Synthesis, biological evaluation and docking analysis of substituted piperidines and (2-methoxyphenyl)piperazines

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Abstract: A series of sixteen novel substituted piperidines and (2-methoxy-phenyl)piperazines were synthesized, starting from the key intermediates 1-(2--methoxyphenyl)-4-(piperidin-4-yl)piperazine and 1-(2-methoxyphenyl)-4--[(piperidin-4-yl)methyl]piperazine. Biological evaluation of the synthesized compounds was illustrated by seven compounds, of which 1-(2-methoxy-phenyl)-4-{[1-(2-nitrobenzyl)piperidin-4-yl]methyl}piperazine had the highest affinity for the dopamine D_2 receptor. For all seven selected compounds, docking analysis was performed in order to establish their structure-to-activity relationship.

Keywords: dopamine D_2 receptor; docking analysis; allosteric; orthosteric binding site.

INTRODUCTION

G-protein-coupled receptors (GPCRs) are transmembrane receptors that mediate most of their intracellular actions through pathways involving an activation of the G-protein.[1] D_2 Dopamine receptors (D_2DAR) are members of this large protein family. Dysfunction of the dopaminergic system in CNS can lead to a number of diseases, such as Parkinson's disease, schizophrenia, some neuro-humoral disturbances, *etc*.[2,3] Therefore it is not surprising that the design and synthesis of new potential dopaminergic drugs is one of the main objectives of organic and medicinal chemistry.

Arylpiperazines are a common structural motif included in various compounds that interact in a specific manner with various GPCRs.[4] Within the scope

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of the program aimed at the discovery of new dopaminergic (DA-ergic) ligands and in order to explore further previously published data, a series of sixteen novel arylpiperazines were synthesized.[5] For all the synthesized ligands, their *in vitro* binding affinities at rat D_2DAR were estimated and compared with the results obtained through docking analysis, using an available D_2DAR molecular model.

EXPERIMENTAL

General

Melting points were measured on a Boetius PHMK apparatus (VEB Analytic, Dresden, Germany) and are uncorrected. The ^{1}H- and ^{13}C-NMR (200 and 50 MHz) spectra of the compounds in deuterochloroform, unless otherwise stated, were recorded on a Gemini 2000 instrument (Varian, Oxford). The chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane. The LC-MS results were acquired on a 6210 time-of-flight LC–MS system (Agilent Technologies, Germany); MassHunter workstation software was used for data analysis. The IR spectra were taken on a Thermo Scientific spectrometer. A MicroSYNTH Milestone and a Biotage Initiator 2.5 EXP were used for the microwave irradiations. Analytical TLC was performed on Polygram SIL G/UV254 plastic-backed thin-layer silica gel plates (Macherey-Nagel, Germany). The chromatographic purifications were realized on Merck-60 silica gel columns (diameter 70 mm, h = 45 mm; the same for all compounds), 230–400 mesh ASTM, medium pressure (dry column flash chromatography). The reagents and solvents (Alfa–Aesar or Sigma–Aldrich) were used without purification. The solutions were routinely dried over anhydrous Na_2SO_4 prior to evaporation.

Chemistry

Ethyl 4-[4-(2-methoxyphenyl)piperazin-1-yl]piperidine-1-carboxylate (3). To a stirred solution of *N*-carbethoxy-4-piperidone (**1**, 1.7 g, 0.01 mol) in methanol (25 mL; the pH value of the solution was adjusted to 7 by addition of CH_3CO_2H), 1-(2-methoxyphenyl)piperazine (**2**, 3.24 g, 0.02 mol) was added, followed by the addition of $NaBH_3CN$ (0.4 g, 0.0072 mol) in portions (Scheme 1). Stirring was continued at room temperature for 24 h. The pH value of the resulting solution was adjusted to 2 by the addition of 10 % HCl solution and the excess of the methanol was removed under vacuum. The pH value of the residue was adjusted to 9 by the addition of 10 % NaOH solution and extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The product was purified by dry-flash chromatography using a gradient of CH_3OH (0–10 %) in dichloromethane as the solvent. Yield: 88 %.

