J. Pharm. Pharmacol. 1997, 49: 1036–1041
Received April 15, 1996
Accepted June 10, 1997

Synthesis of Several Substituted Phenylpiperazines Behaving as Mixed $D_2/5HT_{1A}$ Ligands

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Abstract

Twenty-two different compounds have been synthesized with the aim of creating new, mixed $D_2/5HT_{1A}$ ligands. For this purpose 1-substituted phenylpiperazines attached by the N-4 nitrogen to dopaminergic pharmacophores of the 2-(5-benzimidazole)ethyl-, 2-(5-benztriazole)ethyl-, 2-[5-(benzimidazole-2-thione)]ethyl- and 2-[6-(1,4-dihydroquinoxaline-2,3-dione)]ethyl-type were selected according to known structure–affinity requirements of 1-arylpiperazines.

All the new compounds were evaluated for in-vitro binding affinity at the dopamine (D_1 and D_2) and 5-HT_{1A} receptors. Synaptosomal membranes prepared from fresh bovine caudate nuclei and hippocampi were used as a source of dopamine and 5-hydroxytryptamine receptors, respectively. [^{3}H]SCH 23390 (D_1 selective), [^{3}H]spiperone (D_2 selective) and 8-OH-[^{3}H]DPAT (5-HT_{1A} selective) were employed as the radio-ligands. None of the compounds expressed affinity for binding at D_1 dopamine receptors. Compounds **3b** and **4b** were inactive 8-OH-[^{3}H]DPAT competitors whereas **1b**, **2b** and **4b** were inactive in the [^{3}H]spiperone-binding assay. The other compounds tested showed fair (**1c, 1e, 1f, 2c, 2f, 3b, 3c** and **4c**) to high (**1a, 1d, 2a, 1d, 3a, 3d–3f, 4a,** and **4d**) affinity in the [^{3}H]spiperone-binding assay, the most potent representative being 4-[2-(5-benzimidazole-2-thione)ethyl]-1-(2-methoxyphenyl)piperazine, **3a** ($K_i = 1{\cdot}7$ nM). In the 8-OH-[^{3}H]DPAT-displacement assay compounds **1b, 1d, 1f, 2b, 2f** and **3f** behaved as moderate competitors and **1a, 1c, 1e, 2a, 2c, 2d, 3a, 3c–3e, 4a, 4c, 4d** and **4f** as rather strong competitors; 4-[2-(5-benztriazole)ethyl]-1-(2-methoxyphenyl)piperazine, **2a** had the highest binding affinity at the 5-HT_{1A} receptors ($K_i = 2{\cdot}6$ nM).

Because many antipsychotic and anxiolytic agents behave as mixed dopaminergic and serotonergic ligands, the high affinity of several of these new ligands for binding at both D_2 and 5-HT_{1A} receptors make them promising candidates deserving further pharmacological evaluation as antipsychotic or anxiolytic pharmaceuticals.

Many dopaminergic (Howard & Seeger 1993) and serotonergic (Broekkamp et al 1995) ligands are used in the therapy of a number of psychiatric and neurological diseases and diseases of the endocrine system, and also as useful tools in fundamental research. However, therapeutic application of classical neuroleptics such as haloperidol is associated with severe side-effects including acute extrapyramidal symptoms, tardive dyskinesia and increased prolactin levels (Ben-Jonathans 1985; Reynolds 1992). A lower incidence of extrapyramidal symptoms is observed with members of a new generation of antipsychotic drugs, termed 'atypical' antipsychotics, e.g. clozapine, which are effective in patients who are unresponsive to classical agents. This series includes rather non-selective ligands expressing a moderate to high binding affinity at both dopamine and 5-hydroxytryptamine receptors of different subclasses (Lowe et al 1988). Lowe (1994) suggested that a combination of D_2 and 5-HT_2 antagonism is responsible for the beneficial properties of atypical antipsychotics, and reports of the synthesis of such compounds are accumulating (Howard et al 1994; Strupczevski et al 1995; Dukić et al 1997). Arylpiperazines have been known for some time to have activity profiles similar to those of atypical antipsychotics (Glennon 1992; West & Nemeroff 1993) and are therefore of interest as potential pharmaceuticals.

