Mixed Dopaminergic/Serotonergic Properties of Several 2-Substituted 4-[2-(5-Benzimidazole)ethyl]-1-arylpiperazines

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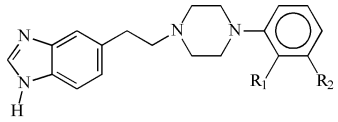
Summary

A series of substituted 4-[2-(5-benzimidazole)ethyl]-arylpiperazines was synthesized by introducing different substituents into position 2 of benzimidazole ring of 4-[2-(*N*,*N*-di-*n*-propylamino)ethyl]-1,2-diaminobenzenes. They were evaluated for *in vitro* binding affinity at the D_1 and D_2 dopamine and 5-HT_{1A} serotonin receptors using synaptosomal membranes of the bovine caudate nuclei and hippocampi, respectively. Tritiated SCH 23390 (D_1 receptor-selective), spiperone (D_2 receptor selective) and 8-OH-DPAT (5-HT_{1A} receptor selective) were employed as the radioligands. Only compound **6** expressed a moderate binding affinity at the dopamine D_1 receptor, while the remaining ligands were inefficient or weak competitors of [^{3}H]SCH 23390. Compound **12** was an absolutely inactive competitor of all three radioligands. Also, compound **7** was an inefficient displacer of [^{3}H]-8-OH-DPAT. Compound **19** with a K_i value of 3.5 nM was the most potent competitor of [^{3}H]spiperone and compound **13** (K_i = 3.3 nM) was the most efficient in displacing [^{3}H]-8-OH-DPAT from the 5-HT_{1A} serotonin receptor. Ligands **5, 6, 8–11**, and **13–20** expressed mixed dopaminergic/serotonergic activity in nanomolar range of concentrations with varying affinity ratios which strongly depended on the properties of the substituents introduced into position 2 of benzimidazole ring of the parent compounds.

Introduction

Undesirable, usually long lasting and irreversible side effects of neuroleptic drugs presently used in the therapy of diseases connected with malfunctioning of the dopaminergic and/or serotonergic receptor system[1–5] led to an intensified research effort in the area of medicinal chemistry focused on design and synthesis of new dopamine and serotonin agonists and antagonists

We have demonstrated previously that benzimidazoles and related heterocyclic systems might be considered as nonclassical catechol bioisosteres that could be used to replace the corresponding catechol moiety in the dopaminergic pharmacophore[6,7]. Linking of this structural motif to 1-arylpiperazines afforded a series of high affinity mixed D_2-dopaminergic/5HT_{1A}-serotonergic ligands (Fig. 1)[8]. Characteristics of substituents introduced into position 2 of benzimidazole ring were shown to be of utmost importance for the specificity of ligand-dopamine receptor interactions[6–8]. In the present study we examined further the influence of properties of these substituents on the binding affinity of the produced ligands at the dopamine (D_1 and D_2) and serotonin (5-HT_{1A}) receptors of the specific mammalian brain structures.

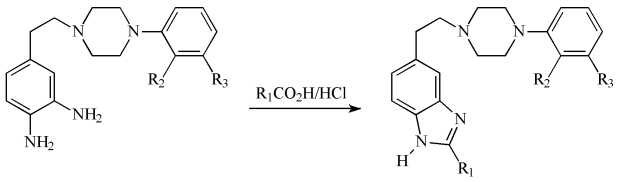


1 R_1,R_2=H
2 R_1=OCH_3, R_2=H
3 R_1=H, R_2=CF_3

Figure 1. Structure of the reference compounds **1–3**.

Chemistry

The chemical structure of the compounds synthesized throughout the present work is depicted in Fig. 2. 4-[2-(*N*,*N*-di-*n*-propylamino)ethyl]-1,2-diaminobenzenes **4a–c** used as a starting material to produce compounds **5–20** were prepared as previously described[8,9]. All novel benzimidazole ligands were synthesized by heating an appropriate diamine with an organic acid in 4 N HCl up to 180 °C for 6 h, according to general procedure of Porai-Košic *et al.*[10]. In this way, a series of different derivatives was obtained. They were purified by silica gel column chromatography and further converted to and crystallized as oxalic acid salts.



