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Original scientific paper

Synthesis of novel piperazino-alkyl-1H-benzo[d]imidazole derivates and assessment of their interactions with the D2 dopamine receptor

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Abstract: A total of 14 novel arylpiperazines were synthesized, and pharmacologically evaluated by measuring their affinities towards the D2 dopamine receptor (D2DR) in a [^{3}H]spiperone competition assay. All herein described compounds consist of a benzimidazole moiety connected to the *N*-(2-methoxyphenyl)piperazine via linkers of various lengths. Molecular docking analysis and molecular dynamics simulations were performed on D2DR-arylpiperazine complexes with an objective to explore the receptor-ligand interactions and properties of the receptor binding site. Crystal structure of D2DR that has been published recently was used throughout this study.

The major finding is that high affinity arylpiperazines must interact with both the orthosteric binding site and the extended binding pocket of D2DR and therefore should contain a linker of 5 or 6 methylene groups long.

Keywords: arylpiperazines; molecular dynamics; molecular docking; receptor bind site

INTRODUCTION

Dopamine receptors belong to rhodopsin-like, aminergic G protein-coupled receptors (GPCRs) group. They are involved in many physiological processes and play important role in the central nervous system (CNS).[1-4]

Targeting the dopamine D2 receptors (D2DR) is a common strategy for the treatment of neurodegenerative diseases, like schizophrenia, Parkinson's disease, dementia and depression.[5-8]

It is a well-documented fact that *N*-substituted arylpiperazines are compounds with pronounced D2DR activity.[9,10] Since arylpiperazines have wide

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spectrum of therapeutic potential and design, synthesis and characterization of new arylpiperazine like drugs is ever growing area of research.[11-14]

In this paper we present the synthesis of 14 new *N*-(2-methoxy-phenyl)piperazines of the general structure **5** (Scheme 1). Their affinities towards D2DR were evaluated in [^{3}H]spiperone competition assay.

Recent discovery of D2DR crystal structure with bound risperidone[15] defined receptor bind site with greater accuracy then existing homology models. This finding, prompted us to investigate D2DR - arylpiperazine binding features, using molecular docking analysis and molecular dynamics simulations in order to define key receptor-ligand interactions and explain the dopaminergic properties of the herein described compounds.

EXPERIMENTAL

Reagents and solvents used in this work were obtained from Alfa–Aesar or Sigma Aldrich and used without further purification. Solvents were routinely dried over anhydrous Na_2SO_4 prior to evaporation.

General

A Boetius PHMK apparatus (VEB Analytic, Dresden, Germany) was used to determine melting points, which are presented here uncorrected. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 200 and 50 MHz, respectively, on a Gemini 2000 (Varian, Oxford). Spectra were recorded in deuterochloroform with tetramethylsilane as the internal standard; the chemical shifts (δ) are reported in parts per million (ppm); all coupling constants (J values) are reported in hertz (Hz). LC/MS was performed on a 6210 Time-of-Flight LC-MS system (Agilent Technologies, Germany). For data analysis, MassHunter Workstation Software was used. The infrared (IR) spectra were obtained on a Thermo Scientific spectrometer. For analytical thin-layer chromatography (TLC), POLYGRAM SIL G/UV$_{254}$ plastic-backed thin layer silica gel plates were used (Macherey-Nagel, Germany). Chromatographic purifications were performed on Merck-60 silica gel columns (230–400 mesh ASTM) under medium pressure (dry column flash chromatography). A MicroSYNTH Milestone and a Biotage Initiator 2.5 EXP were used for the microwave experiments.

Chemistry

General procedure for the synthesis of compounds **3a-g**:

Suspension of 1-(2-methoxyphenyl)piperazine (**1**) (0.084 mol), triethylamine (0.0874 mol), K_2CO_3 (0.175 mol) and bromo-ester **2a-g** (0.084 mol) in 2-butanone (100 mL) was stirred for 24 h at 80°C. After cooling, the mixture was poured into cold water and the organic layer was extracted with CH_2Cl_2 and concentrated *in vacuo*. The resulting ester was purified by silica gel column chromatography using a gradient of methanol (0–5 %) in dichloromethane.

General procedure for the synthesis of compounds **5a-5n**:

Compound **3a-g** (0.0035 mol) and diamine **4a-c** (0.0035 mol) were suspended in 8 mL 50 % methanesulfonic acid in water, transferred into a sealed tube, and microwave irradiated at 180 °C for 45 min at 300 W. After cooling to room temperature, the reaction mixture was poured into ice-cold water and neutralized with a saturated solution of NaOH. The product was extracted with CH_2Cl_2 and concentrated *in vacuo*. The resulting 1*H*-benzimidazoles were

purified by silica gel column chromatography using a gradient of methanol (0–5 %) in dichloromethane.

Biological assays

Membrane preparation

Rat caudate nuclei synaptosomal membranes for D2DR binding experiments were prepared as previously described.[16] Striatal tissue acquired from male Wistar rats (150–200 g) was used as a source of D2DR. Tissue was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2 mM $CaCl_2$ using Potter–Elvehjem homogenizer (6 × 800 rpm). Membrane fraction obtained after centrifugation at 20000 rpm for 15 min was used in binding experiments.

[^{3}H]Spiperone receptor binding assay

[^{3}H]Spiperone (73.36 Ci /mmol, Perkin Elmer LAS GmbH,Rodgau, Germany) binding was assayed in 1.0 mM EDTA, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, 120 mM NaCl, 25 mM Tris-HCl solution, pH 7.4, with rat caudate nuclei synaptosomal membranes (protein concentration 0.6 mg/mL), at 37 °C for 10 min in a total volume of incubation mixture of 0.4 mL. Binding of the radioligand to 5-HT2 receptors was prevented by 50 mM ketanserin. The Ki values of the tested compounds were determined by competition binding at 0.2 nM of the radioligand and eight different concentrations of each compound (10^{-4} - 10^{-10} M). Nonspecific binding was determined in the presence of 10 µM spiperone. The reaction was terminated by rapid filtration through Whatman GF/C filters washed three times with 5.0 mL of ice-cold incubation buffer, and retained radioactivity was measured in a 1219 Rackbeta Wallac scintillation counter (EG&G Wallac, Turku, Finland). Inhibition curve construction and statistical (Student's T-test) analysis were performed by Graph-Pad Prism (GraphPad Software Inc). Hill slope coefficients were fixed to unity during calculation.

