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Investigation of mixed D_2/5-HT_{1A} activity of *N*-heteroarylmethyl-*N*-phenylpiperazines, *N*-heteroarylethyl-*N*-phenylpiperazines and *N*-heteroarylpropyl-*N*-phenylpiperazines

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Eight novel *N*-heteroarylalkyl-*N*-phenylpiperazines have been synthesized, chemically characterized and evaluated for *in vitro* binding affinity at the dopamine and serotonin receptors. Synaptosomal membranes of fresh bovine caudate nuclei (D_1 and D_2), the membranes of COS-7 cells ($D_{4.4}$) and those prepared from fresh bovine hippocampi (5-HT_{1A}) were used as a source of the corresponding receptor subtypes. [^{3}H]SCH 23390 (D_1−selective), [^{3}H]spiperone (D_2- and $D_{4.4}$- selective) and [^{3}H]-8-OH-DPAT (5-HT_{1A}-selective) served as radioligands. None of the compounds expressed the affinity for the binding at the D_1 subtype receptor. Compounds **7–9** containing a single methylene group serving as a bridge between heteroaryl- and *N*-phenylpiperazine part of the molecule were inactive [^{3}H]spiperone and [^{3}H]-8-OH- DPAT competitors. Ligands **15–19** (three methylene groups connecting heteroaryl- and *N*-phenylpiperazine part of the molecule) acted as moderate competitors of [^{3}H]spiperone binding at the D_2 receptor subtype, with the exception of **15** (a thione) which expressed a high binding affinity at the D_2 receptor subtype. Compounds **15–19** behaved as moderate displacers of 8-OH-[^{3}H]DPAT. Among all eight novel ligands only compound **15** expressed a moderate binding affinity at the $D_{4.4}$ receptor subtype.

1. Introduction

Dopaminergic neurotransmission is mediated by five receptor subtypes (D_1–D_5) which can be grouped into two receptor families. D_1 – like receptors include the D_1 and D_5 subtypes, whereas D_2 – like receptors include the D_2, D_3 and D_4 subtypes [1]. Functional or anatomic damages of the central dopaminergic and serotonergic system lead to development of numerous motoric, endocrinologic, neurologic and psychiatric diseases [1–4]. Neuroleptics applied in the treatment of these disorders frequently express undesirable adverse effects deleterious to either CNS or some other systems [5]. Because of that, numerous authors are concentrated on the design and synthesis of dopaminergic and serotonergic agonists and antagonists with adverse effects reduced to a minimum. Ligands with mixed D_2/5-HT_{1A} activity, so called "atypical" antipsychotics showed a lower incidence of extrapyramidal symptoms and were effective in patients unresponsive to classical agents [6]. Also, it has been hypothesised that D_4 subtype selectivity might be a pharmacological requisite in order to obtain antipsychotic drugs devoid of side-efects, or at least possessing limited side-effects, as observed for clozapine [7].

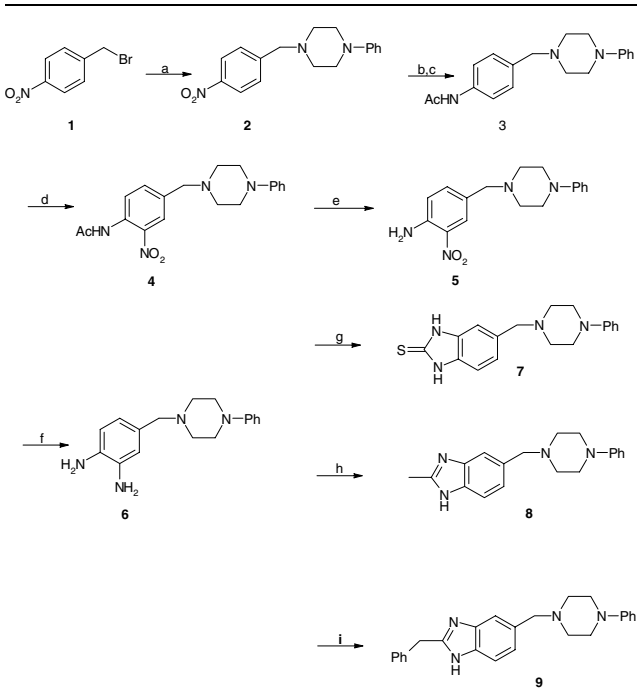
Arylpiperazines have been known for some time to have the activity profiles similar to those of atypical antipsychotics [8]. In our previous studies we have investigated the effects of substitution in position 2 of benzimidazole ring of 4-[2-(5-benzimidazole)ethyl]-1-arylpiperazines and in aryl group of 1-arylpiperazines containing new dopaminergic pharmacophores on mixed D_2/5-HT_{1A} activity of the resulting compounds [9–11]. Based on these results we have found it of interest to examine the effect of the alkyl chain length in heteroarylalkyl-*N*-phenylpiperazines on dopaminergic and serotonergic activity.