General procedure for the hydrolysis of the carbamates 3 and 9. Carbamate **3** or **9** (0.02 mol) was suspended in cc HCl (60 mL), transferred into a sealed tube and placed into a microwave oven. Irradiation at 130 °C was completed after 90 min at an initial power of 300 W (Schemes 2 and 3). The reaction mixture was poured into water, the pH value adjusted to 9 by addition of 10 % NaOH solution and extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The product was purified by dry-flash chromatography using a gradient of methanol (0–10 %) in dichloromethane as the solvent.

1-(Ethoxycarbonyl)piperidine-4-carboxylic acid (6). To a solution of piperidine-4-carboxylic acid (**5**, 20 g, 0.155 mol) in water (200 mL), Na_2CO_3 (20 g) was added, mixture stirred at room temperature for 30 min and a solution of ethyl chloroformate (25.5 g, 0.28 mol) in toluene (240 mL) was added dropwise (Scheme 2). Stirring was continued at room tempe-

rature for 20 h. After separation of the layers, the aqueous layer was acidified with conc. HCl to pH ≈2, and extracted with dichloromethane. The organic layer was dried over anhydrous Na$_2$SO$_4$ and evaporated *in vacuo*. Yield: 78 %.
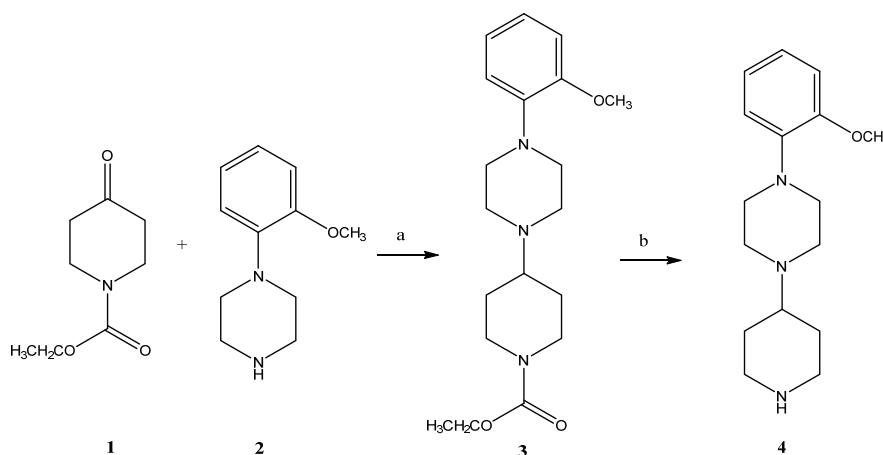
Scheme 1. Synthesis of 1-(2-methoxyphenyl)-4-(piperidin-4-yl)piperazine (**4**); reagents:
a) NaBH$_3$CN, MeOH, pH 7, r.t; b) conc. HCl, MW, 180 °C, 300 W.
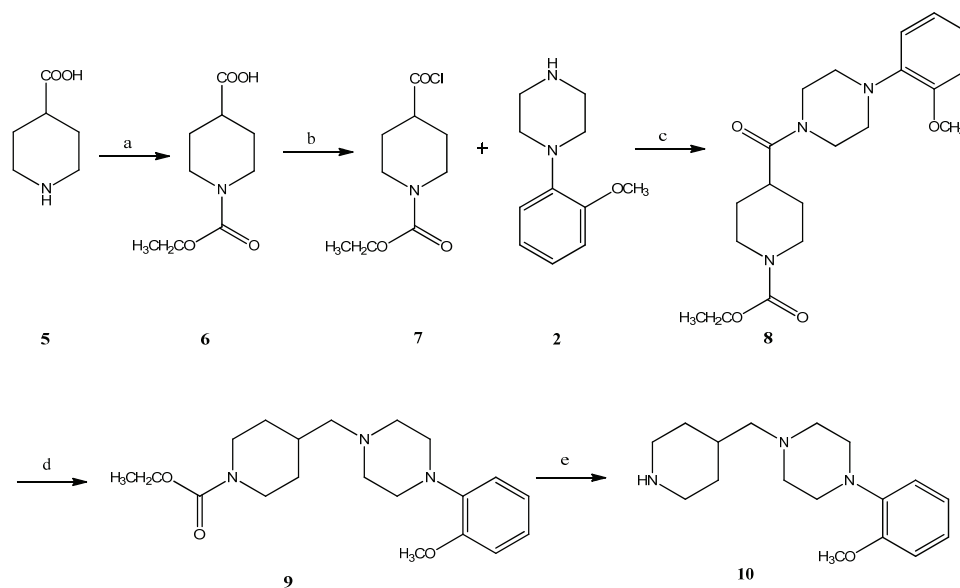
Scheme 2. Synthesis of 1-(2-methoxyphenyl)-4-[(piperidin-4-yl)methyl]piperazine (**10**);
reagents: a) Na$_2$CO$_3$, ethyl chloroformate, toluene, r.t; b) thionyl chloride, CH$_2$Cl$_2$, 0 °C;
c) triethylamine, chloroform, 5 °C; d) NaBH$_4$, boron trifluoride diethyl etherate,
diglyme, −5 °C; e) conc. HCl, MW, 180 °C, 300 W.