4a (R_2,R_3=H)
4b (R_2=OCH_3, R_3=H)
4c (R_2=H, R_3=CF_3)

5-20

R_1:methyl (**5**), phenylmethyl (**6**,**19**,**20**), 4-nitrophenylmethyl (**7**), 4-chlorophenylmethyl (**8**), diphenylmethyl (**9**), 1-naphthylmethyl (**10**), 2-naphthylmethyl (**11**), 4-phenylphenylmethyl (**12**), 2-pyridylmethyl (**13**), 3-pyridylmethyl (**14**), phenyl (**15**), 2-pyridyl (**16**), 3-pyridyl (**17**), 2-phenylethyl (**18**)
R_2: H (**5-18**), methoxy (**19**)
R_3: H (**5-18**), trifluoromethyl (**20**)

Figure 2. Synthesis of new 2-substituted 4-[2-(5-benzimidazolyl)ethyl]-1-arylpiperazines.

Results and Discussion

The final products **5–20** were evaluated for their binding affinity at the D_1 and D_2 dopamine and 5-HT_{1A} serotonin receptors by *in vitro* competition displacement of the specific radioligands from synaptosomal membranes prepared from fresh bovine caudate nuclei and hippocampi, respectively[5,6]. The K_i values for individual compounds calculated from displacement curves are listed in Table 1. Structurally close compounds **1**, **2**, and **3**[8] were run simultaneously in the same test system as references.

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

No	R1	R2	R3	K_i + S.E.M. (nM)		
				D1	D2	5HT1A
5	methyl	H	H	>1000	44.7±2.2	143±17
6	phenylmethyl	H	H	184±11	157±24	21.9±4.2
7	4-NO_2-phenylmethyl	H	H	>1000	231±18	>1000
8	4-Cl-phenylmethyl	H	H	304±24	46.9±5.1	64.2±6.8
9	diphenylmethyl	H	H	910±111	76.3±2.1	4.2±2.0
10	1-naphthylmethyl	H	H	>1000	232±38	481±58
11	2-naphthylmethyl	H	H	>1000	117±8	38.3±1.2
12	4-biphenylmethyl	H	H	>1000	>1000	>1000
13	2-pyridylmethyl	H	H	499±61	3.4±0.5	2.7±0.1
14	3-pyridylmethyl	H	H	479±61	51.9±4.2	149±16
15	phenyl	H	H	>1000	60.2±7.8	10.1±0.7
16	2-pyridyl	H	H	>1000	64.2±4.3	2.78±0.5
17	3-pyridyl	H	H	403±28	10.6±1.2	70.7±9.2
18	2-phenylethyl	H	H	>1000	117±3	31.1±2.2
19	phenylmethyl	MeO	H	>1000	2.9±0.6	5.7±1.3
20	phenylmethyl	H	CF_3	>1000	95.4±21	73.1±4.2
1	H	H	H	>1000	151±11	142±16
2	H	MeO	H	>1000	13.3±1.0	20.3±3.0
3	H	H	CF_3	>1000	273±38	33.1±4.0

Values are the means of three independent experiments done in triplicate performed at eight competing ligand concentrations (10^{-4}–10^{-8} M) and 0.2 nM of [^{3}H]SCH 23390 and [^{3}H]spiperone and 0.6 nM of [^{3}H]-8-OH-DPAT.

As seen from Table 1, the ligands synthesized and evaluated for the dopaminergic/serotonergic activity throughout the present study expressed weak or no affinity for the binding at the D_1 dopamine receptor with the exception of compound **6** which acted as a moderate [^{3}H]SCH 23390 competitor. Compound **12** was an absolutely inactive competitor of both [^{3}H]spiperone and [^{3}H]-8-OH-DPAT, as well. Besides, compound **7** acted as an inefficient competitor of [^{3}H]-8-OH-DPAT binding at the 5-HT_{1A} receptor.

