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Interactions of *N*-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-2-aryl-2-yl-acetamides and 1-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-3-aryl-2-yl-ureas with dopamine D_2 and 5-hydroxytryptamine $5HT_{1A}$ receptors

Vladimir Sukalovic [a], Djurdjica Ignjatovic [b], Gordana Tovilovic [b], Deana Andric [c], Kaveh Shakib [d], Sladjana Kostic-Rajacic [a], Vukic Soskic [e,*]

[a] *ICTM—Department of Chemistry, University of Belgrade, Njegoseva 12, 11000 Belgrade, Serbia*
[b] *Department of Biochemistry, Institute for Biological Research, University of Belgrade, Serbia*
[c] *Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, 11000 Belgrade, Serbia*
[d] *Barnet & Chase Farm Hospital, The Ridgeway, Enfield, Middlesex EN2 8JL, UK*
[e] *Proteosys AG, Carl-Zeiss-Str. 51, 55129 Mainz, Germany*

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ABSTRACT

It is suggested that the ratio of dopamine D_2 to 5-hydroxytryptamine 5-HT_{1A} activity is an important parameter that determines the efficiency of antipsychotic drugs. Here we present the synthesis of *N*-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-2-aryl-2-yl-acetamides and 1-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-3-aryl-2-yl-ureas and their structure–activity relationship studies on dopamine D_2 and 5-hydrohytryptamine 5-HT_{1A} receptors. It was shown that ligand selectivity and affinity strongly depends on their topology and the presence of a pyridyl group in the head of molecules. Molecular modeling studies using homology modeling and docking simulation revealed a rational explanation for the ligand behavior. The observed binding modes and receptor–ligand interactions provided us with a clue for optimizing the optimal selectivity towards 5-HT_{1A} receptors.

Dopamine (DA) and 5-hydrohytryptamine (5-HT) are two of the major neurotransmitters in the central nervous system (CNS) and play crucial roles in behavior and cognition. Receptors for DA and 5-HT are members of the superfamily of G-protein coupled receptors (GPCRs) all shearing a rodopsinelike core structure. Based on their pharmacological profiles and their effects on different signal transduction cascades, these receptors are divided into different subclasses. The focus of this study is the D_2 DA and $5HT_{1A}$ receptors and their ligands, that are suggested to be a potential target in the treatment of neurological disorders such as schizophrenia, depression, anxiety and drug abuse.[1,2] It has been considered that D_2 DA receptors are the main mechanism responsible for the efficacy of antipsychotics. That assumption was challenged by the discovery of atypical antipsychotics with their activities at multiple receptors. This notion was supported by clinical evidence that multi-target drugs are more effective than single target agents in the treatment of CNS conditions.[3,4] Among others, $5HT_{1A}$ receptors have been proposed as one of the targets for atypical antipsychotic drugs. Some works suggest that the $D_2/5HT_{1A}$ ratio is an important parameter that determines the efficiency of antipsychotic drugs.[5] Despite a rather high homology among D_2 DA and $5HT_{1A}$ receptors there is a high diversity in ligands specificity towards these two receptor subtypes. An understanding of molecular mechanisms of ligand receptor interaction would contribute significantly to the drug discovery process. Reliable molecular modeling of interactions of ligands with D_2 DA as well as $5HT_{1A}$ receptor is facilitated by the recent publishing of 3D structures of various eukaryotic GPCRs.[6–8] Starting from that point we published recently our results on molecular modeling of D_2 DA[9] and the $5HT_{1A}$ receptor.[10] In the model proposed by us D_2 DA receptor can easily adopt radar long linear ligands and form a stable complex through interactions with two binding pockets, one located in the helical part and the second in the extracellular loop (ecl2) of the receptor. Similarly, for high affinity binding of large ligands two binding pockets are required for the $5HT_{1A}$ receptor. Both of these binding pockets are located in the helical part of the $5HT_{1A}$ receptor and require a curved shaped ligand to access each of them. To confirm the quality of those two receptor models we designed two sets of ligands of different molecular shape (Fig. 1) and performed the biological and molecular docking test on the D_2 DA and $5HT_{1A}$ receptors. Newly designed ligands consist of: a tail part (phenylpiperazine), a linker

* Corresponding author. Tel.: +49 61315019247.
E-mail address: vukic.soskic@proteosys.com (V. Soskic).