Ethyl 4-(chlorocarbonyl)piperidine-1-carboxylate (7). A solution of 1-(ethoxycarbonyl) piperidine-4-carboxylic acid (**6**, 7.5 g, 0.0375 mol), thionyl chloride (5.35 g, 0.045 mol) and chloroform (200 mL) was stirred for 2 h at 0 °C (Scheme 2). The reaction mixture was evaporated *in vacuo* and the product was used immediately, without further purification. Yield: 67 %.

Ethyl 4-{[4-(2-methoxyphenyl)piperazin-1-yl]carbonyl}piperidine-1-carboxylate (8). A solution of chloride **7** in chloroform (50 mL) was added dropwise to a solution of triethyl-amine (3.78 g, 0.0375 mol), 1-(2-methoxyphenyl)piperazine (**2**, 6.07 g, 0.0375 mol) in chloro-form (150 mL) at 5 °C (Scheme 2). The reaction mixture was stirred at room temperature for 20 h, the resulting mixture extracted with 10 % Na_2CO_3 and the organic layer was extracted with 10 % HCl solution. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in *vacuo*. The product was purified by dry-flash chromatography eluting with dichloro-methane containing increasing amounts of methanol (0–10 %). Yield: 85 %.

Ethyl 4-{[4-(2-methoxyphenyl)piperazin-1-yl]methyl}piperidine-1-carboxylate (9). Mix-ture of compound **8** (0.01 mol) and $NaBH_4$ (1 g, 0.025 mol) in diglyme (1-methoxy-2-(2--methoxyethoxy)ethane, 25 mL) was stirred for 40 min at –5 °C under argon, during which time, boron trifluoride diethyl etherate (3.9 g, 3.4 mL, 0.025 mmol) was added dropwise (Scheme 2). After stirring for 1 h at 5 °C, the reaction mixture was heated to 80–90 °C, followed by stirring for additional I h. The mixture was cooled to room temperature, carefully poured into 10 mL of water and then 20 mL cc HCl was added. Mixture was heated at 60–80 °C on aqueous bath for 3 h and concentrated under *vacuo*. To the residue, 10 % NaOH solution was added until pH 9 and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The product was purified by dry-flash chromatography using the dichloromethane/methanol system as eluent. Yield: 85 %.