All other novel compounds acted as competitors of both [^{3}H]spiperone and [^{3}H]-8-OH-DPAT expressing binding affinity at the corresponding receptors in a nanomolar range of concentrations. Compound **19** with a K_i value of 3.5 nM was the most potent in displacing [^{3}H]spiperone from the D_2 receptor and compound **13** with a K_i value of 3.3 nM was the most active competitor of [^{3}H]-8-OH-DPAT binding at the 5-HT_{1A} serotonin receptor.

In our previous studies we have successfully used the approach of Ariens *et al.*[11] to design new ligands expressing mixed dopaminergic/serotonergic properties. The main idea was to anchor a part of the molecule in the agonist binding pocket and the other one in the lipophilic accessory binding site of the D_2 dopamine receptor. Our earlier results[8] together with the data of some other authors[5] demonstrated that the affinity for the receptors, as well as D_2/5-HT_{1A} affinity ratios could be precisely tuned by minor structural modifications of this kind of ligands. We have also postulated the existence of a rather spacious domain within the D_2 receptor molecule that could accommodate voluminous groups such as those introduced into position 2 of substituted benzimidazoles [12, 13]. Because the affinity and selectivity of this class of ligands strongly depend on even minute structural changes, the goal of this work was to examine the influence of modifications in position 2 of benzimidazole ring of benzimidazole-ethyl-1-arylpiperazines on their binding at the D_2 and 5-HT_{1A} receptors. The choice of substituents was based on their electronic and stereochemical features and earlier experience with 5-[2-(*N*,*N*-di-*n*-propylamino)ethyl]benzimidazole series of ligands[12,13].

Neither of the new compounds acted as a strong competitor in [^{3}H]SCH 23390 binding assays which is a characteristic of all ligands of this class tested so far[7,8].

In [^{3}H]spiperone binding assay only compounds with very voluminous groups like 4-nitrophenylmethyl (**7**), 1-naphthylmethyl (**10**), 2-naphthylmethyl (**11**), and 4-biphenylmethyl (**12**) expressed a reduced binding affinity for the D_2 receptor in comparison with compound **1**. However, this was not the case with diphenylmethyl derivative **9**, suggesting that although sufficiently spacious to accommodate the diphenylmethyl group, this accessory binding pocket in the D_2 receptor molecule is not deep enough to accommodate the 4-biphenylmethyl group. Introduction of phenylmethyl or phenylethyl groups in compounds **1–3** afforded either ligands with an approximately equal (**6**, **18**) or 7- to 4-fold increased binding affinity (compounds **19** and **20**, respectively) at the D_2 receptor. The ligands discussed above behaved similarly in [^{3}H]-8-OH-DPAT binding assay, but opposite to their binding affinity at the D_2 receptor, the introduction of the phenylmethyl, phenylethyl, or 2-naphthylmethyl group affording compounds **6, 18**, and **11**, respectively, led to an increased binding affinity at the serotonin 5-HT_{1A} receptor comparing to structurally related compounds **1–3** run as references.

By introducing a nitro or chloro group into the 2-benzylmethyl group of ligand **6,** compounds **7** and **8** were obtained, respectively, the former being a moderate competitor of [^{3}H]spiperone and an inefficient [^{3}H]-8-OH-DPAT displacer. Ligand **8** showed a 2.7-fold increased affinity for the D_2 receptor and about a 4-fold decreased affinity for 5-HT_{1A} receptor in comparison with compound **6**. This suggests that

besides steric interactions, electronic effects on ligand-receptor binding should be considered in the design of this kind of ligands.

