JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 209–218

Received July 27, 2005
Accepted October 24, 2005
DOI 10.1211/jpp.58.2.0008
ISSN 0022-3573

Synthesis and binding profile of constrained analogues of *N*-[4-(4-arylpiperazin-1-yl)butyl]-3-methoxybenzamides, a class of potent dopamine D_3 receptor ligands

Marcello Leopoldo, Enza Lacivita, Nicola A. Colabufo, Francesco Berardi and Roberto Perrone

Abstract

We recently reported on a series of *N*-[4-(4-arylpiperazin-1-yl)butyl]-3-methoxybenzamides, endowed with high affinity for dopamine D_3 receptors, but lacking of selectivity over D_4, D_2, 5-HT_{1A}, and α_1-receptors. To improve the D_3-receptor affinity and selectivity, without causing a considerable increasing in the lipophilicity, the flexible butyl linker was replaced by a more conformationally constrained cyclohexyl linker. The new *cis*- and *trans*-*N*-[4-(4-aryl-1-piperazinyl)cyclohexyl]-3-methoxybenzamides (Aryl = 2,3-di-Cl-Ph, 2-CH_3O-Ph, 4-Cl-Ph, 2,3-di-CH_3-Ph) were tested in-vitro for their binding affinity for D_3, D_4, D_2, 5-HT_{1A}, and α_1-receptors. The *trans*- derivatives were found to be more potent at D_3 receptor than the corresponding *cis*- isomers, but less potent than the opened counterparts. This reflected negatively on the selectivity over the other studied receptors. Derivative *trans*-*N*-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*trans*-**7**) showed high D_3-receptor affinity (K_i = 0.18 nM) and a relevant selectivity over D_4, D_2, 5-HT_{1A}, and α_1-receptors (>200-fold). This compound was characterized as a full agonist at D_3 receptor when tested in the Eu-GTP binding assay.

Introduction

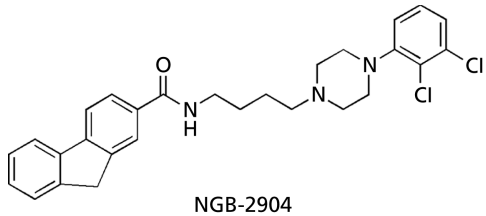
The five known mammalian dopamine receptor subtypes (D_1–D_5), which, on the basis of protein homology and function, can be divided into two receptor families, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4), are all G-protein coupled receptors. The D_3-receptor subtype was first cloned and characterized by Sokoloff et al (1990). The greatest density of D_3 receptor mRNA in rat brain were found primarily in limbic brain areas (islands of Calleja, ventral striatum/nucleus accumbens, dentate gyrus, and striate cortex). Expression of D_3 receptor mRNA in the human brain follows a similar pattern as in the rodent brain. The distribution pattern of D_3 receptors in rodent and human brain is compatible with a major role in emotion, cognition, and processing of motor and sensory information (Levant 1997). On the basis of such distribution the D_3 receptor has been proposed as an appropriate target for the treatment of neuropsychiatric disorders. Furthermore, it has been shown that D_3 receptor participated in the therapeutic action and unwanted side-effects (dyskinesia) of levodopa. Thus, the therapeutic use of D_3 agents for Parkinson's disease has been proposed (Bezard et al 2003). Moreover, pramipexole, a D_2/D_3 agent, is effective in early stages of Parkinson's disease and an effective adjunct therapy to levodopa in treating late Parkinson's disease (Biglan & Holloway 2002). Recent studies have strengthened the likehood that the D_3 receptors are significantly involved in the mechanisms of drug dependence and abuse. In fact, selective D_3 receptor antagonists can reduce cocaine-, nicotine-, ethanol-, and heroin-seeking behaviours (Heidbreder et al 2005).

During the last decade a considerable research effort has been made toward the identification of potent and selective dopamine D_3 receptor ligands. One of the most thoroughly studied class of dopamine D_3 receptor agents is represented by *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamide

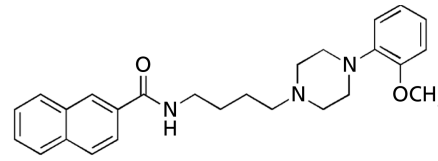
Università degli Studi di Bari, Dipartimento Farmaco-Chimico, via Orabona, 4, 70125 Bari, Italy

Marcello Leopoldo, Enza Lacivita, Nicola A. Colabufo, Francesco Berardi and Roberto Perrone

Correspondence: M. Leopoldo, Università degli Studi di Bari, Dipartimento Farmaco-Chimico, via Orabona, 4, 70125 Bari, Italy. E-mail: leopoldo@farmchim.uniba.it



NGB-2904



BP-897

Figure 1 Structures of D_3 receptor agents.

derivatives (Hackling & Stark 2002; Luedtke & Mach 2003). The well-known antagonist NGB 2904 and the partial agonist BP-897 (Figure 1) belong to this class. In a previous paper (Leopoldo et al 2002) we reported the structure–affinity relationships of a series of *N*-[4-(4-arylpiperazin-1-yl)butyl]-3-methoxybenzamides, including the high-affinity dopamine D_3 receptor ligands **1–4** (Table 1). We have shown that the replacement of the 3-methoxyphenyl ring of compounds **1–4** with a bicyclic aromatic system increased the specificity for the D_3 receptor, as for compounds **5** and **6** (Table 1). However, this modification caused a considerable increase in the lipophilicity. In fact derivatives **5** and **6** displayed higher ClogP values (calculated logarithm of the *n*-octanol/water partition coefficient) than compounds **1–4** (Table 1). In a recent paper, Newman et al (2003) have pointed out that the high lipophilicity of *N*-{4-[4-(2,3-dichlorophenyl) piperazin-1-yl]butyl} arylcarboxamides could represent a limit concerning their bioavailability. With the aim to obtain new, potent, and selective D_3-receptor ligands we have performed structural modifications on *N*-[4-(4-arylpiperazin-1-yl)butyl]-3-methoxybenzamides **1–4**. In particular, because the tetramethylene spacer of **1–4** can adopt various conformations, allowing the interaction with different receptors, it was blocked within a cyclohexane ring originating the compounds *cis*- and *trans*-**7–10** (Table 2). The proposed modification led to a limited increase in lipophilicity. In fact, the constrained compounds displayed ClogP values (Table 2) slightly higher as compared with the opened counterparts **1–4**, but significantly lower than those of derivatives **5** and **6** (Table 1).

