Interaction of Arylpiperazines with the Dopamine Receptor D_2 Binding Site

Vladimir Šukalović[a], Mario Zlatović[b], Deana Andrić[b], Goran Roglić[b], Sladjana Kostić-Rajačić[a], and Vukić Šoškić[a,c]

Centre for Chemistry, Institute for Chemistry, Technology and Metallurgy[a], Belgrade (Serbia and Montenegro), Faculty of Chemistry, University of Belgrade[b], Belgrade (Serbia and Montenegro), and ProteoSys AG[c], Mainz (Germany)

Summary

The docking of several 1-benzyl-4-arylpiperazines to the dopamine receptor (DAR) D_2 was examined. The results demonstrated that the interaction of protonated N1 of the piperazine ring with Asp 86 (III.32) and edge-to-face interactions of the aromatic ring of the arylpiperazine part of the ligand with Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58) of the receptor, represent the major stabilizing forces. Besides, the hydrogen bond acceptor group in position 2 of the phenylpiperazine aromatic ring could build one more hydrogen bond with Trp 182 (VI.48). Bulky substituents in position 4 were not tolerated due to the unfavorable sterical interaction with Phe 178 (VI.44). Substituents in position 2 and 3 were found to be sterically well tolerated. Introduction of electron attractive -NO_2 group in position 3 of arylpiperazines decreased, while electron donors (-OMe) and the second aromatic ring (naphthyl) increased the binding affinity comparing to that of the phenylpiperazine 1. This can be explained in terms of favoured edge-to-face interactions in ligands with a high negative electrostatic surface potential (ESP) in the centre of aromatic residue of arylpiperazines. Thus, besides the salt bridges and hydrogen bonds, edge-to-face interactions significantly contribute to arylpiperazine ligands to form complexes with the DAR D_2. Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58) can be considered as a part of the ancillary DAR D_2 pocket preserved in most G protein-coupled receptors of the A class and obviously, the arylpiperazine structural motif represents one of the privileged structures that bind to this pocket.

For private or internal corporate use only

Key words

- **Arylpiperazines, binding pocket, D_2 receptor, interaction, modelling**
- **Dopamine D_2 agonists**

Arzneim.-Forsch./Drug Res. 55, Nr. 3, 145–152 (2005)

Zusammenfassung

Interaktion von Arylpiperazinen mit der Dopamin D_2-Rezeptorbindungsstelle

Die Bindung von mehreren 1-Benzyl-4-arylpiperazin-Derivaten an den Dopamin D_2-Rezeptor wurde untersucht. Die Ergebnisse zeigen, daß die Interaktion des protonierten N1 des Piperazin-Rings mit Asp 86 (III.32) und „Edge-to-Face"-Interaktionen des aromatischen Rings des Acrylpiperazin-Anteils mit Phe 178 (VI.44), Trp 182 (VI.48) und Tyr 216

© ECV · Editio Cantor Verlag, Aulendorf (Germany)

(VII.58) des Rezeptors die größte stabilisierende Kraft darstellt. Neben der Wasserstoffbrücken-Akzeptorgruppe in Position 2 von Phenylpiperazin kann der aromatische Ring eine zusätzliche Wasserstoffbrücke mit Trp 182 bilden. Große Substituenten in Position 4 wurden aufgrund sterischer Interaktionen mit Phe 179 (VI.44) nicht toleriert. Die Einführung der Elektronenakzeptorgruppe - NO_2 in Position 3 von Arylpiperazin verminderte die Bindungsaffinität im Vergleich zu Phenylpiperazin 1, während die Elektronendonatorgruppe -OMe und der zweite aromatische Ring (Naphthyl) die Bindungsaffinität verstärkte. Dies kann mit einer bevorzugten „Edge-to-Face"-Interaktion bei Liganden mit stark negativem elektrostatischem Oberflächenpotential (ESP) im Zentrum des aromatischen Teils von Arylpiperazin erklärt werden. Neben Ionen- und Wasserstoffbrükken sind daher „Edge-to-Face"-Interaktionen maßgeblich mit an der Komplexbildung zwischen Arylpiperazin-Liganden und dem Dopamin D_2-Rezeptor beteiligt. Phe 178 (VI.44), Trp 182 (VI.48) und Tyr 216 (VII.58) können als Teil einer zusätzlichen Dopamin D_2-Rezeptortasche angesehen werden, die in den meisten GPRCs (G protein-coupled receptors) der A-Klasse erhalten ist. Offensichtlich repräsentiert das Strukturmotiv von Arylpiperazin eine begünstigte Struktur, die an diese Tasche bindet.

1. Introduction

For many years, the dopamine receptor (DAR) D_2 was a major target for neurobiological research and drug development, since DA antagonists have been proven to be efficient antipsychotics [1]. Drug design strategies devoted so far to the design and synthesis of novel dopamine D_2 receptor agonists have been predominantly based on the dopamine itself [2–4]. Traditional dopamine agonists closely resemble the dopamine, most of them having the '3-OH phenethylamine' dopamine pharmacophore or a bioisosteric surrogate, embedded within their molecular structure. Using similar approach, we have synthesized a series of benzimidazoles that could be considered as non-catechol bioisosteres of catecholamines [5]. The most active compounds of this type were obtained by connecting the benzimidazole-like rings through the flexible ethylene linker with arylpiperazine tail. It was noticed that the binding affinity of the prepared ligands for the DAR D_2 depends on both benzimidazole structure and the structure of arylpiperazine part of the molecule, but the effect of the latter was more pronounced. Studies from other laboratories had a similar outcome what resulted in a new generation of DA-ergic agents that no longer rely upon the '3-OH-phenethylamine' framework [6–8]. Using methods of homology modelling Teeter and DuRand [9] came to the same conclusion and postulated the existence of an ancillary pocket of the DAR D_2 separated from the catechol binding site of this receptor. The results of Homan et al. [10] confirmed and supported the data of the above authors [9]. However, the physicochemical basis of the ligand-receptor interactions is still far from being fully understood. This prompted us to study the effect of electron density distribution (electrostatic surface potential; ESP) in the arylpiperazine class of ligands on their binding affinity at the DAR D_2. To limit the number of receptor-ligand interactions in arylpiperazine binding pocket, in the present study, structurally simple ligands were chosen. Binding pocket of the D_2 receptor was defined according to Teeter and DuRand [9]. A special attention has been paid to the nonpolar type of interactions (e.g. stacking or edge-to-face interactions) which play a significant role in the formation of the receptor-ligand complexes [9, 11]. Namely, it is known that aromatic-aromatic interactions involve more than just the entropic contribution provided by the desolvation of nonpolar surfaces. Due to edge-to-face and π–π interactions, there is an enthalpic contribution, as well. These can be of comparable strength to hydrogen bonds and likewise they are directional in character [12, 13]. Edge-to-face interactions between receptors and the corresponding 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 [12, 13]. Complementarities of negative ESP in the centre of aromatic residues of the ligands and positive ESP of the protons in receptor aromatic residues, 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 the dopamine receptor D_2 ligands possibly representing potent pharmacological agents.