Further evidence along this line comes from the results obtained with ligands **13** and **14** which have 2- and 3-pyridylmethyl substituents in position 2 of benzimidazole ring, respectively. Although isosteric with the phenylmethyl group, these groups influenced binding properties of the resulting ligands in a different way. Compound **13** was the most active in both D_2 and 5-HT_{1A} binding assay while compound **14** expressed some 2.5-fold higher affinity only in [^{3}H]-spiperone binding assay in comparison with phenylmethyl derivative **6.** Compound **14** was about 6-fold less efficient displacer of [^{3}H]-8-OH-DPAT than compound **6**. One of the explanations for the effect of 2-pyridylmethyl group may be connected to the fact that there is a possibility for an intermolecular hydrogen bond formation between benzimidazole and pyridyl part of the molecule that could additionally influence the interaction with the receptor molecules.

All three derivatives with aromatic groups directly linked to benzimidazole ring (**15–17**) were more active in both D_2 and 5-HT_{1A} binding assays in comparison with compound **1**. Interestingly, 2-pyridyl (**16**) and 3-pyridyl derivatives (**17**) expressed different profile of D_2/5-HT_{1A} ratio further pointing to the differences in D_2 dopamine *vs.* 5-HT_{1A} serotonin receptor binding site. This divergence of dopaminergic *vs.* serotonergic activity is one of the important criteria in the design of new pharmacologically active agents of this category[14,15].

In conclusion, these data demonstrate a high level of tolerance of both the D_2 and 5-HT_{1A} receptor molecules towards the introduction of relatively bulky groups into the parent compounds used in this study. Besides, during the choice of a substituent to be introduced into position 2 of the parent compound with an aim of producing new dopaminergic/serotonergic ligands, it should be kept in mind that the electronic properties of a substituent, as well as the possibilities of hydrogen bond formation could strongly affect receptor-ligand interactions. Further elaboration of chemical structure of the ligands described herein focused on the improvement of their dopaminergic/serotonergic properties that would add to our knowledge of the topography of the binding site for this class of derivatives is in progress.

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Experimental

General

A Boetius PHMK apparatus (VEB Analytic, Dresden, Germany) was used to determine melting points, presented here as uncorrected. The results of microanalyses were within 0.4% of the calculated values. ^{1}H-NMR spectra recorded on a Gemini 2000 spectrometer (Varian, Palo Alto, CA, U.S.A.) with [D_6]DMSO as a solvent unless otherwise stated are reported in ppm (δ) downfield from the internal standard tetramethylsilane. The IR spectra were run on a Perkin Elmer 457 Grating Infrared Spectrophotometer (Perkin Elmer, Beaconsfield, England). The mass spectra were determined by a Finnigan Mat 8230 mass spectrometer (Finnigan, Brehmen, Germany). For analytical thin-layer chromatography E. Merck (Darmstadt, Germany) F-256 plastic-backed thin-layer silica gel plates were used. Chromatographic purification was performed on Merck-60 silica gel columns, 230–400 mesh ASTM, under medium pressure (MPLC). Solutions were routinely dried over anhydrous Na_2SO_4 *prior* to evaporation.

Chemistry

*General procedure for the synthesis of 2-substituted 4-[2-(5-benzimidazole)-ethyl]-1-arylpiperazines (**5–20**)*

3.0 mmol of diamines **4a–c**[8,9], 3.3 mmol of an appropriate organic acid and 4.0 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 $CHCl_3$. The solvent was removed *in vacuo* and crude benzimidazoles **5–20** were purified by silica gel column chromatography (0–2% MeOH gradient in CH_2Cl_2). The characteristics given below are referring to purified compounds crystallized from EtOH as oxalic acid salts.