Materials and Methods

Synthesis

Column chromatography was performed with 1:30 ICN silica gel 60A (63–200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H, N) were performed on Eurovector Euro EA 3000 analyser; the analytical results were within ±0.4% of the theoretical values for the formula given. ^{1}H NMR spectra were recorded either on a Varian EM-390 where indicated 90 MHz (TMS as internal standard) or on a Varian Mercury-VX spectrometer (300 MHz), with $CDCl_3$ as solvent. All chemical shift values were reported in ppm (δ). 2-D NMR experiments (COSY and NOESY) of compounds *cis*- and *trans*-**8** were performed on a Varian NMR 300 Mercury-VX (300 MHz) instrument. Recording of mass spectra was done on an HP6890–5973 MSD gas chromatograph/mass spectrometer; only significant *m/z* peaks, with their percentage of relative intensity in parentheses, were reported. All spectra were in accordance with the assigned structures. The purity of new compounds that were essential to the conclusions drawn in the text was determined by HPLC on a Perkin-Elmer series 200 LC instrument using a Phenomenex Prodigy ODS-3 RP-18 column, (250 × 4.6 mm, 5 μm particle size) and equipped with a Perkin-Elmer 785A UV/vis detector setting $\lambda = 254$ nm. All compounds were eluted with $CH_3OH/H_2O/Et_3N$, 4:1:0.01, v/v, at a flow rate of 1 mL min^{-1}. A standard procedure was used to transform final compounds into their hydrochloride salts.

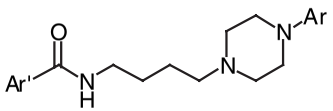
trans-N-*(4-Hydroxycyclohexyl)-3-methoxybenzamide (**11**)*

To a cooled mixture containing *trans*-4-aminocyclohexanol (3.00 g, 26.0 mmol) in 1.2% aqueous NaOH (104 mL) was added dropwise under vigorous stirring a CH_2Cl_2 solution (50 mL) of 3-methoxybenzoylchloride, prepared from 3-methoxybenzoic acid (4.74 g, 31.2 mmol) and $SOCl_2$ (5 mL). The aqueous layer was separated and extracted twice with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude residue was chromatographed ($CHCl_3$/AcOEt, 1:1 as eluent) to give pure **11** as a white semisolid (4.80 g, 74% yield). ^{1}H NMR: δ 1.20–1.35 and 1.87–1.96 (m, 8H, cyclohexylic CH_2), 2.61 (br s, 1H, OH, D_2O exchanged), 3.42–3.52 (m, 1H, C*H*OH), 3.73 (s, 3H, CH_3), 3.75–3.87 (m, 1H, NHC*H*), 6.55 (br d, 1H, NH), 6.88–6.91 and 7.18–7.26 (m, 4H, aromatic). GC-MS *m/z* 250 ($M^+ + 1$, 6), 249 (M^+, 36), 152 (43), 151 (30), 135 (100).

trans-N-*(4-Hydroxycyclohexyl)-3-methoxybenzamide methanesulfonate (**12**)*

Triethylamine (4.0 mL, 29 mmol) and methanesulfonyl chloride (1.6 mL, 21 mmol) were added to a solution of alcohol **11** (4.73 g, 19 mmol) in CH_2Cl_2 cooled to −10°C. The mixture was stirred at room temperature for 6 h. The reaction mixture was washed first with a saturated aqueous solution of $NaHCO_3$ and then with 3 M HCl. The separated organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was chromatographed ($CHCl_3$/AcOEt, 1:1 as eluent) to give pure **12** as a white semisolid (1.34 g, 21% yield). ^{1}H NMR: δ 1.32–1.48 and 1.69–1.91 (m, 4H, cyclohexylic CH_2), 2.15–2.21 (m, 4H, cyclohexylic CH_2), 3.02 (s, 3H, SO_2CH_3), 3.84 (s, 3H, OCH_3), 3.93–4.04 (m, 1H, NHC*H*), 4.61–4.71 (m, 1H, $CHOSO_2$), 5.97 (br d, 1H, NH), 7.01–7.05 and 7.21–7.34 (m, 4H, aromatic).

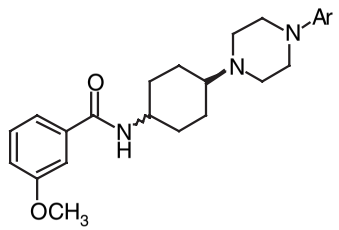
Table 1 Binding affinities of the reference compounds[a]



Compound	Ar′	Ar	ClogP[a]	K_i, nM[b]					Selectivity, K_i ratio			
				D_3	D_4	D_2	5-HT_{1A}	α_1	D_4/D_3	D_2/D_3	5-HT_{1A}/D_3	α_1/D_3
1	3-CH_3O-Ph	2,3-di-Cl-Ph	4.50	0.27 ± 0.03	9.0 ± 0.8	1410 ± 80	123 ± 15	134 ± 9	33	5222	456	496
2	3-CH_3O-Ph	2-CH_3O-Ph	3.00	1.7 ± 0.5	3.1 ± 0.7	680 ± 18	7.5 ± 1.1	26.4 ± 2.2	1.8	400	4.4	15
3	3-CH_3O-Ph	4-Cl-Ph	3.86	0.41 ± 0.05	25 ± 2	2350 ± 270	397 ± 92	406 ± 40	61	5732	968	990
4	3-CH_3O-Ph	2,3-di-CH_3-Ph	3.92	0.17 ± 0.05	63.6 ± 8.0	77.0 ± 5.2	268 ± 12	717 ± 24	374	453	1576	4218
5	1,1′-Biphenyl	2,3-di-Cl-Ph	6.00	1.15 ± 0.30	283 ± 15	>1000 (32%)[c]	>1000 (41%)	>1000 (43%)	246	>870	>870	>870
6	2-Naphthyl	2,3-di-Cl-Ph	5.28	0.58 ± 0.02	370 ± 80	5200 ± 350	335 ± 21	57 ± 5	638	8966	578	98

[a]Calculated using the ClogP 4.0 software (version for Windows), BioByte Corp., Claremont, CA. [b]Data taken from Leopoldo et al (2002). The values are the means ± s.e.m. from three independent experiments in triplicate ($P < 0.0001$). Individual difference between the various compounds have been examined using Tukey's post-hoc test ($P < 0.0001$). Difference in the *K*i values between the receptors for each compound have been analysed using the MannWhitney U test ($P = 0.005$, U = 13.50). Hill plot of listed compounds was between 0.9 and 1.2. [c]Full K_i not obtained, percentage inhibition at the concentration shown given in parentheses.