2. Investigations, results and discussion

2.1 Synthesis of the derivatives

Chemical structure of the compounds synthesized in the present study is shown in Schemes 1 and 2. 4-Nitro-benzyl bromide readily alkylates *N*-phenyl-piperazine in the presence of Na_2CO_3 and KI in dimethyl formamide (DMF) as a solvent. The resulting 1-(4-nitrobenzyl)-4-phenyl-piperazine was reduced with Ra-Ni hydrazine and the obtained amine was acylated without purification with acetanhydride to produce **3**. Nitration of 1-(4-phenyl-piperazin-1-yl-methyl)-phenyl-acetamide (**3**) in acetanhydride with H_2SO_4/

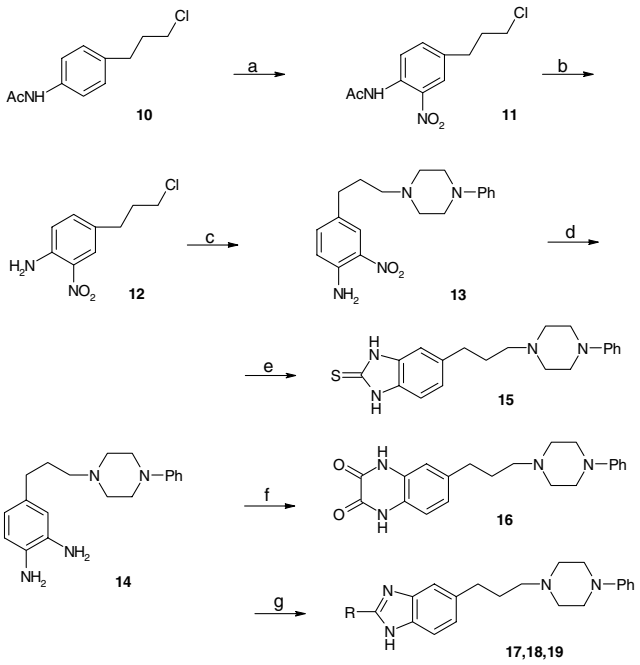
Scheme 1



Synthesis of substituted *N*-heteroarylmethyl-*N*-phenylpiperazines

a) Na_2CO_3, KI, phenyl piperazine, 80 °C; b) Ra-Ni, $N_2H_4 \cdot H_2O$, EtOH, $(CH_2)_2Cl_2$ c) Ac_2O, pyridine, CH_2Cl_2; d) HNO_3/H_2SO_4, Ac_2O; e) 6N HCl, reflux; f) Ra-Ni, $N_2H_4 \cdot H_2O$, EtOH, $(CH_2)_2Cl_2$; g) CS_2, KOH, EtOH, reflux; h) **8**(R=CH_3) CH_3COOH, 4N HCl, reflux; i) **9** (R=$PhCH_2$) $PhCH_2COOH$, 4N HCl, reflux

Scheme 2



Scheme 2. Synthesis of substituted *N*-heteroarylpropyl-*N*-phenylpiperazines

a) HNO_3/H_2SO_4, Ac_2O; b) 6N HCl, reflux; c) Na_2CO_3, KI, phenyl piperazine, 80 °C; d) Ra-Ni, $N_2H_4 \cdot H_2O$, EtOH, $(CH_2)_2Cl_2$; e) CS_2, KOH, EtOH, reflux; f) oxalic acid, 4N HCl, reflux; g) **17** (R=H) HCOOH, 100 °C; **18** (R=Ph) PhCOOH, 4N HCl, reflux; **19** (R=$PhCH_2$) $PhCH_2COOH$, 4N HCl, reflux

100% HNO_3 afforded *N*-[2-nitro-4-(4-phenyl-piperazin-1-yl-methyl)-phenyl]-acetamide. Upon the hydrolysis with 6 N HCl compound **5** was obtained and diamine **6** was produced reducing **5** with Ra-Ni/hydrazine.