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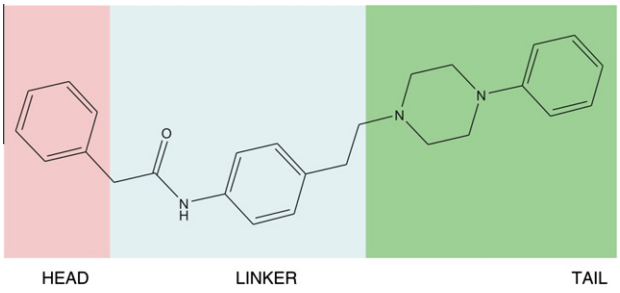
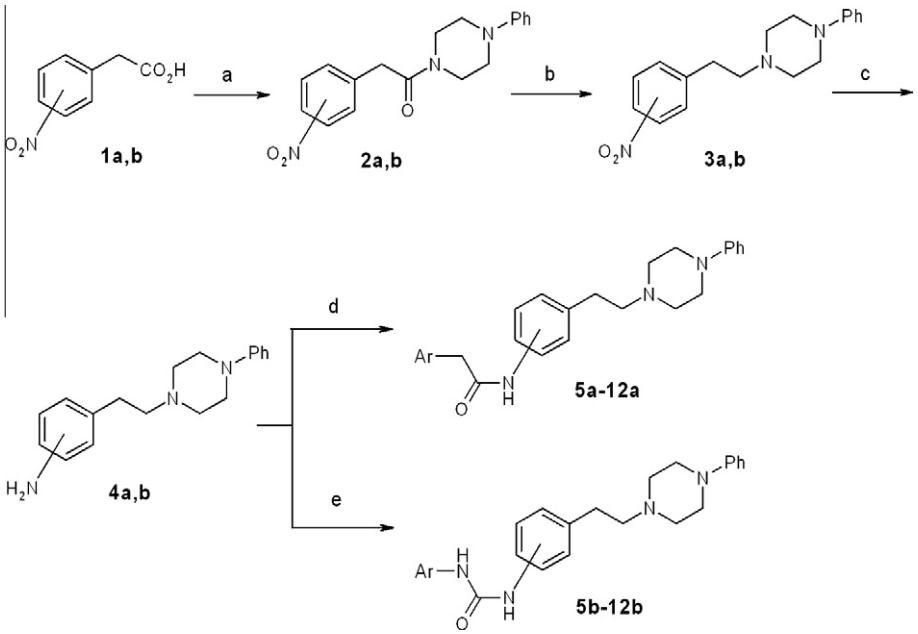


Figure 1. Chemical structure of investigated *N*-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-2-aryl-2-yl-acetamides and 1-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-3-aryl-2-yl-ureas. Molecules are divided into three substructures: head part (red), linker part (blue) and tail part (green) for clarity of discussion.

part (phenylcarbamate or phenylacetamid) and a head part (phenyl or pyridyl group), Figure 1. Those structural motifs are chosen because: mechanism of binding of phenylpiperazine to both types of receptors is well studied,[9,10] the linker part has optimal length and rigidity properties and phenyl or pyridyl head can take part in aryl–aryl but also polar type of interaction in the accessory receptor binding pocket.

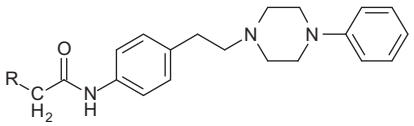
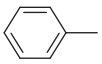
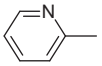
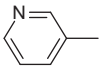
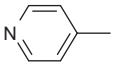
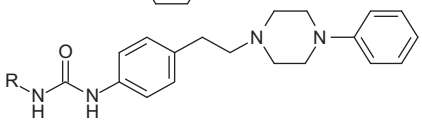
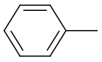
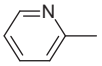
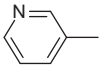
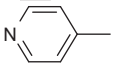
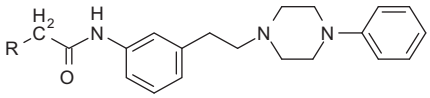
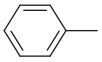
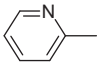
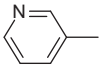
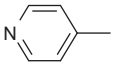
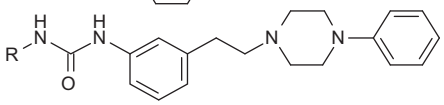
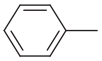
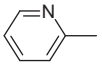
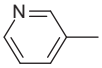
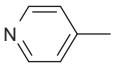
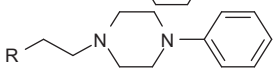
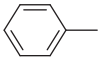
Synthetic route and chemical structures of the compounds synthesized in the present study are shown in Scheme 1. Acylation of *N*-phenylpiperazine using 4-nitrophenylacetic acid gave rise to 2-(nitrophenyl)-1-(4-phenyl-piperazin-1-yl)-ethanones (**2a**,**b**). Amides **2a**,**b** were converted to 1-nitrophenethyl-4-phenyl-piperazines **3a**,**b** using diborane in tetrahydrofuran (THF). Reduction of nitro compounds **3a**,**b** by Ra-Ni/hydrazine provided 1-aminophenethyl-4-phenyl-piperazines **4a**,**b**. Target arylacetamides **5a**–**12a** were obtained by condensation of anilines **4a** and **4b** with corresponding arylacetic acid in presence of propylphosphonic acid anhydride (PPAA) in *N*,*N*-dimethylformamide (DMF). Arylureas **5b–12b** were obtained by reacting anilines **4a**,**b** with bis(trichloromethyl)-carbonate in pyridine, following by addition of arylamine.[11] All compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy and mass spectroscopy.

