and the solution was allowed to warm to room temperature for 3 h. After evaporation and treatment with an aqueous solution of 2.5 N NaOH, the solution was extracted three times with dichloromethane. The organic fractions were dried and then evaporated. Recrystallisation in ethyl acetate gave 0.65 g (yield: 73%) of (±)-(*trans*)-4-amino-5-chloro-2-methoxy-*N*-(2-[*N*′-(*tert*-butyloxycarbonyl)amino]-1-cyclopropyl)benzamide. ^{1}H NMR ($CDCl_3$) δ 7.7 (s, 1H, *H*CCCl), 6.4 (m, 2H, NH and *H*C$COCH_3$), 3.9 (s, 3H, OCH_3), 3.2 (m, 1H, C*H*NHCOPh), 2.9 (m, 1H, C*H*NHBoc), 1.5 (m, 2H, CH_2), 1.4 (s, 9H, CH_3). 0.65 g (1.8 mmol) of the previous compound were treated according to the process described for *trans*-**8** to give 0.5 g (yield: 95%) of *trans*-**3a** after recrystallisation in diethyl ether, mp 92 °C. ^{1}H NMR (CD_3OD) δ 7.7 (s, 1H, *H*CCCl), 6.4 (s, 1H, *H*C$COCH_3$), 3.9 (s, 3H, OCH_3), 3.2 (m, 1H, C*H*NH), 2.9 (m, 1H, C*H*NH2), 1.5 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 168 (CONH), 159 ($C_{ar}$$OCH_3$), 142 ($C_{ar}NH_2$), 133 ($C_{ar}$HCCl), 117 ($C_{ar}$Cl and C_{ar}CONH), 104 (C_{ar}H$COCH_3$), 57 (OCH_3), 31 (CHNH and $CHNH_2$), 13 (CH_2). Anal. calcd for $C_{11}H_{14}ClN_3O_2.H_2O$: C, 48.27; H, 5.89; N, 15.35. Found: C, 48.24; H, 5.86; N, 15.43.

(±)-(*cis*)-4-Amino-*N*-(2-amino-1-cyclopropyl)-5-chloro-2-methoxybenzamide (*cis*-3a). Following the procedure described for *trans*-**3a**, 4-amino-5-chloro-2-methoxybenzoic acid (0.58 g, 2.9 mmol) and the amine *cis*-**8** (0.6 g, 2.9 mmol) gave 0.6 g (yield: 71%) of *cis*-**3a** after recrystallization in diethyl ether, mp 82 °C. ^{1}H NMR (CD_3OD) δ 7.7 (s, 1H, *H*CCCl), 6.5 (s, 1H, *H*C$COCH_3$), 3.9 (s, 3H, OCH_3), 2.8 (m, 1H, C*H*NH), 2.5 (m, 1H, C*H*NH_2), 1.0 (m, 1H, CH_{2trans}), 0.4 (m, 1H, CH_{2cis}). ^{13}C NMR ($CDCl_3$) δ 169 (CONH), 160 ($C_{ar}$$OCH_3$), 150 ($C_{ar}$NH2), 133 ($C_{ar}$HCCl), 111 ($C_{ar}$Cl and C_{ar}CONH), 99 (C_{ar}H$COCH_3$), 57 (OCH_3), 29 (CHNH and $CHNH_2$), 15 (CH_2). Anal. calcd for $C_{11}H_{14}ClN_3O_2$: C, 51.67; H, 5.52; N, 16.43. Found: C, 51.61; H, 5.58; N, 16.31.

