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Modeling of the D_2 Dopamine Receptor Arylpiperazine Binding Site for 1-{2-[5-(*1H*-benzimidazole-2-thione)]ethyl}-4-arylpiperazines

Docking of several 1-{2-[5-(*1H*-benzimidazole-2-thione)]ethyl}-4- and 1-benzylarylpiperazines to the D_2 dopamine receptor (DAR) was examined. The binding pocket of the D_2 DAR defined according to Teeter and DuRand [1] was extended using the Insight II software. It was found that (i) the interaction of the protonated N1 of the piperazine ring with Asp86, (ii) the hydrogen bond formation between the benzimidazole part of the ligand and Ser141, as well as Ser122, and (iii) the edge-to-face interactions of the aromatic ring or arylpiperazine part of the ligand with Phe178, Tyr216 and Trp182 of the receptor represent the mayor stabilizing forces. Besides, the hydrogen bond acceptor group in position 2 of the phenylpiperazine aromatic ring could form one more hydrogen bond with Trp182. Bulky substituents in position 4 are not tolerated, due to the unfavorable sterical interaction with Phe178. Substituents in positions 2 and 3 are sterically well tolerated. Electron-attractive groups (F, Cl, CF_3, and NO_2) decreased, while electron donors (-OMe) and the second aromatic ring (naphthyl) increased the binding affinity, as compared to that of the parent compound **1**. This can be explained by strong edge-to-face interactions of negative electrostatic surface potential (ESP) in the center of aromatic residues of the ligand with positive-ESP protons in the aromatic residues of the receptor. Thus, besides the salt bridges and hydrogen bonds, edge-to-face interactions significantly contribute to arylpiperazine ligands forming complexes with the D_2 DAR.

Keywords: Arylpiperazines; D_2 receptor; Modeling; Interaction; Binding pocket

Received: April 30, 2004; Accepted: July 1, 2004 [FP901]
DOI 10.1002/ardp.200400901

Introduction

During the recent years, the identification of multiple dopamine (DA) receptor subtypes has been accompanied by the development of agents that alter DA neurotransmission [2]. For many years, the D_2 DAR was a major target for neurobiological research and drug development, since dopamine antagonists have been proven to be effective antipsychotics [3]. In the course of a program aimed at the discovery of new dopaminergic ligands, we have synthesized a series of benzimidazoles that could be considered as non-catechol bioisosteres of catecholamines [4]. The most active compounds of this type were obtained by connecting the benzimidazolethione ring through the flexible ethylene linker with N-arylpiperazines, which afforded compounds of the general structure I (Figure 1). It was noticed that the binding affinity of the prepared ligands for the D_2 DAR depends on both the benzimidazole structure and the structure of the arylpiperazine part of the molecule, but the effect of the latter was more pronounced. However, the physicochemical basis of the above interactions is still far from being fully understood. This prompted us to study the effect of the electron density distribution (electrostatic surface potential; ESP) in the arylpiperazine part of this class of ligands on their binding affinity for the D_2 DAR. The binding pocket of the D_2 receptor was de-

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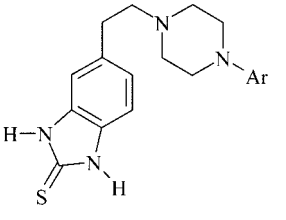
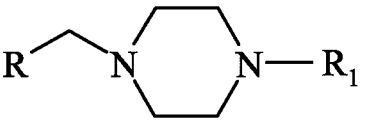


Figure 1. Structure of 1-{2-[5-(1H-benz-imidazole-2-thione)]ethyl}-4-arylpiperazines.

Table 1. Chemical structures of 1-{2-[5-(1*H*-benzimidazole-2-thione)]ethyl}-4-arylpiperazine and 1-benzyl-4-arylpiperazine ligands tested for docking in the D_2 DAR binding pocket. Compounds **9**–**12** were newly synthesized, while all others were already described. For references, see text..



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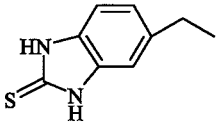
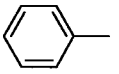
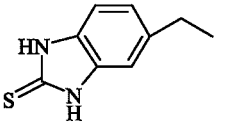
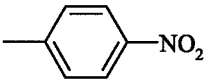
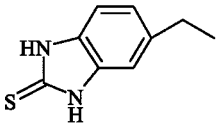
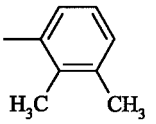
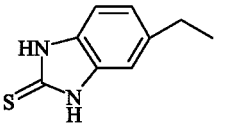
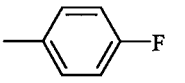
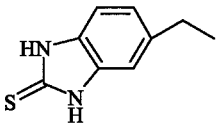
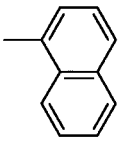
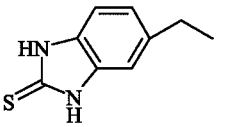
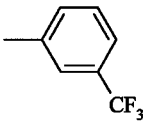
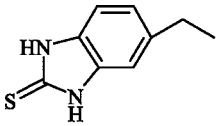
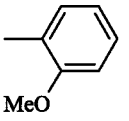
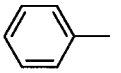
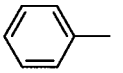
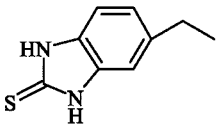
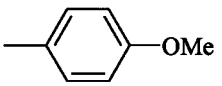
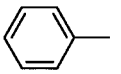
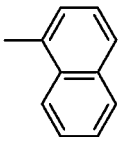
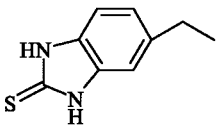
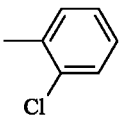
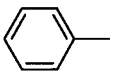
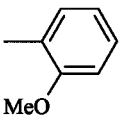
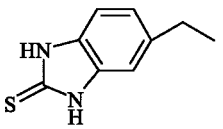
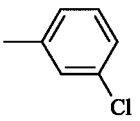
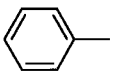
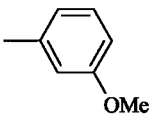
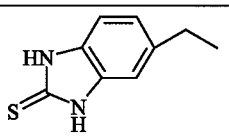
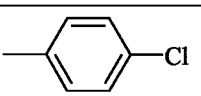
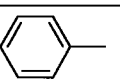
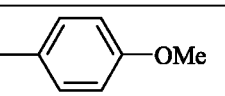
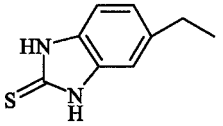
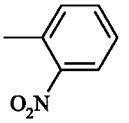
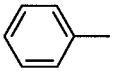
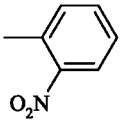
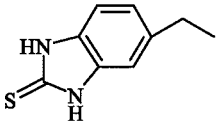
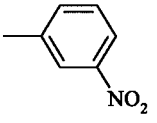
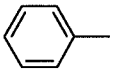
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		