The affinity of the new compounds for cloned human D_2 DA and $5HT_{1A}$ receptors was evaluated in in vitro binding assays using [^{3}H]spiperone for labeling D_2 DA receptors and [^{3}H]8-OH-DPAT for labeling $5HT_{1A}$ receptors, according to our previously described procedures.[12] In vitro receptor binding data are summarized in Table 1. In this series of compounds, studies were carried out to examine the effect of the phenyl ring substitution on the binding affinity for the D_2 DA and $5HT_{1A}$ receptors. This molecular scaffolding was identified on the basis of the unsubstituted compound 1-phenethyl-4-phenyl-piperazine (**13**) having K_i values of 478 and 43.2 nM for the D_2 DA and 5-HT_{1A} receptor, respectively.[13] The compounds with arylureas and arylacetamides substituents at the *para*-position of the phenyl ring (compounds **5a**–**8a** and **5b**–**8b**) were active for both D_2 DA and 5-HT_{1A} with K_i values within the range of 3.6–4189 nM for the D_2 DA receptor and 95.3–2264 nM for $5HT_{1A}$ receptors. *Para*-substituted, linear arylureas, such as the 4-pyridyl (compound **8b**), showed the best D_2 DA activity in this series, with K_i values of 3.6 and 226.8 nM for D_2 DA and 5-HT_{1A}, respectively. Meanwhile, the phenyl derivatives, compounds **5a** and **5b**, exhibited reduced affinities for both DA and 5HT receptors (482.5–4189 nM). Arylureas derivatives **5b**–**8b** have generally higher affinity (3.60–482.5 nM) towards D_2 DA receptors than arylacetamides **5a**–**8a** (48.6–4189 nM). There is an opposite but less clear trend in $5HT_{1A}$ affinities as far as *para*-derivatives are concerned. When the *para*-substitution was changed to *meta*-substitution, the affinities were generally reduced for D_2 DA receptors and increased for 5-HT_{1A}; $5HT_{1A}$ K_i values of arylacetamides **9a**–**12a** and arylureas **9b**–**12b** drop in the low nM range (0.50–7.53 nM), while D_2 DA receptor affinity deceased several fold (29.7–3575 nM). Phenylarylureas and phenylarylacetamides (compounds **5a**–**5b** and **9a**–**9b**) have diminished affinities for both D_2 DA receptors. In $5HT_{1A}$ receptor assay *para*-derivatives (**5a** and **5b**) are less active, while *meta*-substituted compounds (**9a** and **9b**) are expressing 74 and sevenfold increase in affinity when compared to 1-phenethyl-4-phenyl-piperazine. Pyridylarylureas and pyridylarylacetamides (compound **6a**–**8a, 6b**–**8b, 10a**–**12a** and **10b**–**12b**) all have higher D_2 DA and $5HT_{1A}$ affinity than parent



Scheme 1. Synthetic route and chemical structures of the *N*-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-2-aryl-2-yl-acetamides and 1-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-3-aryl-2-yl-ureas. Reagents and conditions: (a) Phenylpiperazine, *N*,*N*′-dicyclohexylcarbodiimide, 4-dimethylaminopyridine, THF, rt, o/n; (b) B_2H_6, THF, 0 °C for 6 h, rt for 1 h then reflux for 2 h; (c) Ra-Ni, $N_2H_4{\cdot}H_2O$, EtOH, 1,2-dichloroethane, 30 °C, 45 °C, 1 h; (d) $ArCH_2CO_2H$, PPAA, DMF; (e) bis(trichloromethyl)-carbonate, dioxane, pyridine, 0 °C for 2 min. rt for 30 min, following by addition of arylamine and triethylamine, 80 °C, o/n.