Computational study

Docking simulations

Docking procedure was carried out using Forecaster software.[17] Receptor model PDB code 6CM4[18] was used together with 2D structures of ligands, prepared in ChemDraw.[19] All structures were prepared in software, using default procedures. Rigid receptor, flexible ligand docking was carried out. Obtained docking structures were examined and structures with maximum number of receptor – ligand interactions were selected for further analysis.

Binding poses metadynamics

Docking pose quality was assessed in terms of the fluctuations of the ligand RMSD (the root-mean-square deviation of atomic positions), and the persistence of important contacts between the ligand and the receptor (Metadynamics Binding PoseScore and Metadynamics Binding Persistence) using Desmond software and default parameters.[20] One docking pose with the lowest RMSD and best overall score was selected for MD simulations.

Construction of protein-membrane system for molecular dynamics

Protein protonation state was adjusted using Schrodinger Protein Preparation module, at physiological pH (pH=7.4). Prepared protein was embedded into POPC membrane bilayer using Desmond system builder module,[20] and oriented according to data from Orientations of Proteins in Membranes (OPM) server.[21] The embedded protein was solvated with TIP3P explicit water model, and system was neutralized via counter ions and salt solution of 0.15 M

KCl. In this way, we obtained the systems that were subjected to membrane relaxation protocol.[20]

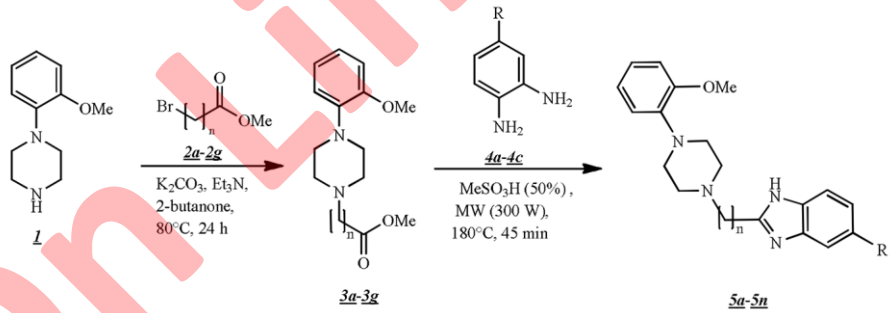
MD simulations

Molecular dynamics (MD) simulations of the system were performed using Schrodinger Desmond software packages.[20] OPLS 2003 forcefield[22] was used to calculate the interactions between all the atoms. For the calculation of the long-range Coulombic interactions, particle-mesh Ewald (PME) method was used, with the cut-off radius of 9 Å for the short-range Van der Waals (VdW) and electrostatic interactions.

During the course of the simulation, constant temperature of 310 K and a pressure of 1.01235 bars were maintained, using the Nose–Hoover thermostat,[23] and the Martyna-Tobias-Klein method.[24] In the simulations 2.0 fs time increments were used. Finally, 100 ns MD simulation for the each ligand-D2DR complex was performed and the collected trajectory frames used in the MD analysis to quantify the protein-ligand interactions.

RESULTS AND DISCUSSION

Compounds **5a**-**5n** were synthesized according to the Scheme 1. The synthesis started with *N*-(2-methoxyphenyl)piperazine (**1**) that was alkylated with a series of homologous bromo-esters **2a**-**2g**, providing *N*-alkylated products **3a**-**3g**. Counterpart diamines **4a**-**4c** were obtained by reduction of the corresponding 2-nitro precursors, using Raney-Ni and hydrazine hydrate under condition described in our earlier publications.[25,26] Microwave assisted condensation of piperazines **3a**-**3g** and diamines **4a**-**4c**, under forcing, strongly acidic conditions, secured the desired benzimidazoles **5a**-**5n**.



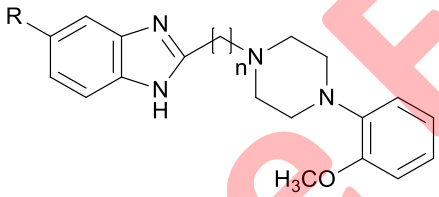
Scheme 1. Synthesis of the compounds 5a–5n
n= 1-7 for the compounds **2a**-**2g**, **3a**-**3g** and **5a**-**5g**; ethyl esters of the general structure **2** were also used in synthesis **3b**, **3c**, **3e** and **3f**; **4a** (R= H); **4b** (R= OMe); **4c** (R= Cl); structures **5a**-**5n** are presented in Table1.

D2DR binding affinities of compounds **5a**-**5n** were evaluated *in vitro* using [^{3}H]spiperone as a standard dopaminergic radioactive ligand (Table I).[27]

Molecular docking simulation of herein described {[4-(2-methoxy-phenyl)piperazin-1-yl]alkyl}-1*H*-benzo[d]imidazoles on D2DR was performed on the D2DR crystal structure published recently by Wang et al.[15] They reported

that the benzisoxazole moiety of risperidone interact with D2DR through Cys118$^{3.36}$, Thr119$^{3.37}$, Ser197$^{5.46}$, Phe198$^{5.47}$, Phe382$^{6.44}$, Phe390$^{6.52}$ and Trp386$^{6.48}$ in the orthosteric binding site (OBS). OBS of D2DR is defined by the amino acid side chains of helices III, V and VI and also harbor Asp114$^{3.32}$. Asp114$^{3.32}$ forms an essential salt-bridge with protonated piperidine nitrogen of risperidone molecule. In addition D2DR has a secondary binding pocket, extended binding pocket (EBP) that enclose risperidone's tetrahydropyrido-pyrimidinone moiety. EBP is bordered by the extracellular part of TM VII consisting of an extracellular loop 1 (EL1) and the junction of helices I, II and VII.[15]