Table 2 Binding affinities of the target compounds *cis*- and *trans*-**7**–**10**



Compound	Ar	ClogP[a]	K_i, nM[b]					Selectivity, K_i ratio			
			D_3	D_4	D_2	5-HT_{1A}	α_1	D_4/D_3	D_2/D_3	5-HT_{1A}/D_3	α_1/D_3
cis-**7**	2,3-di-Cl-Ph	4.76	117 ± 15	336 ± 20	>800 (36%)[c]	867 ± 80	484 ± 10	2.9	>6.7	7.4	4.1
trans-**7**	2,3-di-Cl-Ph	4.76	0.18 ± 0.025	>850 (31%)	38.1 ± 5.0	499 ± 25	62 ± 8.0	>4722	212	2772	344
cis-**8**	2-CH_3O-Ph	3.23	>850 (42%)	635 ± 30	>800 (26%)	873 ± 45	502 ± 25	NC[d]	NC	NC	NC
trans-**8**	2-CH_3O-Ph	3.23	8.4 ± 0.60	>850 (27%)	79 ± 2.5	36.5 ± 5.4	117 ± 30	>101	9.4	4.3	14
cis-**9**	4-Cl-Ph	4.11	970 ± 40	3.4 ± 0.55	>800 (40%)	940 ± 65	382 ± 25	0.004	NC	1	0.4
trans-**9**	4-Cl-Ph	4.11	31 ± 2.2	>850 (25%)	>800 (44%)	2.84 ± 0.70	46 ± 6.0	>27	>26	0.09	1.5
cis-**10**	2,3-di-CH_3-Ph	4.18	730 ± 45	1346 ± 200	>5000 (5%)	NT[e]	NT	1.8	>6.8	NC	NC
trans-**10**	2,3-di-CH_3-Ph	4.18	38.1 ± 9.1	>5000 (12%)	>5000 (15%)	NT	NT	>131	>131	NC	NC

[a]Calculated using the ClogP 4.0 software (version for Windows), BioByte Corp., Claremont, CA. [b]The values are the means ± s.e.m. from three independent experiments in triplicate ($P < 0.0001$). Individual difference between the various compounds have been examined using Tukey's post hoc test ($P < 0.0001$). Difference in the K_i values between the receptors for each compound have been analysed using the MannWhitney U test ($P = 0.005$, U = 13.50). Hill plot of listed compounds was between 0.9 and 1.2. [c]Full K_i not obtained, percentage inhibition at the concentration shown given in parentheses. [d]Not calculated. [e]Not tested.

trans-N-*(4-Aminocyclohexyl)-3-methoxybenzamide (**13**)*

A solution of 3-methoxybenzoylchloride in CH_2Cl_2, prepared by refluxing 3-methoxybenzoic acid (1.98 g, 13.0 mmol) in $SOCl_2$ (5 mL), was added dropwise to a solution of *trans*-1,4-diaminocyclohexane (2.96 g, 26.0 mmol) in CH_2Cl_2, cooled to −60°C. The reaction mixture was allowed to warm at room temperature. The solid was filtered off and the filtrate was extracted twice with 3 M HCl. The combined aqueous layers were alkalized with aqueous 10% NaOH and the resulting suspension was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness under reduced pressure to give pure **13** as a white semisolid (0.80 g, 25% yield). ^{1}H NMR (90 MHz): δ 1.20–1.35 and 1.87–1.96 (m, 10H, cyclohexylic CH_2, NH_2, 2H D_2O exchanged), 3.42–3.52 (m, 1H, NH_2CH), 3.73–3.78 (m, 1H, NHC*H*), 3.80 (s, 3H, CH_3), 6.53 (br d, 1H, NH), 7.18–7.24 (m, 4H, aromatic). GC-MS *m/z* 249 (M^++1, 5), 248 (M^+, 31), 135 (100).

*General procedure for preparation of alcohols **14a–d***

A mixture of the appropriate aniline (1.0 mmol), 2-chloroethanol (5.0 mmol) and $CaCO_3$ (1.2 mmol) in H_2O (10 mL) was refluxed for 7 h. The hot solution was filtered and the residue was washed with hot H_2O. The filtrate was saturated with NaCl and extracted twice with diethyl ether. The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed as detailed below to give compounds **14a–d** as brown oils.

*2,2′-[(2,3-Dichlorophenyl)imino]bis-ethanol (**14a**)*

Eluted with $CHCl_3$/AcOEt, 1:1. Yield 31%. ^{1}H NMR: δ 2.62 (s, 2H, 2 OH, D_2O exchanged), 3.28 [t, 4H, $N(CH_2)_2$, J = 5.1 Hz], 3.60 (t, 4H, 2 CH_2OH, J = 5.2 Hz), 7.14–7.29 (m, 3H, aromatic).

*2,2′-[(2-Methoxyphenyl)imino]bis-ethanol (**14b**)*

Eluted with $CHCl_3$/MeOH, 19:1. Yield 65%. ^{1}H NMR (90 MHz): δ 2.95 (s, 2H, 2 OH, D_2O exchanged), 3.05–3.20 [m, 4H, $N(CH_2)_2$], 3.40–3.60 (m, 4H, 2 CH_2OH), 3.80 (s, 3H, CH_3), 6.80–7.30 (m, 4H, aromatic).

*2,2′-[(4-Chlorophenyl)imino]bis-ethanol (**14c**)*

Eluted with $CHCl_3$/AcOEt, 1:1. Yield 27%. ^{1}H NMR (90 MHz): δ 3.02 (s, 2H, 2 OH, D_2O exchanged), 3.30–3.80 [m, 8H, $N(CH_2CH_2)_2$], 6.50–6.70 (m, 4H, aromatic).

*2,2′-[(2,3-Dimethylphenyl)imino]bis-ethanol (**14d**)*

Eluted with $CHCl_3$/AcOEt, 1:1. Yield 47%. ^{1}H NMR: δ 2.27 and 2.28 (2 s, 6H, 2 CH_3), 2.87 (br s, 2H, 2 OH, D_2O exchanged), 3.16 [t, 4H, $N(CH_2)_2$, J = 5.2 Hz], 3.58 (t, 4H, 2 CH_2OH, J = 5.2 Hz), 6.95–7.08 (m, 3H, aromatic).

*2,2′-[(2,3-Dichlorophenyl)imino]bis-ethanol methanesulfonate (**15a**)*

Compound **15a** was prepared from alcohol **14a** following the procedure described above for preparation of **12**. The crude residue was chromatographed ($CHCl_3$/AcOEt, 9:1 as eluent) to give pure **15a** as a brown oil in 51% yield. ^{1}H NMR: δ 2.96 and 2.97 (2 s, 6H, 2 CH_3), 3.56–3.61 [m, 4H, $N(CH_2)_2$], 4.21–4.24 (m, 4H, 2 CH_2O), 7.19–7.29 (m, 3H, aromatic).

N,N-*bis-(2-Bromoethyl)-2-methoxybenzenamine (**15b**)*

PBr_3 (2.0 mL, 21.3 mmol) was added dropwise to alcohol **14b** (1.50 g, 7.1 mmol), then the mixture was heated under reflux for 3 h. After cooling, H_2O (10 mL) was cautiously added and the mixture was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed (petroleum ether/AcOEt, 4:1 as eluent) to give the title compound as a colourless oil (0.75 g, 31% yield). ^{1}H NMR (90 MHz): δ 3.20–3.65 (m, 8H, CH_2), 3.85 (s, 3H, CH_3), 6.80–7.25 (m, 4H, aromatic). GC-MS *m/z* 339 (M^++2, 15), 337 (M^+, 30), 244 (100), 242 (99), 135 (48).