2. Materials and methods

2.1. 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, England). The mass spectra were determined by a Finnigan Mat 8230 mass spectrometer (Finnigan, Bremen, Germany). For analytical thin-layer chromatography E. 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

For private or internal corporate use only

Arzneim.-Forsch./Drug Res. 55, No. 3, 145–152 (2005)
© ECV · Editio Cantor Verlag, Aulendorf (Germany)

pressure (MPLC). Solutions were routinely dried over anhydrous Na_2SO_4 prior to evaporation.

2.2. Molecular modelling

Ligand models were constructed using a Hyperchem v. 7.0 software (Hypercube, Gainesville, USA) and inbuilt PM3 routine for molecule geometry optimization. It was postulated that the ligands are bound to the receptors in protonated form [14, 15], therefore, a formal charge of +1 was added to the piperazine nitrogen (1N). The obtained results were further optimized in a Gaussian 98, Rev. A.9 (Gaussian, Pittsburgh, USA). The ESPs were calculated in a Gaussian G 98W using the DFT B3LYP method and a 6–31g* basis set [16, 17]. The ESP cube output from a Gaussian G 98W was visualized in a gOpenMol software [18] following the recommended Gaussian procedure to display the calculated properties.

Modelling of the ligand-DAR D_2 complexes was done using the Docking module within an INSIGHT II software (Accelerys, Cambridge, UK) on a SGI Octane 2 workstation (Silicon Graphics, Mountain View, USA). Docking of the ligands described here was performed as it 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 were reached. Minimization was performed by fixing all protein backbone atoms and 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 one with the shortest salt bridge between the ligand and Asp 86, and with a maximum number of hydrogen bonds with the DAR D_2. The obtained results were visualized using a DS View software (Accelerys, Cambridge, UK).

2.3. Chemistry

2.3.1. Genaral procedure for the synthesis of 1-benzyl-4-arylpiperazine and 1-(2-phenylethyl)-aryl-piperazine

To the solution of 5 mmol arylalkylhalide I and 7.5 mmol aryl piperazine I (7.5 mmol) with arylalkylhalide II (5 mmol) in 10 mL methyl ethyl katone, 10 mmol potassium carbonate was added and the mixture was stirred (24 h, 80 °C). After cooling, the reaction mixture was poured into the water and extracted with dichloromethane. After drying and evaporation, products III were purified by dry-flesh chromatography with $MeCl_2$/MeOH as a solvent.

2.3.2. Physicochemical data for arylpiperazines

(**1**): Yield: 82 %; m.p. 42 °C; IR (cm^{-1}): 2936, 2817, 1599, 1504, 1453, 1238; ^{1}H NMR: d 2.57–2.62 (m, 4 H), 3.16–3.21 (m, 4 H), 3.55 (s, 2 H), 6.79–6.93 (m, 3 H, ArH), 7.19–7.35 (m, 7 H, ArH). MS: m/e 252 (M^+); $C_{17}H_{20}N_2$.

(**2**): Yield: 88 %; m.p. 88 °C; IR (cm^{-1}): 2943, 2822, 1592, 1575, 1494, 1451, 1398; ^{1}H NMR: δ 2.72–2.83 (m, 4 H), 3.12–3.20 (m, 4 H), 3.59 (s, 2 H), 7.08 (dd, 1 H, J = 6.2 Hz, J = 2.4 Hz, ArH), 7.24–7.52 (m, 9 H, ArH), 7.77–7.85 (m, 1 H, ArH), 8.17–8.22 (m, 1 H, ArH). MS: m/e 302 (M^+); $C_{21}H_{22}N_2$.

(**3**): Yield: 85 %; oil; IR (cm^{-1}): 2941, 2813, 1677, 1594, 1500, 1451, 1241; ^{1}H NMR: δ 2.63–2.68 (m, 4 H), 3.06–3.09 (m, 4 H), 3.58 (s, 2 H), 3.83 (s, 3 H, OCH_3), 6.82–7.01 (m, 4 H, ArH), 7.21–7.35 (m, 5 H, ArH). MS: m/e 282 (M^+); $C_{18}H_{22}N_2O$.

(**4**): Yield: 81 %; m.p. 48 °C; IR (cm^{-1}): 2961, 2835, 2822, 1610, 1575, 1498, 1447, 1436, 1348, 1218; ^{1}H NMR: δ 2.57–2.62 (m, 4 H), 3.16–3.21 (m, 4 H), 3.56 (s, 2 H), 3.77 (s, 3 H, OCH_3), 6.38–6.45 (m, 2 H, ArH), 6.53 (dd, 1 H, J = 5.8 Hz, J = 2.4 Hz, ArH), 7.12–7.20 (m, 1 H, ArH), 7.23–7.35 (m, 5 H, ArH). MS: m/e 282 (M^+); $C_{18}H_{22}N_2O$.