TABLE I. Chemical structures and D2DR binding constants of {[4-(2-methoxyphenyl)piperazin-1-yl]alkyl}-1*H*-benzo[d]imidazoles (**5a**-**5n**)



Ligand	n	R	Ki ± S.E.M.[nM]
5a	1	H	>1000
5b	2	H	>1000
5c	3	H	>1000
5d	4	H	>1000
5e	5	H	24±1
5f	6	H	16±2
5g	7	H	>1000
5h	4	OCH_3	124±5
5i	5	OCH_3	12±3
5j	6	OCH_3	76±8
5k	7	OCH_3	>1000
5l	4	Cl	109±9
5m	5	Cl	25±3
5n	6	Cl	102±3

D2DR binding constants (Ki) was determined in a [^{3}H]spiperone displacement assay. The values are the mean of three independent experiments done in triplicate performed at eight competing ligand concentrations.

Molecular docking simulations on the binding of {[4-(2-methoxyphenyl)piperazin-1-yl]alkyl}-1*H*-benzo[d]imidazoles into the crystal structure of D2DR show that (2-methoxyphenyl)piperazin moiety occupies D2DR OBS, and interacts with Asp114$^{3.32}$, Cys118$^{3.36}$, Trp386$^{6.48}$ and Phe390$^{6.52}$, while the benzimidazole part interacts with Leu94$^{2.64}$, Ile 184^{EL2},Trp100^{EL1}, Phe389$^{6.51}$, Thr412$^{7.39}$ and Tyr408$^{7.35}$ in the EBP (Figure 1.).

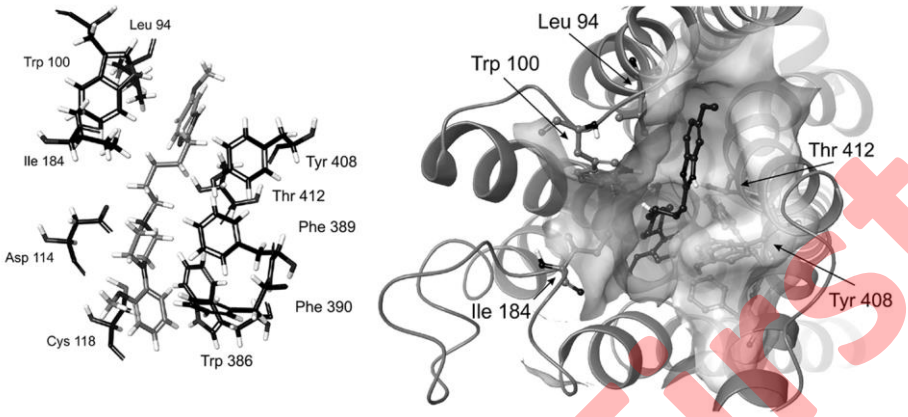


Figure 1. Docking of ligand 5i to D2DR is presented. View of the interaction between 3D model of the D2DR binding site and ligand **5i**.
Images are showing only key amino acid residues of the receptor binding pocket. Figures (side view-left and top-view right) shows docking of 5i viewed from different angles. Bind site ligand accessible surface is shown in the top view.

Compounds with optimal linker length (five or six methylene groups in linker) allow benzimidazole moiety to reach EBP and to interact with Leu94$^{2.64}$, Trp100^{EL1}, Phe389$^{6.51}$, Thr412$^{7.39}$ and Tyr408$^{7.35}$ (Figure 2). Compounds with shorter linker (**5a-5d**) do not reach into the EBP, while ligands with seven methylene groups in linker (**5g**, **5k**) are too long to fit optimally into the D2DR binding cleft and protrude into extracellular space.

Those results are in agreement with experimental data: compound **5d** (with 4 methylene groups linker) has affinity of over 1000 nM, while compounds **5e** and **5f** (with 5 and 6 methylene groups linker) have 24 nM and 16 nM, respectively. Compound **5g** shows sharp drop in affinity because the length of the linker, that cannot be accommodate in D2DR bind cleft.

In series of compounds substituted with methoxy and chloro groups, highest D2DR affinity was obtained with compounds **5i** and **5m.** Linker with 5 methylene groups facilitates optimal positioning of substituted benzimidazole part in the receptor EBP (Figure 1). Shorter linkers, as it is obvious in series **5h-5k** and **5l-5n**, lead to decrease in receptor affinity due to suboptimal placement of benzimidazole part in regard to interacting residues Trp100^{EL1} and Tyr408$^{7.35}$.

To test the stability of obtained docking poses, MD simulations of the D2DR and selected ligands were performed on inactive receptor state for 100ns for each ligand. Obtained trajectories were analyzed with a focus on residues that form OBS and EBP (Table 1. in Supplement).