*4-{2-[2-Methyl-5-benzimidazolyl]ethyl}-1-phenylpiperazine (**5**)*

For the synthesis acetic acid and diamine **4a** were used.– IR (KBr): ν 3088, 2931, 2890, 1599, 1495, 1235, 1009, 790, 767 cm^{-1}.– ^{1}H NMR: δ 2.45 (s, 3H, methyl), 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.20–7.60 (m, 5H, N-phenylpiperazine, H-4, H-6, H-7, benzimidazole).– MS (70 eV); *m/z* (%) = 175 (100), 320 [M$^+$]; mp 163 °C. Anal. ($C_{20}H_{24}N_4 \cdot 2C_2H_2O_4 \cdot 3/2H_2O$) C, H, N.

*4-{2-[2-(Phenylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine (**6**)*

Phenylacetic acid and diamine **4a** were used.– IR (KBr): ν 2817, 1599, 1495, 1238, 725 cm^{-1}.– ^{1}H NMR: δ 2.60–2.80 (m, 4H, piperazine), 2.80–2.95 (m, 4H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.20 (s, 2H, CH_2, benzyl), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.10–7.50 (m, 10H, benzyl, H-4, H-6 and H-7 benzimidazole, N-phenylpiperazine), 12.00 (s, 1H NH).– MS (70 eV); *m/z* (%) = 175 (100), 396 [M$^+$]; mp 214 °C; Anal. ($C_{26}H_{28}N_4 \cdot 2C_2H_2O_4 \cdot 2H_2O$) C, H, N.

*4-{2-[2-(4-Nitrophenylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine (**7**)*

4-Nitrophenylacetic acid and diamine **4a** were employed.– IR (KBr): ν 3436, 2921, 1600, 1519, 1346, 1237 cm^{-1}.– ^{1}H NMR: δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.30 (s, 2H, CH_2-4-nitrophenyl), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.20–7.50 (m, 5H, N-phenylpiperazine, H-4, H-6 and H-7 benzimidazole), 7.50 (d, 2H, *J* = 7 Hz, 4-nitrophenyl), 8.19 (d, 2H, *J* = 7 Hz, 4-nitrophenyl), 12.3 (s, broad, 1H, NH, benzimidazole).– MS (70 eV); *m/z* (%) = 175 (100), 441 [M$^+$]; mp 139 °C; Anal. ($C_{26}H_{27}N_5O_2 \cdot 2C_2H_2O_4 \cdot 2H_2O$) C, H, N.

*4-{2-[2-(4-Chlorophenylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine (**8**)*

Synthesized using 4-chlorphenylacetic acid and diamine **4a**.– IR (KBr): ν 3414, 1599, 1493, 1027, 1008 cm^{-1}.– ^{1}H NMR: δ 2.50–2.65 (m, 6H piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.20 (s, 2H CH_2-4-chlorobenzyl), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.00 (d, 1H, *J* = 6 Hz, H-6 benzimidazole), 7.15–7.40 (m, 8H N-phenylpiperazine, H-4, H-6 and H-7 benzimidazole, 4-chlorophenyl).– MS (70 eV); *m/z* (%) = 175 (100), 430 [M$^+$]; mp 225 °C; Anal. ($C_{26}H_{27}ClN_4 \cdot C_2H_2O_4 \cdot 2H_2O$) C, H, N.

*4-{2-[2-(Diphenylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine (**9**)*

Diphenylacetic acid and diamine **4a** were used.– IR (KBr): ν 3435, 2813, 1599, 1496, 1236, 695 cm^{-1}.– ^{1}H NMR: δ 2.60–2.80 (m, 4H, piperazine), 2.80–2.95 (m, 4H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 5.72 (s, 1H, CH, diphenylmethyl), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.10–7.60 (m, 15H, N-phenylpiperazine, diphenylmethyl, H-4, H-6 and H-7 benzimidazole), 12.2 (s, broad, 1H, NH).– MS (70 eV); *m/z* (%) = 175 (100), 472 [M$^+$]; mp 170 °C; Anal. ($C_{32}H_{32}N_4 \cdot 3C_2H_2O_4 \cdot H_2O$) C, H, N.