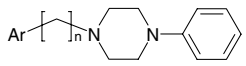
Nitration of 4-(3-chloropropyl)-acetanilide (**10**) with nitric acid and subsequent hydrolysis of *o*-nitroacetanilide intermediate with boiling HCl afforded 2-nitro-4-(chloropropyl)-aniline (**11**) as the main product. Compound **11** readily alkylates *N*-phenyl-piperazine in DMF in the presence of Na_2CO_3 and KI at elevated temperature producing **13** (2-nitro-4-[3-(4-phenyl-piperazin-1-yl)-propyl]-phenylamine) and upon its reduction with Ra-Ni/hydrazine the *o*-phenylendiamine **14** was obtained.

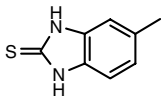
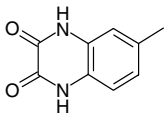
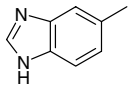
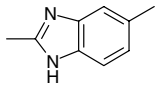
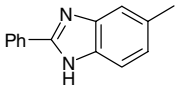
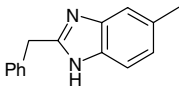
Target benzimidazole-2-thiones **7** and **15**, 1,4-dihydroquinoxalin-2,3-dione **16**, benzimidazole-2-methyl- **8**, benzimidazole-2-phenyl- **17**, and benzimidazole-2-phenylmethyls **9** and **18** were prepared analogously to the corresponding phenylethylamines described previously [9, 14].

2.2. Binding studies

Final products **7–9** and **15–19** were evaluated for the binding affinity at the D_1, D_2 and $D_{4.4}$ dopamine and 5-HT_{1A} serotonin receptors subtypes by *in vitro* competitive displacement of the specific radioligands from the synaptosomal membranes prepared from bovine caudate nuclei, bovine hippocampi and the membranes prepared from COS-7 cells expressing a recombinant human dopamine $D_{4.4}$ receptor [7, 11, 12]. Compounds **20–25** 14 were run in the same assay as references. Binding parameters of the novel compounds are listed in the Table. As seen, none of the novel compounds except for compound **15,** expressed the binding affinity either at the D_1 or $D_{4.4}$ dopamine receptor subtypes.

Table: Affinity and selectivity of the new ligands for the binding at the D_1, D_2 and $D_{4.4}$ dopamine and 5-HT_{1A} serotonin receptors



Ar	No	n	Ki(nM) ± S.E.M.			
			D_1	D_2	$D_{4.4}$	5-HT_{1A}
	**7**	1	>1000	>1000	>1000	>1000
	20	2	>1000	15.7 ± 2.0	N.T.	13.4 ± 1.8
	15	3	>1000	3.0 ± 0.2	92.8 ± 23	177 ± 29
	**21**	2	>1000	23.3 ± 4.2	N.T.	25.4 ± 3.8
	16	3	>1000	20.8 ± 2.8	>1000	93.3 ± 16
	**22**	2	>1000	138 ± 23	N.T.	197 ± 52
	17	3	>1000	129 ± 19	>1000	71.1 ± 8.2
	**8**	1	>1000	>1000	>1000	>1000
	23	2	>1000	44.7 ± 2.2	N.T.	143 ± 17
	**24**	2	>1000	60.2 ± 7.8	N.T.	10.1 ± 0.7
	18	3	>1000	74.2 ± 6.3	>1000	83.9 ± 9.1
	**9**	1	>1000	>1000	>1000	>1000
	25	2	184 ± 11	157 ± 24	N.T.	21.9 ± 4.2
	19	3	>1000	113 ± 18	>1000	98.1 ± 9.4

Ki values were calculated from competition binding experiments. Each values represents the mean ± S.E.M. from at least three independent experiments done in triplicate. N.T. – not tested.

Compounds **7–9** were completely inactive competitors of both [^{3}H]spiperone and [^{3}H]-8-OH-DPAT. Ligands **16–19** expressed a moderate binding affinity at the D_2 and 5-HT_{1A} receptor subtypes, while **15** expressed a clear D_2 receptor subtype selectivity.