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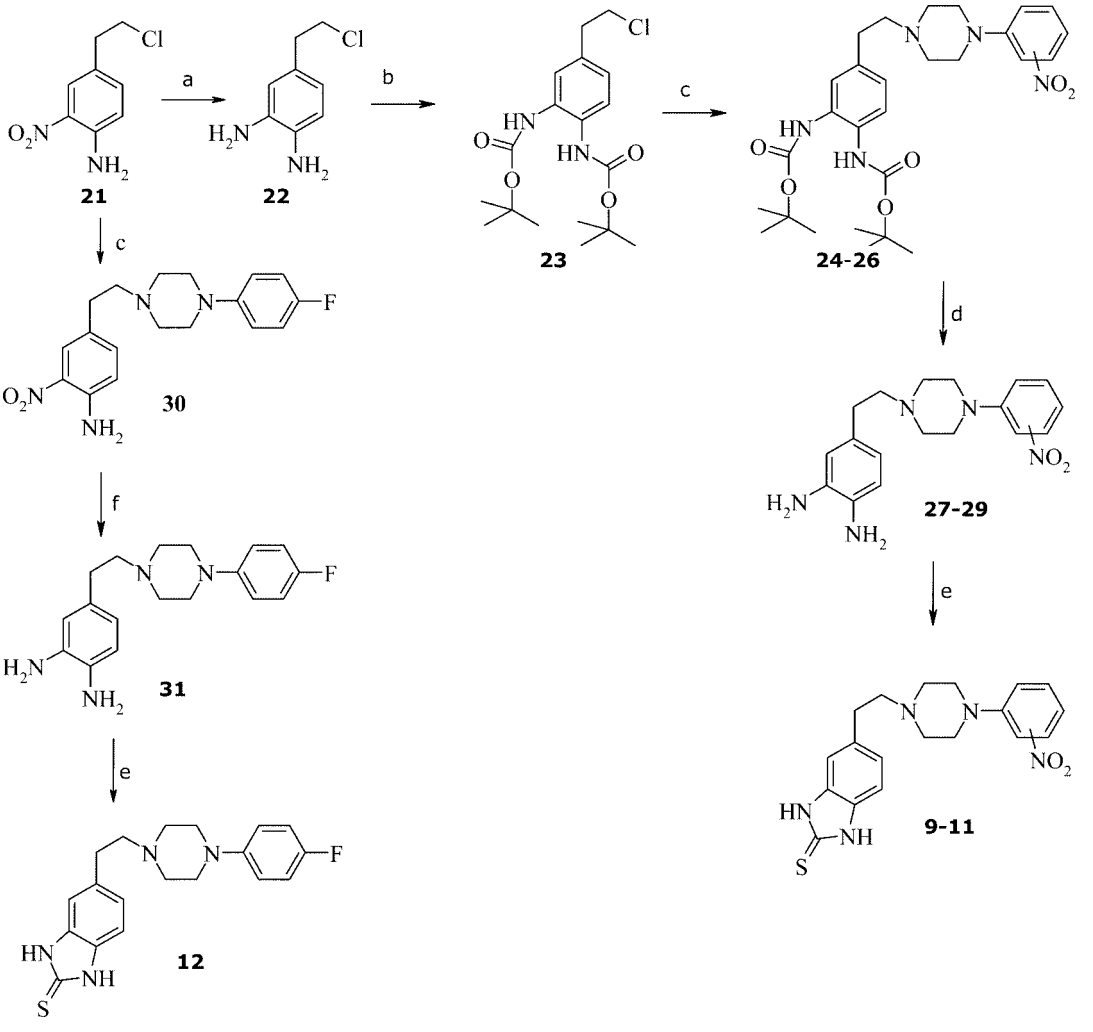

a) EtOH, $SnCl_2$, reflux; b) dioxane, 1N NaOH, di-tert-butyl-dicarbonate; c) DMF, K_2CO_3, KI, substituted piperazines, 80 °C; d) EtOH, 4N HCl; e) EtOH, KOH, CS_2, reflux; f) EtOH, N_2H_4, Ra-Ni

Scheme 1. Synthesis of 1-{2-[5-(*1H*-benzimidazole-2-thione)]ethyl}-4-arylpiperazines (**9**–**12**).

fined according to Teeter and DuRand [1]. Special attention has been paid to hydrophobic-type interactions (e.g. stacking or edge-to-face interactions), which play a significant role in the formation of the receptor-ligand complexes [1, 5]. These attractive interactions occur between aromatic moieties devoid of polar substituents. “Edge-to-face” interactions, though modest in energy terms, can play an important role in diverse areas such as protein folding, base pair stacking in DNA, host-guest binding in supramolecular assemblies, crystal engineering, drug-receptor interactions, and other molecular recognition processes [6]. Energetically, they can stabilize the system by up to −2.5 kcal/mol [7] Edge-to-face interactions between receptors and their ligands should be exclusively dependent on the shape of the ligand molecule and its ability to interact with the aromatic residues in the binding pocket of the receptor [6, 7]. Complementarities of negative ESP in the center of aromatic residues of the ligands and positive ESP of the protons in aromatic residues of the receptor, as well as a proper orientation of molecular entities forming the complex, are prerequisites for this type of interactions. The data obtained throughout the present study could serve as a useful basis for further rational design of D_2 receptor ligands.

Results and discussion

Several new 1-{2-[5-(*1H*-benzimidazole-2-thione)]-ethyl}-4-arylpiperazines (compounds **9**–**12**; Table 1) were synthesized as shown in Scheme 1, and their affinity for binding the D_2 DAR was determined. Shortly, 4-(2-chloroethyl)-2-nitroaniline (**21**) was reduced with stannous chloride in absolute ethanol, and the resulting diamine **22** was converted into di-tBOC derivative **23**, using di-tertbutyldicarbonate. Compound **23** readily alkylated substituted piperazines in the presence of sodium carbonate and potassium iodide in DMF. Compound **30** was prepared in the same manner, directly from nitroaniline **21**. Diamines **27**–**29** were obtained by hydrolyzing di-tBOC derivatives **24**–**26** with 4 N HCl in ethanol. Diamine **31** was produced by reducing nitroaniline **30** with Ra-Ni/hydrazine. Benzimidazole-2-thiones **9**–**12** were synthesized from the corresponding diamines (**27**–**29** and **30**) with CS_2/KOH in EtOH.