(**5**): Yield: 85 %; m.p. 69 °C; IR (cm^{-1}): 2939, 2831, 1511, 1451, 1252, 1148, 1039; ^{1}H NMR: δ 2.59–2.64 (m, 4 H), 3.07–3.12 (m, 4 H), 3.57 (s, 2 H), 3.76 (s, 3 H, OCH_3), 6.80–6.92 (m, 4 H, ArH), 7.25–7.36 (m, 5 H, ArH). MS: m/e 282 (M^+); $C_{18}H_{22}N_2O$.

(**6**): Yield: 85 %; oil; IR (cm^{-1}): 2820, 1604, 1521, 1492, 1453, 1349, 1225, 1133, 1008; ^{1}H NMR: δ 2.58–2.63 (m, 4 H), 3.05–3.10 (m, 4 H), 3.57 (s, 2 H), 6.97–7.06 (m, 1 H, ArH), 7.12 (d, 1 H, J = 8.2 Hz, ArH), 7.26–7.35 (m, 5 H, ArH), 7.42–7.50 (m, 1 H, ArH), 7.77 (dd, 1 H, J = 6.4 Hz, J = 1.6 Hz, ArH). MS: m/e; MS: m/e 297 (M^+); $C_{17}H_{19}N_3O_2$.

(**7**): Yield: 89 %; m.p. 49 °C; IR (cm^{-1}): 2819, 1615, 1524, 1450, 1342, 1232, 1150, 1010; ^{1}H NMR: δ 2.60–2.65 (m, 4 H), 3.26–3.31 (m, 4 H), 3.58 (s, 2 H), 7.14–7.19 (m, 1 H, ArH), 7.30–7.38 (m, 6 H, ArH), 7.62–7.71 (m, 2 H, ArH). MS: m/e 297 (M^+); $C_{17}H_{19}N_3O_2$.

(**8**): Yield: 88 %; m.p. 112 °C; IR (cm^{-1}): 2819, 2778, 1601, 1587, 1506, 1488, 1448; 1333, 1259, 1240, 1119, 1100; ^{1}H NMR: δ 2.56–2.62 (m, 4 H), 3.40–3.45 (m, 4 H), 3.57 (s, 2 H), 6.78–6.82 (m, 2 H, ArH), 7.26–7.36 (m, 5 H, ArH), 8.07–8.15 (m, 2 H, ArH). MS: m/e 297 (M^+); $C_{17}H_{19}N_3O_2$.

(**9**): Yield: 82 %; m.p. 117 °C; IR (cm^{-1}): 2946, 2823, 1589, 1531, 1502, 1448, 1354, 1247, 1212, 1139, 1027; ^{1}H NMR: δ 2.65–2.70 (m, 4 H), 3.08–3.13 (m, 4 H), 3.67 (s, 2 H), 3.85 (s, 3 H, OCH_3), 6.84–7.03 (m, 4 H, ArH), 7.49 (t, 1 H, J = 7.8 Hz, ArH), 7.71 (d, 1 H, J = 7.8 Hz, ArH), 8.09–8.15 (m, 1 H, ArH), 8.25 (s, 1 H, ArH). MS: m/e 312 (M^+); $C_{19}H_{24}N_2O_2$.

(**10**): Yield: 89 %; m.p. 101 °C; IR (cm^{-1}): 2929, 2809, 1595, 1520, 1498, 1458, 1349, 1242, 1141, 1024; ^{1}H NMR: δ 2.65–2.70 (m, 4 H), 3.09–3.13 (m, 4 H), 3.67 (s, 2 H), 3.86 (s, 3 H, OCH_3), 6.85–7.06 (m, 4 H, ArH), 7.56 (d, 2 H, J – 8.6 Hz, ArH), 8.20 (dd, 2 H, J = 4.8 Hz, J = 1.8 Hz, ArH). MS: m/e 227 (M^+); $C_{18}H_{21}N_3O_3$.

(**11**): Yield: 62 %, oil; IR (cm^{-1}): 2932, 2821, 1593, 1500, 1458, 1232; ^{1}H NMR: δ 2.65–3.62 (m, 12 H), 6.89–7.43 (m, 10 H, ArH. MS: m/e 175 (100 %), 266 (M^+); $C_{18}H_{22}N_2$.

(**12**): Yield: 58 %; oil; IR (cm^{-1}): 2938, 2817, 1671, 1586, 1497, 1438, 1238; ^{1}H NMR (d_6DMSO): δ 3.12-4.02 (m, 12 H), 3.75 (s, 3 H, OCH_3), 6.90–7.10 (m, 4 H, ArH), 7.15–7.40 (m, 5 H, ArH). MS: m/e 296 (M^+); $C_{19}H_{24}N_2O$.

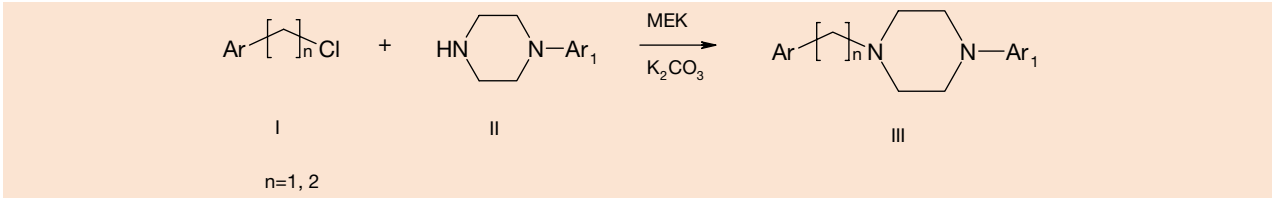
(**13**): Yield: 55 %; oil; IR (cm^{-1}): 2945, 2820, 1589, 1571, 1497, 1456, 1394; ^{1}H NMR: δ 2.62–3.61 (m, 12 H), 6.85–7.43 (m, 12 H, ArH). MS: m/e 225 (100 %), 316 (M^+); $C_{22}H_{24}N_2$.