Our previous results together with the data of some other authors demonstrated that classical catecholamine structure is not crucial for dopaminergic activity and that heterocyclic benzimidazoles which can provide hydrogen bond formation with the receptor represent efficient dopaminergic ligands [15–17]. It was also shown that 2-substituted benzimidazoles and related heterocyclic systems might be considered as non-classical catechol bioisosteres [15, 18] and that could be used to replace the corresponding catechol moiety in the dopaminergic pharmacophore and that the introduction of semirigid *N*-phenyl-piperazine group in the side chain leads to a significant increase of dopaminergic/serotonergic activity of thus obtained ligands. It was also demonstrated that the presence of lipophilic groups in the molecule of active compounds is necessary for the interaction of the pharmacophore with the receptor, while the other part of the molecule is accomodated in an accessory binding pocket of the receptor [18, 19].

The synthesis of the compounds presented in this paper was aimed at the investigating the effect of the length of the alkyl group that connects *N*-phenyl-piperazine part of the molecule with heterocyclic dopaminergic pharmacophores on the dopaminergic and serotonergic activity.

Comparison of the binding parameters of the novel compounds **7–9** and **14–18** with those of previously synthesized ethyl-type ligands led to the following conclusions: 1. Shortening of the alkyl chain (compounds **7–9**) led to a total loss of the binding affinity at both dopamine and 5-HT_{1A} serotonin receptor, probably due to steric hindrances preventing *N*-phenylpiperazine lipophilic group to reach the accessory binding pocket of the receptor; 2. In the case of ligands with propyl group (compounds **15–19**) it is obvious that the increase of the alkyl chain length led to a moderate decrease of binding affinity at the 5-HT_{1A} receptor and to the loss of the mixed D_2/5-HT_{1A} activity accompanied by a slight increase of selectivity for the D_2 receptor subtype which is especially conspicuous in compound **15**; 3. These results suggest that the molecule of the D_2 receptor subtype tolerates certain modifications in the side chain of the ligands, while this is not the case when 5-HT_{1A} receptor is concerned. It is obvious that an increased alkyl chain length enabling the rotation around C–C bond of the alkyl bridge provides more favourable conformation of the ligand for the interaction with the binding pocket of the D_2 receptor subtype.

3. Experimental

M.p.: Boetius PHMK apparatus (WEB Analytic, Dresden, Germany) – uncorrected. MS were recorded in positive mode on a Bruker Biflex MALDI time-of-flight mass spectrometer (Bruker, Bremen, Germany). ^{1}H NMR: Gemini 2000 spectrometer (Varian, Palo Alto, CA, U.S.A.); solvent $CDCl_3$, unless otherwise stated; ppm (δ) downfield from the internal standard tetramethylsilane. IR spectra: Perkin Elmer457 Grafting Spectrophotometer (Perkin Elmer, Beaconsfield, England). Analytical TLC: E. Merck (Darmstadt, Germany) F-256 plastic backed thin-layer silica gel plates. Purifications: Merck-60 silica gel CC, 230–400 mesh ASTM, medium pressure (MPLC). Solutions: routinely dried over anh. Na_2SO_4 prior to evaporation.

3.1. Chemistry

3.1.1. General procedure for the nitration of **3** *and* **10**

To 40 mmol solution of either acetanilide **3** or **10** in 60 ml of acetanhydride, 0.6 ml conc. H_2SO_4 were added at 25 °C. The reaction mixture was cooled to −5 °C and 2 ml of 100% HNO_3 were introduced dropwise. After the mixture reached room temperature, 80 g of crashed ice were added and stirred for 3 h. The crystals were separated by filtration and recrystallized from 90% EtOH.

3.1.1.1. N-[4-(4-phenylpiperazinyl-1-methyl)-2-nitrophenyl]-acetamide (**4**)

Yield: 80%; m.p. 115 °C ; IR (cm^{-1}): 1741, 1459, 1377; ^{1}H NMR (ppm): 2.18 (s, 3 H), 2.59 (m, 4 H), 3.19 (m, 4 H), 3.57 (s, 2 H) 6.90 (m, 3 H), 7.23 (m, 2 H), 7.67 (d, 1 H, J = 8.6 Hz), 8.20 (s, 1 H), 8.71 (d, 1 H, J = 8,6 Hz), 10.28 (NH), MS m/e 353.1619.
$C_{19}H_{21}N_4O_3$