In binding experiments, synaptosomal membranes of the bovine caudate nuclei as a source of the D_2 DAR and [^{3}H]spiperone as a specific radioligand were used. The new compounds, along with a number of previously described ligands (Table 1), were tested for their docking in the D_2 DAR binding site.

The binding pocket of the D_2 DAR was defined according to the model of D_2 DAR proposed by Teeter and DuRand [1]. Initially, the model of the D_2 DAR transmembrane helices was constructed directly from the bacteriorhodopsin (bR) coordinates derived from two-dimensional electron diffraction experiments, but the orientations of all TM domains were subsequently adjusted in order to mimic the topology of the TM domains of rhodopsin [8]. This model was tested for its ability to accommodate rigid agonist and semi-rigid antagonist molecules which were docked into the putative binding pocket with stabilizing interactions. The model is consistent with structure-activity relationships of agonists and antagonists that interact with the receptor [9] and with site-directed mutagenesis data [9–11].

Docking of 1-{2-[5-(1H-benzimidazole-2-thione)]ethyl}-4-arylpiperazine to the thus defined binding site could not explain the experimentally obtained values for the corresponding ligands. Therefore, the binding pocket was enlarged using the Insight II software, by taking into account all receptor amino acid side groups (Table 2) that could interact after initial positioning of the ligands against amino acid residues Asp86 and Ser141. The binding pocket designed in this way provided results matching the obtained experimental results.

Table 2. List of amino acids considered to be part of the 1-{2-[5-(1*H*-benzimidazole-2-thione)]ethyl}-4-arylpiperazine binding site in the D_2 DAR.

Residue	Position	Residue	Position	Residue	Position
Asp	46	Ser	118	His	189
Trp	56	Ser	122	Tyr	208
Phe	82	Ser	141	Phe	211
Val	83	Ser	144	Thr	212
Asp	86	Phe	145	Gly	215
Met	89	Phe	178	Tyr	216
Cys	90	Cys	181	Ser	219
Ser	93	Phe	185	Asn	222
Trp	115	Phe	186		

The main features of the D_2 DAR model shown in Figure 2a, using compound **1** as a ligand, were (i) close interaction of protonated N1 of the piperazine ring with Asp86 (calculated distance 1.68 Å), (ii) hydrogen bond formation between the benzimidazole part of the ligand and Ser141 and Ser122, and (iii) edge-to-face interactions of the aromatic ring or the arylpiperazine part of the ligand with Phe178, Tyr216 and Trp182 of the receptor. Similar results were obtained with 2,3-dimethylphenyl and naphthyl substituents in the piperazine ring (compounds **2** and **3**, respectively). Generally, introduction of the substituent in position 2 of the phenyl ring in the piperazine part of a ligand led to the same docking to the receptor as with ligand **1**. This holds true for all ligands tested in the present study (compounds **4**–**6**, as well as ligand **2**). In addition, 2-methoxy derivative **4** could form one more hydrogen bond with Trp182.

Ligands with substituents in position 4 of the arylpiperazine ring (**5**, **8** and **11**) could not dock to the receptor as previously described for compound **1**. This is the result of an unfavorable steric interaction of bulky substituents with Phe178 in the receptor binding pocket (Figure 2c). As a consequence, formation of a salt bridge between Asp86 and the protonated N1 of the piperazine ring is hindered. The calculated distance between these two entities was increased to 3.51 Å. 4-Fluorophenylpiperazine derivative **12** did not fit into this scheme, since fluorine is similar in size to a hydrogen atom. The decrease in affinity of ligand **12** in comparison with that of compound **1** can be explained in terms of a strong negative inductive effect of the fluorine atom, reducing the energy of edge-to-face interactions.