N,N-*bis-(2-Bromoethyl)-4-chlorobenzenamine (**15c**)*

As described above, the title compound was obtained from alcohol **14c** in 43% yield. ^{1}HNMR (90 MHz): δ 3.20–3.45 (m, 4H, 2 CH_2Br), 3.50–3.85 [m, 4H, $N(CH_2)_2$], 6.45—6.70 and 7.05–7.30 (m, 4H, aromatic). GC-MS *m/z* 343 (M^++2, 20), 341 (M^+, 28), 250 (33), 248 (100), 246 (83).

*2,2′-[(2,3-Dimethylphenyl)imino]bis-ethanol methanesulfonate (**15d**)*

This compound was prepared from alcohol **14d** as described above for preparation of **12**. The crude residue was chromatographed ($CHCl_3$/AcOEt, 1:1 as eluent) to give pure **15d** as a brown oil in 50% yield. ^{1}H NMR: δ 2.25 and 2.26 (2 s, 6H, 2 CH_3), 2.96 and 2.97 (2 s, 6H, 2 SO_2CH_3), 3.56–3.61 [m, 4H, $N(CH_2)_2$], 4.21–4.24 (m, 4H, 2 CH_2O), 6.85–7.15 (m, 3H, aromatic).

General procedure for the synthesis of compounds cis-***7–10***

A stirred mixture of methanesulfonate **12** (2.0 mmol), the appropriate 1-arylpiperazine (2.4 mmol), and a slight excess of K_2CO_3 in acetonitrile was refluxed for seven days. After cooling, the mixture was evaporated to dryness and H_2O was added to the residue. The aqueous phase was extracted twice with $CHCl_3$. The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed ($CHCl_3$/AcOEt, 1:1 as eluent) to give the target compounds *cis*-**7**–**10** as pale yellow semisolids in 9% yield.

cis-N-*{4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide* (cis-***7***)

^{1}H NMR: δ 1.61–2.00 (m, 8H, cyclohexylic CH_2), 2.31–2.36 [m, 1H, C*H*$N(CH_2)_2$], 2.75 [br s, 4H, $CHN(CH_2)_2$], 3.08 [br s, 4H, $(CH_2)_2NAr$], 3.85 (s, 3H, CH_3), 4.21–4–30 (m, 1H, NHC*H*), 6.20 (br d, 1H, NH), 6.94–7.35 (m, 7H, aromatic). GC-MS *m/z* 463 (M^++2, 4), 461 (M^+, 7), 271 (64), 269 (100), 261 (37), 135 (43). The hydrochloride salt melted at 224–225°C (from CH_3OH/diethyl ether). Anal. ($C_{24}H_{29}Cl_2N_3O_2$•HCl•0.2H_2O) C, H, N.

cis-N-*{4-[4-(2-Methoxyphenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*cis-***8***)*
^{1}H NMR: δ 1.63–1.74 and 1.86–1.95 (m, 8H, cyclohexylic CH_2), 2.31–2.36 [m, 1H, C*H*N$(CH_2)_2$], 2.79 [br s, 4H, CHN(C*H*$_2)_2$], 3.13 [br s, 4H, (C*H*$_2)_2$NAr], 3.85 and 3.86 (2 s, 6H, 2 CH_3), 4.21–4.30 (m, 1H, NHC*H*), 6.25 (br d, 1H, NH), 6.85–6.98 and 7.25–7.36 (m, 8H, aromatic). GC-MS *m/z* 424 (M^+ + 1, 13), 423 (M^+, 47), 261 (27), 231 (100), 135 (43). The free base melted at 164–165°C (from $CHCl_3$/petroleum ether). Anal. ($C_{25}H_{33}N_3O_3$) C, H, N.

cis-N-*{4-[4-(4-Chlorophenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*cis-***9***)*
^{1}H NMR: δ 1.59–1.94 (m, 8H, cyclohexylic CH_2), 2.25–2.29 [m, 1H, C*H*N$(CH_2)_2$], 2.71 [app. t, 4H, CHN(C*H*$_2)_2$], 3.17 [app. t, 4H, (C*H*$_2)_2$NAr], 3.89 (s, 3H, CH_3), 4.21–4.24 (m, 1H, NHC*H*), 6.17 (br d, 1H, NH), 6.81–6.86, 7.00–7.04, and 7.18–7.35 (m, 8H, aromatic). GC-MS *m/z* 429 (M^+ + 2, 12), 427 (M^+, 33), 261 (68), 237 (34), 235 (100), 135 (43). The free base melted at 169–170°C (from $CHCl_3$/*n*-hexane). Anal. ($C_{24}H_{30}ClN_3O_2$) C, H, N.

cis-N-*{4-[4-(2,3-Dimethylphenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*cis-***10***)*
^{1}H NMR: δ 1.61–1.76 and 1.83–1.94 (m, 8H, cyclohexylic CH_2), 2.22 and 2.27 (2 s, 6H, 2 CH_3), 2.28–2.33 [m, 1H, C*H*N$(CH_2)_2$], 2.73 [br s, 4H, CHN(C*H*$_2)_2$], 2.93 [br t, 4H, (C*H*$_2)_2$NAr], 3.86 (s, 3H, OCH_3), 4.21–4.30 (m, 1H, NHC*H*), 6.21 (br d, 1H, NH), 6.89–6.94, 7.01–7.10, and 7.25–7.35 (m, 7H, aromatic). GC-MS *m/z* 422 (M^+ + 1, 9), 421 (M^+, 32), 406 (24), 261 (54), 229 (100), 135 (37). The hydrochloride salt melted at 223–225°C (from CH_3OH/diethyl ether). Anal. ($C_{26}H_{35}N_3O_2$•HCl•0.8H_2O) C, H, N.

General procedure for the preparation of compounds trans-***7–10***
A stirred mixture of amine **13** (2.0 mmol), the appropriate alkylating agent **15a–d** (2.4 mmol), and a slight excess of Na_2CO_3 in acetonitrile was refluxed overnight. After cooling, the mixture was evaporated to dryness and H_2O was added to the residue. The aqueous phase was extracted twice with $CHCl_3$. The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed ($CHCl_3$/MeOH, 19:1 as eluent) to give the target compounds *trans*-**7–10** as semisolids.

trans-N-*{4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*trans-***7***)*
Yield 77%. ^{1}H NMR: δ 1.22–1.34 [m, 2H, NCH(C*H*H)$_2$], 1.42–1.54 [m, 2H, NCH(CH*H*)$_2$], 2.00–2.03 [m, 2H, NHCH(C*H*H)$_2$], 2.18–2.21 [m, 2H, NHCH(CH*H*)$_2$], 2.33–2.40 [m, 1H, NC*H*(CHH)$_2$], 2.76 [br t, 4H, CHN(C*H*$_2)_2$], 3.07 [br s, 4H, (C*H*$_2)_2$NAr], 3.84 (s, 3H, CH_3), 3.85–3.97 [m, 1H, NHC*H*(CHH)$_2$], 5.95 (br d, 1H, NH), 6.93–7.35 (m, 7H, aromatic). GC-MS *m/z* 463 (M^+ + 2, 7), 461 (M^+, 11), 310 (23), 271 (65), 269 (100), 135 (50). The free base melted at 199–200 °C (from $CHCl_3$/*n*-hexane). Anal. ($C_{24}H_{29}Cl_2N_3O_2$) C, H, N.