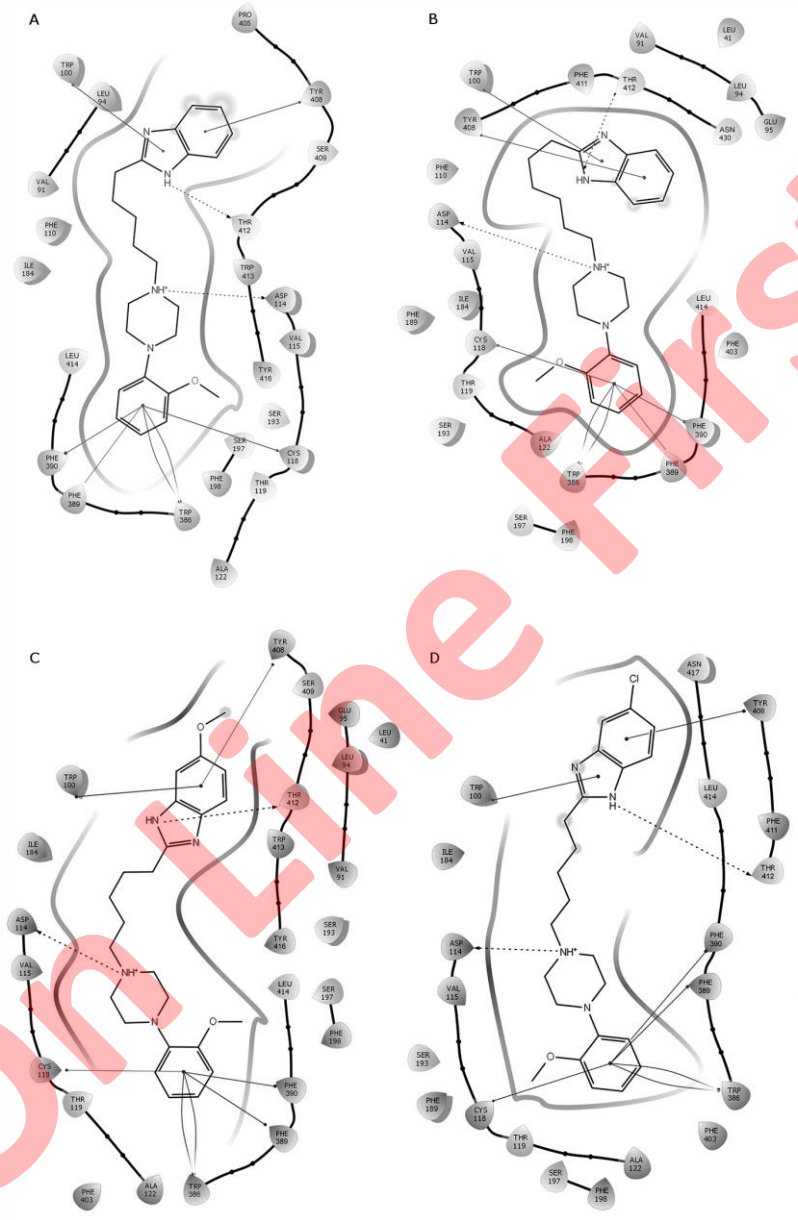


Figure 2. – Results of docking simulations for ligand **5e** (A) , **5f** (B), **5i** (C) and **5m** (D) are presented.
Schematic representation of the best docking pose for all ligands are provided. Only amino acid residues in close contact with ligands are shown for clarity. Solid lines represent aromatic, while dotted lines represent electrostatic interactions

Most of the receptor-ligand interactions in OBS, observed in molecular docking simulations persisted for a significant portion of MD run (>20% total simulation time). Compounds with significant D2DR affinity (**5e**-**5f**, **5h**-**5j** and **5l**-**5n**) had a salt bridge between the protonated piperazine nitrogen of the ligand and Asp114$^{3.32}$ of D2DR preserved for more than 79%-84% of the simulation time. Additional interactions in OBS are aromatic interaction with Cys118$^{3.36}$ (32-75% of simulation time), and edge-to-face interactions with Trp386$^{6.48}$ (76-98% of simulation time) and Phe390$^{6.52}$ (20-49% of simulation time). In the EBP, significant interactions are aromatic interactions (edge-to-face type) with Trp100^{EL1}, Phe389$^{6.51}$ and Tyr408$^{7.35}$. Compounds **5e**, **5f**, **5i** and **5m**, form additional hydrogen bond with Thr412$^{7.39}$.

CONCLUSION

Molecular docking and MD simulation provide important information that explains how the receptor-ligand complexes are formed. High affinity {[4-(2-Methoxyphenyl)piperazin-1-yl]alkyl}-1*H*-benzo[d]imidazoles must simultaneously occupy both OBS and EBP.

To establish key interactions both in OBS (salt bridge formation and aromatic interactions) and EBP (aromatic interactions and hydrogen bond formation), ligands should have a linker of five or six methylene groups. Linker flexibility and substituent size in the benzimidazole moiety determine ligand positioning inside the EBP and brings it in close contact with Trp100^{EL1} and Tyr408$^{7.35}$, that are key interacting residues. Also, as can be concluded from the results of molecular dynamics, affinity of the arylpiperazine ligands benefit greatly from possible formation of the interactions of arylpiperazine part of ligands with Thr412$^{7.39}$ in EBP.

It is clear that both Trp100^{EL1} and Tyr408$^{7.35}$ can form aromatic interactions and/or hydrogen bonds. To establish exact nature of interactions in EBP, modification of presented ligands, in terms of target synthesis of the compounds which can strictly form only one of these interactions, represent a guideline for further investigation.

SUPPLEMENTARY MATERIAL

Supplementary Material are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Conflict of Interest: Authors declare that there are no conflicts of interest

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И З В О Д

СИНТЕЗА НОВИХ ДЕРИВАТА ПИПЕРАЗИНО-АЛКИЛ-1Н-БЕНЗО[d]ИМИДАЗОЛА И ПРОУЧАВАЊЕ ИНТЕРАКЦИЈА СА Д2 ДОПАМИНСКИМ РЕЦЕПТОРОМ

ЈЕЛЕНА З. ПЕЊИШЕВИЋ[1], ДЕАНА Б. АНДРИЋ[2], ВЛАДИМИР Б. ШУКАЛОВИЋ[1], ГОРАН М. РОГЛИЋ[2], ВУКИЋ ШОШКИЋ[3], СЛАЂАНА В. КОСТИЋ-РАЈАЧИЋ[1]

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У овом раду је презентована синтеза 14 нових арилпиперазина и одређен је њихов афинитет везивања за Д2 допаминski рецептор (Д2ДР) тестовима компетиције са [^{3}H]спипероном. По својој хемијској структури ова једињења представљају супституисане бензимидазоле повезане са *N*-(2-метоксифенил)пиперазинским делом, линкерима различитих дужина. У циљу испитивања лиганд-рецептор интеракција и особина везивног места Д2ДР, урађена је докинг анализа новосинтетисаних једињења и симулација молекулске динамике, користећи кристалну структуру рецептора.

Резултати добијени у овом раду указују да арилпиперазини високог афинитета остварују интеракције у ортостерном везивном месту и у екстензији ортостерног места везивања Д2ДР и да стога треба да поседују линкер оптималне дужине, од 5 или 6 метиленских група.