3.1.1.2. N-[4-(3-Chloropropyl)-2-nitrophenyl]-acetamide (**11**)

Yield: 78%; m.p. 98 °C; IR (cm^{-1}): 1749, 1463, 1382; ^{1}H NMR (ppm): 2.18 (m, 2 H), 2.35 (s, 3 H), 2.93 (t, 2 H, J = 7 Hz), 3.63 (t, 2 H, J = 7 Hz), 7.58 (d, 2 H, J = 8 Hz), 8.14 (s, 1 H), 8.78 (d, 2 H, J = 8 Hz), 10.35 (NH) MS: m/e 213.0571.
$C_{10}H_{12}ClNO_2$

3.1.2. General procedure for the alkylation of **1** *and* **12**

To solution of 4.6 mmol of either **1** or **12**, 0.75 ml (3.8 mmol) *N*-phenyl piperazine, 4 ml of dimethylformamide (DMF), Na_2CO_3 and KI (1.0 g of each) were added and the mixture was stirred (24 h, 80 °C). After cooling, the precipitate was discarded and the filtrate evaporated in vacuo. The residue was chromatographed on silica gel.

3.1.2.1. 1-(4-Nitrobenzyl)-4-phenylpiperazine (**1**)

Yield: 89%; m.p. 129 °C ; IR (cm^{-1}): 1500, 1349; ^{1}H NMR (ppm): 2.63 (m, 4 H), 3.22 (m, 4 H), 3.66 (s, 2 H), 6.95 (m, 3 H), 7.25 (m, 2 H), 7.55 (d, 2 H, J = 8.8 Hz), 8.20 (d, 2 H, J = 8.8 Hz); MS m/e 297.1469.
$C_{17}H_{19}N_3O_2$

3.1.2.2. 2-Nitro-4-3-[(4-phenylpiperazin-1-yl)-propyl]-phenylamine (**13**)

Yield: 60%; m.p. 99 °C; IR (cm^{-1}): 3475, 1520, 1343; ^{1}H NMR (ppm): 1.83 (m, 2 H), 2.45 (t, 2 H, J = 7 Hz), 2.60 (m, 6 H), 3.24 (m, 4 H), 6.05 (NH_2), 6.75 (d, 1 H, J = 8 Hz), 6.84 (t, 2 H, J = 8 Hz), 6.92 (d, 1 H, J = 8 Hz), 7.24 (m, 3 H), 7.98 (s, 1 H) MS: m/e 340.1888.
$C_{19}H_{24}N_4O_2$

3.1.3. General procedure for the hydrolysis of compounds **4** *and* **11**

Either **4** or **11** 0.5 mmol were resuspended in 43 ml of 6 N HCl and refluxed for 4 h. The solution was cooled to room temperature and diluted with 150 ml of ice-water mixture. The excess acid was neutralized with 10% NaOH keeping the temperature below 30 °C and the product was extracted with CH_2Cl_2. After drying over anh. Na_2SO_4 and evaporation in vacuo compounds **5** or **12** were obtained.

3.1.3.1. 2-Nitro-4-(4-phenylpiperazin-1-yl-methyl)-phenylamine (**5**)

Yield: 90%; m.p. 134 °C ; IR (cm^{-1}): 2945, 1515, 1342; ^{1}H NMR (ppm): 2.60 (m, 4 H), 3.19 (m, 4 H), 3.47 (s, 2 H), 6.04 (NH_2), 6.81 (t, 1 H, J = 7 Hz), 6.92 (d, 2 H, J = 8 Hz), 7.26 (m, 3 H), 7.42 (d, 1 H, J = 8.8 Hz), 8.06 (s, 1 H), MS m/e 312.1575.
$C_{17}H_{29}N_4O_2$

3.1.3.2. 2-Nitro-4-(3-chloropropyl)-phenylamine (**12**)

Yield: 93%; m.p. 47 °C; IR (cm^{-1}): 2959, 1522, 1347; ^{1}H NMR (ppm): 1.98 (m, 2 H), 2.62 (t, 2 H, J = 7 Hz), 3.61 (t, 2 H, J = 7 Hz), 6.10 (NH_2), 6.99 (d, 1 H, J = 8 Hz), 7.30 (d, 1 H, J = 8 Hz), 7.80 (s, 1 H).

