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MODELING INTERACTIONS OF α_{1A} ADRENERGIC RECEPTOR AND DIFFERENT ARYLPIPERAZINE LIGANDS

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Molecular modelling studies were undertaken in order to identify key interactions of selected ligands with α_{1A} adrenergic receptor, responsible for their binding and presumably receptor activation. The previously made model of α_{1A} adrenergic receptor was optimized by molecular dynamics and different arylpiperazine ligands were docked. The results show a high correlation to the experimentally determined binding affinities. Ligand orientations and its interactions with specific amino acid residues in the binding site explain trends in its structure-activity relationship. The key interactions for those trends are mainly aromatic, which are suggested by the calculation of their ESP surfaces.

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Keywords: α_{1A} adrenergic receptor, molecular modeling, arylpiperazine, molecular docking, explicit membrane simulations

1. Introduction

The adrenoreceptors are subdivided into 3 families: α_1, α_2 and β, based on their pharmacology, structure, and signaling mechanisms [1, 2]. Each family contains three or more subtypes, all of which are members of the G protein coupled receptor superfamily. These receptors consist of single polypeptide chains having 7 membrane spanning domains. The α_1-adrenoceptor family is of particular therapeutic interest because of its important role in the control of blood pressure [2,3,4]. These receptors are also abundant in the brain, where their functional role is not yet clear, but it is known that they play critical roles in controlling contraction and growth of smooth and cardiac muscle. Historically, the discovery of drugs acting at GPCRs has been extremely successful with 50% of all recently launched drugs targeting GPCRs [5]. Especially the subfamily of biogenic amine binding GPCRs has provided excellent drugs for the treatment of several CNS diseases such as schizophrenia (mixed $D_2/D_1/5\text{-}HT_2$), psychosis (mixed $D_2/5\text{-}HT_{2A}$), depression ($5\text{-}HT_1$), or migraine ($5\text{-}HT_1$). This GPCR subfamily has also proven to be a drugable target for other disease areas such as allergies (H1), asthma (β_2), ulcers (H_2), or hypertension (α_1 antagonist, β_1 antagonist). Antagonists of the α_1 adrenergic receptors such as indoramin and prazos are used as antihypertensive agents. In addition, α_{1A} antagonists such as alfuzosin and prazosin are thought to be effective in the management of benign prostatic hypertrophy [6].

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An enormous amount of work has been done towards the development of various new GPCR's ligands as potential therapeutic agents. Among the many investigated substances, arylpiperazines showed considerable affinity towards multiple GPCR. So far pharmacophore of arylpiperazines has been well studied in experiments and molecular simulations [7-13].

Here we report results of binding studies of some previously synthesized arylpiperazine ligands [39-42], showing affinity to α_{1A} receptor. Molecular docking was performed to determine structure – activity relationship. The goal is to explain the binding affinities which depend on variation in the ligand's structure and identify the major interactions with aminoacid residues in the binding pocket of α_{1A} receptor.

2. Experimental

Ligand Construction

Ligand stuctures were drawn in ACD ChemSketch 11.0 [14] and their 3D structures were generated using Avogadro 1.0.0 [15]. Assuming physiological conditions, the basic aliphatic nitrogen atom of the piperazine was protonated. The geometry was optimized using the MMFF94 force field [16] followed by the PM6 [17] semiempirical method implemented in MOPAC 2009 [18]. ESP surfaces, projected to total electronic density were calculated in Gaussian 03W [19], using 6-31g+ basis set [20,21].

Receptor construction

For docking analysis we used α_{1A} (α_{1D}) adrenergic receptor model [22], AC P25100, based on crystal β_2 adrenergic receptor structure, PDBID 2RH1 [23]. After comparing the aminoacids in the binding site with those found in α_{1A} [24,25], we concluded that there were no crucial differences in binding site between α_{1A} and α_{1D} adrenergic receptors, so we decided to use α_{1D} adrenergic receptor model for further calculations.

