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Investigation of key interactions between the second extracellular loop of the dopamine D2 receptor and several hydroxy-*N*-{[2-(4-phenylpiperazin-1-yl)ethyl]phenyl}-nicotinamides

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Abstract: The dopaminergic receptor system has been the focus for the development of new pharmacotherapeutic agents targeting a number of central nervous system related disorders, such as drug addiction, schizophrenia, depression, and Parkinson's disease, to name just a few. To date, the crystal structure for the human D2 receptor is not known, despite its vital function and importance as a therapeutic target. Herein, a recent advancement in the determination of key receptor–ligand interactions for the available arylpiperazine-like ligands, using a D2 receptor model based on the crystal structure of the D3 receptor is presented. To determine key interactions responsible for high dopaminergic activity, computer-docking analysis was used together with experimental data. A total of 4 dopaminergic ligands showing moderate to high affinity were tested and the obtained results rationalized using ligand structures docked into the proposed D2 receptor model.

Keywords: arylpiperazine; G protein-coupled receptors; docking; molecular modeling.

INTRODUCTION

The second extracellular loop (ECL) of dopamine D2 (D2DA) receptors is a part of the ligand-binding site. The exact 3D structure of this receptor domain is so far unknown.[1] The crystal structure for the ECL receptor area of G protein-coupled receptors (GPCRs) is not available because of the high flexibility of that

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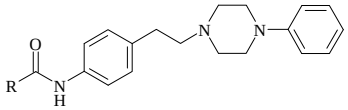
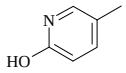
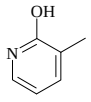
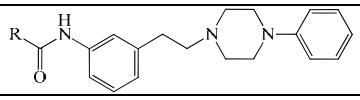
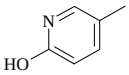
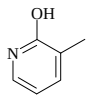
receptor domain. Still ECLs plays a significant role in ligand–receptor interactions and therefore it is important to learn more about the molecular processes involved in the ligand binding. A study of the structural and functional roles of extracellular loops may help in the understanding of certain aspects of ligand binding affinity that cannot be fully explained by interactions with the canonical binding site located in the transmembrane part of the receptor.

Several attempts were made to establish key receptor–ligand interactions in the ECL area[1,2] by using the methods of molecular modeling. In this paper, results are published that were obtained through an investigation of receptor––ligand interactions in the ECL area of D2DAR, and a set of already available four hydroxy-*N*-{[2-(4-phenylpiperazin-1-yl)ethyl]phenyl]nicotinamides[3] (Table I). For the sake of clarity, the ligand molecule was considered as a tail part (phenylpiperazine), a linker part (*meta*- and *para*-amino-phenylethyl) and a head part (heteroaryl group). The tail part was kept constant, while the linker and head parts were varied as described below:

– Ligand **1** is a long linear molecule able to protrude and to bind into the ECL receptor area. It contains polar groups in the head area to allow hydrogen

TABLE I. Structures of the tested hydroxy-*N*-{[2-(4-phenylpiperazin-1-yl)ethyl]phenyl]nicotinamides, *i.e.*, 6-hydroxy-*N*-{4-[2-(4-phenylpiperazin-1-yl)ethyl]phenyl}nicotinamide (**1**), 2--hydroxy-*N*-{4-[2-(4-phenylpiperazin-1-yl)ethyl]phenyl}nicotinamide (**2**), 6-hydroxy-*N*-{3--[2-(4-phenylpiperazin-1-yl)ethyl]phenyl}nicotinamide (**3**), 2-hydroxy-*N*-{3-[2-(4-phenylpiperazin-1-yl)ethyl]phenyl}nicotinamide (**4**), for binding to the D2DA receptors and the determined K_i values that are the mean of three independent experiments performed in triplicate at 7 competing ligand concentrations

No.	R	K_i / nM
		
1		19.5
2		154.3
		
3		95.9
4		120.1

bonding with D2DAR.

– Ligand **2** is the same as ligand **1** but with an altered polar group arrangement in the head part.

– Ligand **3** is a *meta*-regioisomer of ligand **1**, which makes it shorter and therefore suboptimal for binding into the ECL D2DAR area.

– Ligand **4** is the same as ligand **3** but with an altered polar group arrangement in the head part.

In this way, it was assumed that only the hydrogen bond interaction between the ligand and receptor ECL area could be studied, while keeping all other interactions constant.