(±)-(*trans*)-4-Amino-*N*-(2-diethylamino-1-cyclopropyl)-5-chloro-2-methoxybenzamide (*trans*-3b). A solution of *trans*-**3a** (0.73 g, 2.5 mmol) in anhydrous DMF (10 mL) was treated overnight with potassium carbonate (1.35 g, 9.8 mmol) and ethyl iodide (0.81 g, 5 mmol). After filtration and evaporation, water (50 mL) and ethyl acetate (100 mL) were added. The organic phase was extracted by brine, dried over Na_2SO_4, filtered and evaporated. The resulting product was treated with a solution of HCl in ethyl acetate and then recrystallized in isopropanol/toluene mixture to give 0.4 g (yield: 46%) of *trans*-**3b** hydrochloride, mp 190 °C. ^{1}H NMR (CD_3OD) δ 7.7 (s, 1H, *H*CCCl), 6.5 (s, 1H, *H*C$COCH_3$), 3.9 (s, 3H, OCH_3), 3.7 (m, 4H, NCH_2), 3.3 (m, 1H, C*H*NH), 2.9 (m, 1H, C*H*N), 1.5 (m, 2H, CH_2), 1.4 (m, 6H, CH_3); ^{13}C NMR (CD_3OD) δ 169 (CONH), 160 ($C_{ar}$$OCH_3$), 151 ($C_{ar}NH_2$), 133 ($C_{ar}$HCCl), 111 ($C_{ar}$Cl and C_{ar}CONH), 98 (C_{ar}H$COCH_3$), 57 (OCH_3), 44 (NCH_2), 29 (CHN), 24 (CHNH), 13 (CH_3), 9 (CH_2). Anal. calcd for $C_{15}H_{22}ClN_3O_2$.HCl: C, 51.73; H, 6.66; N, 12.07. Found: C, 51.35; H, 6.80; N, 11.84.

(±)-(*cis*)-4-Amino-*N*-(2-diethylamino-1-cyclopropyl)-5-chloro-2-methoxybenzamide (*cis*-3b). Following the previous procedure, the benzamide *cis*-**3a** (0.4 g, 1.37 mmol) gave 0.2 g (yield: 42%) of *cis*-**3b** hydrochloride, mp >200 °C. ^{1}H NMR (CD_3OD) δ 7.7 (s, 1H, *H*CCCl), 6.5 (s, 1H, *H*CCOCH3), 3.9 (s, 3H, OCH_3), 3.4–3.2 (m, 5H, NCH_2 and C*H*NH), 2.9 (m, 1H, C*H*N), 1.5–1.0 (m, 8H, CH_2 and CH_3); ^{13}C NMR ($CDCl_3$) δ 169 (CONH), 160 ($C_{ar}$$OCH3$), 151 ($C_{ar}NH_2$), 133 ($C_{ar}$HCCl), 111 ($C_{ar}$Cl and C_{ar}CONH), 98 (C_{ar}H$COCH_3$), 57 (OCH_3), 41 (CHN), 28 (CHNH), 11 (CH_2), 9 (CH_3). Anal. calcd for $C_{15}H_{22}ClN_3O_2$.HCl.H_2O: C, 49.19; H, 6.88; N, 11.47. Found: C, 48.63; H, 7.10; N, 11.18.

(±)-(*trans*)-4-Amino-*N*-[2-(1-piperidine)-1-cyclopropyl]-5-chloro-2-methoxy benzamide (*trans*-3c). A solution of *trans*-**3a** (0.29 g, 1 mmol) in acetonitrile (150 mL) was