General procedure for the alkylation of compounds 4 and 10. A mixture of compound **4** or **10** (0.0018 mol), benzyl halides **11**–**14** (0.0018 mol), K_2CO_3 (0.0036 mol) and acetonitrile (25 mL) was stirred at room temperature for 48 h, poured into water and extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The product (**15**–**22**, Scheme 3) was purified by dry-flash chromatography using a gradient of methanol (0–10 %) in dichloromethane as the solvent.

General procedure for the synthesis of compounds 27–30 and 31–34. A solution of benzoyl or nitrobenzoyl chloride **23**–**26** (0.0017 mol) and CH_2Cl_2 (0.34 mL) was added dropwise to a solution of **4** or **10** (0.0017 mol), CH_2Cl_2 (1.7 mL) and Et_3N (0.24 mL, 0.0017 mol) at 0 °C. The reaction mixture was stirred at room temperature for 72 h. The resulting mixture was extracted with 10 % Na_2CO_3 solution, the separated organic layer was washed with 10 % K_2CO_3 solution, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The product (**27**–**34**, Scheme 3) was purified by dry-flash chromatography eluting with CH_2Cl_2 containing increasing amounts of MeOH (0–10 %).

Analytical and spectral data for the synthesized compounds are given in the Supple-mentary material to this paper.

Membrane preparation, radio-ligand binding assays and data analysis

Synaptosomal membranes from rat striatum were prepared for radio-ligand binding assays as previously described.[6] [^{3}H]Spiperone (specific. activity: 9.25 MBq $mmol^{-1}$) used to label D_2DAR were purchased from Perkin Elmer LAS GmbH, Rodgau, Germany.
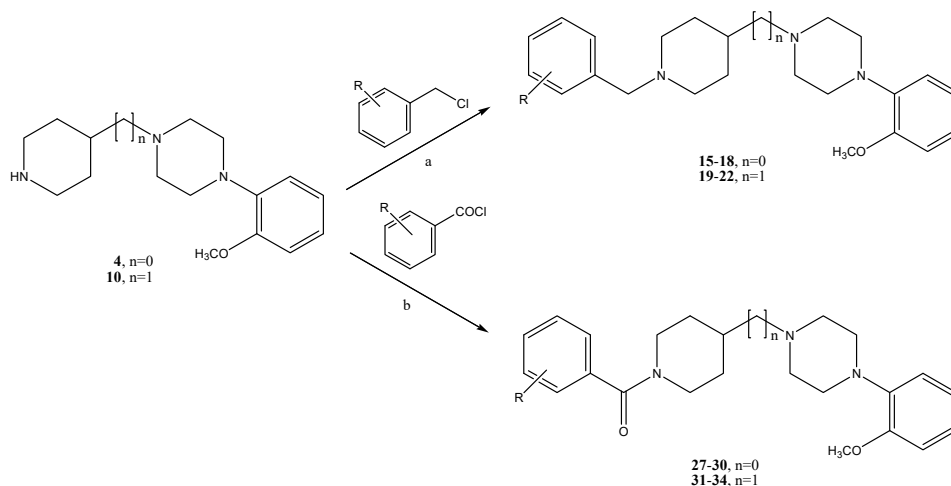
Scheme 3. Synthetic route and chemical structures of the (2-methoxyphenyl)piperazine dopaminergic ligands; reagents: a) compounds **11–14** (R = H, 2-NO_2, 3-NO_2, 4-NO_2, respectively), K_2CO_3, CH_3CN, r.t; b) compounds **23–26** (R = H, 2-NO_2, 3-NO_2, 4-NO_2, respectively), triethylamine, CH_2Cl_2, 0 °C; yields for **15–22** and **27–34**: 68–89 %.