2.2.3. Synaptosomal membrane preparation, binding assays and data analysis

Synaptosomal membranes of the bovine caudate nuclei used as a source of the dopamine D_2 receptor were prepared exactly as described previously [19]. [^{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. The binding of the radioligand to the 5-HT_2 receptors was prevented by 50 nmol/L ketanserin. Ki values were determined by competition binding at 0.2 nmol/L of the radioligand and 8 to 10 concentrations of each tested ligand (0.1 nmol/L – 0.1 mmol/L). Nonspecific binding was measured in the presence of 1.0 mmol/L (+)-buta-

For private or internal corporate use only

© ECV · Editio Cantor Verlag, Aulendorf (Germany)



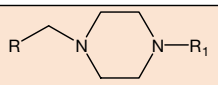
Scheme 1: Synthesis of arylpiperazines.

clamol (CAS 51152-91-1). The reaction was terminated by a rapid filtration through Whatman GF/C filters (Whatman, Maidstone, UK) which were further washed three times with 5.0 mL of 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 [20].

3. Results

All arylpiperazines considered here (compounds **1**–**13,** Table 1) were synthesised by the alkylation of the corresponding arylpiperazines with arylalkylhalides in MEK in the presence of potassium carbonate as a base (Scheme 1) and their affinity for the binding at the DAR D_2 was determined. In binding assays, synaptosomal membranes of the bovine caudate nuclei as a source of the DAR D_2 and [^{3}H]spiperone as a specific radioligand were used. (R)- and (S)-butaclamol were run simultaneously in the same test system as references.

Table 1: Chemical structure and affinity of the examined arylpiperazine ligands for the binding at the dopamine D_2 receptor.

No.			
	R	R_1	K_i ± S.E.M. (nmol/L) D_2
1	phenyl	phenyl	639 ± 43
2	phenyl	1-naphthyl	580 ± 36
3	phenyl	2-metoxyphenyl	28 ± 4.2
4	phenyl	3-metoxyphenyl	577 ± 37
5	phenyl	4-metoxyphenyl	>1000
6	phenyl	2-nitrophenyl	198 ± 26
7	phenyl	3-nitrophenyl	>1000
8	phenyl	4-nitrophenyl	>1000
9	4-metoxyphenyl	2-metoxyphenyl	98 ± 9
10	4-nitrophenyl	2-metoxyphenyl	118 ± 11
11	benzyl	phenyl	478 ± 56
12	benzyl	2-metoxyphenyl	37.5 ± 4.2
13	benzyl	1-naphthyl	132 ± 17
(S)-Butaclamol			2.5 ± 0.1
(R)-Butaclamol			>1000

Structures of 1-benzyl-aryl-piperazine and 1-(2-phenylethyl)-arylpiperazine ligands tested for the docking in the DAR D_2 binding pocket are shown. K_i values are the means of three independent experiments done in triplicate performed at eight competing ligand concentrations (0.1 nmol/L–0.1 mmol/L) and 0.2 nmol/L [^{3}H]spiperone.

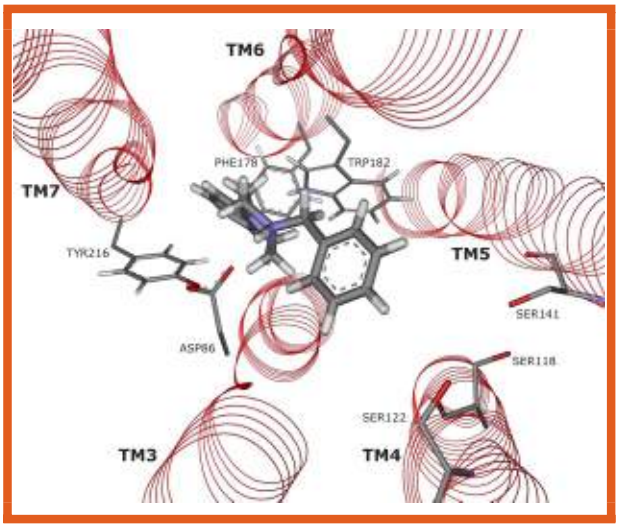


Fig. 1: Schematic representation of ligand 1 interaction with the dopamine D_2 receptor. Schematic model of the proposed interaction of the studied compound 1 with the dopamine D_2 receptor. The 3D model describes a possible interaction of ligand 1 and the theoretical dopamine D_2 receptor model.

The binding pocket of the DAR D_2 was defined according to Teeter and DuRand [9]. In our modelling studies, all receptor amino acid side groups that could interact with the ligands were taken into account (Table 2). Applying this approach, ligand **1** was docked in the DAR D_2 binding site, thus forming the complex shown in Fig. 1**.** In general, the following features of the ligand-receptor complex emerged: i. close interaction of protonated N1 of the piperazine ring with Asp 86 (III.32) (calculated distance 1.67 Å) and ii. edge-to-face interac-

Table 2: List of amino acids considered to be a part of binding site in the dopamine D_2 receptor. TM = transmembrane helices.