treated overnight with potassium carbonate (0.5 g, 3.6 mmol) and 1,5-dibromopentane (0.23 g, 5 mmol). After refluxing for 96 h, the solution was evaporated and then treated with water and extracted several times with dichloromethane. After washing with brine, the organic phase was dried over Na_2SO_4, filtered and evaporated. The resulting product was purified by flash chromatography (CH_2Cl_2/methanol, 95/5), treated with a solution of HCl in ethyl acetate and recrystallized in ethanol/ethyl acetate to give 0.085 g (yield: 22%) of *trans*-**3c** hydrochloride, mp >200°C. ^{1}H NMR (CD_3OD) δ 7.8 (s, 1H, *H*CCCl), 6.5 (s, 1H, *H*CCOCH$_3$), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 4H, NCH$_2$), 3.1 (m, 1H, C*H*NHCO), 2.5 (m, 1H, C*H*N), 1.8 (m, 4H, NCH$_2$C*H*$_2$), 1.6 (m, 2H, NCH$_2$CH$_2$C*H*$_2$), 1.3 (m, 2H, CH$_2$); ^{13}C NMR (CD_3OD) δ 169 (CONH), 160 (*C*$_{ar}$OCH$_3$), 151 (C$_{ar}$NH$_2$), 133 (*C*$_{ar}$HCCl), 111 (C$_{ar}$Cl and *C*$_{ar}$CONH), 99 (*C*$_{ar}$HCOCH$_3$), 57 (OCH$_3$), 55 (NCH$_2$), 47 (NCH), 30 (*C*HNHCO), 25, 24 and 13 (CH$_2$). Anal. calcd for $C_{16}H_{22}ClN_3O_2.HCl.H_2O$: C, 50.79; H, 6.61; N, 11.11. Found: C, 50.76; H, 6.5; N, 11.35.

(±)-(*cis*)-4-Amino-*N*-[2-(1-piperidine)-1-cyclopropyl]-5-chloro-2-methoxy benzamide (*cis*-3c). Following the procedure described for *trans*-**3c**, *cis*-**3a** (0.2 g, 0.54 mmol) gave 0.12 g (yield: 53%) of *cis*-**3c** 2-methoxybenzamide which was isolated as oxalate (MeOH/diethyl ether). ^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1H, *H*CCCl), 6.5 (s, 1H, *H*CCOCH$_3$), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 4H, NCH$_2$), 3.1 (m, 1H, C*H*NHCO), 2.8 (m, 1H, C*H*N), 1.8 (m, 4H, NCH$_2$ C*H*$_2$), 1.7 (m, 2H, NCH$_2$CH$_2$C*H*$_2$), 1.3 (m, 2H, CH$_2$); ^{13}C NMR (CD_3OD) δ 169 (CONH), 160 (*C*$_{ar}$OCH$_3$), 151 (C$_{ar}$NH$_2$), 133 (*C*$_{ar}$HCCl), 111 (C$_{ar}$Cl and *C*$_{ar}$ CONH), 98 (*C*$_{ar}$HCOCH$_3$), 57 (OCH$_3$), 56 (NCH$_2$), 43 (NCH), 29 (*C*HNHCO), 24, 23 and 10 (CH$_2$). Anal. calcd for $C_{16}H_{22}ClN_3O_2.2C_2H_2O_4$: C, 47.66; H, 5.16; N, 8.34. Found: C, 48.01; H, 4.85; N, 8.12.

(±)-(*trans*)-4-Amino-*N*-(2-benzylamino-1-cyclopropyl)-5-chloro-2-methoxybenzamide (*trans*-3d). Benzaldehyde (0.48 g, 4.5 mmol) in methanol (10 mL) was added dropwise to a solution of *trans*-**3a** (0.72 g, 2.5 mmol) in methanol (40 mL) at 0 °C. After 15 min, sodium cyanoborohydride (0.47 g, 7.5 mmol) were added and the solution was allowed to warm to room temperature for 1 h with a limited addition of acetic acid to keep the pH at 7. After evaporation, the solution was extracted with water and then diethyl ether. The organic phase was extracted with brine, dried over Na_2SO_4, filtered and then evaporated. Purification of the residue by flash chromatography (CH_2Cl_2/methanol/NH_4OH, 90:9:1) gave 0.4 g (yield: 46%) of *trans*-**3d**, mp 130 °C. ^{1}H NMR ($CDCl_3$) δ 8.1 (s, 1H, *H*CCCl), 7.6 (m, 1H, NH), 7.3 (m, 5H, H$_{ar}$), 6.3 (s, 1H, *H*CCOCH$_3$), 4.6 (m, 2H, NH$_2$), 3.9 (s, 2H, C*H*$_2$NH), 3.8 (s, 3H, OCH$_3$), 2.8 (m, 1H, C*H*NHCO), 2.5 (m, 1H, NH), 2.2 (m, 1H, C*H*NHCH$_2$), 1.0 and 0.7 (m, 2H, CH$_2$); ^{13}C NMR ($CDCl_3$) δ 166 (CONH), 157 (*C*$_{ar}$OCH$_3$), 147 (C$_{ar}$NH$_2$), 140 (C$_{ar}$CH$_2$), 133 (*C*$_{ar}$HCCl), 128 (C$_{ar}$), 111 (C$_{ar}$Cl and *C*$_{ar}$CONH), 98 (*C*$_{ar}$HCOCH$_3$), 56 (OCH$_3$), 53 (NHCH$_2$Ph), 38 (*C*HNHCH$_2$), 31 (*C*HNHCO), 15 (CH$_2$). Anal. calcd for $C_{18}H_{20}ClN_3O_2.1/2H_2O$: C, 60.93; H, 5.97; N, 11.84. Found: C, 61.03; H, 6.04; N, 11.86.