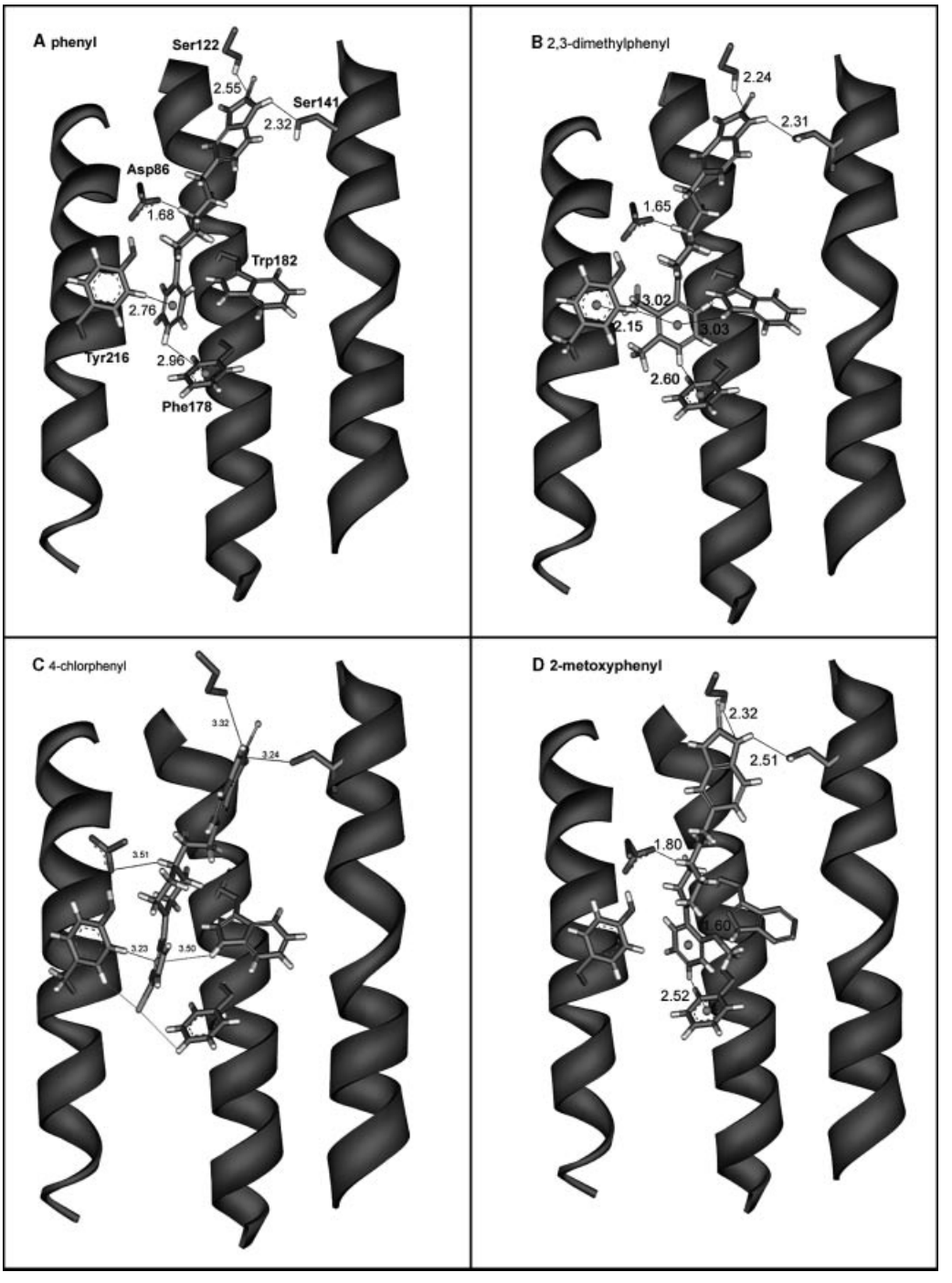

Figure 2. Schematic representation of the interaction of ligands **1**−**4** with the D_2 dopamine receptor. The 3D model describes a possible interaction of compounds **1** (**A**), **2** (**B**), **3** (**C**) and **4** (**D**) and the theoretical dopamine D_2 receptor model.

Docking analyses of the ligands with substituents in position 3 of the piperazine phenyl ring (**7**, **10**, **13** and **17**) revealed that the substituents in this position are tolerated, since no large reduction of affinity was observed. In contrast, substituents with electron withdrawal effect in this position, like trifluoromethyl (**13**),

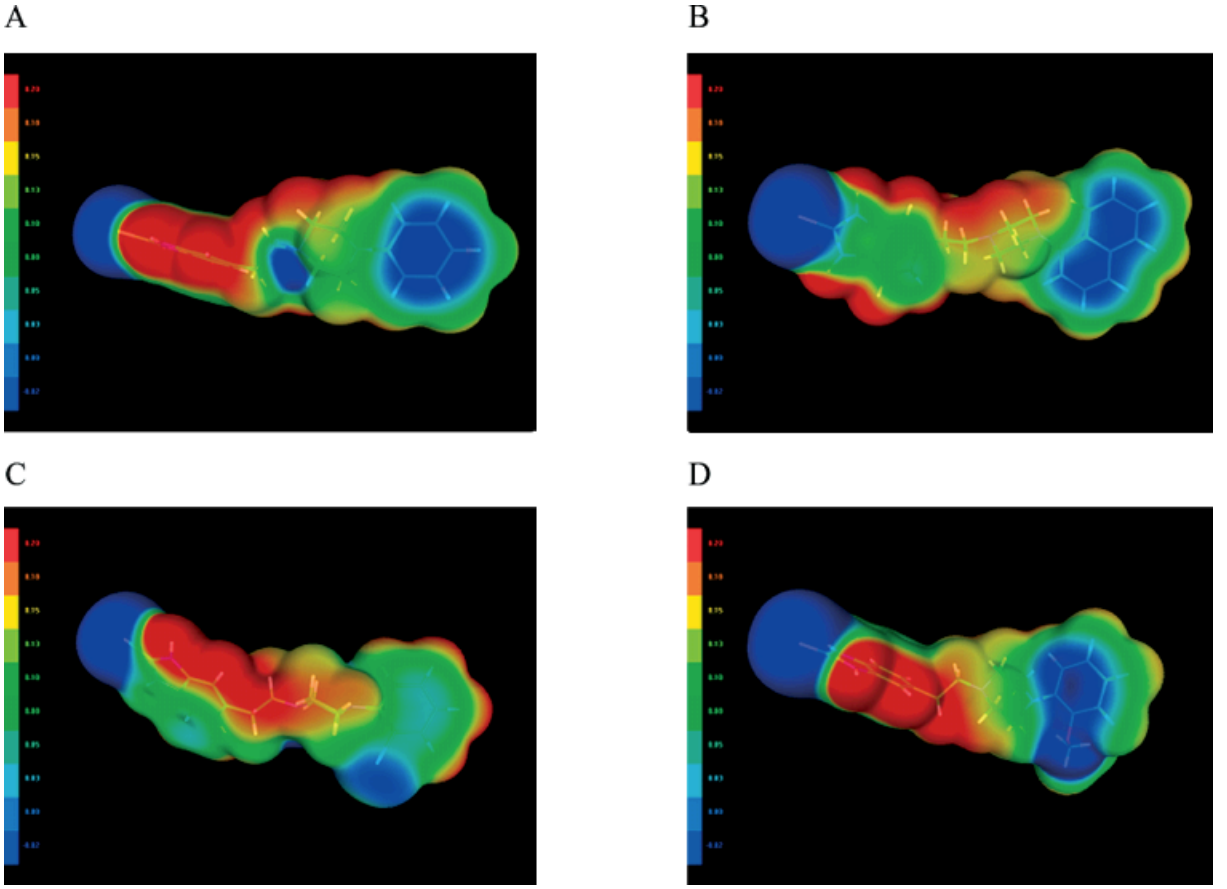

Figure 3. Electrostatic surface potentials of several 1-{2-[5-(1H-benzimidazole-2-thione)]ethyl}-4-arylpiperazines. For simpler comparisons, the ESP values were mapped on the electron density surface. Values in blue indicate a strong negative ESP, whereas those in red correspond to a strong positive ESP. Compounds **1** (**A**), **3** (**B**), **6** (**C**), and **4** (**D**).

chloro (**7**) and nitro groups (**10**), affect the affinity by decreasing the electron density in the benzene ring of these ligands.

From data presented in the literature, it is obvious that the receptor-ligand complexes presented here are in agreement with the published site-directed mutagenesis data, as far as the benzimidazole D_2 DAR binding domain and Asp86 are concerned [9–11]. To our knowledge, such data are not available for the arylpiperazine binding part of D_2 DAR.

ESP calculations on compounds **1**–**4** demonstrated that they were involved in edge-to-face interactions with the receptor molecule (Figure 3a, b, d). Exchange of the 2-methoxy group of ligand **4** with the isosteric chlorine atom (compound **6**) partially reduced the electron density in the aromatic ring, thereby reducing the energy of edge-to-face interactions (Figure 3c). As a consequence, the affinity of ligand **6** was 12 times lower compared to that of compound **4** (Table 2). On the other hand, ligand **6** shows the same activity as compounds **1** and **2**, pointing out that some more factors, apart from edge-to-face interactions, are playing a role in the explanation of structure-activity relationships in this part of molecule.

