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Electrostatic Surface Potential Calculation on Several New Halogenated Benzimidazole-like Dopaminergic Ligands

We examined the effects of the electron density distribution (electrostatic surface potential; ESP) of several new benzimidazole-type ligands on their binding affinity for the D_1 and D_2 dopamine receptors (DAR). Receptors were prepared from synaptosomal membranes of bovine caudate nuclei. [^{3}H]SCH 23390 and [^{3}H]spiperone were used as specific radiolabels for the D_1 and D_2 receptors, respectively. The ESP of these compounds was calculated using Gaussian 98 W software. Calculations performed with known dopaminergic ligands showed that the electron density charge in the aromatic ring of these compounds favors a higher binding affinity for the D_2 DAR. This was confirmed by the synthesis of halogenated analogues of several known dopaminergic ligands. Halogenation resulted in an increase in the positive charge of the aromatic part of the molecule. None of the newly synthesized compounds was efficient in displacing [^{3}H]SCH 23390 from the D_1 DAR. The introduction of chlorine into the molecule led to a higher binding affinity for the D_2 DAR of the new ligands in comparison to both parent compounds and brominated ligands. This difference probably originates from the difference in the sizes of chlorine and bromine atoms, which could influence the interaction of a ligand with the receptor binding site. However, among the new ligands with bromine as a substituent, two compounds (**8b** and **10b**) expressed a higher binding affinity and two of them (**9b** and **11b**) a lower binding affinity for the D_2 DAR, when compared to unsubstituted parent compounds. These results indicate that the electrostatic surface potential of a ligand is an important factor in its interaction with the D_2 DAR and that this should be taken into account during design and synthesis of dopaminergic compounds.

Keywords: Benzimidazole; Electrostatic surface potential; Electron density distribution; Dopaminergic; Dopamine receptors; D_2 receptor

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Introduction

Benzimidazoles and their structural analogues can be considered as non-catechol bioisosteres of catecholamines. As a result, numerous new compounds with varying binding affinity for catecholamine receptors have been designed and synthesized [1, 2]. However, the physicochemical basis of the above bioisosterism is still far from being fully understood. This prompted us to study the effects of the electron density distribution (molecular electrostatic surface potential; ESP) [3, 4] around the heterocyclic nuclei of several known dopaminergic (DA-ergic) ligands of benzimidazole type and their halogenated derivatives on their binding affinity for the D_1 and D_2 dopamine receptors (DAR). The influence of ESP on the biological activity of different classes of compounds has been well documented [5–7]. Wilcox and Teeter [8] provided a theoretical model of the ESP distribution of various DA-ergic ligands. We have also observed previously that electrostatic potential in the benzene part of several benzimidazole type ligands affects D_2 DAR affinity [4].

In the present work, we have further evaluated this observation. For this purpose, starting from four known DA-ergic benzimidazole type ligands as parent compounds [3], we prepared halogenated derivatives with the halogen atom directly attached to the benzene ring (Figure 1). Due to their high electron withdrawal effect, it was expected that the halogen atoms would increase positive ESP in the aromatic part of the ligands. Upon ESP mapping, *i.e.* ESP calculation of parent and halogenated ligands, the halogenated compounds

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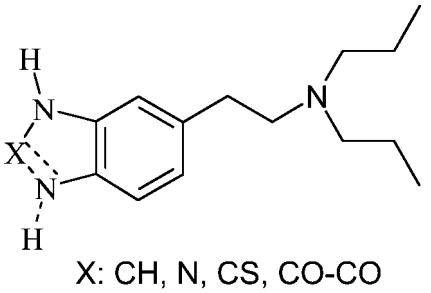

Figure 1. Structure of benzimidazole-like dopaminergic bioisosteres.

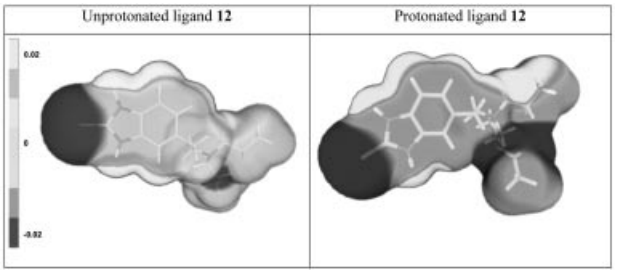

Figure 2. ESP values were mapped on electron density surface for simpler comparisons. Values in dark gray indicate strong, negative ESP, whereas those in light grey correspond to a strong, positive ESP.

were synthesized and their affinities for binding to the D_1 and D_2 DAR were estimated. Correlations between ESP maps in the heterocyclic part of the molecule and the D_2 DAR binding properties are discussed.