In our previous paper (Dukić et al 1997) we reported the synthesis of several new 4-(2-heteroarylethyl)-1-arylpiperazines and their binding affinities at D_1, D_2 and 5-HT_{1A} receptors. Among the compounds described therein 4-(2-heteroarylethyl)-1-phenylpiperazines showed remarkable affinity for binding both at D_2 and at 5-HT_{1A} receptors. Taking the structures of these molecules as 'leads' and with the aim of studying structure–affinity relationship of this class of ligand we now report the synthesis of new 4-(2-heteroarylethyl)-1-(substituted-phenyl)piperazines and their binding affinities at D_1, D_2 and 5-HT_{1A} receptors.

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Materials and Methods

Chemical analysis

Melting points were determined with a Boetius PHMK apparatus (VFB Analytic, Dresden, Germany) and are uncorrected. The results of microanalyses were within 0·4% of the calculated values. ^{1}H NMR spectra were recorded with a Varian (Palo Alto, CA) FT-80A spectrometer, with CDCl3 as solvent unless otherwise stated; chemical shifts (δ) are reported in ppm down-field from the internal standard tetramethylsilane. IR spectra were run on a Perkin-Elmer (Beaconsfield, UK) 457 grating infrared spectrophotometer. Mass spectra were determined with a Finnigan Mat (Bremen, Germany) 8230 mass spectrometer. Analytical thin-layer chromatography was performed on Merck (Darmstadt, Germany) plastic-backed silica gel $60F_{254}$ plates. Chromatographic purification was accomplished by column chromatography on Merck 230-400 mesh ASTM silica gel 60 under medium pressure (MPLC). Solutions

were routinely dried over anhydrous Na_2SO_4 before evaporation.

Chemicals and radio-ligands

1-(2-Methoxyphenyl)piperazine, 1-(4-methoxyphenyl)piperazine, 1-(3-trifluoromethylphenyl)piperazine, 1-(2-chlorophenyl)piperazine, 1-(3-chlorophenyl)piperazine and 1-(4-chlorophenyl)piperazine were from Aldrich (Milwaukee, WI). All other chemicals were obtained from Sigma (St Louis, MO) and were of analytical grade.

[^{3}H]SCH 23390, [^{3}H]spiperone and 8-OH-[^{3}H]DPAT (specific activity 80, 70·5 and 223 Ci mmol^{-1}, respectively) were purchased from Amersham Buchler GmbH (Braunschweig, Germany).

Chemical synthesis

4-[2-(3-Nitro-4-aminophenyl)ethyl]-1-arylpiperazines (**7a–f**; Table 1) and 4-[2-(3,4-diaminophenyl)ethyl]-1-arylpiperazines (**8a–f**: Table 1) were prepared as already described for the analogous compounds (Dukić et al 1997). The physical properties of these intermediates are given in Table 1.

Synthesis of 4-[2-(5-benzimidazole)ethyl]-1-arylpiperazines ***1a–1f***

Diamines **8a–8f** (3·0 mmol) and 98% formic acid (0·44 mL; 7·3 mmol) were heated on an oil bath (100°C, 2 h). After cooling to ambient temperature, $NaHCO_3$ (10%; 15 mL) was added and the product was extracted with $CHCl_3$. The solvent was removed in-vacuo and crude benzimidazoles **1a–1e** were purified by column chromatography on silica gel with a gradient of methanol (0–2%) in CH_2Cl_2. Purified compounds were recrystallized from CH_2Cl_2–hexane as free bases or from ethanol as tartaric and oxalic acid salts.

1a. ^{1}H NMR δ 8·05 (s, 1H, H-1 benzimidazole), 7·60–7·45 (m, 2H, H-4 and H-7 benzimidazole), 7·30-6·80 (m, 5H, aromatic), 3·75 (s, 3H, OCH_3), 3·3–2·5 (m, 12H); mass spectrum m/e 205 (100%), 336 (M+); yield 68%; m.p. 99-100°C; anal. $C_{20}H_{24}N_4O$ (C, H, N).

1b. ^{1}H NMR δ 8·05 (s, 1H, H-1 benzimidazole), 7·60–7·45 (m, 2H, H-4 and H-7 benzimidazole), 7·30–6·80 (m, 5H, aromatic), 3·73 (s, 3H, OCH_3), 3·3–2·5 (m, 12H); mass spectrum m/e 205 (100%), 336 (M^+); yield 60%; m.p. 195–198°C; anal. $C_{20}H_{24}N_4O$ (C, H, N).

Table 1. Principal physicochemical data for the intermediates.