Table 1
Chemical structure and binding affinity of the examined arylpiperazine ligands for the D_2 DA and 5-HT_{1A} receptors

No.	R	K_i D_2 (nM)	K_i 5HT_{1A}(nM)
			
5a		4189	902.6
6a		70.7	95.3
7a		69.6	159.8
8a		48.6	122.5
			
5b		482.5	2264
6b		14.2	125.8
7b		8.2	397.9
8b		3.6	226.8
			
9a		3575	0.58
10a		277.8	4.73
11a		82.4	1.39
12a		201.6	2.26
			
9b		3250	5.78
10b		364	7.53
11b		29.7	0.95
12b		48.7	0.50
			
13		478.0	43.2

Structure of *N*-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-2-aryl-2-yl-acetamides and 1-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-3-aryl-2-yl-ureas tested for the binding to the D_2 DA and 5-HT_{1A} serotonin receptors is shown. K_i values are the mean of three independent experiments done in triplicate performed at eight to 8 competing ligand concentrations.

phenethyl-4-phenyl-piperazine. With regard to the selectivity between D_2 DA and 5-$HT_{1A,}$ the compound arylacetamides (**5a**–**8a** and **5b**–**8b**) showed better affinity for D_2 DA over 5-HT_{1A} receptors. Compound **8b** had the highest selectivity value (63) in this series (226.8 vs 3.60 nM). However, opposite results were observed with arylureas **9a**–**12a** and **9b**–**12b**, the most selective was compound **9a** having 6163 times higher 5HT_{1A} receptor affinity (3575 vs 0.58 nM).

To find the structural basis for further optimization of selectivity, we set out to use molecular modeling methods. For the sake of clarity we divided the ligand structure into three distinctive parts: the tail part formed by aryl-piperazine structure, the linker part connecting the aryl-piperazine structure and the head part that is shown by the arylurea and arylacetamide structural motive (Fig. 1). In this series of compounds the tail part was kept constant while the linker and head parts were altered to investigate the structure to activity relationship.

Receptor–ligand interactions were investigated by structure-based molecular modeling methods. Because crystal structures for our target proteins are not published so far, homology modeling is used for building the three-dimensional protein structures. The human D_3 DA receptor was used as a template crystal structure (PDB code: 3PBL) for modeling the D_2 DA receptor as described in our previous publication.[9] The Serotonin 5HT_{1A} model was adopted from our previous publication.[10]

Ligand 3D structures were generated using the Discovery Studio program.[14] Assuming physiological conditions, the basic aliphatic nitrogen atom of the 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.[15]

Docking of the selected ligands as presented in Table 1 was done by simulated annealing using the LIBDOCK module from Discovery Studio. All ligands were docked as protonated, using the CHARMM force field. The initial position of the ligand in the binding site, was arbitrary, while protonated nitrogen on the ligand part was kept in close proximity to Asp 114 of the D_2 receptor and Asp 116 of the 5HT_{1A} receptor. After initial ligand placement, no further ligand constraints were applied and the docking procedure based on Monte–Carlo methodology was carried out. 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 or 4000 optimization steps. The obtained docked structures were examined, and 10 of those with the lowest total energy were further filtered to obtain docking structures with the best ligand fit. We selected structure based on the following criteria: lowest total energy of the complex, shortest salt bridge formed between Asp 114 of the D_2 DA receptor (Asp 116 of the 5HT_{1A} receptor) and protonated piperazine nitrogen, chair conformation of arylpiperazine ring, and aryl part of the molecule positioned in the rear hydrophobic pocket of the receptors; Phe 386, Trp 390 and Tyr 420 for D_2 DA, and Phe 112 and Tyr 390 for 5HT_{1A} receptors.[16,10] After those initial criteria were satisfied, a second step was performed to examine other interactions that can be formed between receptor and ligand (hydrogen bonds, aromatic–aromatic interactions, etc.). In that way, the best possible docking structures were selected. Structures were visualised using DS Visualise v2.5.1[14] and the obtained images were rendered using PovRay Raytracer v3.6.[17]

Docking analysis results on D_2 DA receptor (Fig. 2) show common interactions, shared by all investigated ligands. Those interactions are: salt bridge formed between Asp 114 and protonated piperazine nitrogen, hydrophobic interactions between Phe 386, Trp 390, Tyr 420 and aryl-piperazine, Ser 194 or Ser 167 hydrogen bond bridges with ligand carbamide as for amide function, and aromatic type interactions of Phe 189 and His 397