Calculations

Ligand models were constructed using the Hyperchem program [9]. Initial geometry optimization was undertaken using Vega software [10] with the PM3 method [11].

The results obtained were further optimized in a Gaussian G 98W [11]. ESP were calculated in G 98W using the DFT B3LYP method and a 6−31 g basis set [12, 13]. The ESP cube output from Gaussian G 98W was visualized in a gOpenMol program [14] following the recommended Gaussian procedure to display the calculated properties**.** The data from the available literature pointed to the fact that DA-ergic ligands bind to the corresponding receptors in a protonated state [15]. We performed ESP calculations using 5-[2-(*N,N*-di-n-propylamino)ethyl]benzimidazol-2-thione (compound **12**) in both the protonated and the unprotonated form (Figure 2). In the protonated form, formiate anion was used to mimic ASP 86 present in the D_2 DAR binding site.

Chemistry

The chemical structures of the compounds synthesized in the present study are shown in Scheme 1. 1-(2-Chloro-ethyl)-4-nitro-benzene (**1**) was reduced with stannous chloride in absolute ethanol and the resulting amine was acylated without purification with acetanhydride to produce N-[4-(2-chloro-ethyl)-phenyl]-acetamide (**2**) [16]. Compound **2** was converted either into N-[2-chloro-4-(2-chloro-ethyl)-phenyl]-acetamide (**3a**) or N-[2-bromo-4-(2-chloro-ethyl)-phenyl]-acetamide (**3b**) using sulfuryl chloride or bromine in acetic acid, respectively. Nitration of N-[2-halogen-4-(2-chloro-ethyl)-phenyl]-acetamides **3a** and **3b** in acetanhydride with sulfuric acid/100% nitric acid afforded the corresponding N-[2-halogen-4-(2-chloro-ethyl)-6-nitro-phenyl]-acetamides **4a** and **4b**. After hydrolysis in 4 N HCl, the resulting 2-halogen-4-(2-chloro-ethyl)-6-nitro-phenylamines **5a** and **5b** readily alkylated dipropylamine in the presence of sodium carbonate and potassium iodide in dimethylformamide. The obtained 2-halogen-4-(2-dipropylamino-ethyl)-6-nitro-phenylamines **6a** and **6b** were reduced with Ra-Ni/hydrazine to produce 3-halogen-5-(2-dipropyl-amino-ethyl)-benzene-1,2-diamines **7a** and **7b**. 4-Chloro-6-(2-dipropylamino-ethyl)-1,3-dihydro-benzimidazole-2-thione (**8a**), 4-bromo-6-(2-dipropylamino-ethyl)-1,3-dihydro-benzimidazole-2-thione (**8b**)**,** [2-(7-chloro-1H-benzimidazol-5-yl)-ethyl]-dipropyl-amine (**9a**), [2-(7-bromo-1H-benzimidazol-5-yl)-ethyl]-dipropyl-amine (**9b**), [2-(7-chloro-1H-benzotriazol-5-yl)-ethyl]-dipropyl-amine (**10a**), [2-(7-bromo-1H-benzotriazol-5-yl)-ethyl]-dipropyl-amine (**10b**), 5-chloro-7-(2-dipropyl-amino-ethyl)-1,4-dihydro-quinoxaline-2,3-dione (**11a**), and 5-bromo-7-(2-dipropylamino-ethyl)-1,4-dihydro-quinoxaline-2,3-dione (**11b**)**,** used as target ligands, were prepared analogously to the corresponding phenylethylamines described previously [17].

Results and discussion

All compounds were evaluated for their binding affinity for the D_1 and D_2 dopamine receptor subtypes from the synaptosomal membranes, prepared from bovine caudate nuclei, by the *in vitro* competitive displacement of specific radioligands [18]. The binding param-