3.1.4. General procedure for the reduction of compounds **5** *and* **13**

Ra-Ni (0.4–0.5 g) was added in small portions to a stirring solution of 12 mmol of either nitro compound (**5** or **13**) in 12 ml EtOH, 24 ml 1,2-dichloro-ethane and 4 ml (40 mmol) hydrazine hydrate at 30 °C. After the addition of Ra-Ni was completed, the mixture was heated in a water bath (50 °C, 60 min) and filtered through celite. The filtrate was evaporated in vacuo and crude products were used for further syntheses.

3.1.4.1. 1-(4-Phenylpiperazin-1-yl-methyl)-phenylacetamide (**3**)

Compound **2** was reduced according to the procedure described for **5** and **13.**
The amine was dissolved in 90 ml CH_2Cl_2 and 12 mmol of pyridine were added. The solution was cooled to 0 °C and then 12 mmol of acetanhydride were introduced dropwise. After the stirring (room temperature, 24 h), 120 ml of 10% Na_2CO_3 were added. Upon the separation, organic layer was dried over anh. Na_2SO_4 and evaporated.
Yield: 80%; IR (cm^{-1}): 2980, 1741, 1377; m.p. 213 °C ; ^{1}H NMR (ppm): 2.04 (s, 3 H), 2.48 (m, 4 H), 3.10 (m, 4 H), 3.44 (s, 2 H), 6.75 (t, 1 H,

J = 7 Hz), 6.90 (d, 2 H, J = 8 Hz), 7.21 (m, 4 H), 7.55 (d, 2 H, J = 8.6 Hz), 9.93 (NH), MS m/e 308.1769.
$C_{19}H_{22}N_3O$

3.1.5. General procedure for the synthesis of benzimidazole-2-thiones **7** *and* **15**

Carbon disulfide (0.36 ml, 6 mmol) and KOH (0.37 g in 0.9 ml water) were added to 3 mmol of either diamine **6** or **14** previously dissolved in 5 ml of EtOH. After refluxing for 3 h, activated charcoal was added and the suspension filtered through celite. The solvent was removed in vacuo and the residue resuspended in 10 ml of 10% $NaHCO_3$, extracted with CH_2Cl_2 and concentrated in vacuo. The resulting benzimidazole-thiones were purified by chromatography or recrystallized from hot EtOH.

3.1.5.1. 5-[(4-Phenylpiperazin-1-ylmethyl)]-1,3-dihydrobenzoimidazole-2-thione (**7**)

Yield: 56%; m.p. > 225 °C ; IR (cm^{-1}): 2922, 1626, 1485, 1469, 1189; ^1H NMR (ppm): 2.51 (m, 4 H), 3.12 (m, 4 H), 3.54 (s, 2 H), 6.75 (t, 1 H, J = 7 Hz), 6.90 (d, 2 H, J = 8 Hz), 7.15 (m, 5 H). MS: m/e 324.1420.
$C_{18}H_{20}N_4S$

3.1.5.2. 5-[(4-Phenylpiperazin-1-yl)-propyl]-1,3-dihydrobenzoimidazole-2-thione (**15**)

Yield: 72%; m.p. > 225 °C ; IR (cm^{-1}): 2927, 1620, 1493, 1465, 1183; ^1H NMR (ppm): 1.75 (m, 2 H), 2.31 (t, 2 H, J = 6.8 Hz), 2.48 (m, 4 H), 2.64 (t, 2 H, J = 6.8 Hz), 3.12 (m, 4 H), 6.76 (t, 1 H, J = 7.4 Hz), 6.89–7.07 (m, 5 H), 7.20 (t, 2 H, J = 8 Hz). MS: m/e 352.1734.
$C_{20}H_{24}N_4S$

3.1.6. General procedure for the synthesis of 2-alkyl-substituted benzimidazoles **8, 9, 18** *and* **19**

3.0 mmol of diamine (**6** or **14**), 3.3 mmol of the appropriate organic acid and 40 ml of 4 N HCl were heated in an oil bath (180 °C, 6 h). After cooling to ambient temperature, 15 ml of 10% $NaHCO_3$ were added and the product was extracted with CH_2Cl_2.. The compounds were purified as oxalates in EtOH or *iso*-PrOH.