(Примљено 29. октобра; ревидирано 3. децембра; прихваћено 4. децембра 2018)

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SUPPLEMENTARY MATERIAL TO

Synthesis of novel piperazino-alkyl-1H-benzo[d]imidazole derivates and assessment of their interactions with the D2 dopamine receptor

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ANALYTICAL AND SPECTRAL DATA FOR THE SYNTHESIZED COMPOUNDS

*Methyl 2-[4-(2-methoxyphenyl)piperazin-1-yl]acetate (**3a**):*

Yield: 95.3 %; orange crystals m.p. 50 °C; IR (ATR, cm^{-1}): 2820.2, 1724.3, 1500.9, 1451.7, 1241.4, 1027.1, 751.3; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.75-2.80 (m, 4H, piperazine), 3.12-3.17 (m, 4H, piperazine), 3.29 (s, 2H, CH_2), 3.74 (s, 3H, -$COCH_3$), 3.85 (s, 3H, OCH_3), 6.84-7.01 (m, 4H, ArH); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 50.22, 51.56, 53.20, 55.16, 59.43, 111.01, 118.11, 120.86, 122.86, 141.03, 152.10, 170.64; MS: m/z $[M+H]^+$ calculated for $C_{14}H_{20}N_2O_3$ 265.15467, found 265.15493.

*Ethyl 3-[4-(2-methoxyphenyl)piperazin-1-yl]propanoate (**3b**):*

Yield: 84.2 %; oil; IR (ATR, cm^{-1}): 2818.6, 1734.0, 1501.0, 1452.7, 1241.3, 1026.5, 749.6; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.28 (t, 3H, J=7.4 Hz, CH_3), 2.51-2.59 (m, 2H, CH_2), 2.66-2.71 (m, 4H, piperazine), 2.75-2.83 (m, 2H, CH_2), 3.08-3.10 (m, 4H, piprazine), 3.86 (s, 3H, OCH_3), 4.16 (q, J=7.4 Hz, J=11.4 Hz, 2H, $COCH_2$), 6.84-7.03 (m, 4H, ArH); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 14.04, 32.13, 50.39, 52.94, 53.45, 55.13, 60.17, 110.99, 118.00, 120.80, 122.73, 141.12, 152.08, 172.35; MS: m/z $[M+H]^+$ calculated for $C_{16}H_{24}N_2O_3$, 293.18597, found 293.18584.

*Ethyl 4-[4-(2-methoxyphenyl)piperazin-1-yl]butanoate (**3c**):*

Yield: 86.6 %; oil; IR (ATR, cm^{-1}): 2817.0, 1732.8, 1501.1, 1452.5, 1241.3, 1028.1, 750.6; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.26 (t, 3H, J= 7.4 Hz, CH_3), 1.78-1.93 (m, 2H, CH_2), 2.33-2.47 (m, 4H, CH_2), 2.63-2.66 (m, 4H, piperazine), 3.09

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(s, 4H, piperazine), 3.86 (s, 3H, OCH_3), 4.13 (q, 2H, J= 6.6 Hz, J= 7.4 Hz, $COCH_2$), 6.83-7.04 (m, 4H, ArH); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 14.11, 22.03, 32.19, 50.52, 53.23, 55.18, 57.64, 60.12, 111.01, 118.03, 120.84, 122.73, 141.25, 152.16, 173.49; MS: m/z $[M+H]^+$ calculated for $C_{17}H_{26}N_2O_3$ 307.20162, found 307.20152.

*Methyl 5-[4-(2-methoxyphenyl)piperazin-1-yl]pentanoate (**3d**):*

Yield: 69.0 %; oil; IR (ATR, cm^{-1}): 2818.4, 1737.6, 1500.9, 1450.9, 1241.1, 1027.2, 751.1; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.67-1.71 (m, 4H, CH_2), 2.35-2.38 (m, 2H, CH_2), 2.51-2.54(m, 2H, CH_2), 2.76(s, 4H, piperazine), 3.17 (s, 4H, piperazine), 3.67 (S, 3H, CH_3), 3.86 (m, 3H, OCH_3), 6.85-7.02 (m, 4H, ArH); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 22.41, 25.80, 33.35, 50.10, 50.90, 52.93, 54.78, 57.70, 110.64, 117.63, 120.47, 122.33, 140.85, 151.75, 173.40; MS: m/z $[M+H]^+$ calculated for $C_{17}H_{26}N_2O_3$ 307.20162, found 307.20075.

*Ethyl 6-[4-(2-methoxyphenyl)piperazin-1-yl]hexanoate (**3e**):*

Yield: 91.0%; oil; IR (ATR, cm^{-1}): 2814.2, 1734.5, 1501.2, 1452.5, 1240.9, 1029.8, 748.0; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.22 (t, 3H, J=7.4 Hz, CH_3), 1.29-1.39 (m, 2H, CH_2), 1.45-1.71 (m, 4H, CH_2), 2.24-2.41 (m, 4H, CH_2), 2.61 (s, 4H, piperazine), 3.07 (s, 4H, piperazine), 3.82 (s, 3H, OCH_3), 4.09 (q, 2H, J=6 Hz, J= 8 Hz, $COCH_2$), 6.80-7.00 (m, 4H, ArH); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 14.07, 24.72, 26.42, 26.96, 34.08, 50.47, 53.33 55.13, 58.42, 60.01, 110.95, 118.00, 120.80, 122.70, 141.23, 152.10, 173.59; MS: m/z $[M+H]^+$ calculated for $C_{19}H_{30}N_2O_3$ 335.23292, found 335.23342.