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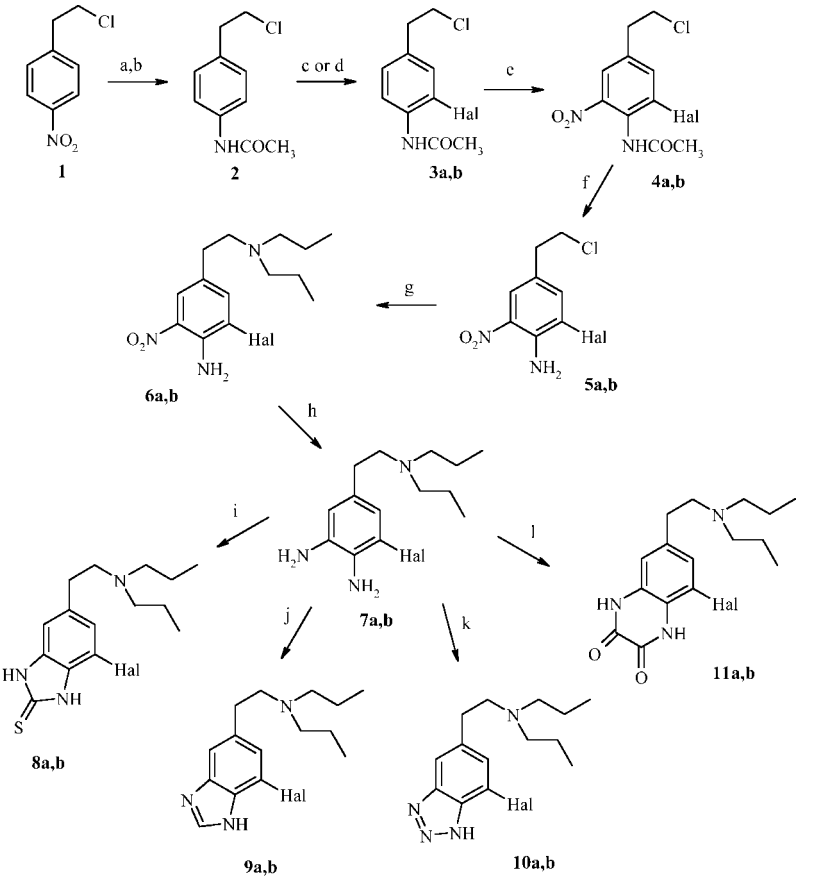
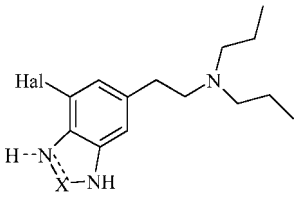


Scheme 1. Pathways for the synthesis of the ligands.

eters of the compounds are listed in Table 1. The distribution of electrostatic charges in these compounds was calculated and the results for parent compounds in protonated and unprotonated form are shown in Figure 2. In the heterocyclic part of the ligands no significant differences were observed between the ESP of protonated and unprotonated forms. Therefore, we decided to rely on the ESP calculations of the unprotonated form of the novel ligands considered in the present study.

As seen from Table 1, none of the compounds examined were active displacers of [^{3}H]SCH 23390 from the D_1 DAR. D_2 DAR binding studies revealed an interesting profile of activities of the novel ligands. Chlorinated ligands **8a**-**11a** had a higher affinity for binding to the D_2 DAR in comparison with both parent compounds and their brominated analogues. Among the bromine-substituted ligands, two compounds (**8b** and **10b**) had a higher binding affinity, while the other two (**9b** and **11b**) had a lower binding affinity for the D_2 DAR when compared to the parent compounds. This can be ascribed to the size of the bromine atom, which could prevent sterically favorable interactions of the brominated ligand with the receptor ligand binding pocket. On the other hand, the introduction of halogen atoms into the benzene ring did not significantly affect the relative order of magnitude of DA-ergic activity of the different types of ligands, *i.e.* benzimidazolethiones > quinoxaline-2,3-diones ~ benzotriazoles > benzimidazoles.

Table 1. Affinity of the new ligands for binding to the D_1 and D_2 dopamine receptors. Values are the means of three independent experiments done in triplicate performed at eight competing ligand concentrations ($10^{-4}-10^{-9}$ M) and 0.2 mM [^{3}H]SCH23390 or [^{3}H]spiperone.



No	X	Hal	Ki ± S.E.M. (μM) D_1	D_2
8a	C=S	Cl	>1000	0.05 ± 0.003
8b	C=S	Br	>1000	0.2 ± 0.04
9a	CH	Cl	>1000	4.0 ± 0.1
9b	CH	Br	>1000	>100
10a	N	Cl	>1000	0.3 ± 0.01
10b	N	Br	>1000	0.7 ± 0.02
11a	CO-CO	Cl	>1000	0.9 ± 0.04
11b	CO-CO	Br	>1000	>100
12	C=S	H	>1000	1.0 ± 0.2
13	CH	H	>1000	50 ± 9
14	N	H	>1000	5.0 ± 2.0
15	CO-CO	H	>1000	3.0 ± 0.1