(±)-(*cis*)-4-Amino-*N*-(2-benzylamino-1-cyclopropyl)-5-chloro-2-methoxybenzamide (*cis*-3d). Following the procedure described for *trans*-**3d**, benzamide *cis*-**3a** (0.6 g, 2 mmol) gave 0.4 g (yield: 56%) of *cis*-**3d**, mp 140 °C. ^{1}H NMR ($CDCl_3$) δ 8.2 (m, 1H, NH), 8.1 (s, 1H, *H*CCCl), 7.3 (m, 5H, Har), 6.3 (s, 1H, *H*CCOCH$_3$), 4.4 (m, 2H, NH$_2$), 3.9 (s, 2H, C*H*$_2$NH), 3.8 (s, 3H, OCH$_3$), 3.3 (m, 1H, C*H*NHCO), 2.4 (m, 1H, C*H*NHCH$_2$), 1.0 (m, 1H, CH$_{2trans}$), 0.4 (m, 1H, CH$_{2cis}$). Anal. calcd for $C_{18}H_{20}ClN_3O_2.1/2H_2O$: C, 60.93; H, 5.97; N, 11.84. Found: C, 60.90; H, 5.95; N, 11.75.

(±)-(*trans*)-4-Amino-5-chloro-2-methoxy-*N*-{2-[*N*′,*N*′-bis-(phenylmethyl)amino]-1-cyclopropyl}benzamide (*trans*-3e). A solution of *trans*-**3a** (0.5 g, 1.7 mmol) in DMF (5 mL) was treated overnight with potassium carbonate (0.3 g, 2.16 mmol), benzyl chloride (0.25 g, 2 mmol) and potassium iodide (0.01 g). After refluxing for 96 h, the solution was evaporated and then treated with water and extracted with ethyl acetate. After washing with brine, the organic phase was dried over $MgSO_4$, filtered and evaporated to give 0.44 g (yield: 59%) of *trans*-**3e**, mp 154 °C. ^{1}H NMR ($CDCl_3$) δ 8.1 (s, 1H, *H*CCCl), 7.6 (m, 1H, NH), 7.3 (m, 10H, H$_{ar}$), 6.3 (s, 1H, *H*CCOCH$_3$), 4.4 (m, 2H, NH$_2$), 3.8 (s, 3H, OCH$_3$), 3.7,(4H, C*H*$_2$N), 2.8 (m, 1H, C*H*NH), 2.0 (m, 1H, CHN), 1.0 and 0.7 (m, 2H, CH$_2$). Anal. calcd for $C_{25}H_{26}ClN_3O_2$: C, 68.12; H, 6.19; N, 9.41. Found: C, 68.5; H, 6.01; N, 9.64.