No.	Yield	m.p. (°C)	Recrystallization solvent	Formula
7a	75	Oil	CH_2Cl_2-hexane	$C_{19}H_{24}N_4O_3$
7b	72	141-142	CH_2Cl_2-hexane	$C_{19}H_{24}N_4O_3$
7c	78	Oil	CH_2Cl_2-hexane	$C_{19}H_{21}N_4O_2F_3$
7	65	102-103	CH_2Cl_2-hexane	$C_{18}H_{21}N_4O_2Cl$
7e	68	133-135	CH_2Cl_2-hexane	$C_{18}H_{21}N_4O_2Cl$
7f	70	155-156	CH_2Cl_2-hexane	$C_{18}H_{21}N_4O_2Cl$
8a	Quantitative*	–	–	$C_{19}H_{26}N_4O$
8b	Quantitative*	–	–	$C_{19}H_{26}N_4O$
8c	Quantitative*	–	–	$C_{19}H_{23}N_4F_3$
8d	Quantitative*	–	–	$C_{18}H_{23}N_4Cl$
8e	Quantitative*	–	–	$C_{18}H_{23}N_4Cl$
8f	Quantitative*	–	–	$C_{18}H_{23}N_4Cl$

*Yields were calculated relative to the raw products 8b–8f.

1c. ^{1}H NMR (d_6DMSO) δ 8·06 (s, 1H, H-1 benzimidazole), 7·7–7·5 (m, 2H, H-4 and H-7 benzimidazole), 7·4–7·0 (m, 5H, aromatic), 3·3–2·5 (m, 12H); mass spectrum m/e 243 (100%), 374 (M^+); yield 80%; m.p. of tartaric acid salt 177–179°C; anal. $C_{20}H_{21}N_4F_3 . C_4H_6O_6$ (C, H, N).

1d. ^{1}H NMR δ 8·05 (s, 1H, H-1 benzimidazole), 7·6–7·4 (m, 2H, H-4 and H-7 benzimidazole), 7·2–6·8 (m, 5H, aromatic), 3·3–2·5 (m, 12H); mass spectrum m/e 209 (100%), 340 (M^+); yield 67%; m.p. 194–197°C; anal. $C_{19}H_{21}N_4Cl$ (C, H, N).

1e. ^{1}H NMR (d_6DMSO) δ 8·07 (s, 1H, H-1 benzimidazole), 7·6–7·4 (m, 2H, H-4 and H-7 benzimidazole), 7·2–6·8 (m, 5H, aromatic), 3·3–2·5 (m, 12H); mass spectrum m/e 209 (100%), 340 (M^+); yield 27%; m.p. of oxalic acid salt 231–233°C; anal. $C_{19}H_{21}N_4Cl . C_2H_2O_4$ (C, H, N).

1f. ^{1}H NMR δ 8·06 (s, 1H, H-1 benzimidazole), 7·7–7·4 (m, 2H, H-4 and H-7 benzimidazole), 7·2–6·8 (m, 5H, aromatic), 3·3–2·5 (m, 12H); mass spectrum m/e 209 (100%), 340 (M^+); yield 61%; m.p. 221–222°C; anal. $C_{19}H_{21}N_4Cl$ (C, H, N).

Synthesis of 4-[2-(5-benztriazole)ethyl]-1-arylpiperazines ***2a–2d, 2f***

Diamines **8a–8d** and **8f** (3·1 mmol) were dissolved in a mixture of acetic acid (0·7 mL) and water (1·4 mL) and a solution of $NaNO_2$ (0·24 g, 3·47 mmol) in water (0·36 mL) was added at 0°C. The mixture was warmed in a water bath (70°C, 10 min) and upon cooling to ambient temperature the solvent was removed in-vacuo. The residue was re-suspended in $NaHCO_3$ (10%; 20 mL), extracted with CH_2Cl_2, concentrated in-vacuo and purified by column chromatography on silica gel with a gradient of methanol (0–2%) in CH_2Cl_2. Purified compounds were recrystallized from CH_2Cl_2-hexane as the free bases.

2a. ^{1}H NMR δ 7·8–7·6 (m, 2H, H-4 and H-7 benztriazole), 7·25 (d, J = 8·0 Hz, 1H, H-6 benztriazole), 7·0–6·7 (m, 4H, aromatic), 3·75 (s, 3H, OCH_3), 3·2–2·4 (m, 12H); mass spectrum m/e 205 (100%), 337 (M^+); yield 53%; m.p. 155–157°C; anal. $C_{19}H_{23}N_5O$ (C, H, N).

2b. ^{1}H NMR δ 7·8–7·6 (m, 2H, H-4 and H-7 benztriazole), 7·25 (d, J = 8·0 Hz, 1H, H-6 benztriazole), 7·0–6·7 (m, 4H, aromatic), 3·72 (s, 3H, OCH_3), 3·2-2·4 (m, 12H); mass spectrum m/e 205 (100%), 337 (M^+); yield 54%; m.p. 201–203°C; anal. $C_{19}H_{23}N_5O$ (C, H, N).