EXPERIMENTAL

Biological assays

Radioligand [^{3}H]spiperone was purchased from Amersham Biosciences (Amersham, UK). CHO-hD2S cells stably expressing the native human D2DA receptor were obtained from Professor Phillip G. Strange (University of Reading, UK). The cell lines maintain the isolation of membranes with the D2DA receptor. Competition binding assays at the D2 DA receptor were performed using [^{3}H]spiperone (0.4 nM) by the protocol provided in previous publications.[4] Competition binding studies were performed with seven varied concentrations of the test compounds run in triplicate tubes, and isotherms from three assays were calculated by computerized nonlinear regression analysis to yield K_i values.

Docking analysis

A D2DR homology model, used in the docking analysis, was built using the crystal structure of D3DR as the template. The receptor binding site was defined to include all key amino acid residues, as described previously.[2,5]

The 3D structures of the ligand were generated using the Discovery Studio program.[6] Assuming physiological conditions, the basic aliphatic nitrogen atom of piperazine was protonated. The geometry was optimized using the CHARMM force field applying the conjugate gradient method until the energy difference between successive cycles was below 0.0042 kJ mol^{-1}.[7]

Docking of the selected ligands as presented in Table 1 was realized by simulated annealing using the LIBDOCK module from Discovery Studio, employing the CHARMM force field. The initial position of the ligand in the binding site was arbitrary, while the protonated nitrogen on the ligand part was kept in close proximity to Asp 114 of the receptor. No further ligand constraints were applied. Up to 100 structures were produced in every run and each finally optimized in order to remove steric interaction with a gradient limit of 0.0042 kJ mol^{-1} or 4000 optimization steps. A structure was selected based on the following criteria: lowest total energy of the complex, shortest salt bridge formed between Asp 114 of the D2DR and protonated piperazine nitrogen, chair conformation of the arylpiperazine ring and the aryl part of the molecule positioned in the rear of the hydrophobic pocket of the receptor Phe 386, Trp 390 and Tyr 420.[8] After these initial criteria were satisfied, a second step was performed to examine other interactions that could be formed between the receptor and ligand (hydrogen bonds, aromatic–aromatic interactions, *etc*.). In this way, the best possible docking structures were selected. The structures were visualized using DS Visualise, v. 2.5.1[6], and the obtained images were rendered using PovRay Raytracer, v. 3.6.[9]

Electronic surface potential (ESP) calculation

The geometry of the ligands was further optimized in Gaussian 03W software[10] using the DFT B3LYP method with a 6-31g* basis set. The geometry obtained in this way was used for calculation of the ESP. Only the head and linker part of the ligand were subjected to calculation, since the tail segment cannot influence the ESP distribution. The obtained results were visualized in gOpenMol.[11]

RESULTS AND DISCUSSION

The experimental results showed that all ligands bind to D2DR with moderate to high affinity (Table I). The D2DAR receptor cavity is defined by two binding sites, *i.e.*, the orthosteric binding site (OBS) located in the receptor interior and the second binding pocket (SBP).[5] The OBS consists of the following amino acid residues: Asp 114, Ser 194, Ser 197, Ser 167, Trp 386, Phe 390 and Tyr 420. During ligand binding, the role of residue Asp 114 is to anchor *via* a salt bridge with the charged nitrogen atom. Residues Trp 386, Phe 390 and Tyr 420 form a hydrophobic pocket inside the OBS. The hydrophobic tail segment of the ligand forms multiple edge-to-face (ETF) interactions with the pocket amino acid residues, resulting in correct orientation of the ligand inside the OBS. Ser 194/197 and Ser 167 form additional hydrogen bonds that result in a high affinity complex.[5,8]

The head segment of ligands **1**–**4** can interact with amino acid residues located in the SBP, including the ECL area (Ile 166, Leu 170, Leu 171, Ile 184, Phe 189, Val 190, His 397 and Ile 398). Amino acid residues Ile 166, Leu 170, Leu 171, Ile 184, Val 190 and Ile 398 can form hydrophobic interactions. Phe 189 and His 397 are oriented in a way that allows edge-to-face interactions to occur. Ligand **1** can establish an additional hydrogen bond with Asn 186 (Fig. 1).