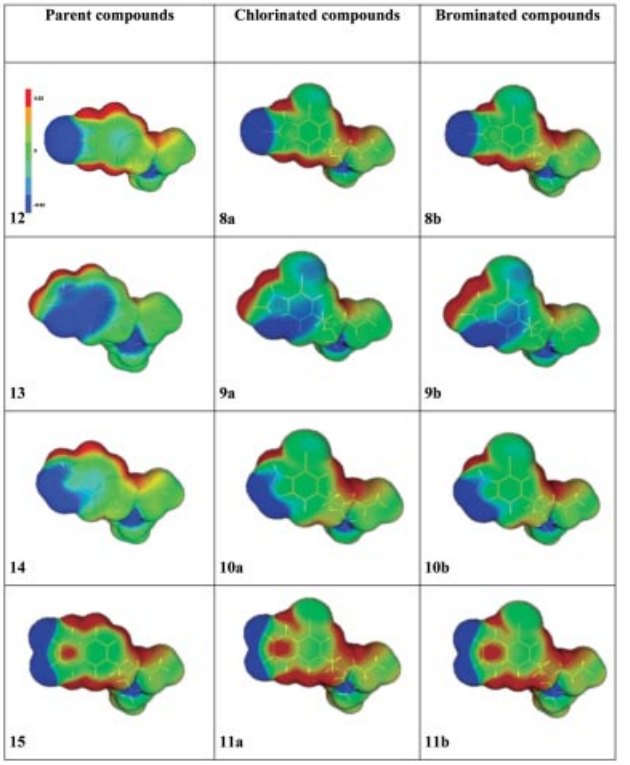


Figure 3. ESP values of benzimidazoles, benzimidazolethiones, benzotriazoles, quinoxalinediones and their halogenated derivatives. ESP values were mapped on the electron density surface for a simpler comparison. Values in blue indicate a strong, negative ESP, whereas values in red correspond to a strong, positive ESP.

For the G protein-coupled receptors, whose native ligands are catecholamines, it seems that the catechol moiety of the ligands interacts with the receptor via hydrogen bonds to serine residues in the fifth transmembrane spanning region (TMV) of the receptors [19]. For the D_2 dopamine receptor, that contains three conserved serines in the TMV [Ser^{193} (V8), Ser^{194} (V9), and Ser^{197} (V12)] [20, 21], mutation of these residues to alanines affects the binding of certain antagonists and agonists. These studies indicated that the conserved serine residues clearly act together to alter ligand binding and effector coupling. Results of the ESP calculations for the novel ligands are shown in Figure 3. Examination of the ESP maps suggested that high electron density areas could be necessary for hydrogen bond formation between the central front end of a ligand and the serines in the binding pocket of the D_2 DAR.

Benzimidazolethiones **8a**, **8b** and **12** are the most potent DA-ergic agents among the ligands tested. We believe this is because they have the highest centrally located negative ESP, which might favor hydrogen bonding with Ser^{193} (V8), Ser^{194} (V9), and Ser^{197} (V12) of the D_2 receptor. Benzotriazoles **10a, 10b** and **14** and quinoxaline-2,3-diones **11a**, **11b** and **15** were less active displacers of [^{3}H]spiperone than the above benzimidazolethiones. Benzimidazoles **9a**, **9b** and **13** were found to have a laterally located negative ESP on only one side of the molecule (Figure 3), which could render the formation of the hydrogen bond between the ligand and the receptor binding site difficult.

Comparison of the ESP maps of parent compounds with the halogenated ligands (Figure 3) clearly demonstrated that the introduction of a halogen atom in the benzene part of the former molecules resulted in a decrease in electron density in the benzene ring, just as expected. The increasing ESP in the benzene part of the heterocyclic ring was accompanied by an increased affinity for binding to the D_2 DAR, and **8a**,**b**,

10a,b and **11a** were the most active DA-ergic ligands among the newly synthesized compounds. Since the decrease in electron density in the benzene ring of chlorinated (**8a**−**11a**) and brominated (**8b**−**11b**) ligands was similar, the differences in the DA-ergic activity between these two groups of compounds can be ascribed to the difference in size between the chlorine and bromine atoms. At present, the details of the interaction between the part of ligand characterized by low electron density and the D_2 receptor are insufficiently known, although the presence of an electron-rich counterpart in the receptor ligand binding pocket could be postulated. Generally, a compound with the lowest electron density in the aromatic part and the highest negative ESP in the central front end of the molecule should express the highest DA-ergic activity.

As alternative explanation for the results obtained, the observed differences may be due to the fact that not all of the compounds interact in the same way with the binding site or the receptor. This would influence hydrogen bond geometry and, therefore, the energy of binding in general. Without exact 3D crystal data it is difficult to distinguish between the two models of receptor-ligand interaction presented above.

Our current hypothesis is that the overall affinity of the compounds synthesized during the present study and evaluated for the DA-ergic activity represents a sum of electronic and bulk interactions as well as of hydrogen bonding. However, the influence of some other factors such as ESP cannot be excluded. A full understanding of the way in which ESP influences the overall DA-ergic activity of a ligand might be an important step in the direction of design and synthesis of new DA-ergic ligands.