*4-{2-[2-(1-Naphthylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine **(10)***

1-Naphthylacetic acid and diamine **4a** were used.– IR (KBr): ν 3436, 2814, 1600, 1451, 1238, 803 cm^{-1}.– ^1H NMR: δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.70 (s, 2H, CH_2-naphthyl), 6.80–8.10 (m, 15H, phenyl, H-4, H-6 and H-7 benzimidazole, naphthyl).– MS (70 eV); *m/z* (%) = 175 (100), 446 [M^+]; mp 189 °C; Anal. ($C_{30}H_{30}N_4 \cdot 2C_2H_2O_4 \cdot 2H_2O$) C, H, N.

*4-{2-[2-(2-Naphthylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine **(11)***

2-Naphthylacetic acid and diamine **4a** were used.– IR (KBr): ν 3441, 1599, 1452, 1236, 758 cm^{-1}.– ^1H NMR ($CDCl_3$): δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.30 (s, 2H, CH_2-naphthyl), 6.80–7.80 (m, 15H, phenyl, H-4, H-6 and H-7 benzimidazole, naphthyl).– MS (70 eV); *m/z* (%) = 175 (100), 446 [M^+]; mp 216 °C; Anal. ($C_{26}H_{27}ClN_4 \cdot 2C_2H_2O_4 \cdot 2H_2O$) C, H, N.

*4-{2-[2-(4-Biphenylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine **(12)***

4-Biphenylacetic acid and diamine **4a** were used.– IR (KBr): ν 2946, 2819, 1602, 1488, 1242 cm^{-1}.– ^1H NMR: δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.18 (s, 2H, CH_2-biphenyl), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.10–7.20 (m, 2H, N-phenylpiperazine), 7.20–7.70 (m, 12H, H-4, H-6 and H-7 benzimidazole, biphenyl), 12.20 (s, broad, 1H, NH benzimidazole).– MS (70 eV); *m/z* (%) = 175 (100), 472 [M^+]; mp 215 °C; Anal. ($C_{32}H_{32}N_4$) C, H, N.

*4-{2-[2-(2-Pyridylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine **(13)***

2-Pyridylacetic acid and diamine **4a** were used.– IR (KBr): ν 3402, 1657, 1495, 1051, 1027, 1006 cm^{-1}.– ^1H NMR: δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.30 (s, 2H, CH_2-pyridyl), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.05 (d, 1H, *J* = 6Hz, H-6 benzimidazole), 7.20 (m, 3H, N-phenylpiperazine, H-5 pyridyl), 7.30–7.50 (m, 2H, H-4 and H-7 benzimidazole), 7.72 (d, 1H, *J* = 4 Hz, H-4 pyridyl), 8.30 (s, 1H, H-3 pyridyl), 8.49 (d, 1H, *J* = 2 Hz, H-6 pyridyl).– MS (70 eV); *m/z* (%) = 175 (100), 397 [M^+]; mp 128 °C; Anal. ($C_{25}H_{27}N_4 \cdot 3C_2H_2O_4 \cdot 3/2H_2O$) C, H, N.

*4-{2-[2-(3-Pyridylmethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine **(14)***

3-Pyridylacetic acid and diamine **4a** were used.– IR (KBr): ν 3436, 1656, 1050, 1026, 1006 cm^{-1}.– ^1H NMR: δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.20 (s, 2H, CH_2-pyridyl), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.00 (d, 1H, *J* = 6Hz, H-6 benzimidazole), 7.20 (m, 3H, N-phenylpiperazine, H-5 pyridyl), 7.30–7.50 (m, 2H, H-4 and H-7 benzimidazole), 7.66 (d, 1H, *J* = 4 Hz, H-4 pyridyl), 8.40 (d, 1H, *J* = 2 Hz, H-6 pyridyl), 8.60 (s, 1H, H-2 pyridyl).– MS (70 eV); *m/z* (%) = 175 (100), 397 [M^+]; mp 129 °C; Anal. ($C_{25}H_{27}N_5 \cdot 3C_2H_2O_4 \cdot 3H_2O$) C, H, N.