3.1.6.1. 2-Methyl-5-(4-phenylpiperazin-1-ylmethyl)-1*H*-benzoimidazole (**8**)

Acetic acid and diamine **6** were used. Yield: 68%; m.p. 163 °C; IR (cm^{-1}): 3086, 2925, 2890, 1611, 1495, 1235, 1018, 790 cm^{-1}; ^1H NMR (ppm): 2.60–2.65 (m, 4 H), 3.20 (m, 4 H), 3.67 (s, 2 H), 6.84 (t, 1 H, J = 7.4 Hz), 6.91 (d, 2 H, J = 8 Hz), 7.25 (m, 5 H); MS: m/e 306.1838.
$C_{19}H_{22}N_4$

3.1.6.2. 2-Benzyl-5-[4-phenylpiperazin-1-yl)methyl])-1*H*-benzoimidazole (**9**)

Phenylacetic acid and diamine **6** were used. Yield: 62%; m.p. 148 °C; IR (cm^{-1}): 3102, 2942, 2870, 1612, 1492, 1232, 1021, 782 cm^{-1}; ^1H NMR (ppm): 3.01 (m, 4 H), 3.07 (m, 4 H), 3.98 (s, 2 H), 4.24 (s, 2 H), 6.85 (m, 2 H), 7.10 (m, 3 H), 7.28 (m, 8 H). MS: m/e 382.2163.
$C_{25}H_{26}N_4$

3.1.6.3. 2-Phenyl-5-[3-(4-phenylpiperazin-1-yl)-propil]-1*H*-benzoimidazole (**18**)

Benzoic acid and diamine **14** were used. Yield: 56%; m.p. 133 °C; IR (cm^{-1}): 3150, 3003, 2858, 1650, 1380, 1028 cm^{-1}; ^1H NMR (d_6DMSO, ppm) of oxalate salt: 2.05 (m, 2 H), 2.76 (t, 2 H, J = 6.8 Hz), 3.09 (t, 2 H, J = 6.8 Hz), 3.30 (m, 4 H), 3.38 (m, 4 H), 6.85 (t, 1 H, J = 7.4 Hz), 6.99 (d, 2 H, J = 8 Hz), 7.09–7.29 (m, 3 H), 7.52 (m, 5 H), 8.18 (d, 2 H, J = 7 Hz). MS: m/e 396.2323.
$C_{26}H_{28}N_4$

3.1.6.4. 2-Benzyl-5-[3-(4-phenylpiperazin-1-yl)-propil]-1*H*-benzoimidazole (**19**)

Phenylacetic acid and diamine **14** were used. Yield: 65%; m.p. 183 °C; IR (cm^{-1}): 3108, 2953, 2858, 1603, 1506, 1231, 1021 cm^{-1}; ^1H NMR (d_6DMSO, ppm) of oxalate salt: 2.03 (m, 2 H), 2.73 (t, 2 H, J = 7.6 Hz), 3.08 (t, 2 H, J = 7.6 Hz), 3.28 (m, 4 H), 3.37 (m, 4 H), 4.20 (s, 2 H), 6.80–7.58 (m, 13 H). MS: m/e 410.2462.
$C_{27}H_{30}N_4$

3.1.6.5. 6-[3-(4-Phenylpiperazin-1-yl)-propyl]-1,4-dihydroquinoxaline-2,3-dione (**16**)

The mixture containing 4 mmol of diamine **14**, 0.55 g (4.4 mmol) of oxalic acid and 2.5 ml of 4 N HCl was refluxed for 30 min. Upon cooling to ambient temperature the solvent was removed in vacuo. The residue was suspended in 20 ml of 10% $NaHCO_3$, extracted with CH_2Cl_2, concentrated in vacuo and chromatographed on silica gel.

Yield 45%; m.p. > 230 °C; IR (cm^{-1}) 3505, 2939, 1675, 1402; ^1H NMR (d_6DMSO, ppm) 1.73 (m, 2 H), 2.31 (t, 2 H, J = 7.2 Hz), 2.48 (m, 4 H), 2.64 (t, 2 H, 7.2 Hz), 3.12 (m, 4 H), 6.76 (t, 1 H, J = 7.2 Hz), 6.89–7.07 (m, 5 H), 7.20 (t, 2 H, J = 7 Hz) ; MS: m/e 364.1918.
$C_{21}H_{24}N_4O_2$

3.1.6.6. 5-[3-(4-Phenylpiperazin-1-yl)-propyl]-1*H*-benzoimidazole (**17**)

