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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

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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-

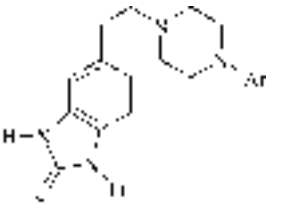

Figure 1. Structure of 1-{2-[5-(1H-benz-imidazole-2-thione)]ethyl}-4-arylpiperazines.

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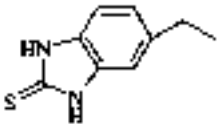
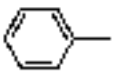
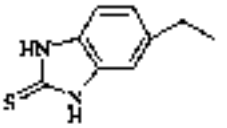
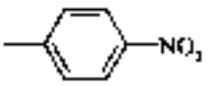
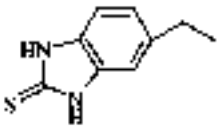
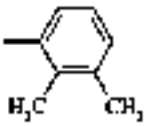
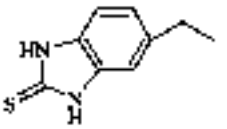
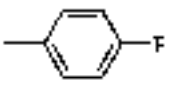
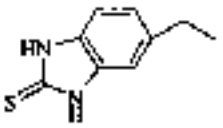
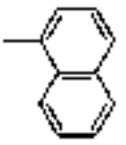
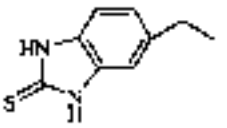
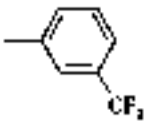
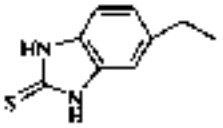
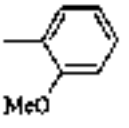
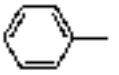
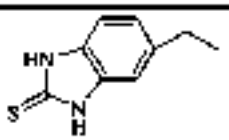
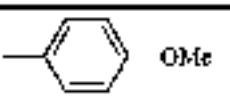
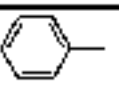
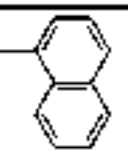
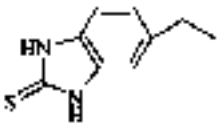
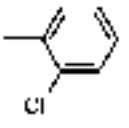
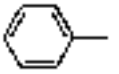
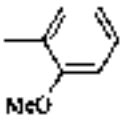
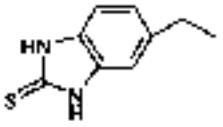
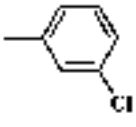
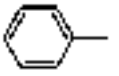
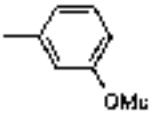
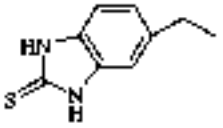
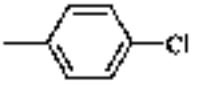
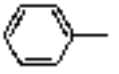
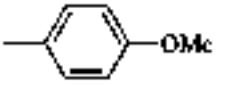
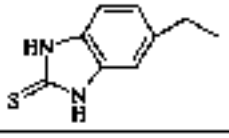
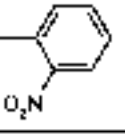
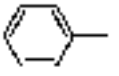
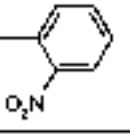
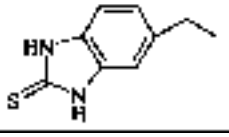
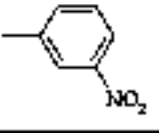
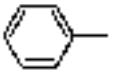
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		