2c. ^{1}H NMR δ 7·8–7·6 (m, 2H, H-4 and H-7 benztriazole), 7·25 (d, J = 7·5 Hz, 1H, H-6 benztriazole), 7·2–6·9 (m, 4H, aromatic), 3·2–2·5 (m, 12H); mass spectrum m/e 243 (100%), 375 (M^+); yield 38%; m.p. 129–131°C; anal. $C_{19}H_{20}N_5F_3$ (C, H, N).

2d. ^{1}H NMR δ 7·8–7·6 (m, 2H, H-4 and H-7 benztriazole), 7·25 (d, J = 8·0 Hz, 1H, H-6 benztriazole), 7·2–6·9 (m, 4H, aromatic), 3·2–2·6 (m, 12H); mass spectrum m/e 209 (100%), 341 (M^+); yield 90%; m.p. 178–179°C; anal. $C_{18}H_{20}N_5Cl$ (C, H, N).

2f ^{1}H NMR δ 7·8–7·6 (m, 2H, H-4 and H-7 benztriazole), 7·4–7·1 (m, 3H, aromatic), 6·90 (d, J = 6·5 Hz, 1H, H-6 aromatic), 3·3-2·6 (m, 12H); mass spectrum m/e 209 (100%), 341 (M^+); yield 48%; m.p. 196–199°C; anal. $C_{18}H_{20}N_5Cl$ (C, H, N).

Synthesis of 4-{2-[5-(1H-benzimidazole-2-thione)]ethyl}-1-arylpiperazines 3a–3e

Carbon disulphide (0·36 mL, 6·0 mmol) and a solution of KOH (0·37 g) in water (0·90 mL) were added to solutions of **8a–8e** (3 mmol) in ethanol (5 mL). After heating under reflux for 3 h the solvent was removed in-vacuo. The reaction mixture was diluted with ice-cold water (300 mL) and the precipitate separated by filtration. The resulting crude benzimidazole-2-thiones **3a–3f** were purified by recrystallization from hot ethanol.

3a. ^{1}H NMR δ 7·1–6·9 (m, 3H, benzimidazole-2-thione), 6·9–6·7 (m, 4H, aromatic), 3·75 (s, 3H, OCH3), 3·3–2·5 (m, 12H); mass spectrum m/e 205 (100%), 368 (M^+); yield 70%; m.p. 250–251°C; anal. $C_{20}H_{24}N_4OS$ (C, H, N).

3b. ^{1}H NMR δ 7·1–6·9 (m, 3H, benzimidazole-2-thione), 6·9–6·7 (m, 4H, aromatic), 3·75 (s, 3H, OCH_3), 3·3–2·5 (m, 12H); mass spectrum m/e 205(100%), 368 (M^+); yield 70%; m.p. 259–260°C; anal. $C_{20}H_{24}N_4OS$ (C, H, N).

3c. ^{1}H NMR δ 7·2–6·8 (m, 7H, aromatic), 3·3–2·5 (m, 12H); mass spectrum m/e 243 (100%), 406 (M^+); yield 82%; m.p. 265–268°C; anal. $C_{20}H_{21}N_4SF_3$ (C, H, N).

3d. ^{1}H NMR δ 7·3–6·7 (m, 7H, aromatic), 3·2–2·4 (m, 12H); mass spectrum m/e 209 (100%), 372 (M^+); yield 86%; m.p. 236–237°C; anal. $C_{19}H_{21}N_4SCl$ (C, H, N).

3e. ^{1}H NMR δ 7·2–6·8 (m, 7H, aromatic), 3·3–2·5 (m, 12H); mass spectrum m/e 209 (100%), 372 (M^+); yield 37%; m.p. 256–259°C; anal. $C_{19}H_{21}N_4SCl$ (C, H, N).

3f. ^{1}H NMR δ 7.20 (d, J = 7·0 Hz, 1H aromatic), 7·0–6·8 (m, 6H, aromatic), 3·3–2·5 (m, 12H); mass spectrum m/e 209 (100%), 372 (M^+); yield 72%; m.p. 256–257°C; anal. $C_{19}H_{21}N_4SCl$ (C, H, N).