For a further evaluation of the effects of electron withdrawing groups on dopaminergic activity, several new benzimidazole arylpiperazines (compounds **9**–**13**, Scheme 1) were synthesized. Groups that differ in size and electron withdrawal properties (fluoro, nitro, and trifluoromethyl) were chosen. These substituents were introduced at positions 2, 3 and 4 of the phenyl group attached to the piperazine ring of parent compound **1**. Regardless of the position of substitution, reduction of the binding affinity was expected. All new compounds behaved as predicted, with the exception of the 2-nitro derivative **9**. This might have been expected for the 2-

nitro substitution, since this group forms one additional hydrogen bond with Trp182 (similar to the one proposed for the 2-OMe group of compound **4**). This additional hydrogen bond can compensate for the negative effect of the nitro group on edge-to-face interactions.

The results of the docking analyses, along with the biological activities of all tested compounds, are summarized in Table 3.

To determine the contribution of the arylpiperazine part of the ligands to D_2 DAR binding, 1N-benzyl compounds **14**−**20** were prepared, according to previously published procedures (for references see Experimental). These ligands lack the benzimidazole part, which eliminates the contribution of hydrogen bonds through serines in TM IV and V. Driving forces for the formation of the complex with the D_2 receptor molecule were (i) a salt bridge with Asp86, (ii) edge-to-face interactions with aromatic residues (Tyr216, Phe178, and Trp182), and (iii) hydrogen bonds formed between Trp182 and 2-OMe (i.e. the 2-nitro substituent of the ligands).

The results of the docking analyses, along with the biological activities, are listed in Table 3. Generally, all compounds of this class had a lower activity than their benzimidazole counterparts. Benzyl ligands (**14**−**20**) docked according to the proposed model. A salt bridge between the protonated N1 of the piperazine ring and Asp86, edge-to-face interactions of the aromatic ring or the arylpiperazine part of the ligand and Phe178, Tyr216 and Trp182 of the receptor, and a hydrogen bond between Trp182 and 2-OMe or 2-nitro substituents (compounds **16** and **19,** respectively) represent the interacting points with the receptor (Figure 4). Introduction of bulky substituents at position 4 (4-OMe-

Table 3. Correlation of the arylpiperazine-D_2 DAR binding affinity and the type of ligand-receptor interactions.

Ligand No.	Asp86	Ser141	Ser122	Phe178	Tyr216	Trp182	Ki (nM)
1	salt bridge	hydrogen bond	hydrogen bond	ETF	ETF	ETF	15.7 ± 2.0
2	salt bridge	hydrogen bond	hydrogen bond	ETF	ETF	ETF	15.8 ± 2.3
3	salt bridge	hydrogen bond	hydrogen bond	ETF	ETF	ETF	3.4 ± 0.4
4	salt bridge	hydrogen bond	hydrogen bond	ETF	ETF	hydrogen bond	1.7 ± 0.4
5	salt bridge	hydrogen bond	hydrogen bond	bulk	ETF	ETF	110 ± 12
6	salt bridge	hydrogen bond	hydrogen bond	ETF	n/a	n/a	20.7 ± 2.2
7	salt bridge	hydrogen bond	hydrogen bond	n/a bulk	n/a	n/a	250 ± 35
8	salt bridge	hydrogen bond	n/a	bulk	n/a	n/a	500 ± 37
9	salt bridge	hydrogen bond	hydrogen bond	n/a	n/a	hydrogen bond	11.79 ± 0.9
10	salt bridge	hydrogen bond	hydrogen bond	n/a	n/a	n/a	159.3 ± 15
11	salt bridge	hydrogen bond	n/a	bulk	n/a	n/a	>1000
12	salt bridge	hydrogen bond	hydrogen bond	n/a	n/a	n/a	73.8 ± 2.1
13	salt bridge	hydrogen bond	hydrogen bond	n/a	n/a	n/a	134 ± 15
14	salt bridge	n/a	n/a	ETF	ETF	ETF	639 ± 43
15	salt bridge	n/a	n/a	ETF	ETF	ETF	580 ± 36
16	salt bridge	n/a	n/a	ETF	ETF	hydrogen bond	28 ± 4.2
17	salt bridge	n/a	n/a	ETF/bulk	ETF	ETF	577 ± 37
18	salt bridge	n/a	n/a	bulk	ETF	ETF	>1000
19	salt bridge	n/a	n/a	n/a	n/a	hydrogen bond	198 ± 26
20	salt bridge	n/a	n/a	bulk	n/a	n/a	>1000

K_i values are the means of three independent experiments done in triplicate performed at eight competing ligand concentrations (0.1 nM−0.1 mM) and 0.2 nM [^{3}H]spiperone. The observed salt bridge lengths ranged from 1.65 to 1.95 Å for active compounds and exceeded 1.95 Å for inactive compounds. For effective hydrogen bonds, the observed lengths were under 2.5 Å, while the criterion for ETF interactions was a length under 3 Å measured from the phenyl centered part of the ligand to the nearest hydrogen atom of the listed amino acid residues. *ETF is the abbreviation for edge-to-face interactions.