3.2 mmol of diamine **14** and 0.44 ml (7.3 mmol) of 98% formic acid were heated in an oil bath at 100 °C for 2 h. After cooling to ambient temperature, 15 ml of 10% $NaHCO_3$ were added and the product was extracted with CH_2Cl_2. The solvent was removed in vacuo and the oily residue purified as oxalate salt.
Yield 70%; m.p. 199–200 °C; IR (cm^{-1}) 3503, 2948, 1677, 1408; ^1H NMR (d_6DMSO, ppm) of oxalate salt: 2.02 (m, 2 H), 2.75 (t, 2 H, J = 7.6 Hz), 3.04 (t, 2 H, J = 7.6 Hz), 3.23 (m, 4 H), 3.29 (m, 4 H), 6.84 (t, 1 H, J = 7.2 Hz), 6.98 (d, 2 H, J = 8 Hz), 7.09 (d, 1 H, J = 8 Hz), 7.25 (t, 2 H, 7.8 Hz) 7.45 (s, 1 H), 7.53 (d, 1 H, J = 8 Hz), 8.23 (s, 1 H). MS: m/e 320.1989.
$C_{20}H_{24}N_4$

3.2. Biochemical tests

3.2.1. Synaptosomal membrane preparation and binding assays

Synaptosomal membranes of the bovine caudate nuclei and hippocampi were used for preparation of the dopamine D_1 and D_2 and serotonin 5-HT_{1A} receptors subtypes exactly according to described procedures [10, 11]. As a source of dopamine $D_{4.4}$ receptor subtype membranes of COS-7 cells tranfected with human $D_{4.4}$ were used [7]. Transfected COS-7 cells were washed with PBS (8.1 mM NaH_2 PO_4, 1.5 mM KH_2 PO_4, 138 mM NaCl, 2.7 mM KCl, pH 7.2), briefly treated with PBS containing 1 mM EDTA, and then dissociated in PBS. Cells were pelleted at 1000 × g for 5 min at 4 °C and resuspended in binding buffer (120 mM NaCl, 5.0 mM KCl, 25 mM TRIS HCL, 1 mM EDTA, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$ pH 7.4). Cells were then disrupted on ice with a Polytron homogenizer at a setting of 6 for 12 s. The membranes were collected by centrifugation at 40,000 × g for 15 min at 4 °C. The pellet was resuspended in binding buffer and used immediately for binding or stored at −80 °C until use.
[^{3}H]SCH 23390 (spec. act. 80 Ci $mmol^{-1}$), [^{3}H]spiperone (spec. act. 70 Ci $mmol^{-1}$) and [^{3}H]-8-OH-DPAT (spec. act. 223 Ci $mmol^{-1}$) used to label D_1, D_2, $D_{4.4}$ and 5-HT_{1A} receptors subtypes, respectively, were purchased from Amersham Buchler GmbH (Braunschweig, Germany). Shortly assay conditions were: [^{3}H]Spiperone binding was assayed in binding buffer at 37 °C for 20 min in a total volume of 0.5 ml. Binding of the radioligand to 5-HT_2 receptors was prevented by 50 nM ketanserin. Ki values were determined by competition binding at 0.2 nM of the radioligand and eight to ten concentrations of each novel compound (0.1 μM–0.1 mM). Nonspecific binding was measured in the presence of 1.0 mM (+)-butaclamol. The reaction was terminated by rapid filtration through Whatman GF/C filters, further washed three times with 5.0 ml of ice-cold incubation buffer. Each point was determined in triplicate. Retained radioactivity was measured by introducing dry filters into 10 ml of toluene-based scintillation liquid and counting in a 1219 Rackbeta Wallac scintillation. Binding of [^{3}H]SCH 23390 was examined by the same rapid filtration assay discussed for [^{3}H]spiperone in the absence of ketanserin. [^{3}H]-8-OH-DPAT binding was assayed in a solution containing binding buffer containing 0.1% ascorbic acid at 0.6 nM of the radioligand and various concentrations (0.1 nM–0.1 mM) of the tested ligands in a final volume of 0.5 ml. The incubation, termination of the reaction and handling of the filters were done as described in [^{3}H]spiperone binding assay. Specific binding at 5-HT_{1A} receptor was defined as the difference between binding in the absence and in the presence of 10 μM 5-hydroxytryptamine.

3.2.2. Data analysis

Competition binding data were analyzed by the non-linear least-squares curve-fitting program LIGAND [13].

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