Synthesis of 4-{2-[6-(1H,4H-quinoxaline-2,3-dione)]-ethyl}-1-arylpiperazines, 4a–4d, 4f

Diamines **8a–8d** and **8f** (3 mmol) and oxalic acid dihydrate (0·75 g; 8 mmol) of were heated to 200°C (oil bath, nitrogen atmosphere, 30 min). Upon cooling to ambient temperature the residue was resuspended in boiling dil. ethanol and the pH of the solution adjusted to 3·0 with 4 N HCl. The warm solution was filtered through cotton wool. Quinoxalinediones **4a–4d** and **4f** recrystallized from the filtrate as hydrochloric acid salts. Analytical samples were obtained after recrystallization from dil. ethanol.

4a. ^{1}H NMR (d_6DMSO) δ 12·3–12·0 (m, 2H, NH), 7·3–6·7 (m, 7H, aromatic), 3·75 (s, 3H, OCH_3), 3·3–2·5 (m, 12H); mass spectrum m/e 205 (100%), 380 (M^+); yield 54%; m.p. 198–199°C; anal. $C_{21}H_{24}N_4O_3 \cdot 2HCl$ (C, H, N).

4b. ^{1}H NMR (d_6DMSO) δ 12·3–12·0 (m, 2H, NH), 7·3–6·7 (m, 7H, aromatic), 3·75 (s, 3H, OCH_3), 3·3–2·5 (m, 12H); mass spectrum m/e 205 (100%), 380 (M^+); yield 67%; m.p. 279–282°C; anal. $C_{21}H_{24}N_4O_3 \cdot 2HCl$ (C, H, N).

4c. ^{1}H NMR (d_6DMSO) δ 12·3–12·0 (m, 2H, NH), 7·3–6·7 (m, 7H, aromatic), 3·5–2·5 (m, 12H); mass spectrum m/e 243 (100%), 418 (M^+); yield 65%; m.p. 218–221°C; anal. $C_{21}H_{21}N_4O_2F_3 \cdot 2HCl$ (C, H, N).

4d. ^{1}H NMR (d_6DMSO) δ 12·3-12·0 (m, 2H, NH), 7·3–6·7 (m, 7H, aromatic), 3·4–2·5 (m, 12H); mass spectrum m/e 209 (100%), 384 (M^+); yield 63%; m.p. 227–229°C; anal. $C_{20}H_{21}N_4O_2F_3 \cdot 2HCl$ (C, H, N).

4f. ^{1}H NMR (d_6DMSO) δ 12·3–12·0 (m, 2H, NH), 7·3–6·7 (m, 7H, aromatic), 3·3–2·5 (m, 12H); mass spectrum m/e 209 (100%), 384 (M^+); yield 71%; m.p. 270–273°C; anal. $C_{20}H_{21}N_4O_2 \cdot 2HCl$ (C, H, N).

Synthesis of 4-(phenylethyl)-1-(2-methoxyphenyl)piperazine (9)

1-Chloro-2-phenylethane (14·2 mmol), Na_2CO_3 (6·0 g) and KI (0·2 g) were added to a solution of 1-(2-methoxyphenyl)piperazine (10·0 mmol) in DMF (50 mL), and the mixture was stirred (90°C, 12 h). After cooling, the precipitate was removed and the filtrate evaporated in-vacuo. The residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent. Compound **9** was isolated as a gum and was converted to the hydrochloride with ethereal HCl. Recrystallization from ethanol gave 3·2 g of pure **9** 2HCl.

9. ^{1}H NMR (d_6DMSO) δ 7·40–7·15 (m, 5H, aromatic), 7·10–6·90 (m, 4H, aromatic), 3·75 (s, 3H, OCH_3), 4·0–3·1 (m, 12H); m.p. 234–235°C; anal. $C_{19}H_{23}N_{20} \cdot 2HCl$ (C, H, N).

Synaptosomal membrane preparation

Caudate nuclei and hippocampi were cut, minced and homogenized in 8 vols 0·32 M sucrose, 2 mmol EDTA, 5 mmol Tris-HCl, pH 7·4, in a Teflon homogenizer (900 rev min^{-1}, 2 min). The homogenates were centrifuged (10 000 *g*, 30 min, 4°C). The pellet was made hypotonic by adding two vols water. After homogenization the suspensions were centrifuged (20 000 *g*, 30 min, 4°C), the pellets were resuspended in 100 mmol NaCl, 5 mmol EDTA, 50 mmol Tris-HCl, pH 7·4 and the resulting suspensions stored at −80°C until used. Further details are given by Tuce et al (1994).