*4-[2-(2-Phenyl-5-benzimidazolyl)ethyl]-1-phenylpiperazine **(15)***

Synthesized using benzoic acid and diamine **4a**.– IR (KBr): ν 3418, 1720, 1635, 1600, 1402, 1231 cm^{-1}.– ^1H NMR (oxalic acid salt): δ 3.00–3.60 (m, 12H, piperazine, CH_2CH_2N), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.16 (d, 1H, *J* = 6 Hz, H-6 benzimidazole), 7.20 (m, 2H, N-phenylpiperazine), 7.40–7.60 (m, 5H, phenyl), 8.18 (d, 2H, *J* = 6 Hz, H-4 and H-7 benzimidazole).– MS (70 eV); *m/z* (%) = 175 (100), 382 [M^+]; mp 132 °C; Anal. ($C_{25}H_{26}N_4 \cdot 2C_2H_2O_4 \cdot 1/2H_2O$) C, H, N.

*4-{2-[2-(2-Pyridyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine **(16)***

2-Picolinic acid and diamine **4a** were used.– IR (KBr): ν 3436, 1658, 1052, 1027, 1007 cm^{-1}.– ^1H NMR: δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.20–3.40 (m, 4H, piperazine), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.00–7.60 (m, 6H, H-4, H-6 and H-7 benzimidazole, N-phenylpiperazine, H-5 pyridyl), 8.0 (m, 1H, H-4 and pyridyl), 8.3 (d, 1H, *J* = 4 Hz , H-3 pyridyl), 8.65 (d, 1H, *J* = 2 Hz, H-6 pyridyl).– MS (70 eV); *m/z* (%) = 175 (100), 383 [M^+]; mp 116 °C; Anal. ($C_{24}H_{25}N_5 \cdot 3C_2H_2O_4 \cdot 2H_2O$) C, H, N.

*4-{2-[2-(3-Pyridyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine **(17)***

Synthesized using nicotinic acid and diamine **4a**.– IR (KBr): ν 3423, 1626, 1600, 1232 cm^{-1}.– ^1H NMR: δ 3.00–3.60 (m, 12H, N-phenylpiperazine and CH_2CH_2N), 6.80–7.30 (m, 6H, N-phenylpiperazine and H-6 benzimidazole), 7.50–7.70 (m, 3H, H-4 and H-7 benzimidazole, H-4 pyridyl), 8.24 (s, 1H and H-2 pyridyl), 8.49 (m, 1H, H-5 pyridyl), 8.68 (d, 1H, *J* = 2 Hz, H-6 pyridyl), 9.35 (s, broad, 1H, NH, benzimidazole).– MS (70 eV); *m/z* (%) = 175 (100), 383 [M^+]; mp 125 °C; Anal. ($C_{24}H_{25}N_5 \cdot 3C_2H_2O_4 \cdot 2H_2O$) C, H, N.

*4-{2-[2-(Phenylethyl)-5-benzimidazolyl]ethyl}-1-phenylpiperazine **(18)***

2-Phenylpropionic acid and diamine **4a** were used.– IR (KBr): ν 2947, 2819, 1602, 1495, 1244, 697 cm^{-1}.– ^1H NMR: δ 2.50–2.70 (m, 6H, piperazine, CH_2CH_2N), 2.80 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 8H, piperazine, CH_2CH_2-phenyl), 6.70–7.00 (m, 3H, N-phenylpiperazine), 7.10–7.40 (m, 10H, benzyl, H-4, H-6 and H-7 benzimidazole, N-phenylpiperazine), 12.10 (s, broad, 1H, NH, benzimidazole).– MS (70 eV); *m/z* (%) = 175 (100), 410 [M^+]; mp 162 °C ; Anal. ($C_{27}H_{30}N_4$), C, H, N.