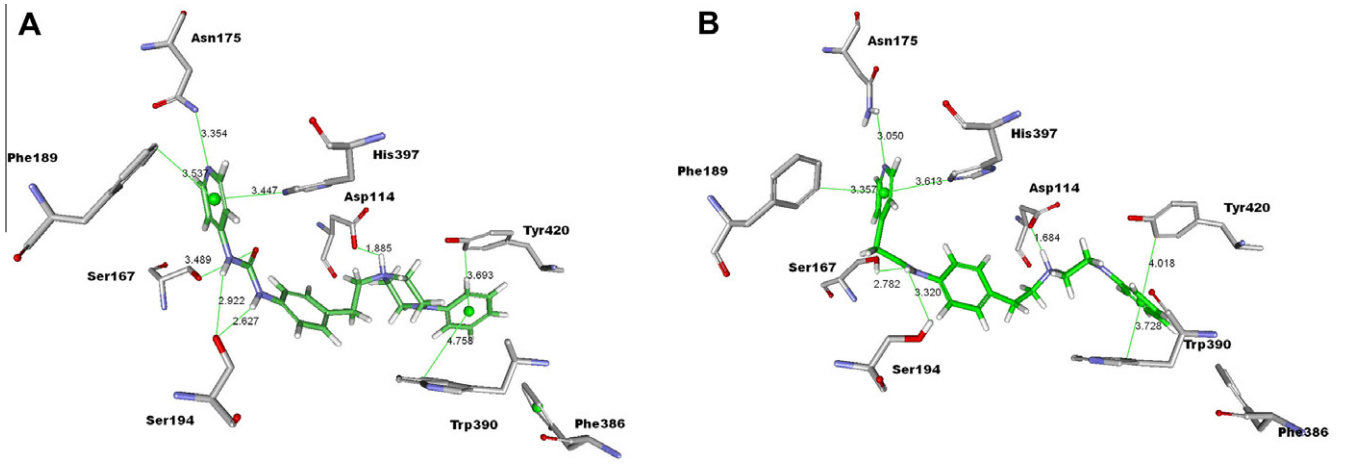


Figure 2. Results from docking compound **8a** and **8b** in binding pocket of the homology model of D_2 DA receptor. (**A**) Docking of ligand **8a** in homology model of D_2 DA receptor active site. (**B**) Docking of ligand **8a** in homology model of D_2 DA receptor active site. Only key amino acid residues are shown.

and polar type of interaction of Asn 175 with head of ligands. Amino acid residues Asp 114, Phe 386, Trp 390 and Tyr 420 are all located in the transmembrane domain of while Ser 194, Ser 167, Phe 189 and His 397 are part of the extracellular loops of the D_2 DA receptor.

In the $5HT_{1A}$ receptor (Fig. 3) ligand–receptor interactions are: salt bridge between Asp 116 and protonated piperazine nitrogen, hydrophobic interactions between ligand aryl-piperazine and receptor hydrophobic pocket formed by Phe 112 and Tyr 390, hydrogen bonds between ligand and Ser 199 and Thr 200 and aromatic type of interaction between Phe 204 and Phe 362 and the head of ligands. All listed amino acid residues are located in the transmembrane domain of receptor molecules and are described in our earlier publications.[13]

Ligand affinity towards the D_2 or $5HT_{1A}$ receptor is in the first place influenced by the shape and length of the ligand (Fig. 4). *para*-Substituted ligands **5a**–**8b** have an elongated, linear shape (Fig. 4), that protrudes into the extracellular part of the D_2 DA receptor wherein additional stabilizing interactions are taking place. These stabilizing effects are absent in $5HT_{1A}$ receptor binding because the binding pocket of the $5HT_{1A}$ receptor is deeper than the binding pocket of the D_2 DA receptors and therefore ligands do not protrude into the extracellular part. Stabilizing effect of the D_2 DA receptor ecl2 domain is also absent in *meta*-substituted ligands **9a**–**12b** since they are bent in shape (Fig. 4), and therefore not long enough to occupy extracellular

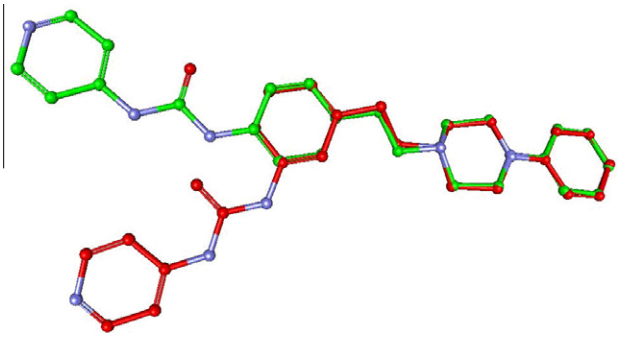


Figure 4. Overlay of compounds **8b** (green) and **12b** (red). Overlaying the tail part of the compounds **8b** and **12b** that occupies the same space in the receptor hydrophobic pocket, shows the difference in overall shape of *para*- (**5a**–**8a** and **5b**–**8b**) and *meta*-substituted (**9a**–**12a** and **9b**–**12b**) ligands. Ligand overlay was obtained using targeted overlay method for flexible compounds in DISCOVERY STUDIO 2.5 software.