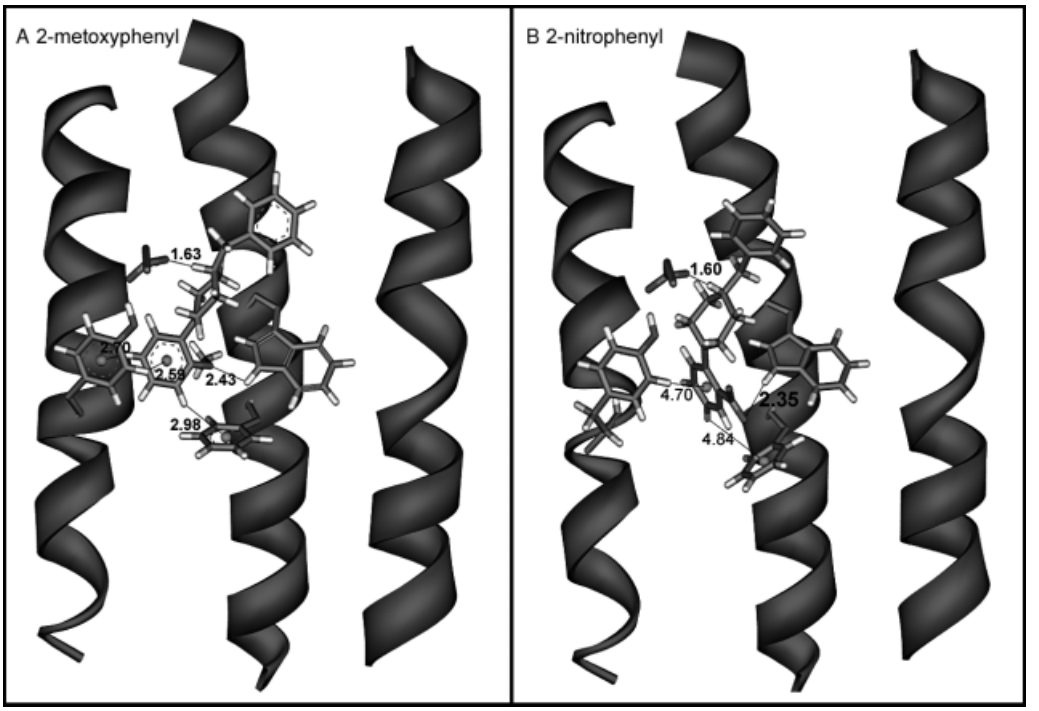

Figure 4. Schematic representation of the interaction of 1-benzyl-4-arylpiperazine ligands and the D_2 DAR. Schematic model of the proposed interaction of the studied compounds **16** (**A**) and **19** (**B**) with the D_2 receptor.

phenyl in **18** and 4-nitro-phenyl in **20**) completely blocked the interaction of the ligands with the receptor. The ligands that can form a hydrogen bond with Trp182 (2-OMe-phenyl, **16**, and 2-nitro-phenyl, **19**) were the most active. They were followed by those that can take part in edge-to-face interactions (phenyl, naphthyl and 3-MeO-phenyl; *i.e.* **14**, **15** and **17**, respectively), whereas the ligands with bulky substituents introduced at position 4 (**18** and **20**) were inactive.

Löber et al. [12] used a similar strategy for rationally based efficacy tuning of 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-*a*]pyridines of D_4 DAR activity, which resulted in a different docking model from the one presented in our paper. This is probably due to the different subtype of DAR and the different arylpiperazine ligands considered. Beyond that, a vast amount of literature about structure-activity relationship of the arylpiperazine class of dopaminergic ligands exists (as an example, see the paper of Cha et al. [13] and references cited therein). The aim of this paper is not to give a general explanation for all arylpiperazine-D_2 DAR interactions, but is limited to the family of arylpiperazines presented, which are currently under study in our laboratory.

Conclusions

The results of our docking studies on 1-{2-[5-(1H-benzimidazole-2-thione)]ethyl}-4-aryl-piperazine-D_2 DAR complexes revealed that (i) close interaction of the protonated N1 of the piperazine ring with Asp86, (ii) hydrogen bond formation between the benzimidazole part of the ligand and Ser141, as well as Ser 122, and (iii) edge-to-face interactions of the aromatic ring or the arylpiperazine part of the ligand and Phe178, Tyr216 and Trp182 of the receptor represent the main stabilizing forces. In addition, the 2-methoxy derivative **4** could form one additional hydrogen bond with Trp182.

Bulky substituents in position 4 of the aromatic part of the phenylpiperazine ring are not tolerated because of unfavorable steric interactions with Phe178. This result agrees well with the data of Simpson et al. [14] and Löber et al. [15].

Substituents in position 2 and 3 of phenylpiperazine are sterically well tolerated. Electron-attractive groups such as F, Cl, CF_3 and NO_2 decreased the binding affinity, while electron donors like -OMe and the second aromatic ring (naphthyl) increased the affinity in

comparison with the parent compound **1**. These effects can be explained by strong edge-to-face interactions of negative ESP in the center of aromatic residues of the ligands and positive ESP of the protons of the receptor aromatic residues.

The presented data give us a good explanation of the behavior of this type of ligands, which can be used as the basis for further rational design of active dopaminergic compounds.

Acknowledgments

This work was supported by the Ministry for Science, Technology and Development of Serbia, Grant #1698 (V. Šukalović, M. Z., G. R., S. H., S. K.-R. and D. A.) and ProteoSys AG, Mainz, Germany (V. Šoškić).

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 solvent unless otherwise stated, are reported in ppm downfield from the internal standard tetramethylsilane.

IR spectra were run on a Perkin Elmer 457 Grating Infrared Spectrophotometer (Perkin Elmer, Beaconsfield, UK). Mass spectra were determined by a Finnigan Mat 8230 mass spectrometer (Finnigan, Bremen, Germany). High-resolution mass spectra were acquired on a Bruker Biflex MALDI TOF (Bruker, Bremen, Germany). For analytical thin-layer chromatography, F-256 plastic-backed thin-layer silica gel plates from E. Merck (Darmstadt, Germany) 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.

Molecular modeling

Modeling of the ligands

Ligand models were constructed using Hyperchem version 7.0 software (Hypercube, Inc. Gainesville, USA) and an in-built PM3 routine for molecule geometry optimization. It was postulated that the ligands are bound to the receptors in protonated form [16, 17]; therefore, a formal charge of +1 was added to the piperazine nitrogen (1N). The results obtained were further optimized in a Gaussian 98, Rev. A.9 (Gaussian, Inc., Pittsburgh, USA). ESP were calculated in a Gaussian G 98W using the DFT B3LYP method and a 6-31 g basis set [18, 19]. The ESP cube output from Gaussian G 98W was visualized in a gOpenMol software [20] following the recommended Gaussian procedure to display the calculated properties.

Modelling of the D_2 DAR

The D_2 DAR model proposed by Teeter and DuRand [1, 8] was used to explain structure-activity relationships of our ligands. Initially, the model of the D_2 DAR transmembrane helices was constructed directly from the bR coordinates derived from two-dimensional electron diffraction experiments, but the orientations of all TM domains were subsequently adjusted in order to mimic the topology of the TM domains of rhodopsin [10]. Primary sequences of bR and D_2 DAR were aligned, superimposing conservative amino acid residues. The seven TM helices were arranged using constructed helical wheels, matching hydrophobic and hydrophilic inter-helices interactions. Energy minimization was performed on the obtained model, to relieve all close van-der-Waals contacts. No global energy minimization was performed, nor were molecular dynamics run.