Binding assays

[^{3}H]Spiperone binding was assayed in a mixture containing (mmol) EDTA 1, $MgCl_2$ 5, NaCl 120, Tris-HCl 25, pH 7·4 and 0·7 mg mL^{-1} membrane protein (Markwell et al 1978) at 37°C for 20 min in a total volume of 1·0 mL. Binding of the radioligand to 5-HT_2 receptors was prevented by addition of 50 mmol ketanserin. K_i values of the tested compounds were determined by competition binding at 0·2 nmol of the radioligand and eight to ten different concentrations of each compound (0·1 nmol–0·1 mmol). Nonspecific binding was measured in the presence of 1·0 mmol (+)-butaclamol. The reaction was terminated by rapid filtration through Whatman GF/C filters which were further washed three times with ice-

cold incubation buffer (5·0 mL). Each point was determined in triplicate. Retained radioactivity was measured by introducing dry filters in toluene-based scintillation liquid (10 mL) and counting in a 1219 RackBeta Wallac scintillation counter at an efficiency of 51-55% for tritium.

Binding of [^{3}H]SCH 23390 was assayed by the same rapid filtration method discussed for [^{3}H]spiperone in the absence of ketanserin.

Binding of 8-OH-[^{3}H]DPAT was examined by a slightly modified version of the procedure of Gozlan et al (1983). Briefly, each tube contained the same buffer as was used for [^{3}H]spiperone binding assay supplemented with nialamide (10 μmol), ascorbic acid (0·1%), membrane protein (0·7 mg mL^{-1}), 8-OH-[^{3}H]DPAT (0·6 nmol) and the same range of the tested compounds as above in a final volume of 0·5 mL. Further conditions and handling of the filters were as described for [^{3}H]spiperone binding. 5-HT_{1A}-specific binding was calculated as the difference between binding in the absence and in the presence of 10 μmol 5-HT.

Haloperidol, SCH 23390 and 5-HT were run simultaneously in competition binding assays as references.

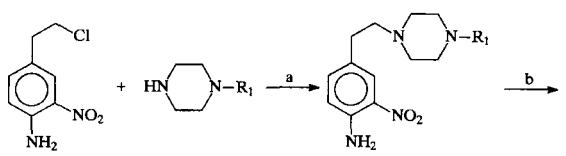
Data analysis

Competition binding data were analysed by the iterative non-linear least-squares curve-fitting program LIGAND (Munson & Rodbard 1980).

Results

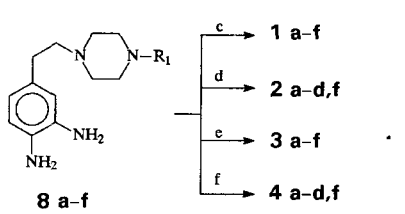
Chemistry

The synthetic pathways of the compounds synthesized during this work are shown in Fig. 1. Physical and chemical characteristics of the compounds prepared in this way are given under 'Materials and Methods'.



5 **6 a–f** **7 a–f**

R_1 : 2-methoxyphenyl; 4-methoxyphenyl; 3-trifluoromethylphenyl; 2-chlorophenyl; 3-chlorophenyl; 4-chlorophenyl.



8 a–f

a) Na_2CO_3, KI, DMF; b) Ra-Ni, $N_2H_4 \cdot H_2O$, EtOH;
c)HCOOH, 100 °C; d) $NaNO_2$, AcOH;
e) CS_2, KOH, EtOH, reflux; f) oxalic acid, 4M HCl, reflux

FIG. 1. Synthetic pathways for the new ligands.

Binding affinity of the new compounds for the D_1, D_2 and 5-HT_{1A} receptors

The binding characteristics of the new ligands evaluated under in-vitro conditions are listed in Table 2. It is apparent that none of the compounds had any affinity for binding at D_1 dopamine receptors. Compounds **3b** and **4b** were inactive 8-OH-[^{3}H]DPAT competitors and **1b, 2b** and **4b** were inactive in [^{3}H]spiperone binding assay. The other compounds tested were fair (**1c, 1e, 1f, 2c, 2f, 3b, 3c** and **4c**) to strong (**1a, 1d, 2a, 2d, 3a, 3d–3f, 4a** and **4d**) competitors for [^{3}H]piperone binding, the most potent being 4-[2-(5-benzimidazole-2-thione)ethyl]-1-(2-methoxyphenyl)piperazine **3a** ($K_i = 1·7$ nM). In 8-OH-[^{3}H]DPAT displacement assay, compounds **1b, 1d, 1f, 2b, 2f** and **3f** behaved as moderate competitors and **1a, 1c, 1e, 2a, 2c, 2d, 3a, 3c–3e, 4a, 4c, 4d, 4f** and **9** as strong competitors, with 4-[2-(5-benztriazole)ethyl]-1-(2-methoxyphenyl)piperazine **2a** being the most active ($K_i = 2·6$ nM).