*[^{3}H]Spiperone–receptor binding assa*y. [^{3}H]Spiperone binding was assayed in 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, 120 mM NaCl, 25 mM Tris–HCl solution, pH 7.4, at a membrane protein concentration of 0.7 mg mL^{-1} at 37 °C for 10 min in a total volume of 0.4 mL of the incubation mixture. Binding of the radioligand to the 5-HT_2 receptors was prevented by 50 μM ketanserin. The K_i values of the tested compounds were determined by competition binding at 0.2 nM of the radio-ligand and eight to ten different concentrations of each compound (10^{-4} to 10^{-10} M). Nonspecific binding was measured in the presence of 1.0 mM spiperone. The reaction was terminated by rapid filtration through Whatman GF/C filters, which were further washed three times with 5.0 mL of ice-cold incubation buffer. Each point was determined in triplicate. The 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 (EG & G Wallac, Turku, Finland) at an efficiency of 51–55 % for tritium. The results were analyzed by nonlinear curve fitting of the inhibition curves of the compounds utilizing the Graph-Pad Prism program.[7] Hill slope coefficients were fixed to unity during the calculation.

Docking analysis

Docking analysis was performed with an already available D_2DAR model based on the D_3DAR crystal structure.[8] The binding site of the receptor was determined by combining results from experimental data[9,10] and the Schrödinger Maestro receptor grid generation module.[11] Amino acid residue charges were adjusted where needed, assuming physiological conditions.

Selected ligands were prepared with the ligprep Maestro module and docked using the Glide module from the Schrödinger Suite 2011.[11] All ligands were docked as protonated, using the OPLS_2005 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 D_2DAR. After initial ligand placement, no further constraints were applied and the

docking procedure was performed. The obtained structures were examined and those meeting the following criteria were selected: best docking score of the complex, shortest salt bridge formed between Asp 114 of the D_2DAR and ligand, chair conformation of arylpiperazine ring and aryl part of the molecule positioned in the rear hydrophobic pocket of the receptor (Phe 386, Trp 390 and Tyr 420).[12] After an initial criterion was satisfied, the second step was the examination of different interactions that could be formed between the receptor and ligand (hydrogen bonds, aromatic–aromatic interactions, *etc*.). In that way, the best possible docking structures were selected. Structures were visualized using DS Visualize v2.5.1[13] and the obtained images were rendered using PovRay Raytracer v3.6.[14]

RESULTS AND DISCUSSION

The general synthetic route and chemical structures of the novel substituted piperidine and (2-methoxyphenyl)piperazine are summarized in Schemes 1–3.

Preparation of the key intermediates, 1-(2-methoxyphenyl)-4-(piperidin-4--yl)piperazine (**4**) and 1-(2-methoxyphenyl)-4-[(piperidin-4-yl)methyl]piperazine (**10**) are described in Schemes 1 and 2. Ethyl 4-[4-(2-methoxyphenyl)piperazin--1-yl]piperidine-1-carboxylate (**3**), produced by the reductive amination of the commercially available ketone **1** was further hydrolyzed, under microwave conditions, and intermediate **4** was obtained (Scheme 1).

Commercially available piperidine-4-carboxylic acid (**5**) was transformed into carbamate **6** and further into chloride **7** by reaction with thionyl chloride. Acylation of (2-methoxyphenyl)piperazine with chloride **7** gave amide **8**, which provided compound **9** by reduction with $NaBH_4$/boron trifluoride ethyl etherate. The carbamate **9** was converted to the secondary amine **10** by hydrolysis with cc HCl under MW conditions (Scheme 2).

Both **4** and **10** intermediates were alkylated with benzyl or nitrobenzyl halogenide to give the final ligands **15**–**18** and **19**–**22**, respectively. Ligands **27**–**30** and **31**–**34** were obtained by acylation with the corresponding acyl chloride (Scheme 3).