D_2 DAR complexes were done using the Docking module within the Insight II software (Accelerys Inc., Cambridge, UK) on an SGI Octane 2 workstation (Silicon Graphics Inc., Mountain View, USA). Docking of the ligands described here was performed as follows: Initially, using SA docking algorithm 100, structures were generated applying a Monte Carlo method. Each structure was further minimized for 4000 cycles or until 0.01 kcal/mol/Å was reached. Minimization was performed by fixing all the protein backbone atoms and by keeping ligand and amino acid residues in the binding site flexible. In this way, the relaxation of van-der-Waals interactions was permitted. Subsequently, all structures were filtered using the general rule that the best structure is the one with the shortest salt bridge between the ligand and Asp86 and with a maximum number of hydrogen bonds with the D_2 DAR. The obtained results were visualized using DS View software (Accelerys Inc., Cambridge, UK).

Chemistry

1-{2-[5-(1*H*-benzimidazole-2-thione)]ethyl}-4-arylpiperazines **1**–**8** were prepared as previously described [21]. 1-Benzyl-4-aryl-piperazines **14**–**20** were synthesized according to the procedures of other authors as follows: compound **14** [22], **15** [23], **16**–**18** [24], **19** [25] and **20** [26].

*Synthesis of 1-chloro-2-(3,4-di-tBOC-phenyl)ethane (**23**)*

Stannous chloride (47.5 g, 0.23 mol) was added to a solution of 4-(2-chloroethyl)-2-nitroaniline (**21**) (9.5 g, 40 mmol) in absolute ethanol (85 mL). After refluxing for 4 h, the solution was poured into ice, made alkaline with 5 M NaOH and extracted with EtOAc. Extracts were dried, the solvent was removed *in vacuo*, and the resulting diamine **22** was immediately used without further purification. Obtained diamine **22** (3.5 g, 21 mmol) was dissolved at 0 °C in a mixture of dioxane (65 mL) and 1 N NaOH (65 mL). To this solution, di-tert-butyl dicarbonate (6.9 g, 31.5 mmol) was added at 0 °C. Cooling was stopped after 2 h, and the reaction mixture was stirred overnight at room temperature. Excess of solvent was evaporated *in vacuo*, and the obtained residue was extracted with CH_2Cl_2. The obtained product was purified by MPLC using CH_2Cl_2 as the eluent. Yield: 4.5 g (60 %), colorless oil; IR (KBr): (cm^{-1}) 773, 1065, 1160, 1276, 1480, 1523, 1684, 2988, 3330. ^{1}H-NMR: δ 1.51 (s, 18H, CH_3); 3.01 (t, J = 7.6 Hz, 2H, CH_2Cl); 3.67 (t, J = 7.6 Hz, 2H, $ArCH_2$); 6.61 (s, 1H, NH); 6.80 (s, 1H, NH); 6.96 (dd, J = 6.2 Hz, J = 2 Hz, 1H, ArH); 7.35–7.43 (m, 2H, ArH). MS *m/z* 370 (M+). Anal. calc. for ($C_{18}H_{27}ClN_2O_4$): C, 58.29; H, 7.34; N, 7.55; found: C, 58.24; H, 7.38; N, 7.51.

*General procedure for the synthesis of 1-{2-[3,4-di(tBOC-amino)phenyl]ethyl}-4-aryl-piperazines (**24**–**26**) and 1-[2-(3-nitro-4-aminophenyl)ethyl]-4-(4-fluorophenyl)piperazine (**30**)*

To a solution of 10.0 mmol of either arylpiperazine in 50.0 mL DMF, 12.0 mmol of 1-chloro-2-arylethane (**21** or **23**), 6.0 g K_2CO_3 and 0.1 g KI were added. The mixture was stirred at 80 °C for 12 h. After cooling, the precipitate was removed and the filtrate was evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2, and the obtained products were purified by MPLC using CH_2Cl_2 as the eluent.

(**24**): Yield: 3.2 g (58 %), yellow oil; IR (KBr): (cm^{-1}) 766, 1048, 1156, 1245, 1332, 1518, 1710, 2977, 3352. ^{1}H-NMR: δ 1.52 (s, 18H, CH_3); 2.65–2.70 (m, 6H); 2.78–2.84 (m, 2H); 3.08–3.13 (m, 4H); 6.60 (s, 1H, NH); 6.76 (s, 1H, NH); 6.94–7.18 (m, 3H, ArH); 7.32–7.52 (m, 3H, ArH); 7.62 (dd, *J* = 6.4 Hz, *J* = 1.6 Hz, 1H, ArH). MS *m/z* 541 (M+). Anal. calc. for ($C_{28}H_{39}N_5O_6$): C, 62.09; H, 7.26; N, 12.93; found: C, 62.07; H, 7.30; N, 12.95.

(**25**): Yield: 3.6 g (66 %), yellow oil; IR (KBr): (cm^{-1}) 760, 1050, 1159, 1244, 1347, 1528, 1725, 2977, 3402. ^{1}H-NMR: δ 1.52 (s, 18H, CH_3); 2.63–2.73 (m, 6H); 2.79–2.86 (m, 2H); 3.30–3.35 (m, 4H); 6.60 (s, 1H, NH); 6.80 (s, 1H, NH); 6.97 (dd, *J* = 6.6 Hz, *J* = 1.8 Hz, 1H, ArH); 7.19 (dd, *J* = 6 Hz, *J* = 1.6 Hz, 1H, ArH); 7.31–7.44 (m, 3H, ArH); 7.63–7.72 (m, 2H, ArH). MS *m/z* 541 (M+). Anal. calc. for ($C_{28}H_{39}N_5O_6$): C, 62.09; H, 7.26; N, 12.93; found: C, 62.12; H, 7.30; N, 12.96.

(**26**): Yield: 4.9 g (90 %), yellow oil; IR (KBr): (cm^{-1}) 754, 1049, 1162, 1246, 1326, 1598, 1730, 2978, 3314. ^{1}H-NMR: δ 1.51 (s, 18H, CH_3); 2.60–2.67 (m, 6H); 2.76–2.83 (m, 2H); 3.41–3.47 (m, 4H); 6.80–6.85 (m, 3H, NH, ArH); 6.96 (dd, *J* = 6.4 Hz, *J* = 1.8 Hz, 1H, ArH); 7.32 (d, *J* = 8.2 Hz, 1H, ArH); 7.45 (s, 1H, ArH); 8.12 (d, *J* = 9.4 Hz, 2H, ArH). MS *m/z* 541 (M+). Anal. calc. for ($C_{28}H_{39}N_5O_6$): C, 62.09; H, 7.26; N, 12.93; found: C, 62.05; H, 7.22; N, 12.96.