receptor domains. Due to the existence of the lateral accessory binding pocket formed by Ser 199, Thr 200, Phe 204 and Phe 362 that can adopt head of only *meta*-substituted ligands, that shape is preferred by the $5HT_{1A}$ receptor (Fig. 3). A similar structure is not present in D_2 DA receptors leading to lower D_2 DA activity of compounds with *meta*-substitution compared to *para*-counterparts

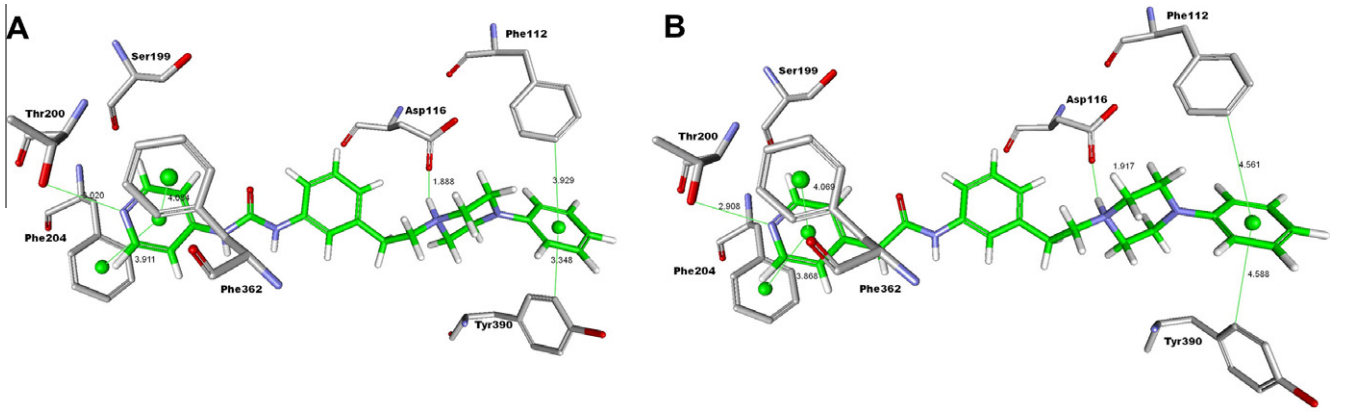


Figure 3. Results from docking compound **12a** and **12b** in binding pocket of the homology model of $5HT_{1A}$ receptor. (**A**) Docking of ligand **12a** in homology model of $5HT_{1A}$ receptor active site. (**B**) Docking of ligand **12b** in homology model of $5HT_{1A}$ receptor active site. Only key amino acid residues are shown.

or the parent compound (**13**). Except for playing a crucial role in defining the overall shape of the molecules, the linker structure itself determines compounds' biological activity through effective hydrogen bonding with Ser 194 and Ser 167 of the D_2 DA receptor. In this prospect higher affinity of arylureas (**5b**–**8b**) compared to arylacetamides (**5a**–**8a**) in the D_2 DA receptor assay (Table 1) can be explained by three hydrogen bonds in arylureas compared to only two in arylacetamides (Fig. 2).

The head part of the ligands plays an influential role in ligand binding to both D_2 DA and $5HT_{1A}$ receptors. The head part of *para*-substituted ligands **5a**–**8a** and **5b**–**8b** can occupy the hydrophobic pocket formed by flexible ecl2 of the D_2 DA receptor but not the lateral hydrophobic pocket of the $5HT_{1A}$ receptors, giving those compounds a more dopaminergic character. On the other hand aromatic heads of *meta*-substituted compounds fit well into the lateral hydrophobic pocket of $5HT_{1A}$ receptors but do not interact with the extracellular domain of D_2 DA receptors. As a onsequence this group of ligands has a more pronounced serotonergic activity.

Higher affinity of arylureas (**5b**–**8b**) compared to arylacetmides (**5a**–**8a**) can be accounted for by stronger hydrogen bonds between urea linker and the receptor molecules as their more rigid structure can contribute to better positioning of the aromatic ring of the head of the molecules into the receptor binding pocket.