trans-N-*{4-[4-(2-Methoxyphenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*trans-***8***)*
Yield 72%. ^{1}H NMR: δ 1.23–1.35 [m, 2H, NCH(C*H*H)$_2$], 1.44–1.56 [m, 2H, NCH(CH*H*)$_2$], 2.02–2.06 [m, 2H, NHCH(C*H*H)$_2$], 2.17–2.21 [m, 2H, NHCH(CH*H*)$_2$], 2.35–2.43 [m, 1H, NC*H*(CHH)$_2$], 2.81 [br s, 4H, CHN(C*H*$_2)_2$], 3.12 [br s, 4H, (C*H*$_2)_2$NAr], 3.85 and 3.87 (2s, 6H, 2 CH_3), 3.86–3.98 [m, 1H, NHC*H*(CHH)$_2$], 5.99 (br d, 1H, NH), 6.85–7.05 and 7.24–7.35 (m, 8H, aromatic). GC-MS *m/z* 424 (M^+ + 1, 14), 423 (M^+, 48), 408 (20), 261 (19) 231 (100), 135 (40). The hydrochloride salt melted at > 250°C (from CH_3OH/diethyl ether). Anal. ($C_{25}H_{33}N_3O_3$•HCl•0.3H_2O) C, H, N.

trans-N-*{4-[4-(4-Chlorophenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*trans-***9***)*
Yield 25%. ^{1}H NMR: δ 1.22–1.33 [m, 2H, NCH(C*H*H)$_2$], 1.42–1.54 [m, 2H, NCH(CH*H*)$_2$], 1.99–2.03 [m, 2H, NHCH(C*H*H)$_2$], 2.18–2.21 [m, 2H, NHCH(CH*H*)$_2$], 2.32–2.43 [m, 1H, NC*H*(CHH)$_2$], 2.74 [br t, 4H, CHN(C*H*$_2)_2$], 3.17 [br t, 4H, (C*H*$_2)_2$NAr], 3.85 (s, 3H, CH_3), 3.86–3.95 [m, 1H, NHC*H*(CHH)$_2$], 5.90 (br d, 1H, NH), 6.82–6.86, 7.00–7.04, and 7.18–7.35 (m, 8H, aromatic). GC-MS *m/z* 429 (M^+ + 2, 14), 427 (M^+, 41), 261 (45), 237 (32), 235 (100), 135 (40). The hydrochloride salt melted at > 250 °C (from CH_3OH/diethyl ether). Anal. ($C_{24}H_{30}ClN_3O_2$•HCl•0.5H_2O) C, H, N.

trans-N-*{4-[4-(2,3-Dimethylphenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*trans-***10***)*
Yield 15%. ^{1}H NMR: δ 1.23–1.34 [m, 2H, NCH(C*H*H)$_2$], 1.44–1.71 [m, 2H, NCH(CH*H*)$_2$], 2.01–2.05 [m, 2H, NHCH(C*H*H)$_2$], 2.15–2.20 [m, 2H, NHCH(CH*H*)$_2$], 2.22 and 2.26 (2 s, 6H, 2 CH_3), 2.33–2.38 [m, 1H, NC*H*(CHH)$_2$], 2.75 [br s, 4H, CHN(C*H*$_2)_2$], 2.93 [app t, 4H, (C*H*$_2)_2$NAr], 3.85 (s, 3H, OCH_3), 3.92–3.96 [m, 1H, NHC*H*(CHH)$_2$], 5.90 (br d, 1H, NH), 6.88–6.94, 7.01–7.10 and 7.23–7.35 (m, 7H, aromatic). GC-MS *m/z* 422 (M^+ + 1, 9), 421 (M^+, 29), 261 (31), 229 (100), 135 (34). The hydrochloride salt melted at 250 °C (dec.) (from CH_3OH/diethyl ether). Anal. ($C_{26}H_{35}N_3O_2$•HCl•H_2O) C, H, N.

Pharmacology

Human recombinant $D_{4.4}$ dopamine receptor expressed in CHO cells, human recombinant D_{2L} dopamine receptor expressed in Sf9 cells, and rat recombinant D_3 dopamine receptor expressed in Sf9 cells were obtained from RBI (Research Biochemicals International, Natick, MA, USA). For receptor binding studies, the compounds were dissolved in absolute ethanol. Male Wistar Hannover rats (200–250 g) were from Harlan (S. Pietro al Natisone, Italy). The animals were handled according to internationally accepted principles for care of laboratory animals (E.E.C. Council Directive 86/609, O.J. No. L358, December 18, 1986). 8-OH-DPAT hydrobromide was from RBI (Research Biochemicals International, Natick, MA, USA). Haloperidol, phentolamine hydrochloride and clozapine were from Sigma-Aldrich (Milan, Italy); [^{3}H]prazosin, [^{3}H]8-OH-DPAT and

[^{3}H]spiroperidol and Eu-GTP were obtained from Perkin-Elmer NEN Life Science Products (Milan, Italy). Quinpirole and GTPγS were from Sigma-Aldrich (Milan, Italy).

Radioligand binding assay at rat cloned D_3 dopaminergic receptors

Binding of [^{3}H]spiroperidol at rat cloned D_3 receptor was performed according to Swarzenski et al (1994) with minor modifications. The reaction buffer consisted of 50 mM Tris, 5 mM $MgCl_2$, 5 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 120 mM NaCl (pH 7.4), including 100 μL of dopamine D_3 diluted membranes, 0.4 nM of [^{3}H]spiroperidol (K_d = 0.60 nM), and 100 μL of the drug solution (six to nine concentrations) for a total volume of 1 mL. Samples were incubated at 27°C for 60 min, then the incubation was stopped by rapid filtration through Whatman GF/C glass fibre filters (pre-soaked in 0.3% polyethylenimine). The filters were washed twice with 1 mL ice-cold buffer (50 mM Tris, pH 7.4). Nonspecific binding was defined in the presence of 10 μM haloperidol. K_i value of haloperidol was 28 ± 2 nM. K_i value of quinpirole was 0.41 ± 0.03 nM.