*4-{2-[2-(Phenylmethyl)-5-benzimidazolyl]ethyl}-4-(2-methoxyphenyl)-piperazine **(19)***

Synthesized using phenylacetic acid and diamine **4b.–** IR (KBr): ν 2937, 1500, 1454, 1241 cm^{-1}.– ^1H NMR: δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 3.80 (s, 3H, methoxy), 4.20 (s, 2H, CH_2 , benzyl), 6.80–7.00 (m, 4H, 2-methoxyphenyl), 7.10–7.50 (m, 8H, benzyl, H-4, H-6 and H-7 benzimidazole), 12.2 (s, broad, 1H, NH, benzimidazole).– MS (70 eV); *m/z* (%) = 205 (100), 426 [M^+]; mp 197 °C; Anal. ($C_{27}H_{30}ON_4 \cdot 2C_2H_2O_4 \cdot 2H_2O$) C, H, N.

*4-{2-[2-(Phenylmethyl)-5-benzimidazolyl]ethyl}-4-(3-trifluoromethyl-phenyl)-piperazine **(20)***

Phenylacetic acid and diamine **4c** were employed.– IR (KBr): ν 3420, 3225, 1588, 1413, 1168 cm^{-1}.– ^1H NMR: δ 2.50–2.65 (m, 6H, piperazine, CH_2CH_2N), 2.70–2.90 (m, 2H, CH_2CH_2N), 3.10–3.20 (m, 4H, piperazine), 4.10 (s, 2H, CH_2 benzyl), 7.00–7.50 (m, 12H, 3-trifluoromethylphenyl, benzyl, H-4, H-6 and H-7 benzimidazole), 12.3 (s, broad, 1H, NH, benzimidazole).– MS (70 eV); *m/z* (%) = 243 (100), 464 [M^+]; mp 127 °C; Anal. ($C_{27}H_{27}N_4F_3 \cdot 3C_2H_2O_4 \cdot 2H_2O$), C, H, N.

Radioligand binding studies

Synaptosomal membranes prepared from bovine caudate nuclei were used in [^{3}H]spiperone and [^{3}H]SCH 23390 binding assays and for [^{3}H]-8-OH-DPAT assay synaptosomal membranes of the bovine hippocampi were employed[8].

[^{3}H]Spiperone (spec. act. 70 Ci mM^{-1}, Amersham Buchler GmbH, Braunschweig, Germany) binding was assayed at membrane protein concentration of 0.7 mg ml^{-1} in a solution containing (in mM): EDTA 1, $MgCl_2$ 4, $CaCl_2$ 1.5, KCl 5, NaCl 120, Tris-HCl 25, pH 7.4, 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 50 nM ketanserin. K_i values were determined by competition binding at 0.2 nM of the radioligand and eight to ten concentrations of each novel compound (0.1nM–0.1mM). 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 counter at an efficiency of 51–55% for tritium. Binding of [^{3}H]SCH 23390 (spec. act. 80 Ci mM^{-1}, Amersham Buchler GmbH, Braunschweig, Germany) was examined by the same rapid filtration assay discussed for [^{3}H]spiperone in the absence of ketanserin. [^{3}H]-8-OH-DPAT (spec.act. 223 Ci mM^{-1}, Amersham Buchler GmbH, Braunschweig, Germany) binding was assayed in a solution containing (in mM): EDTA 1,

$MgCl_2$ 4, $CaCl_2$ 1.5, KCl 5, NaCl 120, Tris-HCl 25, pH 7.4, then 10 mM nialamide and 0.1% ascorbic acid, at membrane protein concentration of 0.7 mg ml^{-1}, 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 mM 5-hydroxytryptamine.

Competition binding data were analyzed by the iterative non-linear least-squares curve-fitting program LIGAND[16].

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