*Ethyl 7-[4-(2-methoxyphenyl)piperazin-1-yl]heptanoate (**3f**):*

Yield: 92.2%; oil; IR (ATR, cm^{-1}): 2818.4, 1734.5, 1501.4, 1450.3, 1240.1, 1028.6, 750.3; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.20 (t, 3H, J=7.4 Hz, CH_3), 1.28-1.34 (m, 4H, CH_2), 1.46-1.63 (m, 4H, CH_2), 2.21-2.35 (m, 4H, CH_2), 2.60 (s, 4H, piperazine), 3.06 (s, 4H, piperazine), 3.80 (s, 3H, OCH_3), 4.07 (q, 2H, J=7.2 Hz, J=7.4 Hz, $COCH_2$), 6.78-6.99 (m, 4H, ArH); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 14.02, 24.65, 26.52, 27.03, 28.82, 34.03, 50.43, 53.29, 55.07, 58.53, 59.90, 110.90, 117.94, 120.75, 122.62, 141.19, 152.05, 173.55; MS: m/z $[M+H]^+$calculated for $C_{20}H_{32}N_2O_3$ 349.24857, found 349.24825.

*Methyl 8-(4-(2-methoxyphenyl)piperazin-1-yl)octanoate **(3g):***

Yield: 82.8 %; oil; IR (ATR, cm^{-1}): 2828.2, 1754.6, 1521.4, 1448.8, 1236.3, 1025.6, 755.4; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.26-1.32 (m, 6H, CH_2), 1.53-1.66 (m, 4H, CH_2), 2.27-2.43 (m, 4H, CH_2), 2.65-2.67 (m, 4H,piperazine), 3.11 (s, 4H, piperazine), 3.67 (s, OCH_3), 3.86 (s, 3H, OCH_3), 6.84-7.04 (m, 4H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 24.79, 26.74, 27.34, 29.09, 33.97, 50.56 , 51.33, 53.43, 55.25, 58.76, 111.06, 118.12, 120.91, 122.79, 141.33, 152.22, 174.24; MS: m/z $[M+H]^+$ calculated for $C_{20}H_{32}N_2O_3$ 349.24857, found 349.24849.

*2-{[4-(2-methoxyphenyl)piperazin-1-yl]methyl}-1H-benzo[d]imidazole (**5a**):*

Yield: 16 %; oil; IR (ATR, cm^{-1}): 2817.2, 1502.0, 1455.7, 1240.3, 1026.5, 743.0; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.75-2.80 (m, 4H, piperazine), 3.08-3.13 (m, 4H, piperazine), 3.84 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2), 6.84-7.06 (m, 4H, ArH), 7.20-7.27 (m, 2H, ArH), 7.57-7.60 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.41 , 53.64, 55.31, 56.46, 111.23, 118.14, 120.93, 122.02, 122.40, 123.11, 140.90, 151.90, 152.23; MS: m/z $[M+H]^+$ calculated for $C_{19}H_{22}N_4O$ 323.18664, found 323.18515.

*2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-1H-benzo[d]imidazole (**5b**):*

Yield: 36.5 %; oil; IR (ATR, cm^{-1}): 2818.4, 1500.6, 1456.7, 1240.0, 1026.3, 745.2; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.77-2.92 (m, 6H, 4H piperazine and CH_2), 3.11-3.18 (m, 6H, 4H piperazine and CH_2) 3.87 (s, 3H, OCH_3), 6.87-7.04 (m, 4H, ArH), 7.17-7.25 (m, 2H, ArH), 7.52-7.57 (m, 2H,ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 25.03, 50.76, 52.94, 55.33, 58.06, 111.23, 114.67, 118.13, 120.95, 122.02, 123.24, 140.78, 152.21, 154.52; MS: m/z $[M+H]^+$ calculated for $C_{20}H_{24}N_4O$ 337.20229, found 337.20205.

*2-{3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl}-1H-benzo[d]imidazole (**5c**):*

Yield: 55.7 %; oil; IR (ATR, cm^{-1}): 2825.5, 1500.7, 1452.9, 1241.4, 1026.5, 746.5; ^{1}H NMR (200, MHz, $CDCl_3$) δ: 1.96-2.08 (m, 2H, CH_2), 2.64 (t, 2H, J=5.6 Hz, CH_2), 2.72 (s, 4H, piperazine), 3.06-3.16 (m, 6H, 4H piperazine and CH_2), 3.85 (s, 3H, OCH_3), 6.86-7.05 (m, 4H, ArH), 7.16-7.18 (m, 2H, ArH), 7.52-7.57 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 23.61, 28.90, 50.49, 53.25, 55.24, 58.72, 111.18, 114.46, 118.03, 120.98, 121.66, 123.18, 140.77, 152.18, 155.58; MS: m/z $[M+H]^+$ calculated for $C_{21}H_{26}N_4O$ 351.21794, found 351.21682.

*2-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-1H-benzo[d]imidazole (**5d**):*

Yield: 90.6 %; oil; IR (ATR, cm^{-1}): 2818.3, 1499.7, 1456.0, 1244.1, 1028.9, 793.3; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.55-1.70 (m, 2H,CH_2), 1.83-1.94 (m, 2H,CH_2), 2.43 (t, 2H, J=6.8 Hz, CH_2), 2.64 (s, 4H, piperazine), 2.98 (t, 2H, J=6.8 Hz, CH_2), 3.12(s, 4H,piperazine), 3.84 (s, 3H, OCH_3), 6.84-7.05 (m, 4H, ArH), 7.14-7.21 (m, 2H,ArH), 7.51-7.56 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ**:** 25.38, 25.81, 28.45, 50.34, 53.18, 55.24, 57.24, 111.19, 114.52, 118.16, 120.98, 121.88, 123.08, 138.57, 140.94, 152.19, 155.38; MS: m/z $[M+H]^+$ calculated for $C_{22}H_{28}N_4O$ 365.23359, found 365.23263.