Radioligand binding assay at human cloned $D_{4.4}$ dopaminergic receptors

Binding of [^{3}H]spiroperidol at human cloned $D_{4.4}$ receptor was performed according to Boyfield et al (1996) with minor modifications. The reaction buffer consisted of 50 mM Tris, 5 mM $MgCl_2$, 5 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$ (pH 7.4), including 500 μL dopamine $D_{4.4}$ diluted membranes, 0.15 nM [^{3}H]spiroperidol (K_d = 0.17 nM), and 100 μL of the drug solution (six to nine concentrations) for a total volume of 1 mL. Samples were incubated at 25 °C for 60 min, then the incubation was stopped by rapid filtration through Whatman GF/A glass fibre filters (presoaked in 0.3% polyethylenimine). The filters were washed twice with 1 mL ice-cold buffer (50 mM Tris, pH 7.4). Nonspecific binding was defined in the presence of 10 μM clozapine. K_i value of haloperidol was 0.74 ± 0.8 nM.

Radioligand binding assay at human cloned D_{2L} dopaminergic receptors

Binding of [^{3}H]spiroperidol at human cloned D_{2L} receptor was performed according to Boyfield et al (1996) with minor modifications. The reaction buffer consisted of 50 mM Tris, 10 mM $MgCl_2$, 1 mM EDTA (pH 7.4), including 500 μL dopamine D_{2L} receptor diluted membranes, 0.2 nM [^{3}H]spiroperidol (K_d = 0.20 nM), and 100 μL drug solution (six to nine concentrations) for a total volume of 1 mL. Samples were incubated at 27°C for 60 min, then the incubation was stopped by rapid filtration through Whatman GF/C glass fibre filters (presoaked in 0.3% polyethylenimine). The filters were washed twice with 1 mL ice-cold buffer (50 mM Tris, pH 7.4). Nonspecific binding was defined in the presence of 10 μM haloperidol. K_i value of haloperidol was 0.12 ± 0.4 nM.

Radioligand binding assay at rat hippocampal membranes 5-HT_{1A} receptors

Binding experiments were performed according to Borsini et al (1995) with minor modifications. Rats were killed by decapitation, the brain was quickly removed, and the hippocampus was dissected. The hippocampus (1.0 g) was homogenized with a Brinkman polytron (setting 5 for 3 × 15 s) in 25 mL 50 mM Tris buffer, pH 7.6. The homogenate was centrifuged at 48000 *g* for 15 min at 4°C. The supernatant was discarded, and the pellet was resuspended in 25 mL buffer, then pre-incubated for 10 min at 37°C. The homogenate was centrifuged at 48000 *g* for 15 min at 4° C. The supernatant was discarded, and the final pellet was stored at −80°C until used. Each tube received in a final volume of 1 mL 50 mM Tris (pH 7.6) hippocampus membrane suspension and 1 nM [^{3}H]8-OH-DPAT. For competitive inhibition experiments various concentrations of drugs studied were incubated. Nonspecific binding was defined using 1 μM 8-OH-DPAT. Samples were incubated at 37°C for 20 min and then filtered on Whatman GF/B glass microfibre filters. The K_d value determined for 8-OH-DPAT was 8.8 nM. K_i value of 8-OH-DPAT was 2.1 ± 0.4 nM.

Radioligand binding assay at rat cortical membranes α_1-adrenoceptors

Binding experiments were performed according to Glossmann & Hornung (1980) with minor modifications. Rats were killed by decapitation, the brain was quickly removed and the cerebral cortex was dissected. The cerebral cortex (1.0 g) was homogenized with a Brinkman Polytron (setting 5 for 3 × 15 s) in 25 mL buffer (50 mM Tris, 0.1 mM PMSF, pH 7.4). The homogenate was centrifuged at 1000 *g* for 15 min at 4°C. The surnatant was recovered and centrifuged at 50000 *g* for 30 min at 4°C. The final pellet was stored at −80°C until used. Each tube received in a final volume of 1 mL 50 mM Tris-Hcl (pH 7.4) rat cerebral cortical membranes suspension and 1 nM [^{3}H]prazosin. For competitive inhibition experiments various concentrations of drugs studied were incubated. Nonspecific binding was defined using 10 μM phentolamine. Samples were incubated at 25°C for 50 min and then filtered on Whatman GF/B glass microfibre filters. The filters were presoaked for 50 min in Tris.HCl-polyethylenimine 0.5%. The K_d value determined for prazosin was 0.5 nM. K_i value of phentolamine was 18 ± 3 nM.

DELFIA (dissociation enhanced lanthanide fluoro immunoassay) Eu-GTP binding assay

This assay was performed according to the technical data sheet by PerkinElmer Life Science. The experimental conditions were optimized, in particular referring to the composition of the incubation buffer, by following the procedure reported by Newman-Tancredi et al (1999). The incubation buffer (20 mM HEPES, 100 mM NaCl, 1 mM $MgCl_2$, 1 μM GDP, saponine 10 μg mL^{-1} pH 7.4) contained 2 μg D_3 receptor membranes, drugs at various concentrations (six to nine points) in a final volume of 100 μL. The samples were equilibrated for 30 min at room temperature using the DELFIA plate shake, then 10 μL 0.1 μM Eu-GTP were added. The samples were incubated for 30 min at room temperature, shaking as described above. The filter plate was washed in a vacuum manifold with ice-cold GTP Wash solution (2 × 300 μL). The basal level was determined in a sample containing dopamine D_3 receptor membranes in incubation buffer. The maximal stimulation was obtained in these conditions in

the presence of 10 μM quinpirole. Nonspecific binding was determined in the presence of 50 μM GTPγS and 10 μM quinpirole. The plates were analysed on a 1420 Multilabel Counter Victor3 (Perkin-Elmer Life Sciences) by time resolved fluorimetry. The emission and excitation wavelengths were 615 nm and 340 nm, respectively.

Statistical methods

The inhibition curves on the different binding sites of the compounds reported in Tables 1 and 2 were analysed by nonlinear curve fitting utilizing the GraphPad Prism program. The value for the inhibition constant, K_i, was calculated by using the Cheng–Prusoff equation (Cheng & Prusoff 1973). The values are means ± s.e.m. from three experiments in triplicate. Individual differences between the various compounds have been examined using Tukey's post-hoc test. Differences in K_i values between the receptors for each compound have been analysed using the Mann-Whitney U test. A difference with $P < 0.05$ was considered statistically significant.

The pEC50 values were obtained from nonlinear iterative curve fitting by GraphPad Prism. One-way analysis of variance was used to estimate the significance of difference. Statistical differences were determined by the Mann-Whitney U test. A difference with $P < 0.05$ was considered statistically significant.