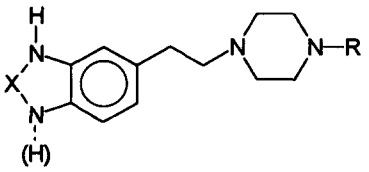
Discussion

In our previous paper (Dukić et al 1997) we reported the synthesis and D_1-, D_2- and 5-HT_{1A}-binding properties of novel 1-arylpiperazines with heterocyclic dopaminergic pharmacophores introduced into position 4 of the piperazine ring. Some of the compounds described therein showed mixed $D_2/5HT_{1A}$ affinity, suggesting that they might be pharmacologically interesting (Lowe et al 1991; Glennon 1992). 4-(2-Heteroarylethyl)-1-phenylpiperazines showed remarkable binding affinity at both D_2 and 5-HT_{1A} receptors (Dukić et al 1997) and the pharmacological properties of 1-arylpiperazines were shown to depend on the nature of their aryl substituents (Glennon 1992; van Steen et al 1993). This prompted us to study the effects of introduction of various substituents into the 1-phenyl ring on the binding properties of the novel compounds obtained in this way. The choice of substituents and the position of their introduction into the phenyl ring were selected according to known 5-HT_{1A} structure–affinity relationships of 1-phenylpiperazines (Glennon 1992; van Steen et al 1993). Dopaminergic pharmacophores of the 2-(5-benzimidazole) ethyl-, 2-(5-benztriazole)ethyl-, 2-[5-(benzimidazole-2-thione)] ethyl- and 2-[6-(1,4-dihydroquinoxaline-2,3-dione)]ethyl-types were attached through the N-4 nitrogen of the piperazine ring.

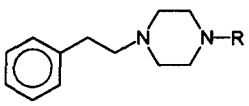
None of the novel compounds showed affinity for the binding at the D_1 subclass of the dopamine receptors. One of the 4-methoxyphenyl derivatives (**4b**) was inactive in the other two competition binding assays also, whereas **3b** was inactive in the 5-HT_{1A} binding assay. Generally, ligands with substituents in position 2 of the phenyl ring, e.g. 2-methoxy- (**1a–4a**) and 2-chloro- (**1d–4d**), showed high binding affinity at both D_2 and 5-HT_{1A} receptors. 2-Methoxyphenyl derivatives were stronger competitors than the corresponding phenyl derivatives (Dukić et al 1997) for both [^{3}H]spiperone and 8-OH-[^{3}H]DPAT binding. On the other hand 2-chlorophenyl derivatives were equipotent in both competition binding assays or slightly less potent competitors for 8-OH-[^{3}H]DPAT (**1d, 3d** and **4d**) than for [^{3}H]spiperone.

Substitution in position 4 of the phenyl ring afforded ligands **1b–4b** and **1f–4f** characterized by low to moderate activity in both [^{3}H]spiperone and 8-OH-[^{3}H]-DPAT binding assays. Compound **3f**, with high binding affinity at the D_2 dopamine

Table 2. Affinity and selectivity of the new ligands for binding to the D_1, D_2 and 5-HT_{1A} receptors.