Acknowledgments

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Experimental

General

A Boetius PHMK apparatus (VEB Analytic, Dresden, Germany) was used to determine melting points, presented here as uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra recorded on a Gemini 2000 spectrometer (Varian, Palo Alto, CA, USA) with $CDCl_3$ as a solvent unless otherwise stated are reported in ppm downfield from the internal standard tetramethylsilane. The IR spectra were run on a Perkin Elmer 457 Grating Infrared Spectrophotometer (Perkin Elmer, Beaconsfield, GB). The mass spectra were determined by a Finnigan Mat 8230 mass spectrometer (Finnigan, Bremen, Germany). For analytical thin-layer chromatography Merck (Darmstadt, Germany) F-256 plastic-backed thin-layer silica gel plates were used. Chromatographic purifications were 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

*Synthesis of N-[2-chloro-4-(2-chloro-ethyl)-phenyl]-acetamide (**3a**)*

The mixture containing 0.56 g (3 mmol) N-[4-(2-chloro-ethyl)-phenyl]-acetamide (**2**), 5 mL $CHCl_3$ and 0.4 mL (5 mmol) sulfuryl chloride was stirred (2 h, 70 °C). The reaction mixture was cooled to ambient temperature, washed with diluted $NaHCO_3$ solution, dried and evaporated *in vacuo*. The residue was chromatographed on silica gel. Yield: 75 %; ^{1}H-NMR (ppm): 2.23 (s, 3H, CH_3), 3.01 (t, 2H, J = 7.2 Hz, CH_2), 3.68 (t, 2H, J = 6.8 Hz, CH_2), 7.13 (dd, 1H, J = 8.4 Hz, ArH), 7.25 (d, 1H, J = 2 Hz, ArH), 7.60 (s, 1H, NH), 8.29 (d, 1H, J = 8.4 Hz, ArH).

*Synthesis of N-[2-bromo-4-(2-chloro-ethyl)-phenyl]-acetamide (**3b**)*

The mixture of 1.0 g (5 mmol) N-[4-(2-chloro-ethyl)-phenyl]-acetamide (**2**) and 3.0 mL CH_3CO_2H was heated to 45 °C, then 0.26 mL (0.82 g, 5.05 mmol) bromine were added dropwise at a rate that maintained the temperature of the well-stirred mixture at 50−55 °C. After all bromine was added, stirring was continued for a further 30 min, then the reaction mixture was poured into a well-stirred mixture of ice and solid sodium metabisulphite, and extracted with CH_2Cl_2. The organic layer was dried and evaporated *in vacuo*. The residue was chromatographed on silica gel. Yield: 60 %; ^{1}H-NMR (ppm): 2.23 (s, 3H, CH_3), 3.00 (t, 2H, J = 7 Hz, CH_2), 3.68 (t, 2H, J = 7.2 Hz, CH_2), 7.17 (dd, 1H, J = 7.4 Hz, ArH), 7.41 (d, 1H, J = 2 Hz, ArH), 7.57 (s, 1H, NH), 8.27 (d, 1H, J = 8.4 Hz, ArH).

*Synthesis of N-[2-chloro-4-(2-chloro-ethyl)-6-nitro-phenyl]-acetamide (**4a**) and N-[2-bromo-4-(2-chloro-ethyl)-6-nitro-phenyl]-acetamide (**4b**)*

Conc. H_2SO_4 (0.6 mL) was added to the cooled solution (0 °C) of 40 mmol of either acetamide **3a** or **3b** in 60 mL acetanhydride. The reaction mixture was cooled to −5 °C and 2 mL 100 % HNO_3 were introduced dropwise. After overnight stirring, the reaction mixture was poured into ice, made alkaline with sodium hydroxide solution and extracted with CH_2Cl_2. The organic layer was dried and evaporated *in vacuo*. The residue was chromatographed on silica gel.

(**4a**): Yield: 70 %; ^{1}H-NMR (ppm): 2.23 (s, 3H, CH_3), 3.20 (t, 2H, J = 6.8 Hz, CH_2), 3.81 (t, 2H, J = 6.8 Hz, CH_2), 7.10 (s, 1H, NH), 7.72 (d, 1H, J = 2 Hz, ArH), 7.90 (d, 1H, J = 2 Hz, ArH).

(**4b**): Yield: 65 %; ^{1}H-NMR (ppm): 2.82 (s, 3H, CH_3), 3.22 (t, 2H, J = 6.6 Hz, CH_2), 3.82 (t, 2H, J = 6.6 Hz, CH_2), 7.27 (s, 1H, NH), 7.93 (d, 1H, J = 2 Hz, ArH), 8.10 (d, 1H, J = 2 Hz, ArH).