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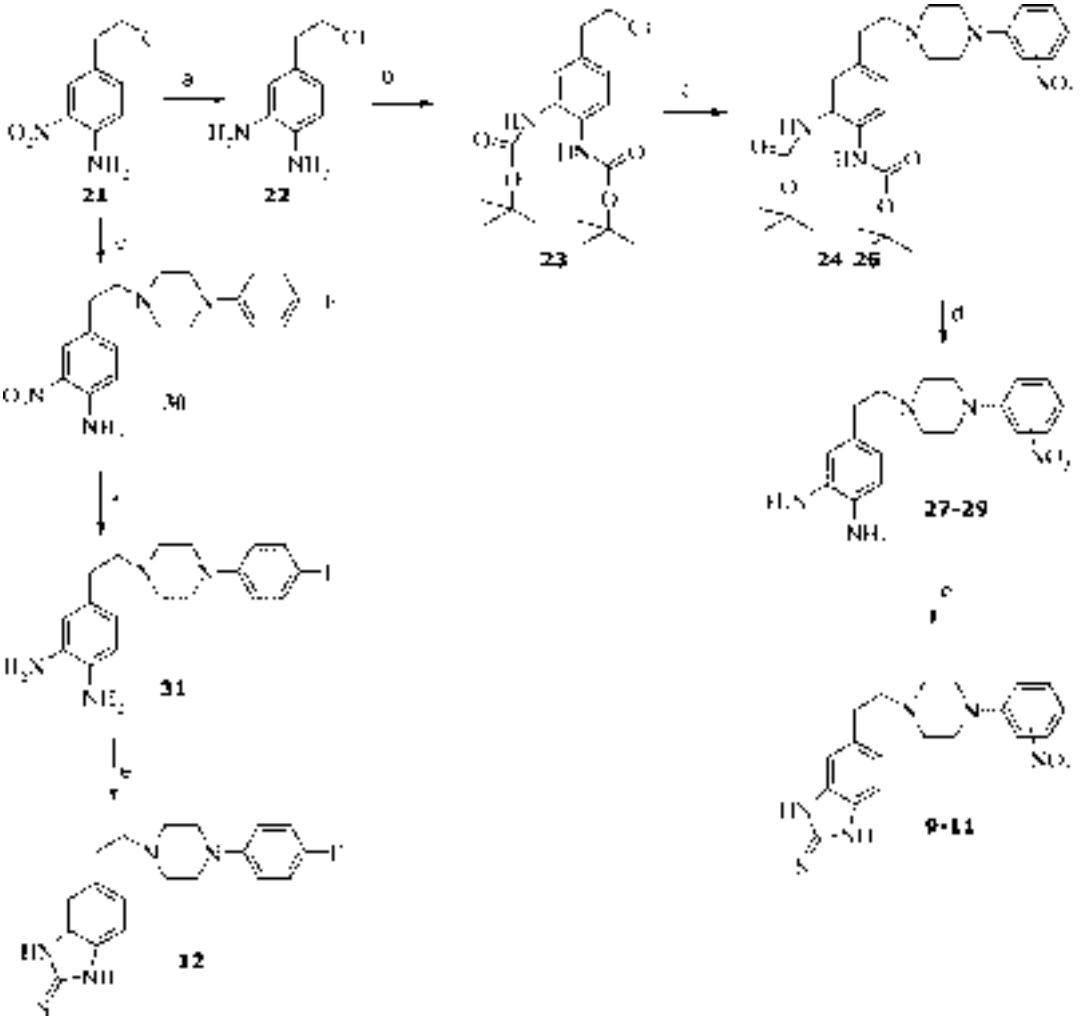

a) EtOH, $SnCl_2$, reflux; b) dioxane, 1N NaOH, di-tert-butyl-dicarbonate; c) DMF, K_2CO_3, KI, substituted piperazines, 80 °C; d) EtOH, 4N HCl; e) EtOH, KOH, CS_2, reflux; f) EtOH, N_2H_4, Ra-Ni

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.