No	X	R	$K_i \pm$ s.e.m. (nM) D_1	D_2	5-HT_{1A}
1a	CH	2-Methoxyphenyl	> 1000	13·3 ± 1·0	20·3 ± 3·0
2a	N	2-Methoxyphenyl	> 1000	18·2 ± 4·1	2·6 ± 1·0
3a	CS	2-Methoxyphenyl	ND	1·7 ± 0·4	2·9 ± 1·1
4a	COCO	2-Methoxyphenyl	> 1000	11·8 ± 2·1	9·9 ± 1·5
1b	CH	4-Methoxyphenyl	> 1000	> 1000	332 ± 39
2b	N	4-Methoxyphenyl	> 1000	> 1000	474 ± 42
3b	CS	4-Methoxyphenyl	> 1000	110 ± 12	> 1000
4b	COCO	4-Methoxyphenyl	> 1000	> 1000	> 1000
1c	CH	3-CF_3-Phenyl	> 1000	273 ± 38	33·0 ± 4·0
2c	N	3-CF_3-Phenyl	> 1000	300 ± 18	26·4 ± 3·7
3c	CS	3-CF_3-Phenyl	> 1000	134 ± 15	10·7 ± 3·2
4c	COCO	3-CF_3-Phenyl	> 1000	105 ± 9	8·0 ± 31
1d	CH	2-Chlorophenyl	1000	59·7 ± 3·2	243 ± 54
2d	N	2-Chlorophenyl	> 1000	49·8 ± 3·0	38·5 ± 1·3
3d	CS	2-Chlorophenyl	ND	20·7 ± 2·2	80·1 ± 8·4
4d	COCO	2-Chlorophenyl	ND	20·4 ± 1·6	54·4 ± 6·2
1e	CH	3-Chlorophenyl	ND	204 ± 18	31·3 ± 6·1
3e	CS	3-Chlorophenyl	ND	46·6 ± 6·2	13·4 ± 3·1
1f	CH	4-Chlorophenyl	> 1000	268 ± 39	372 ± 41
2f	N	4-Chlorophenyl	> 1000	472 ± 37	115 ± 19
3f	CS	4-Chlorophenyl	> 1000	32 ± 4·1	217 ± 38
4f	COCO	4-Chlorophenyl	> 1000	116 ± 11	54·4 ± 6·2



9	–	2-Methoxyphenyl	> 1000	37·5 ± 4·2	7·5 ± 1·2
Haloperidol			420 ± 60	12·3 ± 23	> 1000
SCH 23390			0·83 ± 0·4	> 1000	ND
5-Hydroxytryptamine			ND	> 1000	5·2 ± 1·6

K_i values were calculated from competition binding experiments employing [^{3}H]SCH 23390 (D_1 selective; 0·2 nmol), [^{3}H]spiperone (D_2 selective; 0·2 nmol) or 8-OH-[^{3}H]DPAT (5-HT_{1A} selective; 0·6 nmol) as radio-ligands. Binding of [^{3}H]spiperone to 5-HT_2 receptors was prevented by addition of 50 nM ketanserin. Each value represents the means ± s.e.m. from three independent experiments performed in triplicate at eight ligand concentrations (0·1 nM–0·1 mM) and a membrane protein concentration of 0·7 mg mL^{-1}.

receptor was the only exception. A trifluoromethyl substituent at position 3 of the phenyl ring produced ligands **1c–4c** with moderate and high binding affinity at the D_2 and 5-HT_{1A} receptors, respectively. The same was true for 3-chlorophenyl derivatives **1e** and **3e**. The most conspicuous affinity increase compared with that of the reference compound **9** was observed upon introduction of 2-(5-benztriazole)ethyl- and 2-[5-(benzimidazole-2-thione)]ethyl- heterocyclic dopaminergic pharmacophores affording compounds **2a** and **3a**. Benztriazoles **2a–2d** and **2f** and quinoxaline-2,3-diones **4a, 4c** and **4f** were found to have greater affinity for 5-HT_{1A} receptors than for D_2 receptors, the only exception being ligand **4d**. In contrast, the D_2/5HT_{1A} ratio of benzimidazole-2-thiones and 1,4-dihydroquinoxaline-2,3-diones depended on the position of substitution in the 1-phenylpiperazine ring. So, 3-substituted phenyl derivatives **3c** and **3e** were selective 5-HT_{1A} ligands whereas 2- and 4-substituted phenyl derivatives **3a, 3b, 3d** and **3f** had more pronounced affinity for binding to the D_2 receptors. In this respect benzimidazoles **1a–1f** behaved similarly to benzimidazole-2-thiones.

Taken together our data show that affinity for the D_2 and 5HT_{1A} receptors and the D_2/5HT_{1A} affinity ratio depend both on the nature of the dopaminergic pharmacophores and on the choice of substituents in the phenyl ring of the 1-phenylpiperazine part of the molecule. These parameters could be precisely tuned by proper choice of a substituent, which could make the binding characteristics of this kind of ligand predictable.

It has been reported that many potentially useful antipsychotic and anxiolytic agents behave as mixed dopaminergic and serotonergic ligands (Lowe et al 1991; Glennon 1992). This, together with the high affinity of several of the new ligands for the binding at both D_2 and 5-HT_{1A} receptors, i.e. the affinity profiles described in the current work, make them

promising candidates deserving further pharmacological evaluation as antipsychotic or anxiolytic pharmaceuticals.

Acknowledgements

This work was supported by the Ministry of Science and Technology of Serbia, contracts #03E20 (S. D., J. J., V. S.) and #02E24 (S. K.-R., D. D., V. S.).

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