The introduction of nitrogen atoms in the aromatic ring into the head of ligands, dramatically increases their affinity towards D_2 DA receptors. For example, 4-pyridyl derivatives **8a** and **8b** are 86 and 134 times more active than corresponding phenyl derivatives **5a** and **5b**. Most probably this is due to favorable aromatic type interactions of ligand pyridyl residue with counterpart amino acid residues (e.g., Phe 189 and His 397, Fig. 2) in ecl2 part of D_2 DA receptors. Compounds **8a** and **8b** were also more active in D_2 DA receptor binding assay than 2-pyridyl (**6a** and **6b**) and 3-pyridyl (**7a** and **7b**) derivatives most likely due to their interaction with Asn 175 (Fig. 2). On the other hand there is no clearly obvious correlation between replacing the phenyl ring with a pyridyl one on $5HT_{1A}$ receptor affinity (Table 1). It is postulated that for high $5HT_{1A}$ receptor affinity, aromatic interactions between the head of the ligands and the hydrophobic pocket of receptor formed by Phe 204 and Phe 362, are necessary (Fig. 3, Ref. 13). The introduction of nitrogen into the position 3 and 4 of the phenyl ring of arylacetamide **9a** and arylcarbamates **9b** has the opposite effect on their $5HT_{1A}$ affinity (Table 1). Those results show the complex nature of these interactions, wherein at least two factors play a role; for example rigidity of the linker part of the molecule as well as the electrostatic surface potential in the head part of the molecule that can both favor or disfavor ligand–receptor interactions.

In conclusion, the molecular shape of the herein described arylureas and arylacetmides together with the correct orientation of a number of functional groups determines affinity towards D_2 DA and $5HT_{1A}$ receptors. High affinity ligand must form numerous interactions with different parts of the receptor. In case of the D_2 receptor, the ligand must occupy two hydrophobic pockets, one located deep inside the binding site, and the second formed by an ecl2 loop. Only by forming interactions with both parts of the receptor, together with salt bridge with Asp 144 and hydrogen bonds with Ser 167, 194 or 197, can high affinity be achieved.

A ligand bound to the $5HT_{1A}$ receptor with high affinity also has to fit into two hydrophobic pockets, both placed within the membrane part of the receptor. One of these sites is located near Asp 116, while the second is adjacent to Ser 199 and Thr 200. Salt bridge between ligand and Asp 116, hydrogen bond with Ser 199 or Thr 200 together with aromatic interactions with hydrophobic pockets plays a key role. Extracellular loops in the $5HT_{1A}$ receptor do not play a significant role in ligand binding.