(±)-(*trans*)-4-Amino-*N*-[2-(*N*-benzyl-*N*-methylamino)-1-cyclopropyl]-5-chloro-2-methoxybenzamide (*trans*-3f). Following the procedure described for **3d**, benzamide *trans*-**3d** (0.4 g, 2 mmol) and 0.15 mL (2 mmol) of formaldehyde (37% in methanol) gave 0.2 g (yield: 48%) of *trans*-**3f**, mp 137 °C. ^{1}H NMR ($CDCl_3$) δ 8.1 (s, 1H, *H*CCCl), 7.6 (m, 1H, NH), 7.3 (m, 5H, H$_{ar}$), 6.3 (s, 1H, *H*CCOCH$_3$), 4.5 (m, 2H, NH$_2$), 4.0–3.6 (m, 2H, CH$_2$N), 3.8 (s, 3H, OCH$_3$), 2.9 (m, 1H, C*H*NH), 2.3 (s, 3H, NCH$_3$), 1.9 (m, 1H, CHN), 1.0 and 0.8 (m, 2H, CH$_2$). Anal. calcd for $C_{19}H_{22}ClN_3O_2$: C, 63.42; H, 6.16; N, 11.68. Found: C, 63.75; H, 6.33; N, 11.51.

(±)-(*cis*)-4-Amino-*N*-[2-(*N*-benzyl-*N*-methylamino)-1-cyclopropyl]-5-chloro-2-methoxybenzamide (*cis*-3f). Following the procedure described for **3d**, benzamide *cis*-**3d** (0.2 g, 1 mmol) and 0.1 mL (1.5 mmol) of formaldehyde (37% in methanol) gave 0.08 g (yield: 39%) of *cis*-**3f** by recrystallization in diethyl ether, mp 157 °C. ^{1}H NMR ($CDCl_3$) δ 8.2 (m, 1H, NH), 8.1 (s, 1H, *H*CCCl), 7.3 (m, 5H, H$_{ar}$), 6.2 (s, 1H, *H*CCOCH$_3$), 4.4 (m, 2H, NH$_2$), 3.9 (m, 2H, CH$_2$N), 3.6 (s, 3H, OCH$_3$), 3.4 (m, 1H, C*H*NH), 2.2 (s, 3H, NCH$_3$), 2.0 (m, 1H, CHN), 1.0 (m, 1H, CH$_{2trans}$), 0.4 (m, 1H, CH$_{2cis}$). Anal. calcd for $C_{19}H_{22}ClN_3O_2$: C, 63.42; H, 6.16; N, 11.68. Found: C, 63.75; H, 6.33; N, 11.51.

Binding assays

Human dopamine D_2 and D_3 receptors. Chinese hamster ovary (CHO) cells expressing human dopamine D_3 (hD_3) or D_2 (short) receptors (hD_2) were prepared as described.[22] Binding assays was carried out by incubating

10 μg of membrane protein with [^{125}I]iodosulpride (2000 Ci/mmol, Amersham, 0.1 nM for hD_2 and 0.2 nM for hD_3) and 7 concentrations of competing ligands in triplicate (final volume 0.5 mL) in a buffer containing Tris–HCl 50 mM (pH 7.5), NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 5 mM, bovine serum albumin (0.2% w/v) and ascorbic acid (0.1% w/v) for 30 min at 30°C. Nonspecific binding was defined using 10 μM raclopride in triplicate.

Bovine D_2 receptor. Frozen striata from female bovine brains (Cellubio, France) were used in all studies and were stored at −80 °C prior to use. The tissue was dissected and homogenised in 40 volumes of buffer solution (ice-cold Tris, 50 mM, pH 7.4 at 23 °C) with a Polytron. The homogenate was centrifuged at 3000 *g* for 5 min. The supernatant was then homogenised 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 buffer solution was added, the pellet was homogenised 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 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. Nonspecific binding was determined by the addition of 10 μM butaclamol in duplicate. Total binding was defined in quadruplicate.

5-HT_3 and 5-HT_4 receptors. Binding assays were performed according to the methods previously described.[19]

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