(**30**): Yield: 1.8 g (53 %), colorless oil; IR (KBr): (cm^{-1}) 820, 1189, 1342, 1515, 2945. ^{1}H-NMR: δ 2.64–2.68 (m, 6H); 2.73–2.81 (m, 2H); 3.41–3.45 (m, 4H); 6.05 (s, 2H, NH_2); 6.52–6.81 (m, 5H, ArH); 7.24 (d, *J* = 8 Hz, 1H, ArH); 7.94 (s, 1H, ArH). MS *m/z* 344 (M+). Anal. calc. for ($C_{18}H_{21}FN_4O_2$): C, 62.78; H, 6.15; N, 16.27; found: C, 62.77; H, 6.19; N, 16.30.

*General procedure for the synthesis of 1-[2-(3,4-diaminophenyl)ethyl]-4-arylpiperazines (**27**–**29**)*

Hydrochloric acid (37 %, 10 mL) was added to a stirring solution of 1-{2-[3,4-di(tBOC-amino)phenyl]ethyl}-4-arylpiperazines **24**–**26** (5.0 mmol) in 20 mL EtOH at room temperature. After 60 min, solvent and HCl were evaporated *in vacuo.* The residue was extracted with a mixture of 20 mL 10 % $NaHCO_3$ and 20 mL chloroform; the organic phase was separated, dried over Na_2SO_4 and evaporated *in vacuo*. The products obtained were used without further purification for the synthesis of compounds **9**–**11**.

*Synthesis of 1-[2-(3,4-diaminophenyl)ethyl]-4-(4-fluorophenyl)piperazine (**31**)*

Ra-Ni (0.3 g) was added in small portions to a stirring solution of **30** (6 mmol) in 6 mL EtOH, 12 mL 1,2-dichloroethane, and 2.0 mL (40 mmol) hydrazine hydrate, at 30 °C. After 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 the product was used without further purification for the synthesis of compound **12**.

*General procedure for the synthesis of 1-{2-[5-(1H-benzimidazole-2-thione)]ethyl}-4-arylpiperazines (**9**–**12**)*

Carbon disulfide (0.36 mL, 6.0 mmol) and KOH solution (0.37 g in 0.90 mL water) were added to a solution of 3.0 mmol of either one of diamines **27**–**29** and **31** in 5.0 mL EtOH. After refluxing for 3 h, the solvent was removed *in vacuo*. The reaction mixture was diluted with 100 mL ice-cold water; the obtained suspension was neutralized with 2 M HCl, and the precipitate was collected by filtration. The resulting crude benzimidazolethiones **9**–**12** were purified by recrystallization from hot EtOH.

(**9**): Yield: 0.56 g (50 %); m.p.: 238–240 °C. IR (KBr): (cm^{-1}) 742, 1110, 1188, 1343, 1461, 1490, 1518, 1603, 2834, 2948, 3069. ^{1}H-NMR (DMSO): δ 2.49–2.56 (m, 2H); 2.76–2.83 (m, 4H); 2.97–3.01 (m, 6H); 6.98–7.16 (m, 4H, ArH); 7.32 (dd, *J* = 7.2 Hz, *J* = 1 Hz, 1H, ArH); 7.58 (t, *J* = 2.6 Hz, 1H, ArH); 7.78 (d, *J* = 6.8 Hz, 1H, ArH); 12.47 (s, 2H, NH). MS *m/z* 384, 142 (MH+). Anal. calc. for ($C_{19}H_{21}N_5O_2S$): C, 59.51; H, 5.52; N, 18.26; found: C, 59.54; H, 5.49; N, 18.30.

(**10**): Yield: 0.45 g (40 %); m.p.: 235–237 °C. IR (KBr): (cm^{-1}) 741, 1129, 1188, 1245, 1343, 1461, 1490, 1522, 1617, 2817, 1952, 3129. ^{1}H-NMR (DMSO): δ 2.50–2.60 (m, 6H); 2.78–2.85 (m, 2H); 3.25–3.29 (m, 4H); 6.98–7.08 (m, 3H, ArH); 7.40–7.65 (m, 4H, ArH); 12.46 (s, 2H, NH). MS *m/z* 384, 139 (MH+). Anal. calc. for ($C_{19}H_{21}N_5O_2S$): C, 59.51; H, 5.52; N, 18.26; found: C, 59.49; H, 5.50; N, 18.30.

(**11**): Yield: 0.42 g (39 %); m.p.: 232–234 °C. IR (KBr): (cm^{-1}) 740, 1118, 1240, 1325, 1490, 1520, 1609, 2824, 2950, 3102. ^{1}H-NMR (DMSO): δ 2.41–2.96 (m, 8H); 3.21–3.38 (m, 4H); 6.96–7.43 (m, 5H, ArH); 8.11 (d, *J* = 9.2 Hz, 2H, ArH); 12.46 (s, 2H, NH). MS *m/z* 384, 144 (MH+). Anal. calc. for ($C_{19}H_{21}N_5O_2S$): C, 59.51; H, 5.52; N, 18.26; found: C, 59.50; H, 5.56; N, 18.28.

(**12**): Yield: 0.51 g (42 %); m.p.: 197–199 °C. IR (KBr): (cm^{-1}) 816, 1128, 1193, 1223, 1348, 1461, 1510, 1618, 2837, 2958, 3072. ^{1}H-NMR (DMSO): δ 2.52–2.84 (m, 8H); 3.09–3.20 (m, 4 H); 6.05 (s, 2H, NH_2); 6.92–7.10 (m, 7H, ArH); 12.46 (s, 2H, NH). MS *m/z* 357, 149 (MH+). Anal. calc. for ($C_{19}H_{21}FN_4S$): C, 64.02; H, 5.94; N, 15.72; found: C, 64.06; H, 5.90; N, 15.75.

Synaptosomal membrane preparation, binding assays and data analysis

Synaptosomal membranes of the bovine caudate nuclei used as a source of the dopamine D_2 receptors were prepared exactly as described previously [27]. [^{3}H]spiperone (spec. act. 70 Ci $mmol^{-1}$) used to label the D_2 receptor was purchased from Amersham Buchler GmbH (Braunschweig, Germany). Briefly, [^{3}H]spiperone binding was assayed in a 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 addition of 50 nM ketanserin. Ki values were determined by competition binding at 0.2 nM radioligand, and eight to ten concentrations of each ligand were tested (0.1 nM–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, before being washed three times with 5.0 mL ice-cold incubation buffer. Radioligand binding for each concentration of the tested compounds 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. Competition binding data were analyzed by the non-linear least-squares curve-fitting program LIGAND [28].

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