Results and Discussion

Two synthetic routes were followed to prepare the target compounds. The synthesis of *cis* isomers is depicted in Figure 2. 3-Methoxybenzoyl chloride was reacted with *trans*-1-aminocyclohexanol to give intermediate alcohol **11**. This latter intermediate was transformed into its methanesulfonate derivative **12**. The reaction of the sterically hindered *trans*-methanesulfonate **12** with the appropriate arylpiperazine proceeded with inversion of configuration to provide the *cis* isomers albeit in low yield (Norman et al 1996). The synthesis of *trans* isomers (Figure 3) required the preparation of key

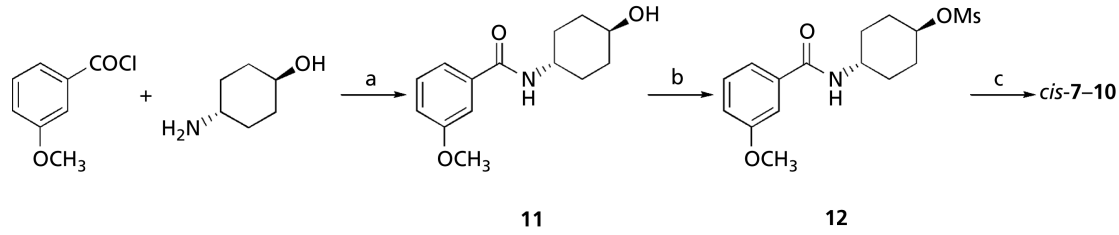


Figure 2 Synthesis of *cis* compounds. Reagents: a, 1.2% NaOH, CH_2Cl_2, 0°C; b, CH_3SO_2Cl, triethylamine, CH_2Cl_2, room temperature; c, 1-arylpiperazine, acetonitrile, Na_2CO_3, seven days reflux.

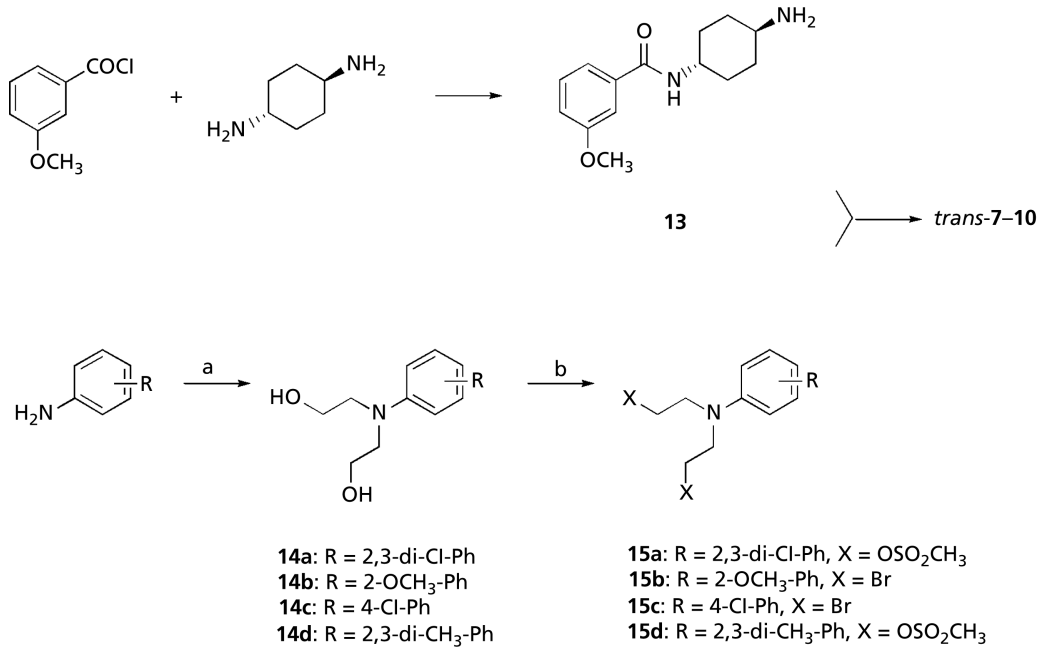


Figure 3 Synthesis of *trans* compounds. Reagents: a, 2-chloroethanol, $CaCO_3$, H_2O, 7 h reflux; b, PBr_3 or CH_3SO_2Cl, triethylamine.

intermediates **13** and **15a–d**. Amine **13** was obtained by condensing 3-methoxybenzoyl chloride with commercially available *trans*-1,4-diaminocyclohexane. The other key intermediates **15a–d** were prepared as follows: the appropriate aniline was alkylated with 2-chloroethanol in the presence of $CaCO_3$ to afford the alcohols **14a–d** (Ross 1949), which reacted with PBr_3 or methanesulfonyl chloride to give derivatives **15a–d**. Condensation of amine **13** and derivatives **15a–d** provided the expected final compounds.

The results of the in-vitro binding studies of the target compounds *cis*- and *trans*-**7–10** are listed in Table 2. Considering the D_3 receptor affinities of the target compounds, it can be noted that the *trans*-isomers showed higher affinity than the *cis*-isomers. Moreover, the D_3 receptor affinities of all constrained derivatives were lower than that of the corresponding opened counterparts **1–4**, except for compound *trans*-**7** that showed D_3 receptor affinity value in the same range as **1** (K_i values 0.18 vs 0.27 nM, respectively). Clearly the replacement of the butyl chain with a cyclohexane ring was detrimental for affinity, probably because of the shortened distance between the piperazine ring and the arylcarboxamide moiety. Moreover, the difference in D_3 receptor affinity between the *trans*- and *cis*-isomers suggested the extended linear arrangement as the most probable bioactive conformation of flexible molecules.

The proposed structural modification was detrimental for D_4 receptor affinity also. In fact, the affinity values of compounds *cis*-and *trans*-**7–10** were lower than that of the corresponding opened counterparts **1–4**. Moreover, the *cis*-isomers showed higher D_4 receptor affinity than the *trans*-isomers, differently from the trend observed for D_3 receptor.

As far as the D_2 receptor affinities are concerned, a clear trend was not shown. In fact, the compounds *trans*-**7** and *trans*-**8** displayed higher D_2 affinities than the corresponding *cis*-isomers, whereas the *cis*- and the *trans*-isomers of derivatives **9** and **10** were devoid of D_2 receptor affinity. Moreover, derivatives *trans*-**7** and *trans*-**8** displayed higher D_2 receptor affinity than the opened counterparts **1** and **2**.

Considering the 5-HT_{1A} receptor affinity values, the blocking of the butyl chain of compounds **1–4** led to a decrease in affinity that was more evident for *cis*-isomers. Only compound *trans*-**9** was significantly more potent than **3** (K_is 2.84 vs 397 nM, respectively).

Also for α_1-receptor affinities, *trans*-**7–9** were more potent than the corresponding *cis*-isomers. Moreover, compounds *trans*-**7–9** showed higher affinity at α_1 receptor than the derivatives **1–3**.

Taken together, the obtained results did not completely meet our initial expectations. As already discussed above, the compounds *trans*-**7–10** displayed lower D_3 receptor affinities than the derivatives **1–4**. Moreover, the substitution of the butyl chain with a cyclohexane ring increased the affinities for D_2, 5-HT_{1A}, and α_1 receptors and reduced significantly the D_4 receptor affinity. Consequently, the selectivity of the new compounds for D_3 receptors was less pronounced than that of compounds **1–4**. One exception was represented by *trans*-**7** that showed D_3 receptor affinity in the subnanomolar range and an overall improved selectivity profile (>200-fold selectivity over D_4, D_2, 5-HT_{1A}, and α_1-receptors).