*Synthesis of 2-chloro-4-(2-chloro-ethyl)-6-nitro-phenylamine (**5a**) and 2-bromo-4-(2-chloro-ethyl)-6-nitro-phenylamine (**5b**)*

Of either acetamide **4a** or **4b,** 0.5 mmol were resuspended in 50 mL of 4 N HCl and refluxed for 4 h. The solution was cooled to ambient temperature and diluted with 150 mL of an ice-water mixture. The excess acid was neutralized with 10% NaOH solution keeping the temperature below 30 °C, and the product was extracted with CH_2Cl_2. After drying and evaporation *in vacuo,* the residue was chromatographed on silica gel.

(**5a**): Yield: 90%; ^{1}H-NMR (ppm): 2.98 (t, 2H, *J* = 6.8 Hz, CH_2), 3.70 (t, 2H, *J* = 6.8 Hz, CH_2), 6.51 (s, 2H, NH_2), 7.52 (d, 1H, *J* = 2 Hz, ArH), 7.96 (d, 1H, *J* = 2 Hz, ArH).

(**5b**): Yield: 93%; ^{1}H-NMR (ppm): 2.93 (t, 2H, *J* = 6.6 Hz, CH_2), 3.71 (t, 2H, *J* = 6.6 Hz, CH_2), 6.60 (s, 2H, NH_2), 7.61 (d, 1H, *J* = 2 Hz, ArH), 8.06 (d, 1H, *J* = 2 Hz, ArH).

*Synthesis of 2-chloro-4-(2-dipropylamino-ethyl)-6-nitro-phenylamine (**6a**) and 2-bromo-4-(2-dipropylamino-ethyl)-6-nitro-phenylamine (**6b**)*

The stirred mixture of 2.28 mmol of either **5a** or **5b**, 0.7 mL (0.5 g, 5 mmol) dipropylamine, finely powdered sodium carbonate (0.5 g) and potassium iodide (0.04 g) in 10 mL dimethylformamide was heated overnight at 80 °C. The reaction mixture was cooled to ambient temperature, filtered, washed with toluene and evaporated *in vacuo*. The residue was chromatographed on silica gel.

(**6a**): Yield: 54%; ^{1}H-NMR (ppm): 1.03 (t, 6H, *J* = 7.6 Hz, CH_3), 1.86 (m, 4H, CH_2), 3.03 (m, 4H, CH_2), 3.17 (s, 4H, CH_2CH_2), 6.58 (s, 2H, NH_2), 7.56 (d, 1H, *J* = 2 Hz, ArH), 7.95 (d, 1H, *J* = 2 Hz, ArH).

(**6b**): Yield: 60%; ^{1}H-NMR (ppm): 0.88 (t, 6H, *J* = 7.4 Hz, CH_3), 1.46 (m, 4H, CH_2), 2.46 (m, 4H, CH_2), 2.67 (s, 4H, CH_2CH_2), 6.50 (s, 2H, NH_2), 7.61 (d, 1H, *J* = 2 Hz, ArH), 7.98 (d, 1H, *J* = 2 Hz, ArH).

*Synthesis of 3-chloro-5-(2-dipropylamino-ethyl)-benzene-1,2-diamine (**7a**) and 3-bromo-5-(2-dipropylamino-ethyl)-benzene-1,2-diamine (**7b**)*

Raney-Ni (0.06−0.08 g) was added in small portions to a stirring solution of 2 mmol of either nitro compound (**6a** or **6b**) in 5 mL EtOH, 10 mL 1,2-dichloro-ethane and 0.9 mL 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.

*Synthesis of 4-chloro-6-(2-dipropylamino-ethyl)-1,3-dihydro-benzimidazole-2-thione (**8a**) and 4-bromo-6-(2-dipropyl-amino-ethyl)-1,3-dihydro-benzimidazole-2-thione (**8b**)*

Carbon disulfide (0.24 mL, 4 mmol) and KOH (0.25 g in 0.6 mL water) were added to 2 mmol of either diamine **7a** or **7b** in 10 mL EtOH. After refluxing for 3 h, 0.3 mL of acetic acid in 3.3 mL water were added. The solvent was removed *in vacuo* and the residue chromatographed on silica gel.

(**8a**): Yield: 340 mg, 52%; m.p. >250 °C; IR (KBr) (cm^{-1}): 3065, 2962, 1567, 1491, 1403; ^{1}H-NMR (DMSO)(ppm): 0.81 (t, 6H, *J* = 7.4 Hz, CH_3), 1.40 (m, 4H, CH_2), 2.45 (m, 4H, CH_2), 2.69 (m, 4H, CH_2CH_2), 6.96 (s, 1H, ArH), 7.09 (s, 1H, ArH); ^{13}C-NMR: 11.92, 20.66, 32.29, 55.34, 55.59, 108.57, 113.16, 122.87, 128.47, 133.76, 136.87, 169.40; MS: *m/z* 114(100), 311(M^+).