The final products **15**–**22** and **27**–**34** were evaluated for their affinity to D_2DAR by the *in vitro* competitive displacement assay of [3H]spiperone (Table I). As a source of D_2DAR, synaptosomal membranes prepared from rat striatum were used.
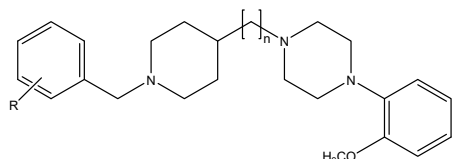
The compound with the highest affinity for D_2DAR was 1-(2-methoxy-phenyl)-4-{[1-(2-nitrobenzyl)piperidin-4-yl]methyl}piperazine (**20**, $K_i = 30.6$ nM). Compounds **19**, **21**, **22** and **31**–**34** expressed moderate binding affinity for D_2DAR, while **15**–**18** and **27**–**30** were completely inactive competitors of bound [3H]spiperone.

Compounds **20**–**22** and **31**–**34** were selected for docking analysis in order to establish their structure-to-activity relationship.
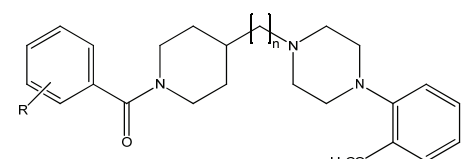
D_2DAR model and selected compounds were prepared as described and docking analysis was performed. The obtained results showed that compound **20** binds to D_2DAR *via* a salt bridge with Asp 114 on TM3. This is followed by

multiple aromatic interactions between the aryl part of the ligand and the hydro-phobic pocket (Phe 386, Trp 390 and Tyr 420).[8,15,16] In this way, the ligand establishes a favorable orientation inside the receptor binding cavity that is a pre-requisite for the formation of hydrogen bonds with Ser 193 on TM5. The stated interactions are formed with D_2DAR inside the orthosteric bind site (OBS). Docking analysis showed possible aromatic interactions with Phe 393 and His 397, both located inside the alosteric bind site (ABS, Fig. 1) and the listed inter-actions lead to high compound activity.

TABLE I. Binding constants of the synthesized compounds for the dopamine D_2 receptor

Compound	n	R	($K_i \pm SEM$) / nM
15	0	H	736±24
16	0	2-NO_2	521.5±13
17	0	3-NO_2	937.5±35
18	0	4-NO_2	1512±30
19	1	H	341.5±12
20	1	2-NO_2	30.6±1.2
21	1	3-NO_2	258 ±11
22	1	4-NO_2	200±12

Compound	n	R	($K_i \pm SEM$) / nM
27	0	H	1583.5±32
28	0	2-NO_2	1205±19
29	0	3-NO_2	755±21
30	0	4-NO_2	905±28
31	1	H	189.5±12.1
32	1	2-NO_2	219.5±14.4
33	1	3-NO_2	300±16.2
34	1	4-NO_2	334.5±17.8

In the case of compounds **21** and **22**, in contrast to compound **20**, the optimal hydrogen bond with the serine residues on TM5 cannot be formed. The reduced binding affinity was a clear consequence of the unfavorable orientation of ligands **21** and **22** inside the receptor binding cavity. Compounds **31**–**34** dock, in the

same manner, with aryl part oriented inside the hydrophobic pocket (Phe 386, Trp 390 and Tyr 420), a salt bridge with Asp 114 and hydrogen bonds with Ser 193 (Fig. 2). The only difference, compared to compound **20**, is the positioning of the head part of the ligand. The reduced flexibility of the head part leads to sub-optimal positioning of the aromatic part inside the ABS and the only obs-erved aromatic interaction is with Phe 394. This leads to reduced binding affinity, with respect to ligand **20**.
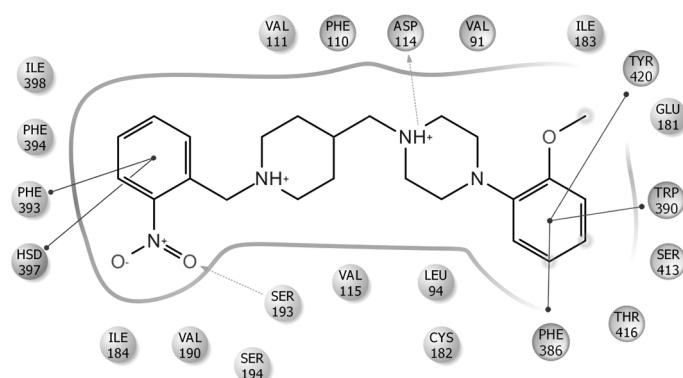