Helix	Residue	Position	Helix	Residue	Position
TM II	Asp	46	TM V	Phe	145
TM II	Trp	56	TM VI	Phe	178
TM III	Phe	82	TM VI	Cys	181
TM III	Val	83	TM VI	Phe	185
TM III	Asp	86	TM VI	Phe	186
TM III	Met	89	TM VI	His	189
TM III	Cys	90	TM VII	Tyr	208
TM III	Ser	93	TM VII	Phe	211
TM IV	Trp	115	TM VII	Thr	212
TM IV	Ser	118	TM VII	Gly	251
TM IV	Ser	122	TM VII	Tyr	216
TM V	Ser	141	TM VII	Ser	219
TM V	Ser	144	TM VII	Asn	222

For private or internal corporate use only

Arzneim.-Forsch./Drug Res. 55, No. 3, 145–152 (2005)
© ECV · Editio Cantor Verlag, Aulendorf (Germany)

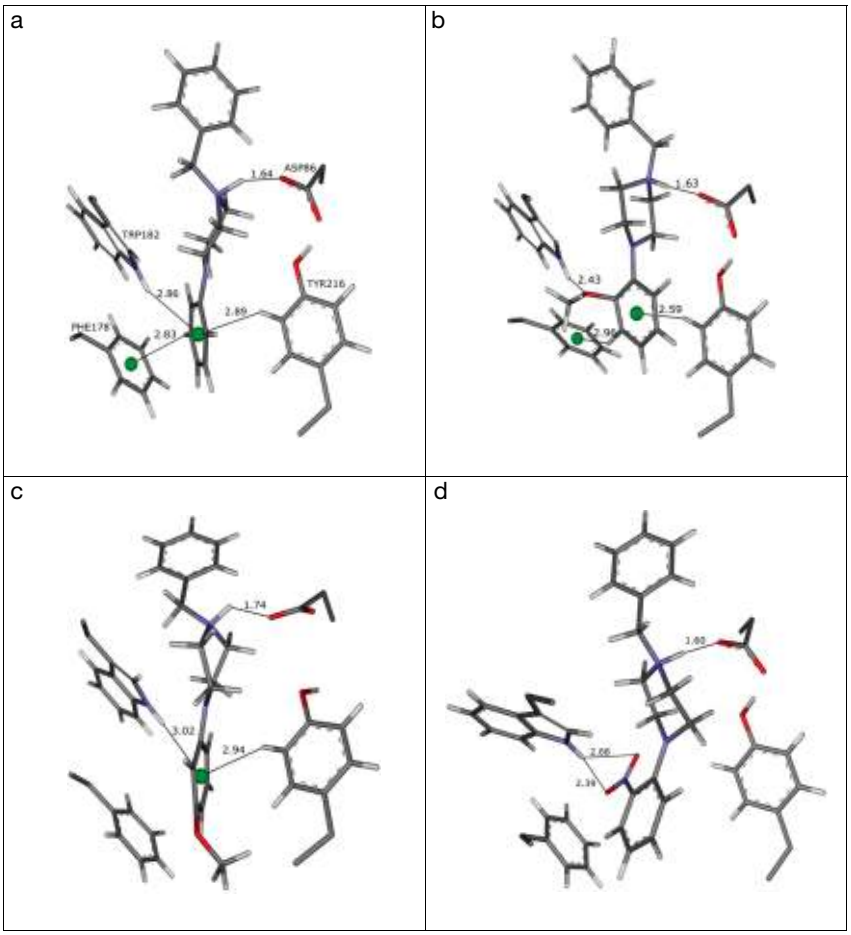


Fig. 2: Schematic representation of interaction of ligands 1, 3, 4 and 6 with the dopamine D_2 receptor binding site. Schematic model of the proposed interaction of the studied compounds 1, 3, 4 and 6 with the dopamine D_2 receptor. The 3D model describes a possible interaction of ligands: 1 (a), 3 (b), 4 (c) and 6 (d) with the ancillary binding pocket of the dopamine D_2 receptor.

tion of the aromatic ring of the phenylpiperazine part of the ligand with Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58) of the receptor.

Detailed images of the complexes of ligands **1**, **3**, **4** and **6** with the interacting amino acid residues of the DAR D_2 binding pocket are shown in Fig. 2. Calculated distance for protonated N1 of the piperazine ring and Asp 86 ranged between 1.67 and 1.85 Å. Molecular counterparts participating in the edge-to-face interactions, distanced by less than 3 Å, were optimally oriented.

The ESPs of 1-benzyl-4-arylpiperazines were calculated using a Gaussian G 98W software and some examples are presented in Fig. 3.

To calculate free energy (ΔG) of the receptor-ligand interactions, the structures obtained in the docking analysis were used. The coordinates of a ligand and Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58) of the receptor were frozen, while all other elements of the receptor were removed. Contribution of ASP 86 (III.32) to the energy of the ligand-receptor interaction was considered constant for all ligands studied here and therefore, it was not used for further calculations. Single Point calculation was employed to define the total energy of the system thus defined. In the following step, the ligand was translated along the x-axis for 10 Å and the calculation was repeated. The difference in the obtained energies was taken as a measure of the system stabilization. For this calculation, the B3LYP method

Table 3: ΔG of 1-benzyl-4-arylpiperazine interaction with the dopamine D_2 receptor.

Compound No.	ΔG of ligand DAR interactions (kcal/mol)
1	4
3	12
4	4
6	7

Free energy (ΔG) of 1-benzyl-4-arylpiperazine interactions with the dopamine D_2 receptor was calculated as follows: coordinates of ligand and Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58) of the receptor were frozen, while all other elements of the receptor were removed. Single Point calculation was used to define the total energy of the system thus defined. In the next step, the ligand was translated along the X-axis for 10 Å and the calculation was repeated. The difference in the obtained energies was taken as a measure of the system stabilization. For this calculation a B3LYP method was used with the basis set 6–31g* in a Gaussian 98W software.