(**8b**): Yield: 430 mg, 60%; m.p. >250 °C; IR (KBr) (cm^{-1}): 3055, 2963, 1570, 1485, 1403; ^{1}H-NMR (DMSO) (ppm): 0.80 (t, 6H, *J* = 7.2 Hz, CH_3), 1.42 (m, 4H, CH_2), 2.66 (m, 4H, CH_2), 3.45 (m, 4H, CH_2CH_2), 6.98 (s, 1H, ArH), 7.20 (s, 1H, ArH), 8.33 (s, 1H, NH); ^{13}C-NMR: 11.94, 20.09, 32.44, 55.43, 55,76, 100.73, 108.92, 125.74, 130.20, 133.39, 137.41, 169.29; MS: *m/z* 114(100), 356(M^{+1}).

*Synthesis of [2-(7-chloro-1H-benzimidazol-5-yl)-ethyl]-dipropyl-amine (**9a**) and [2-(7-bromo-1H-benzimidazol-5-yl)-ethyl]-dipropyl-amine (**9b**)*

Two mmol of either diamine **7a** or **7b** and 0.44 mL (7.3 mmol) 98% formic acid were heated in an oil bath at 100 °C for 2 h. After cooling to ambient temperature, 15 mL 10% $NaHCO_3$ were added and the product was extracted with CH_2Cl_2. The solvent was removed *in vacuo* and the residue chromatographed on silica gel.

(**9a**): Yield: 335 mg, 60%; m.p. 113 °C; IR (KBr) (cm^{-1}): 3029, 2932, 1578, 1477, 1433; ^{1}H-NMR (ppm): 0.89 (t, 6H, *J* = 7.4 Hz, CH_3), 1.50 (m, 4H, CH_2), 2.51 (m, 4H, CH_2), 2.81 (m, 4H, CH_2CH_2), 7.16 (s, 1H, ArH), 7.27 (s, 1H, NH), 7.35 (s, 1H, ArH), 8.08 (s, 1H, CH); ^{13}C-NMR: 11.95, 20.13, 32.80, 55.50, 56.07, 118.90, 122.56, 125.63, 131.24, 134.83, 136.30, 142.77; MS: *m/z* 114(100), 280(M^{+1}).

(**9b**): Yield: 421 mg, 65%; m.p. 141 °C; IR (KBr) (cm^{-1}): 3025, 2958, 1570, 1463, 1434; ^{1}H-NMR (ppm): 0.89 (t, 6H, *J* = 7.2 Hz, CH_3), 1.49 (m, 4H, CH_2), 2.52 (m, 4H, CH_2), 2.84 (m, 4H, CH_2CH_2), 7.32 (s, 1H, ArH), 7.39 (s, 1H, ArH), 8.09 (s, 1H, CH); ^{13}C-NMR: 11.97, 20.03, 32.69, 55.50, 56.50, 117.81, 119.32, 125.56, 135.46, 139.21, 140.52, 142.62; MS: *m/z* 114(100), 324(M^{+1}).

*Synthesis of [2-(7-chloro-1H-benzotriazol-5-yl)-ethyl]-dipropyl-amine (**10a**) and [2-(7-bromo-1H-benzotriazol-5-yl)-ethyl]-dipropyl-amine (**10b**)*

Of either diamine **7a** or **7b**, 2 mmol were dissolved in a mixture of 0.5 mL acetic acid and 0.9 mL water. After that, 0.16 g (2.35 mmol) $NaNO_2$ dissolved in 0.25 mL water were added at 0 °C. The solution was heated (70 °C, 10 min.) and, after cooling to ambient temperature, the solvent was removed *in vacuo*. The residue was resuspended in 10 mL of 5% $NaHCO_3$ and the product was extracted with CH_2Cl_2. The solvent was removed *in vacuo* and the residue chromatographed on silica gel.

(**10a**): Yield: 343 mg, 61%; oil; IR (KBr) (cm^{-1}): 3055, 2965, 1554, 1467, 1417; ^{1}H-NMR (ppm): 0.92 (t, 6H, *J* = 7.6 Hz, CH_3), 1.64 (m, 4H, CH_2), 2.81 (m, 4H, CH_2), 3.05 (d, 4H, *J* = 3.6 Hz, CH_2CH_2), 7.12 (s, 1H, NH), 7.13 (s, 1H, ArH), 7.62 (s, 1H, ArH); ^{13}C-NMR: 11.88, 19.93, 32.56, 55.34, 56.04, 111.64, 120.54, 125.82, 138.29, 138.65, 140.69; MS: *m/z* 114(100), 280(M^+).