*2-{5-[4-(2-methoxyphenyl)piperazin-1-yl]pentyl}-1H-benzo[d]imidazole (**5e**):*

Yield: 43.0 %; brown crystals m.p. 63°C; IR (ATR, cm^{-1}): 2825.2, 1500.3, 1454.9, 1240.7, 1023.6, 752.8; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.33-1.48 (m, 2H,CH_2), 1.60-1.75 (m, 2H, CH_2), 1.80-1.93 (m, 2H, CH_2), 2.54-2.62 (m, 2H, CH_2), 2.84-2.95 (m, 6H, CH_2 and 4H piperazine), 3.17-3.19 (m, 4H, piperazine), 3.86 (s, 3H, OCH_3), 6.85-7.06 (m, 4H, ArH), 7.19-7.22 (m, 2H, ArH), 7.54-7.58 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 25.54, 26.62, 27.82, 28.84, 49.94,

53.00, 55.20, 57.97, 111.15, 114.50, 118.18, 120.95, 121.88, 123.10, 138.52, 140.78, 152.12, 155.31; MS: m/z $[M+H]^+$ calculated for $C_{23}H_{30}N_4O$ 379.24924, found 379.24889.

*2-{6-[4-(2-methoxyphenyl)piperazin-1-yl]hexyl}-1H-benzo[d]imidazole (**5f**):*

Yield: 69.0 %; brown crystals m.p. 134 °C; IR (ATR, cm^{-1}): 2828.5, 1503.0; 1454.6, 1240.6, 1019.5, 750; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.26-1.58 (m, 6H, CH_2), 1.76-1.92 (m, 2H, CH_2), 2.37 (t, 2H, J=7.4 Hz, CH_2), 2.61-2.65 (m, 4H,piperazine), 2.90 (t, 2H, J=8 Hz, CH_2), 3.11 (s, 4H, piperazine), 3.85 (s, 3H, OCH_3), 6.84-7.04 (m, 4H, ArH), 7.18-7.27 (m, 2H, ArH), 7.51-7.58 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 26.12, 26.94, 28.00, 28.96, 29.13, 50.29, 53.20, 55.22, 58.37, 111.13, 114.54, 118.18, 121.97, 121.89, 123.02, 138.55, 141.01, 152.17, 155.38; MS: m/z $[M+H]^+$ calculated for $C_{24}H_{32}N_4O$ 393.26489, found 393.26320.

*2-{7-[4-(2-methoxyphenyl)piperazin-1-yl]heptyl}-1H-benzo[d]imidazole (**5g**):*

Yield: 72.0 %; oil; IR (ATR, cm^{-1}): 2962,9, 1497,9, 1449,6, 1261,7, 1026,4, 744,5; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.26-1.49 (m, 8H, CH_2), 1.79-1.86 (m, 2H, CH_2), 2.38 (t, 2H, J=7.4 Hz, CH_2), 2.68 (s, 4H, piperazine), 2.89 (t, 2H, J=8 Hz, CH_2), 3.13 (s, 4H, piperazine), 3.85 (s, 3H, OCH_3), 6.84-6.94 (m, 4H, ArH), 7.19-7.24 (m, 2H, ArH), 7.52-7.56 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 26.45, 27.21, 28.11, 29.05, 29.29, 50.40, 53.35 , 55.28, 58.65, 111.13, 114.64, 118.22, 120.98, 121.99, 123.01, 138.47, 141.11, 152.22, 155.26; MS: m/z $[M+H]^+$ calculated for $C_{25}H_{34}N_4O$ 407.28054, found 407.28007.

*5-methoxy-2-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-1H-benzo[d]imidazole (**5h**):*

Yield: 88.0%; oil; IR (ATR, cm^{-1}): 2817.0, 1498.4, 1458.2, 1237.6, 1027, 751.2; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.64-1.76 (m, 2H, CH_2), 1.84-1.98 (m, 2H, CH_2), 2.49 (t, 2H , J=7.4 Hz, CH_2), 2.70 (s, 4H, piperazine), 2.96 (t, 2H, J=7.2 Hz, CH_2), 3.16 (s, 4H, piperazine), 3.83 (s, 3H, OCH_3,), 3.87 (s, 3H, OCH_3), 6.81-7.08 (m, 7H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 25.09, 25.87, 28.40, 50.54, 53.31, 55.33, 55.82, 57.09, 111.17, 118.18, 121.02, 123.17, 141.03, 152.26, 155.98; MS: m/z $[M+H]^+$ calculated for $C_{23}H_{30}N_4O_2$ 395.24415, found 395.24331.

*5-methoxy-2-{5-[4-(2-methoxyphenyl)piperazin-1-yl]pentyl}-1H-benzo[d]imidazole (**5i**):*

Yield: 73.0%; oil; IR (ATR, cm^{-1}): 2832.2, 1500.2, 1455.8, 1242.6, 1027.7, 752.9; ^{1}H NMR (200 MHz, $CDCl_3$) δ**:** 1.38-1.45 (m, 2H, CH_2), 1.71-1.90 (m, 4H, CH_2), 2.77-2.94 (m, 4H, CH_2), 3.09 (s ,4H, piperazine), 3.28 (s, 4H,piperazine), 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.82-6.92 (m, 4H, ArH), 7.02-7.08 (m, 2H, ArH), 7.46 (d, 2H, J=8Hz, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 22.59, 22.97, 25.21, 27.00, 48.25, 52.18, 55.38, 55.80, 56.82, 97.42, 111.26, 111.63, 115.19, 118.51, 121.09, 123.92, 138.19, 139.72, 152.10, 154.72, 156.18; MS: m/z $[M+H]^+$ calculated for $C_{24}H_{32}N_4O_2$ 409.25980, found 409.26000.

*5-methoxy-2-{6-[4-(2-methoxyphenyl)piperazin-1-yl]hexyl}-1H-benzo[d]imidazole (**5j**):*

Yield: 67.1%; oil; IR (ATR, cm^{-1}): 2856.0, 1501.3, 1457.2, 1247.5, 1026.2, 753.3; ^{1}H NMR (200 MHz, $CDCl_3$) δ**:** 1.35-1.55 (m, 4H,CH_2), 1.63-1.88 (m, 2H,CH_2), 2.41 (t, 2H, J=8 Hz, CH_2), 2.67-2.72 (m ,4H, piperazine), 2.85 (t, 2H, J=7.4 Hz, CH_2), 3.10-3.15 (m, 4H, piperazine), 3.79 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.21-4.24 (m, 2H, CH_2), 6.81-7.03 (m, 4H, ArH), 7.38-7.43 (m, 1H, ArH), 7.50-7.55 (m, 1H, ArH), 7.67-7.74 (m, 1H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 22.85, 23.59, 25.83, 26.78, 27.82, 50.05, 53.11, 55.24, 55.71, 58.22, 97.59, 111.12, 115.16, 118.18, 120.95, 123.10, 130.85, 140.87, 152.14, 154.98, 155.91; MS: m/z $[M+H]^+$ calculated for $C_{25}H_{34}N_4O_2$ 423.27545, found 423.27570.