For private or internal corporate use only

© ECV · Editio Cantor Verlag, Aulendorf (Germany)

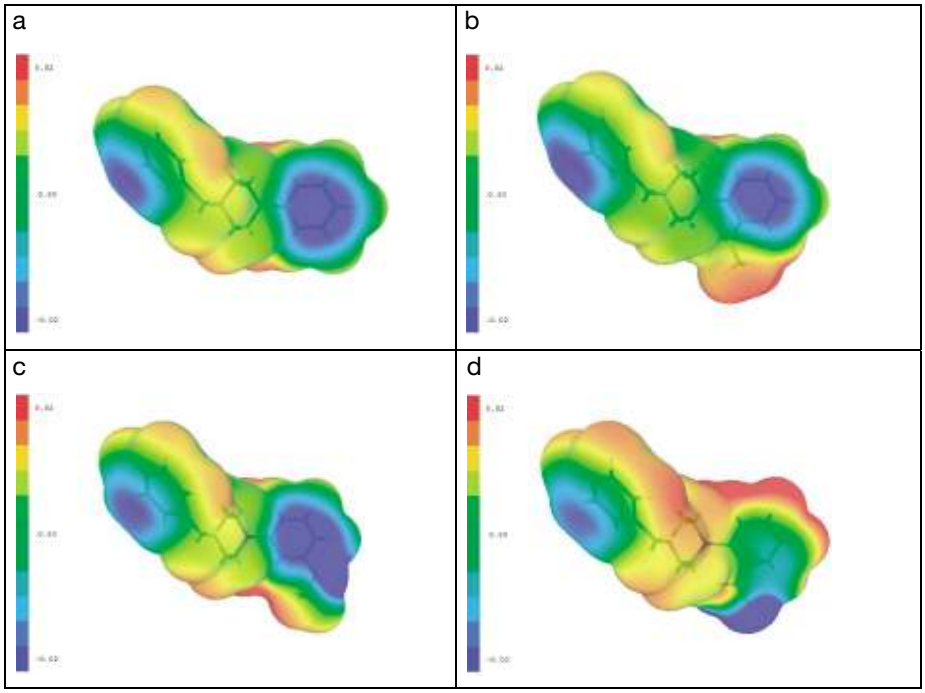


Fig. 3: Electrostatic surface potentials (ESP) of several 1-benzyl-4-arylpiperazines. For simpler comparisons, the ESP values were mapped on the electron density surface. Values in blue indicate a strong, negative ESP, whereas those in red correspond to a strong, positive ESP. Compounds 1 (a), 3 (b), 4 (c) and 6 (d).

was used with the basis set at 6–31 g* in a Gaussian 98W software. The obtained results are summarized in Table 3 and the values reflect the stability of the ligand-receptor complexes of the listed compounds. The values given followed the experimental results presented in Table 1.

4. Discussion

In our previous studies on 1-{2-(benzimidazole)ethyl}-4-arylpiperazine type of DA-ergic ligands, we have noticed that the binding affinity of a compound for the DAR D_2 depends substantially on the structure of arylpiperazine part of the molecule [5]. In order to understand better receptor-ligand interactions at molecular level, a special attention has been paid to the contribution of arylpiperazine part of the ligands to their binding affinity.

Docking of arylpiperazine **1** at the DAR D_2 binding site afforded the complex shown in Fig. 1. In general, the following features of the ligand-receptor complex emerged: i. close interaction of protonated N1 of the piperazine ring with Asp 86 (III.32) (calculated distance 1.67 Å) and ii. edge-to-face interactions of the aromatic ring of the phenylpiperazine part of the ligand with Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58) of the receptor.

Interestingly enough, all these amino acids are highly conserved through the A class of the G protein-coupled receptor (GCPR) family. For example, the residue Asp (III.32) conserved throughout the biogenic transmitter subfamily forms a salt bridge with the amino group present in all these transmitter molecules. Within the binding pocket, the residues oriented toward the lower part of the pocket were more conserved. In particular, the subpocket spanned by the residues Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58) between TM 5 and TM 7 is highly conserved. The residue Phe 178 (VI.44) is either Phe or Tyr in 98 % of the receptors, the residue Trp 182 (VI.48) is Trp in 90 % of the receptors, while Tyr 216 (VII.58) is conserved among 95 % of the A receptor class [14]. Thus, this cluster of aromatic residues, conserved in the vast majority of receptors, seems to mark the borderline between the more conserved central part of the TM 7 domain and more variable part toward the extracellular face. Bondensgaard et al. [15] postulated that this ancillary pocket in GPCRs is responsible for the docking of so-called „privileged structures“. These structures are defined as ligand substructures that are widely used to generate high-affinity ligands for more than one type of the receptors.

As a consequence, it can be hypothesized that there must be some common feature in the target proteins. Following this definition, arylpiperazines can be defined as privileged structures since they are a part of numerous high affinity ligands for different GPCRs [23–25].

It is very likely that some contacts are established between a privileged structure and non-conserved parts of the binding pocket and besides, orientation

For private or internal corporate use only

Arzneim.-Forsch./Drug Res. 55, No. 3, 145–152 (2005)
© ECV · Editio Cantor Verlag, Aulendorf (Germany)

and positioning of conserved amino acid residues in the ancillary binding pocket differ among GPCRs. These factors will make a huge difference in ligand binding affinity to different receptor types, since both edge-to-face and π–π interactions are directional in character.

More detailed inspection of the complexes with ligands **1**, **3**, **4** and **6** is presented in Fig. 2. Generally, all these ligands interact with the same part of the D_2 receptor. Only substituents able to take part in hydrogen bond formation (methoxy- or nitro-) in position 2 of the phenyl ring in the piperazine part of a ligand (ligands **3** and **6,** respectively) formed one more hydrogen bond with Trp 182 (VI.48). Molecular counterparts taking part in edge-to-face interaction (the aromatic ring or arylpiperazine part of the ligand and Phe 178 (VI.44), Tyr 216 (VII.58) and Trp 182 (VI.48) of the receptor) are distanced by less than 3 Å and optimally oriented. There are two prerequisites for edge-to-face interactions: i. complementarities of negative ESP in the centre of aromatic residues (FACE) of the one interacting counterpart and positive ESP of the protons in aromatic residues (EDGE) of the other and ii. proper orientation of molecular entities forming the complex. The results presented in Fig. 3 show that the ligands **1**–**5** and **9**–**13** have negative ESP in the centre of aromatic residues (FACE) that can interact with positive ESP of the protons in aromatic residues of the dopamine receptor binding pocket (EDGE). In the case of ligands with nitro substituents (**7** and **8**), there is no negative ESP in the centre of aromatic residues and therefore, they do not afford edge-to-face interactions.