Results presented in this publication are consistent with our previously presented models of D_2 DA[9] and $5HT_{1A}$[10] receptors. We can understand rather well the influence of the linker structure to the biological activity of ligands but the huge impact of a minor modification in the head part remains to be explained in future studies.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.098. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. *Physiol. Rev.* **1998**, *78*, 189.
2. Hayes, D. J.; Greenshaw, A. J. *Neurosci. Biobehav. Rev.* **2011**, *35*, 1419.
3. Farah, A. *J. Clin. Psychiatry* **2005**, *7*, 268.
4. Nasrallah, H. A. *Mol. Psychiatry* **2008**, *13*, 27.
5. Newman-Tancredi, A.; Assié, B.; Leduc, N.; Ormière, A. M.; Danty, N.; Cosi, C. *Int. J. Neuropsychopharmacol.* **2005**, *8*, 341.
6. Rasmussen, S. G.; Choi, H. J.; Fung, J. J.; Pardon, E.; Casarosa, P.; Chae, P. S.; Devree, B. T.; Rosenbaum, D. M.; Thian, F. S.; Kobilka, T. S.; Schnapp, A.; Konetzki, I.; Sunahara, R. K.; Gellman, S. H.; Pautsch, A.; Steyaert, J.; Weis, W. I.; Kobilka, B. K. *Nature* **2011**, *469*, 175.
7. Chien, E. Y.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G. W.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. *Science* **2010**, *1091*, 330.
8. Shimamura, T.; Shiroishi, M.; Weyand, S.; Tsujimoto, H.; Winter, G.; Katritch, V.; Abagyan, R.; Cherezov, V.; Liu, W.; Han, G. W.; Kobayashi, T.; Stevens, R. C.; Iwata, S. *Nature* **2011**, *475*, 65.
9. Sukalovic, V.; Soskic, V.; Andric, D.; Roglic, G.; Kostić-Rajacic, S. J. *Serb. Chem. Soc.* **2011**. http://dx.doi.org/10.2298/JSC111028212S.
10. Zlatovic, M. V.; Sukalovic, V. V.; Schneider, C.; Roglic, G. M. *Bioorg. Med. Chem.* **2006**, *14*, 2994.
11. General procedure for the synthesis of *N*-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-2-aryl-2-yl-acetamides (**5a**–**5b** and **9a**–**12a**). Arylacetic acids (2.2 mmol), amines **4a** or **4b** (560 mg, 2.0 mmol), 1.0 ml triethyl amine, and 1.8 ml 50% PPAA, ware stirred in 7 ml DMF at room temperature for 16 h, whereupon diluted with 200 ml ethyl acetate and extracted 2 times with 50 ml 8% $NaHCO_3$ and 50 ml H_2O, each. Organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. Obtained products ware purified by silica gel column chromatography using a gradient of methanol (0–5%) in dichloromethane. Obtained amides (**5a**–**5b** and **9a**–**12a**) were crystallized from ethyl acetate as free bases.
General procedure for the synthesis of 1-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-phenyl}-3-aryl-2-yl-ureas (**5b**–**8b** and **9b**–**12b**). Bis(trichloromethyl)-carbonate (200 mg, 0.7 mmol) was added upon stirring into a solution of amine **4a** or **4b** (560 mg, 2.0 mmol) in 2.0 ml dry pyridine and 8.0 ml dry dioxane. Reaction mixture was stirred for 2 min. at ice bath, 30 min. at room temperature, following by addition of 200 mg of arylamine and 1 ml triethylamine. Reaction mixture was transferred to an oil bath at 80 °C and left at that temperature for another 12 h, whereupon diluted with 200 ml ethyl acetate and extracted 2 times with 50 ml 8% $NaHCO_3$ and 50 ml H_2O, each. Organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. Obtained products ware purified by silica gel column chromatography using a gradient of methanol (0–10%) in dichloromethane. Carbamates (**5b**–**8b** and **9b**–**12b**) were crystallized from ethyl acetate as free bases.
12. *Biological assays:* Radioligands [^{3}H]spiperone and [^{3}H]8OH-DPAT were purchased from Amersham Biosciences (Buckinghamshire, UK). CHO-hD2S cells stably expressing native human D2 DA receptor and CHO-K1 clonal cell line that stably expresses 5-HT1A receptors were obtained from Professor Phillip G. Strange (University of Reading, UK) and Dr. Kelly Berg (University of Texas Health Science Center, San Antonio, Texas), respectively. CHO cells were grown in DMEM containing 5% fetal calf serum and 400 μg/ml active geneticin (to maintain selection pressure). The cell lines were maintained at 37 °C in a humidified atmosphere with 5% CO_2. Isolation of membranes with D2 DA and 5-HT1A receptors and competition binding assays at the D2 DA and serotonin 5-HT1A receptor were performed using [^{3}H]-spiperone (0.4 nM) and [^{3}H]8OH-DPAT (0.8 nM), respectively by the protocol provided in.[18,19] Briefly, receptor membranes (50 μg) were incubated at 25 °C for 60 min in a final volume of

1.0 ml reaction mixture containing radioligands and various concentrations of the new compounds in 20 mM HEPES (pH 7.4) buffer containing 1 mM EGTA, 1 mM EDTA, 10 mM $MgCl_2$ and 100 mM NaCl. The samples were incubated at 25° C for 60 min and incubation was followed by rapid filtration through Whatman GF/B filters under vacuum. The filters were washed twice with 3.5 ml of ice-cold phosphate buffer saline and counted using PPO/POPOP/ toluene based cocktail at liquid scintillation counter (LKB 1219 RACKBETA LSC). Nonspecific binding was determined in the presence of 3 μM (+) butaclamol or 10 μM buspirone for D2 DA and 5HT1A receptors, respectively. The analysis of the data was performed using GraphPadPrism 4.0 software for MS Windows (GRAPHPAD PRISM Program, San Diego, USA). Competition binding studies were carried out with 8 varied concentrations of the test compounds run in triplicate tubes, and isotherms from three assays were calculated by computerized non-linear regression analysis to yield K_i values.

13. Soskic, V.; Joksimovic, J. *Curr. Med. Chem.* **1998**, *5*, 493.
14. Accelrys Software Inc.; Discovery Studio Modeling Environment, Release 2.5, San Diego: Accelrys Software Inc.; 2009.
15. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
16. Sukalovic, V.; Andric, D.; Roglic, G.; Kostic-Rajaci, c S.; Schrattenholz, A.; Soskic, V. *Eur. J. Med. Chem.* **2005**, *40*, 481.
17. Pov-Ray; The Persistence of Vision Ray-Tracer, Version 3.6 2003-2011, http://www.povray.org/ (Accessed Jun 12, 2011).
18. Roberts, D. J.; Lin, H.; Strange, P. G. *Mol. Pharmacol.* **2004**, *66*, 1573.
19. Evans, K. L.; Cropper, J. D.; Berg, K. A.; Clarke, W. P. J. *Pharmacol. Exp. Ther.* **2001**, *1025*, 297.