*5-methoxy-2-(7-(4-(2-methoxyphenyl)piperazin-1-yl)heptyl)-1H-benzo[d]imidazole (**5k**):*

Yield: 62.3%; oil; IR (ATR, cm-1): 2927.7, 1501.3, 1499.0, 1453.5, 1240.3, 1026.6, 748.2; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.58 (s, 6H, CH_2), 2.16-2.23 (m, 2H, CH_2), 2.53 (s, 2H, CH_2), 2.85 (s, 6H, 4H piperazine and CH_2), 3.15 (s, 6H, 4H piperazine and CH_2), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.84-7.01 (m 7H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 25.57, 27.13, 29.13, 49.53, 52.60, 55.31, 55.77, 57.9 , 97.67, 111.11, 118.29, 120.99, 123.20, 140.74, 152.14, 154.93, 155.96; MS: m/z $[M+H]^+$ calculated for $C_{26}H_{36}N_4O_2$ 437.29110, found 437.29115.

*5-chloro-2-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-1H-benzo[d]imidazole (**5l**):*

Yield: 88,4%; oil; IR (ATR, cm^{-1}): 2811.0, 1501.1, 1447.5, 1239.6, 1027.4, 751.2; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.59-1.69 (m, 2H, CH_2), 1.82-1.96 (m, 2H,CH_2), 2.39 (t, 2H, J=7.2 Hz, CH_2), 2.65 (s, 4H, piperazine), 2.96 (t, 2H, J=6.6 Hz, CH_2), 3.12 (s, 4H, piperazine), 3.84 (s, 3H, OCH_3), 6.84-7.02 (m, 4H, ArH), 7.12-7.17 (m, 1H, ArH), 7.39-7.50 (m, 2H, ArH); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 25.18, 25.83, 28.45, 50.38, 53.18, 55.24, 57.11, 111.12, 114.48, 115.19, 118.16, 120.97, 122.39, 123.17, 127.43, 140.79, 152.16, 156.67; MS: m/z $[M+H]^+$ calculated for $C_{22}H_{27}ClN_4O$ 399.19462, found 399.19367.

*5-chloro-2-{5-[4-(2-methoxyphenyl)piperazin-1-yl]pentyl}-1H-benzo[d]imidazole (**5m**):*

Yield: 65%; oil; IR (ATR, cm^{-1}): 2824.8, 1500.7, 1450.7, 1241.2, 1027.0, 749.9; ^{1}H NMR (200 MHz, CDCl3) δ: 1.35-1.62 (m, 4H, CH_2), 1.77-1.91 (m, 2H, CH_2), 2.39 (t, 2H, J=8 Hz, CH_2), 2.64 (s, 4H, piperazine), 2.89 (t, 2H, J=8 Hz, CH_2), 3.11 (s, 4H, piperazine), 3.85 (s, 3H, OCH3), 6.84-7.06 (m, 4H, ArH), 7.13-7.18 (m, 1H, ArH), 7.40-7.50 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ**:** 25.96, 26.63, 27.67, 28.98, 50.38, 53.33, 55.27, 58.22, 111.15, 118.20, 121.00, 122.50, 123.13, 127.58, 140.96, 152.19, 156.32; MS: m/z $[M+H]^+$ calculated for $C_{23}H_{29}ClN_4O$ 413.21027, found 413.20891.

*5-chloro-2-{6-[4-(2-methoxyphenyl)piperazin-1-yl]hexyl}-1H-benzo[d]imidazole (**5n**):*

Yield: 74%; oil; IR (ATR, cm^{-1}): 2825.8, 1500.6, 1451.2, 1241.8, 1027.6, 750.4; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.30-1.46 (m, 6H, CH_2), 1.72-1.83 (m, 2H, CH_2), 2.34 (t, 2H, J=8.6 Hz, CH_2), 2.63 (s, 4H, piperazine), 2.86 (t, 2H, J=7.4 Hz, CH_2), 3.09 (s, 4H, piperazine), 3.84 (s, 3H, OCH_3), 6.84-7.06 (m, 4H, ArH), 7.12-7.18 (m, 1H, ArH), 7.38-7.48 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 26.27, 26.96, 27.91, 28.89, 29.09, 50.45, 53.27, 55.22, 58.42, 111.10, 118.16, 120.97, 122.46, 123.08, 127.52, 140.98, 152.14, 156.51; MS: m/z $[M+H]^+$ calculated for $C_{24}H_{31}ClN_4O$ 427.22592, found 427.22408.

SUMMARY RESULTS OF MD SIMULATIONS

Table S1. D2DR-ligand key interactions observed in 100 ns MD simulatios.

Residue / Ligand	Leu 94	Trp 100	Asp 114	Cys 118	Ile 184	Phe 382	Trp 386	Phe 389	Phe 390	Tyr 408	Thr 412	Tyr 416
5e		54	81	68	27		76	65	42	65	37	
5f	21	31	79	36	40		82	74	20	33	42	
5h		67	81	58			84	85	24	36		
5i	50	89	80	75			96	84	42	22	30	
5j	36	94	82	73	24	32	98	53	31	25		
5l		22	82	64	26		95	75	34	37		
5m		75	84	69			78	50	49	63	40	
5n	32	42	80	32			82	68	35	28		32

D2DR-ligand interactions presented in more than 20% of MS simulation time are shown. Numbers provided in the table refers to the percentage of the total simulation time one interaction observed to occur. Interacting residues in OBS are shaded in grey colour; residues found in EBP are white.