(**10b**): Yield: 423 mg, 65%; oil; IR (KBr) (cm^{-1}): 3100, 2970, 1548, 1466, 1413; ^{1}H-NMR (ppm): 0.92 (t, 6H, *J* = 7.2 Hz, CH_3), 1.60 (m, 4H, CH_2), 2.79 (m, 4H, CH_2), 3.03 (s, 4H, CH_2CH_2), 7.28 (s, 1H, ArH), 7.63 (s, 1H, ArH), 10.47 (s, 1H, NH); ^{13}C-NMR: 11.83, 19.76, 32.33, 55.45, 56.11, 108.68, 112.16, 122.17, 129.00, 137.80, 140.86; MS: *m/z* 114(100), 324(M^+).

*Synthesis of 5-chloro-7-(2-dipropylamino-ethyl)-1,4-dihydro-quinoxaline-2,3-dione (**11a**) and 5-bromo-7-(2-dipropylamino-ethyl)-1,4-dihydro-quinoxaline-2,3-dione (**11b**)*

Two mmol of either diamine **7a** or **7b,** 0.55 g (4.4 mmol) oxalic acid and 2.5 mL 4 N HCl were refluxed for 60 min. After cooling to ambient temperature, the solvent was removed *in vacuo*. The residue was resuspended in 20 mL 10% $NaHCO_3$ and the product was extracted with CH_2Cl_2. The solvent was removed *in vacuo* and the residue chromatographed on silica gel.

(**11a**): Yield: 280 mg, 43%; m.p. 230°C; IR (KBr) (cm^{-1}): 3046, 2965, 1699, 1601, 1392; ^{1}H-NMR (DMSO) (ppm): 0.92 (t, 6H, J = 7.2 Hz, CH_3), 1.69 (m, 4H, CH_2), 3.05 (m, 4H, CH_2), 3.13 (m, 2H, CH_2CH_2), 3.46 (m, 2H, CH_2N), 7.01 (s, 1H, ArH), 7.25 (s, 1H, ArH), 11.45 (s, 1H, NH), 12.18 (s, 1H, NH); ^{13}C-NMR: 11.14, 16.60, 28.46, 52.81, 53.34, 114.66, 118.79, 121.94, 123.82, 127.29, 133.17, 155.04, 155.49; MS: *m/z* 114(100), 322(M^{-1}).

(**11b**): Yield: 295 mg, 40%; m.p. 152°C; IR (KBr) (cm^{-1}): 3146, 2967, 1695, 1620, 1395; ^{1}H-NMR (DMSO) (ppm): 0.92 (t, 6H, J = 7 Hz, CH_3), 1.69 (m, 4H, CH_2), 3.04 (m, 4H, CH_2), 3.24 (m, 4H, CH_2CH_2), 7.03 (s, 1H, ArH), 7.40 (s, 1H, ArH), 11.06 (s, 1H, NH), 12.15 (s, 1H, NH); ^{13}C-NMR: 11.84, 16.60, 28.38, 52.83, 53.30, 108.16, 115.33, 123.11, 127.06, 129.72, 133.65, 155.02, 155.57; MS: *m/z* 114(100), 367(M^+).

Synaptosomal membrane preparation, binding assays and data analysis

The synaptosomal membranes of the bovine caudate nuclei that were used as a source of the dopamine D_1 and D_2 receptor subtypes were prepared exactly as described previously [22].

[^{3}H]SCH 23390 (80 Ci $mmol^{-1}$) and [^{3}H]spiperone (70 Ci $mmol^{-1}$) used to label D_1 and D_2 receptor subtypes, respectively, were purchased from Amersham Buchler GmbH (Braunschweig, Germany). Briefly, [^{3}H]spiperone binding was assayed in binding buffer at 37°C for 20 min in a total volume of 0.5 mL. The 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 ligand were tested (0.1 μM–0.1 mM). Nonspecific binding was measured in the presence of 1.0 mM (+)-butaclamol. The reaction was terminated by a rapid filtration through Whatman GF/C filters, before washing three times with 5.0 mL ice-cold incubation buffer. Radioligand binding for each compound concentration was determined in triplicate. Retained radioactivity was measured by introducing dry filters into 10 mL toluene-based scintillation liquid and by counting in a 1219 Rackbeta Wallac scintillation counter. Binding of [^{3}H]SCH 23390 was examined by the same rapid filtration assay discussed for [^{3}H]spiperone but in the absence of ketanserin.

Competition binding data were analyzed by the non-linear least-squares curve-fitting program LIGAND [23].

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