Additionally, ligands **3, 6, 9** and **10** are stabilized with the hydrogen bond between the ligand methoxy- or nitro-group in orto-position of arylpiperazine and Trp 182 (VI.48) of the receptor molecule. The family of the structures examined here have hydrogen bond ranging from 1.85 to 2.8 Å and from 1.75 to 2.65 Å for methoxy derivatives for nitro derivatives, respectively.

Docking of the para-substituted compounds **5** and **8** at the same binding site of the DAR D_2 revealed unfavourable steric interactions of para-substituents with Phe 178 (VI.44) of the receptor (not shown). As a consequence, the distance between protonated N1 of the piperazine ring and Asp 86 (III.32) was increased over 3.27 Å for ligand **8** and over 2.98 Å for ligand **5**. In addition, edge-to-face interactions were hindered due to unfavourable orientation.

Docking analyses of the ligands with substituents in position 3 of the piperazine phenyl ring (ligand **4**) demonstrated that substituents in this position are tolerated, since no large reduction of the binding affinity was recorded. In contrast, substituents with electron withdrawal effect in this position such as the nitro group (ligand **7**) affected the binding affinity by decreasing electron density in the benzene ring of these ligands so that edge-to-face interactions could not be realized.

To obtain a better insight into quantitative aspects of a certain type of interactions within the ligand-receptor complex, the structures obtained by the docking experiments were used. Single Point calculation was employed to define the total energy of the system thus defined. Ligand **3** (12 kcal/mol) built the most stable complex due to the hydrogen bond formation between the methoxy group of the ligand and Trp 182 (VI.48) of the receptor. Apart from this interaction, this ligand was able to interact edge-to-face with aromatic residues in the DAR D_2 binding pocket. It was followed by ligand **6** that it is able to form a hydrogen bond only. Ligand **1** is capable of realizing three edge-to-face interactions, thus providing the stabilization of 4 kcal/mol. Ligand **3**, however, realized just two edge-to-face interactions. It should be emphasized that this calculation deals with a simplified system, since it is very difficult to set properly initial parameters of the system, but it provides a kind of semi-quanitative results.

In order to examine the influence of the benzyl part of ligand molecules on their binding affinities for the DAR D_2, compounds **9**–**13** were synthesized and assayed. The obtained results demonstrated that the affinity of o-methoxyphenylpiperazine derivatives **9, 10** and **12** was decreased comparing to parent compound **3**. On the other hand, the binding affinity of phenylethylpiperazines **11** and **13** was increased in relation to benzyl analogues **1** and **2**, respectively. This effect can not be explained by considering the ligand-receptor interaction solely at the „arylpiperazine“ binding site but the overall set of receptor-ligand interactions which is the subject of our current investigations.

The results obtained throughout this work clearly demonstrate that the arylpiperazine structural motif can be considered as a privileged structure fitting within the ancillary pocket of the DAR D_2 that is preserved in most of the GPCRs. The aryplpiperazine moiety is positioned in the ancillary pocket spanned by the three conserved aromatic residues, i.e. Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58), providing favourable aromatic-aromatic interactions. The results of the docking studies on 1-benzyl-4-aryl-piperazine-DAR D_2 complexes revealed that close interaction of protonated N1 of the piperazine ring with Asp 86 (III.32) and edge-to-face interactions of the aromatic ring or arylpiperazine part of the ligand with Phe 178 (VI.44), Trp 182 (VI.48) and Tyr 216 (VII.58) of the receptor represent the main stabilizing forces. In addition, the 2-methoxy derivative **3** could build one additional hydrogen bond with Trp 182 (VI.48). Bulky substituents in position 4 of the aromatic part of phenylpiperazine ring are not tolerated because of the unfavourable steric interactions with Phe 178 (VI.44). This result is in full accordance with the data of Simpson et al. [26] and Lober et al. [27]. Substituents in position 2 and 3 of phenylpiperazine are sterically well tolerated. Electron attractive groups in position 3 such as -NO_2 decreased the bind-

For private or internal corporate use only

ing affinity, while electron donors like -OMe and the second aromatic ring (naphthyl) increased the affinity for the binding at the DAR D_2 in comparison with the unsubstituted phenylpiperazine **1**. These effects can be explained by stronger edge-to-face interactions of negative ESP in the centre of the aromatic residues of the ligands and positive ESP of the protons of the receptor aromatic residues. Methoxy- and nitro-groups, regardless of the electron properties, increased the affinities of the ligands if attached to position 2, since one additional hydrogen bond could be formed with Trp 182 (VI.48).

5. Literature

[1] Strange, P. G., Antipsychotic drugs: Importance of dopamine receptors for mechanisms of therapeutic actions and side effects. Pharmacol. Rev. **53,** 119 (2001)

[2] Kaiser, C., Jain, T., Dopamine receptors: functions, subtypes and emerging concepts. Med. Res. Rev. **5,** 145 (1985)

[3] Seeman, P., Ulpian, C., Dopamine D_1 and D_2 receptor selectivities of agonists and antagonists. Adv. Exp. Med. Biol. **235,** 55 (1988)

[4] Malmberg, A., Nordvall, G., Johansson, A. M. et al., Molecular basis for the binding of 2-aminotetralins to human dopamine D_{2A} and D_3 receptors. Mol. Pharmacol. **46,** 299 (1994)

[5] Šoškić, V., Joksimović, J., Bioisosteric approach in the design of new dopaminergic/ serotonergic ligands. Curr. Med. Chem. **5,** 493 (1998)