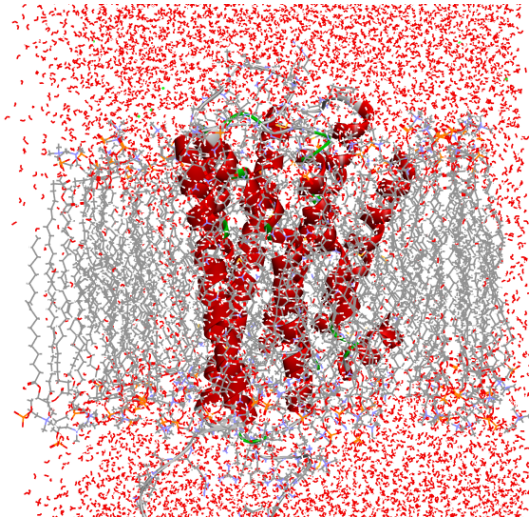


Fig. 1. α_{1A} receptor in membrane bilayer with water, set for molecular dynamics simulation

The receptor was additionally optimized by explicit membrane molecular dynamics simulation. The membrane system of POPC with dimensions 70 x 70 Å was built, the protein was inserted, and additional water molecules and ions (0.15M NaCl) were added, using VMD 1.8.7. (Figure 1) [26]. The system was set to cascade 10000 steps minimization, 250ps equilibration and 5 ns production under PBC conditions on 310K in NVE ensemble running in NAMD 2.7b [27]. After completion of this simulation step, the system was additionally simulated for 1 ns to check RMSD stability of the trajectory (6 ns total). The trajectory was written after each 1ps and the

time-step was 1fs. The average structure was calculated from the trajectory using tcl script [28], after analyzing RMSD plot (Figure 2). All amino-acid residues were named according to Ballesteros-Weinstein nomenclature in superscript [29].

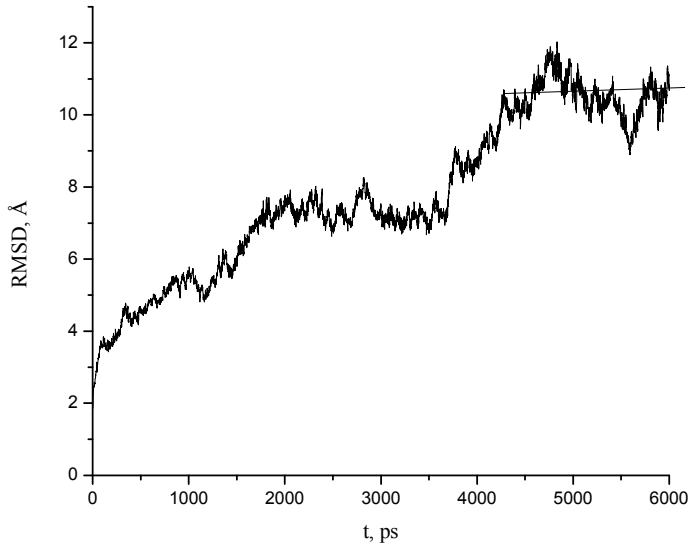


Fig. 2. RMSD graph of molecular dynamics production phase, and marked region for calculation of average structure

Molecular docking

All ligands and receptors were prepared for docking in ADT Tools 1.5.6 [30]. Molecular docking was performed in Autodock Vina 1.1.1 [31]. The grid box dimensions were set to 22x22x22 $Å^3$, and its centre was set to span all aminoacid residues of the binding pocket. The binding site was identified according to previous studies [24,25] Exhaustiveness was set to 100. Number of output conformations was set to 250. The searching seed was random. The preferred conformations were the ones of lowest binding energy with the salt bridge to the receptor aminoacid residue Asp $176^{3.27}$

Results visualization

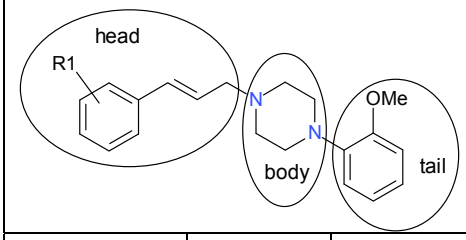
Structures were visualised using DS Visualiser v3.1 [32] and the obtained images were rendered using PovRay Raytracer v3.6 [33], and edited in PhotoScrape v3.6 [34]. ESP surfaces were visualised using Gopenmol program [35,36].

All calculations were performed on PARADOX cluster [37] or Intel Dual Core @ 2.1 GHz personal computer.

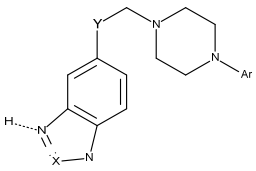
3. Results and discussion

As seen from the ligand structures (Table I), they consist of two aromatic moieties, each specifically involved in binding to the receptor and affecting binding affinity.