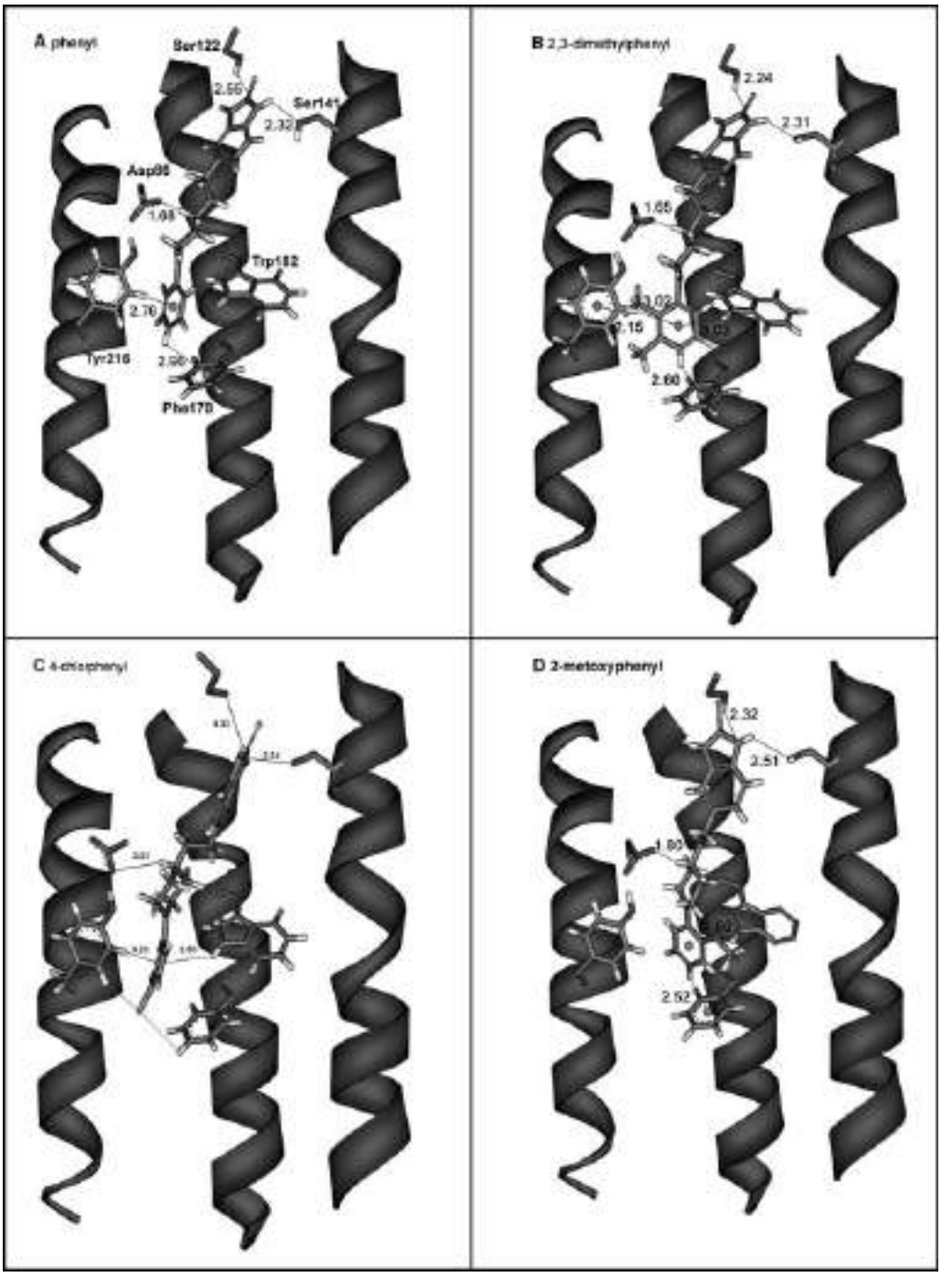

Figure 2. Schematic representation of the interaction of ligands **1**−**4** with the D_2 dopamine receptor. The 3D model describes a possible interaction of compounds **1** (**A**), **2** (**B**), **3** (**C**) and **4** (**D**) and the theoretical dopamine D_2 receptor model.