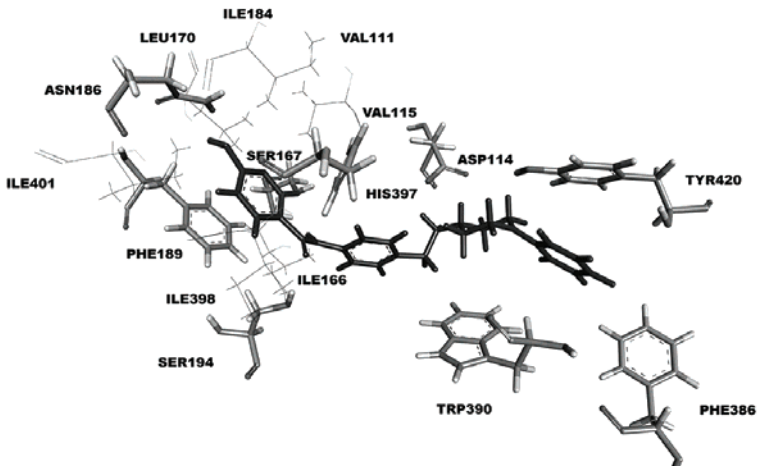


Fig. 1. Docking results – 3D model of D2DR with the position of ligand **1** (black) inside the binding site. Only key amino acid residues are displayed for clarity.

The individual differences in the affinity of ligands could be explained by the obtained docking results.

Ligand **1** was optimally positioned inside the binding site of the receptor, leading to a high affinity complex. Key interactions include a short salt bridge with Asp 114, the ligand tail part oriented between Trp 386, Phe 390 and Tyr 420, forming several edge-to-face interaction, hydrogen bonds with Ser 194 and Ser 167, the ligand head part correctly oriented between Phe 189 and His 397, and a hydrogen bond between the ligand head part and Asn 186 (Fig. 2).

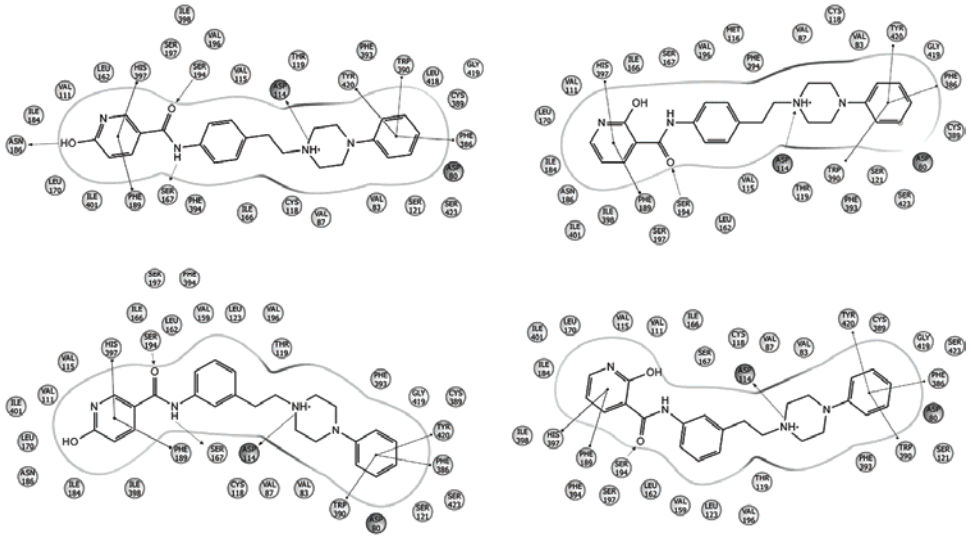


Fig. 2. Schematic representation of the key interaction between D2DR and ligand **1** (top left), ligand **2** (top right), ligand **3** (bottom left) and ligand **4** (bottom right). The aromatic interactions are shown as solid lines, while hydrogen bonds and the salt bridge are denoted by dashed lines.

Ligand **2** occupied the same space inside the D2DR but its head part could not form a hydrogen bond with Asn 186 due to the formation of an intramolecular hydrogen bond with the carbonyl oxygen. This led to reduced affinity for D2DR.

Ligand **3**, being shorter than ligand **1**, could not benefit from the interaction with Asn 186, but retained some interactions in the ECL area. The docking results showed aromatic interactions with Phe 189 and His 397 but simultaneously, the positioning of the hydroxyl group of the head part of the ligand inside the mainly hydrophobic pocket diminished the formation of aromatic interaction with the surrounding hydrophobic groups.