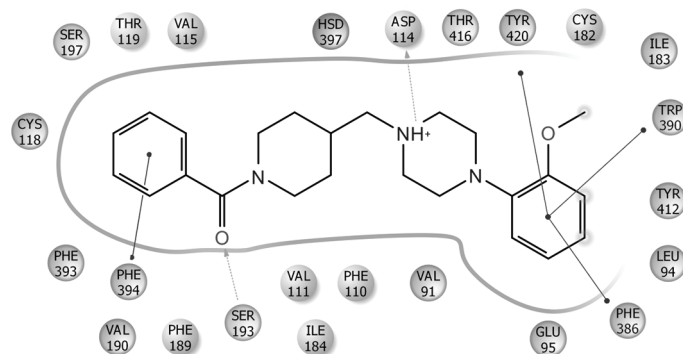Fig. 1. Docking of compound **20** into the D_2DAR binding pocket.

Fig. 2. Docking of compound **31** into the D_2DAR binding pocket.

The other compounds cannot form the interactions discussed above, mostly due to their rigidity. Therefore, they either cannot achieve the correct orientation inside the ABS, or cannot form any hydrogen bond with serine residues on TM5.

CONCLUSIONS

In order to achieve high binding affinity, a D_2DAR ligand has to fulfill several requirements. The formation of a salt bridge with Asp 114 is the crucial interaction that starts the binding process, which is followed by orientation of

arylpiperazine ligand part into the OBS hydrophobic cavity. After these initial requirements are met, the ligand has to establish one or more hydrogen bonds with serine residues on TM5. Failing this, the ligand could still bind to D_2DAR, but with reduced affinity. In order to establish hydrogen bonds, the ligand has to be of considerable length to span the entire OBS between Asp 114 and Ser 193 and/or 197. Since the OBS is not linear, the ligand has to adopt a slightly curved conformation in order to bind successfully. The conformation of the arylpiperazine part is fixed at the chair conformation of the arylpiperazine ring and the rest of the ligand has to be flexible enough to fit into the OBS space. In the case of compounds **15**–**34**, only compounds **21**, **22** and **31**–**34** can adopt the described conformation that leads to high affinity receptor binding. Once the conformational requirements are fulfilled, the affinity is determined by the number and strength of particular receptor–ligand interactions. Ligand **20** has the best overall fit into D_2DAR that, together with the formed interactions, leads to the highest affinity in the group of ligands.

Obtained results suggest that in future studies special attention should be paid to the synthesis of the ligands with a prolonged, flexible bridge that will provide more degrees of rotational freedom of the molecules, which allows a proper orientation of the ligands in the OBS cavity, which is an essential prerequisite for high affinity D_2DAR ligands.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

СИНТЕЗА, БИОЛОШКО ИСПИТИВАЊЕ И ДОКИНГ АНАЛИЗА СУПСТИТУИСАНИХ ПИПЕРИДИНСКИХ И (2-МЕТОКСИФЕНИЛ)ПИПЕРАЗИНСКИХ ЛИГАНАДА

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Синтетисана је серија од шеснаест нових супституисаних пиперидина и (2-метокси-фенил)пиперазина, полазећи од кључних интермедијера 1-(2-метоксифенил)-4-(пипе-ридин-4-ил)пиперазина и 1-(2-метоксифенил)-4-(пиперидин-4-илметил)пиперазина. Биолошко испитивање синтетисаних једињења је истакло седам једињења, од којих 1-(2--метоксифенил)-4-{[1-(2-нитробензил)пиперидин-4-ил]метил}пиперазин има највиши афинитет ка D_2 допаминском рецептору. За свих седам једињења је урађена докинг ана-лиза у циљу утврђивања односа структуре и активности.

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