[6] Mewshaw, R. E., Verwijs, A., Shi, X. et al., New generation of dopaminergic agents. 5. heterocyclic bioisosteres that exploit the 3-OH-N^i-phenylpiperazine dopaminergic template. Bioorg. Med. Chem. Lett. **8,** 2675 (1998)

[7] Mewshaw, R. E., Marquis, K. L., Shi, X. et al., New generation of dopaminergic agents. 4. Exploiting the 2-Methyl Chroman Scaffold. Synthesis and evaluation of two noval series of 2-(aminomethyl)-3,4,7,9-tetrahydro-2H-pyrano[2,3-e]indole and indole-8-one derivatives. Tetrahedron **54,** 7081 (1998)

[8] Mewshaw, R. E., Husbands, M., Gildersleeve, E. S. et al., New generation of dopaminergic agents. 2. Discovery of 3-OH-phenoxyethylamine and 3-OH-N^1-phenylpipe-razine dopaminergic templates. Bioorg. Med. Chem. Lett. **8,** 295 (1998)

[9] Teeter, M. M., DuRand, C. J., Dopamine D_2 receptor model explains binding affinity of neuroleptics: piquindone and its structure/activity relationships. Drug Des. Discov. **13,** 49 (1996)

[10] Homan, E. J., Wikstrom, H. V., Grol, C. J., Molecular modelling of the dopamine D_2 and serotonin 5-HT_{1A} receptor binding modes of the enantiomers of 5-OMe-BPAT. Bioorg. Med. Chem. **7,** 1805 (1999)

[11] Wilcox, R. E., Tseng, T., Brusniak, M. Y. et al., CoMFA-based prediction of agonist affinities at recombinant D_1 vs D_2 dopamine receptors. J. Med. Chem. **41,** 4385 (1998)

[12] Jennings W. B., Farell, B. M., Malone, J. F., Attractive intramolecular edge-to-face aromatic interactions in flexible organic molecules. Acc. Chem. Res. **34,** 885 (2001)

[13] Tsuzuki, S., Honda, K., Uchimaru, T. et al., Origin of attraction and directionality of the π/π interaction: Model chemistry calculations of benzene dimer interaction. J. Am. Chem. Soc. **124,** 104 (2002)

[14] Katerinopoulos, H. E., Schuster, D. I., Structure-activity relationships for dopamine analogs: A review. Drugs Fut. **12,** 233 (1987)

[15] Fausto, R., Ribeiro, M. J. S., Pedroso de Lima, J. J., Elucidation of the conformational properties of dopamine [1,2-benzenediol-4-(2-aminoethyl)] and dopamine cation: A molecular orbital study. J. Mol. Structure **484,** 181 (1999)

[16] Stewart, J. J. P., Optimization of parameters for semiempirical methods. I. Method. J. Comput. Chem. **10,** 209 (1989)

[17] Becke, A. D., Density-functional thermochemistry. III. The role of exact exchange. J. Chem. Phys. **98,** 5648 (1993)

[18] Laaksonen, L., A graphics program for the analysis and display of molecular dynamics trajectories, J. Mol. Graph. **10,** 33 (1992)

[19] Šoškić, V., Maelicke, A., Petrović, G. et al., Synthesis of some phenothiazine derivatives as potential affinity ligands for the central dopamine receptors. J. Pharm. Pharmacol. **43,** 27 (1990)

[20] Munson, P. J., Rodbard, D., Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. **107,** 220 (1980)

[21] Beukers, M. W., Kristiansen, K., IJzerman A. P. et al., Tiny GRAP database: a bioinformatics tool to mine G protein-coupled receptor mutant data. TIPS **20,** 475 (1999)

[22] Bondensgaard, K., Ankersen, M., Thøgersen, H. et al., Recognition of privileged structures by G-Protein coupled receptors. J. Med. Chem. **47,** 888 (2004)

[23] Perrone, R., Berardi, F., Colabufo, N. A. et al., 1-Aryl-4-[(5-methoxy-1,2,3,4-tetrahy-dronaphthalen-1-yl)alkyl]piperazines and their analogues: Influence of the stereochemistry of the tetrahydronaphthalen-1-yl nucleus on 5-HT_{1A} receptor affinity and selectivity versus alpha$_1$ and D_2 receptors, 5. J. Med. Chem. **42,** 490 (1999)

[24] Mouithys-Mickalad, A., Poupaert, J. H., Spampinatoc, S. et al., Synthesis and pharmacological evaluation of 6-piperidino- and 6-piperazinoalkyl-2(3H)-benzothiazolones as mixed sigma/5-$HT_{(1A)}$ ligands. Bioorg. Med. Chem. Lett. **12,** 1149 (2002)

[25] Barakat, K. J., Prendergast, K. J., Cheng, K. et al., Tripeptide growth hormone secretagogues. Bioorg. Med. Chem. Lett. **8,** 1431 (1998)

[26] Simpson, M. M., Ballesteros, J. A., Chiappa, V. et al., Dopamine D_4/D_2 receptor selectivity is determined by a divergent aromatic microdomain contained within the second, third, and seventh membrane-spanning segments. Mol. Pharmacol. **56,** 1116 (1999)

[27] Lober, S., Hübner, H., Gmeiner, P., Azaindole derivatives with high affinity for the dopamine D_4 receptor: synthesis, ligand binding studies and comparison of molecular electrostatic potential maps. Bioorg. Med. Chem. Lett. **9,** 97 (1999)

Acknowledgement

This work was supported by the Ministry for Science, Technology and Development of Serbia, Grant #1698.

Correspondence:
Prof. Vukić Šoškić,
ProteoSys AG, Carl Zeiss Str.51,
55129 Mainz (Germany)
Fax: +49 6131 50 192 11
E-mail: vukic.soskic@proteosys.com

For private or internal corporate use only

Arzneim.-Forsch./Drug Res. 55, No. 3, 145–152 (2005)
© ECV · Editio Cantor Verlag, Aulendorf (Germany)