Ligand **4**, is a *meta*-isomer of ligand **2** and docks in a similar manner. Again the intramolecular hydrogen bond leads to a reduced number of interactions with D2DR, resulting in a receptor affinity in the same range as with ligand **2**.

The ligand affinity depended on the aromatic interactions within the OBS and SBP. These interactions were of the edge-to-face type and depended on the correct ESP distribution. The negative ESP in the aromatic part of the ligand tail segment, which forms the edge for ETF interactions with Trp 386 and Tyr 420 (OBS), was preferred, which led to high ligand affinity.[8]

The SBP amino acid residues Phe 189 and His 397 can establish ETF interactions, contributing to ligand affinity. The ESP distribution in the head segment of the ligands **1**–**4** show that aromatic interactions with Phe 189 and His 397 were possible *via* hydrogen atoms in the nicotinamide part of the molecule and the negative ESP on Phe 189 (the ligand is the edge and Phe 189 is face), and *via* the partially positively charged proton on His 397 and the negative ESP on the nicotinamide nitrogen in the ligand head part (Fig. 3).

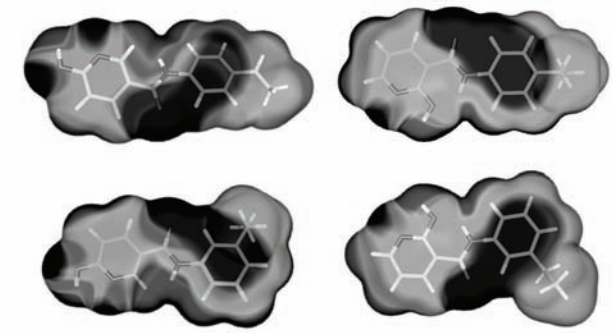


Fig. 3. ESP distribution in ligand **1** (top left), ligand **2** (top right), ligand **3** (bottom left) and ligand **4** (bottom right). Only the head and linker parts are shown for clarity. The negative ESP is shown in dark gray, while the positive ESP is shown in light gray.

CONCLUSION

In previous studies, a 3D model of D2DR was proposed; special focus was on molecular modeling of key receptor–ligand interactions in the ECL area.[2,5] In this publication, the role of Phe 189, His 397 and Asn 186 in ligand binding was stressed. Phe 189 and His 397 provided aromatic type interactions while Asn 186, which is located in the middle of ECL2, provided hydrogen bonding interaction with 6-hydroxy-*N*-{4-[2-(4-phenylpiperazin-1-yl)ethyl]phenyl}nicotinamide.

The obtained results showed that the ECL receptor area plays an important role in the binding of long linear-shaped ligands that cannot entirely fit into the canonical D2DR bind site. The ECL domain is a part of the receptor-binding site, and provides several key receptor–ligand interactions, leading to high affinity complexes.

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И З В О Д

ИСПИТИВАЊЕ КЉУЧНИХ ИНТЕРАКЦИЈА ИЗМЕЂУ ДРУГЕ ЕКСТРАЦЕЛУЛАРНЕ ПЕТЉЕ ДОПАМИНСКОГ D2 РЕЦЕПТОРА И РАЗЛИЧИТИХ ХИДРОКСИ-*N*-{[2-(4--ФЕНИЛПИПЕРАЗИН-1-ИЛ)ЕТИЛ]ФЕНИЛ}-НИКОТИНАМИДА

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Допаминергички систем се већ дуже време налази у фокусу истраживања усмерених ка развоју нових фармакотерапијских супстанци, намењених лечењу обољења централног нервног система (ЦНС), као што су: наркоманија, шизофренија, депресија и Паркинсонова болест. До данас, кристална стуктура допаминског D2 рецептора није позната, упркос његовој битној функцији и важности у циљаној терапији обољења ЦНС. У овом раду, представљамо напредак у одређивању кључних рецептор–лиганд интеракција, између арилпиперазину сличних лиганада и модела D2 рецептора добијеног на основу кристалне струкуре D3 рецептора. Употребом докинг анализе, упоредо са експерименталним резултатима, одређене су кључне интеракције које доприносе високој допаминергичкој активности. Укупно 4 лиганда арилпиперазинске структуре су тестирана, показала су умерени до високи афинитет везивања, а добијени резултати су објашњени путем доковања лиганада у предложени модел D2 рецептора.

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