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**),

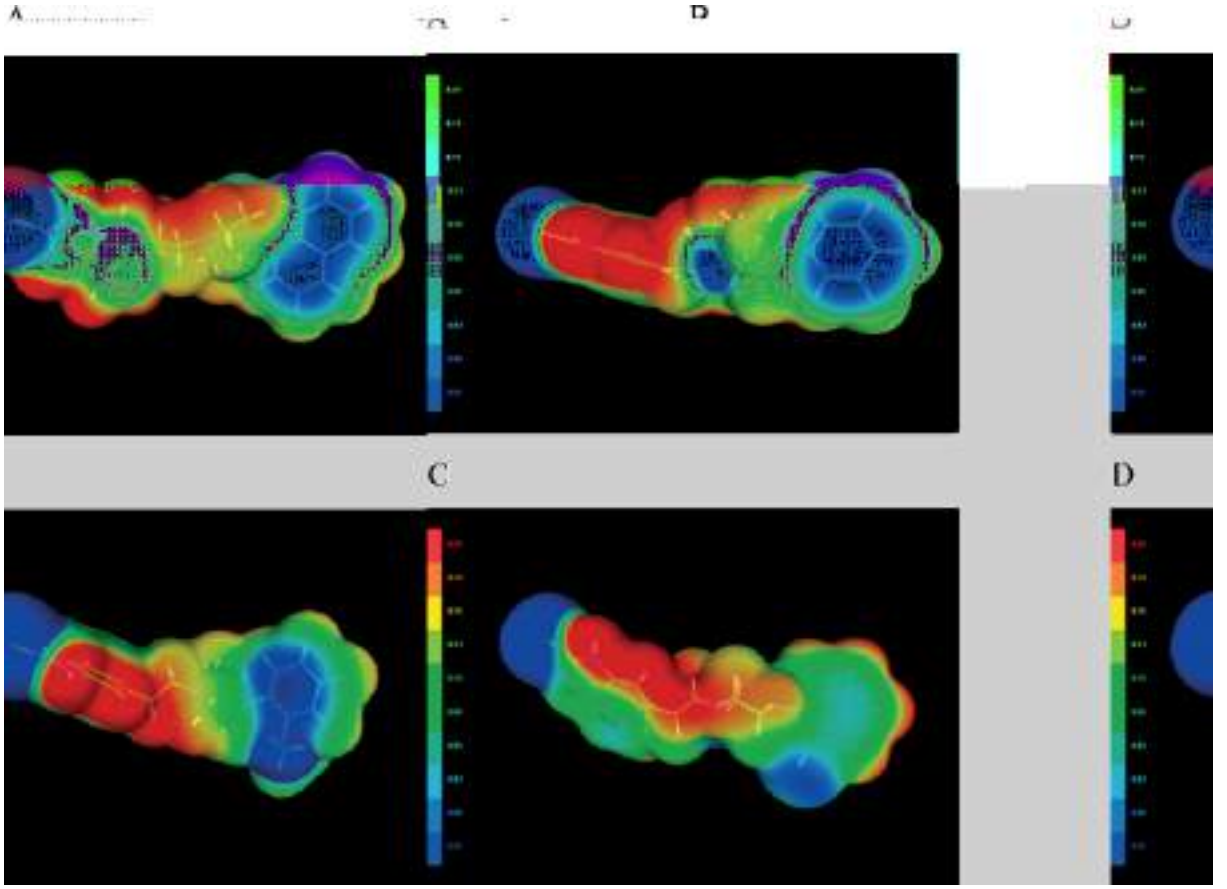

Figure 3. Electrostatic surface potentials of several 1-{2-[5-(1H-benzimidazole-2-thione)]ethyl}-4-arylpiperazines. For simpler comparisons, the ESP values were mapped on the electron density surface. Values in blue indicate a strong negative ESP, whereas those in red correspond to a strong positive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

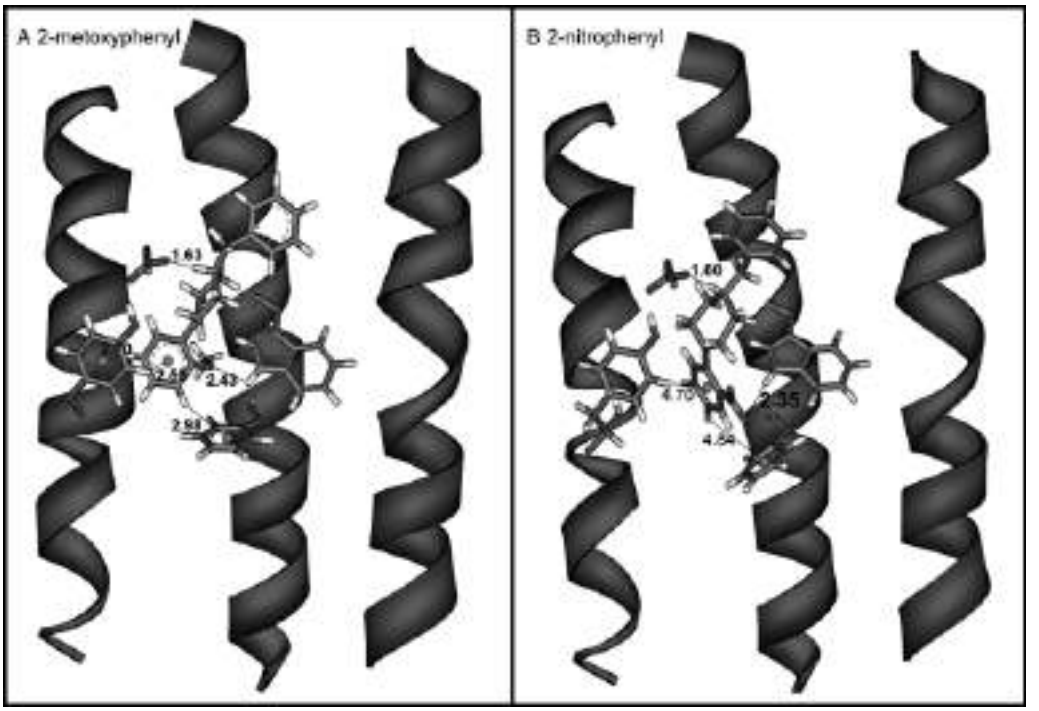

Figure 4. Schematic representation of the interaction of 1-benzyl-4-arylpiperazine ligands and the D_2 DAR. Schematic model of the proposed interaction of the studied compounds **16** (**A**) and **19** (**B**) with the D_2 receptor.

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

*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

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