Compound *trans*-**7**, that showed the highest D_3 receptor affinity among the new compounds reported here, and its opened counterpart **1** were tested for their intrinsic activity at D_3 receptor. For this purpose the recently introduced DELFIA (Dissociation Enhanced Lanthanide Fluoro Immuno Assay) Eu-GTP binding assay was used (Frang et al 2003). This method represents an alternative for use in filtration assays where [^{35}S]GTPγS is used. pEC50 values of quinpirole, *trans*-**7**, and **1** were found to be 7.60 ± 0.25, 6.62 ± 0.30, and 6.13 ± 0.15, respectively ($n = 3$, $P < 0.001$ for each compound with respect to the control). Derivatives *trans*-**7** and **1** behaved as agonists displaying 94% and 90% of the maximal response, respectively. The maximal effect was determined by using quinpirole. pEC50 of quinpirole was in good agreement with literature data (Wicke & Garcia-Ladona 2001).

Conclusion

We have replaced the flexible butyl linker of *N*-[4-(4-arylpiperazin-1-yl)butyl]-3-methoxybenzamides **1–4** with a more conformationally constrained cyclohexyl linker, to improve the D_3 receptor affinity and the selectivity over D_4, D_2, 5-HT_{1A}, and α_1-receptors, without causing a considerable increase in the lipophilicity. The new *trans*-*N*-[4-(4-aryl-1-piperazinyl)cyclohexyl]-3-methoxybenzamides **7–10** were found to be more potent at D_3 receptor than the corresponding *cis*-isomers, but less potent than the opened counterparts **1–4**. Consequently, no great improvement in selectivity was achieved. One exception was compound *trans*-*N*-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]cyclohexyl}-3-methoxybenzamide (*trans*-**7**), which showed high D_3 receptor affinity ($K_i = 0.18$ nM) and an interesting selectivity profile over D_4, D_2, 5-HT_{1A}, and α_1-receptors (>200-fold). This compound was characterized as a full agonist at D_3 receptor when tested in the Eu-GTP binding assay.

References

Bezard, E., Ferry, S., Mach, U., Stark, H., Leriche, L., Boraud, T., Gross, C., Sokoloff, P. (2003) Attenuation of levodopa-induced dyskinesia by normalizing dopamine D_3 receptor function. *Nat. Med.* **9**: 762–767

Biglan, K. M., Holloway, R. G. (2002) A review of pramipexole and its clinical utility in Parkinson's disease. *Expert Opin. Pharmacother.* **3**: 197–210

Borsini, F., Giraldo, E., Monferini, E., Antonini, G., Parenti, M., Bietti, G., Donetti, A. (1995) BIMT 17, a 5-HT_{2A} receptor antagonist and 5-HT_{1A} receptor full agonist in rat cerebral cortex. *Naunyn Schmiedebergs Arch. Pharmacol.* **352**: 276–282

Boyfield, I., Brown, T. H., Coldwell, M. C., Cooper, D. G., Hadley, M. S., Hagan, J. J., Healy, M. A., Jones, A., King, R. J., Middlemiss, D. N., Nash, D. J., Riley, G. J., Scott, E. E., Smith, S. A., Stemp, G. (1996) Design and synthesis of 2-naphthoate esters as selective dopamine D_4 antagonists. *J. Med. Chem.* **39**: 1946–1948

Cheng, Y. C., Prusoff, W. H. (1973) Relationship between the inhibition constant (*K*i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**: 3099–3108

Frang, H., Mukkala, V.-M., Syystö, R., Ollikka, P., Hurskainen, P., Scheinin, M., Hemmilä, I. (2003) Nonradioactive GTP binding assay to monitor activation of G protein-coupled receptors. *Assay Drug Dev. Technol*. **1**: 275–280

Glossmann, H., Hornung, R. (1980) α-Adrenoceptors in rat brain: sodium changes the affinity of agonists for prazosin sites. *Eur. J. Pharmacol*. **61**: 407–408

Hackling, A. E., Stark, H. (2002) Dopamine D_3 receptor ligands with antagonistic properties. *ChemBioChem* **3**: 946–961

Heidbreder, C. A., Gardner, E. L., Xi, Z.-X., Thanos, P. K., Mugnaini, M., Hagan, J. J., Ashby, C. R. (2005) The role of central dopamine D_3 receptors in drug addiction: a review of pharmacological evidence. *Brain Res. Rev*. **49**: 77–105

Leopoldo, M., Berardi, F., Colabufo, N. A., De Giorgio, P., Lacivita, E., Perrone, R., Tortorella, V. (2002) Structure-affinity relationship study on *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamides as potent and selective dopamine D_3 receptor ligands. *J. Med. Chem*. **45**: 5727–5735

Levant, B. (1997) The D_3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol. Rev*. **49**: 231–252

Luedtke, R. R., Mach, R. H. (2003) Progress in developing D_3 dopamine receptor ligands as potential therapeutic agents for neurological and neuropsychiatric disorders. *Curr. Pharm. Des*. **9**: 643–671

Newman, A. H., Cao, J., Bennett, C. J., Robarge, M. J., Freeman, R. A., Luedtke, R. R. (2003) *N*-(4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl, butenyl and butynyl)arylcarboxamides as novel dopamine D_3 receptor antagonists. *Bioorg. Med. Chem. Lett*. **13**: 2179–2183

Newman-Tancredi, A., Cussac, D., Audinot, V., Millan, M. J. (1999) Actions of roxindole at recombinant human dopamine D_2, D_3 and D_4 and serotonin 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors. *Naunyn Schmiedebergs Arch. Pharmacol*. **359**: 447–453

Norman, M. H., Minick, D. J., Rigdon, G. C. (1996) Effect of linking bridge modifications on the antipsychotic profile of some phthalimide and isoindolinone derivatives. *J. Med. Chem*. **39**: 149–157

Ross, W. C. J. (1949) Aryl-2-haloalkylamines. I. *J. Chem. Soc*. 183–191

Sokoloff, P., Giros, B., Martres, M. P., Bouthenet, M. L., Schwarz, J. C. (1990) Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target of neuroleptics. *Nature* **347**: 72–76

Swarzenski, B. C., Tang, L., Oh, Y. J., O'Malley, K. L., Todd, R. D. (1994) Morphogenic potentials of D_2, D_3, and D_4, dopamine receptors revealed in transfected neuronal cell lines. *Proc. Natl. Acad. Sci. USA* **91**: 649–653

Wicke, K., Javier Garcia-Ladona, J. (2001) The dopamine D3 receptor partial agonist, BP 897, is an antagonist at human dopamine D3 receptors and at rat somatodendritic dopamine D3 receptors. *Eur. J. Pharmacol*. **424**: 85–90