Table I. Structures of docked compounds and experimentally determined Ki values

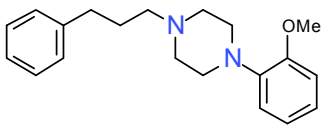


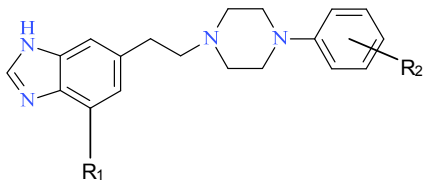
Compounds	R_1	Ki(nM)
1	H	47.1
2	2-NO_2	48.6
3	3-NO_2	43.1
4	4-NO_2	158
5	2-OCH_3	33.2
6	3-OCH_3	66.7
7	4-OCH_3	80
8	2-Cl	300
9	3-Cl	>1000
10	4-Cl	>1000

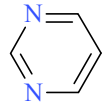


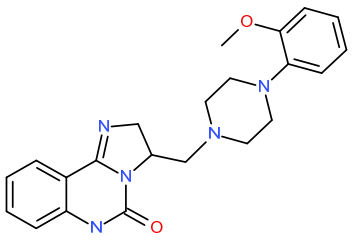
Compounds	X	Y	Ar	Ki (nM)
12	C=S	-O-CH_2-	Phe	29.15
13	C=S	-O-CH_2-	2-MeOPhe	1.95
14	C=S	-O-CH_2-	naphthyl	429
15	C=S	-O-CH_2-	3-CF_3Phe	493
16	=CH-	-O-CH_2-	Phe	26.35
17	=CH-	-O-CH_2-	2-MeOPhe	5.95
18	=CH-	-O-CH_2-	naphthyl	62.5
19	=CH-	-O-CH_2-	3-CF_3Phe	121
20	CH	-CH_2-	naphtyl	93.3
21	N	-CH_2-	2,3-dimethylphenyl	67.6

Compounds	Ki(nM)
11	7.8

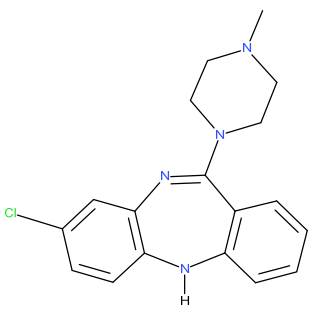




Compounds	R_1	R_2	Ki(nM)	Compounds	R_1	R_2	Ki(nM)
22	H	2- NO_2	11.4	31	H	2-Cl	15.5
23	Cl		19.3	32	Cl		3.02
24	Br		9.8	33	Br		1.1
25	H	-	42.7	34	H	3-CF_3	204.1
26	Cl		15.5	35	Cl		154.9
27	Br		5.13	36	Br		123
28	H	2-OMe	4.7	37	H		676.1
29	Cl		3.24				
30	Br		0.42				



Compound	Ki(nM)



Compound	Ki(nM)

38	0.13	39 (clozapine)	22.4

The key amino-acids in the binding site of α_{1A} were found to be Asp $176^{3.27}$, Val $177^{3.28}$, Thr $181^{3.32}$, Tyr $254^{5.38}$, Ser $258^{5.42}$, Ser $262^{5.46}$, Phe $388^{7.39}$, Tyr $392^{7.43}$, Leu $145^{2.53}$, Trp $361^{6.48}$, Phe $263^{5.47}$, Phe $365^{6.52}$ and Trp $172^{3.23}$. They can be divided into two binding pockets, one aromatic and one polar, mostly hydrophilic. The favoured protein-ligand stabilizing interactions are the following: salt bridge between protonated aliphatic nitrogen of a ligand and Asp $176^{3.27}$ (the anchor interaction), combined edge to face aromatic interactions (in aromatic pocket), hydrogen bond and CH-π interactions (via amino-acid residues in hydrophilic pocket). The docking results of our 37 compounds and the 2 antagonists, (taken from literature), clozapine and compound [38] show high overlying of docked ligands in their preferred conformations. They all have specific orientations in the binding site (Figures 3,4). The docked conformations were chosen on criteria of having the lowest binding energy and salt bridge with the receptor. Important ligand-receptor interactions, besides the formation of a salt bridge, include aromatic edge-to-face interactions of aromatic ligand groups with Trp $361^{6.48}$, Phe $388^{7.39}$, Trp $361^{6.48}$ and Tyr $392^{7.43}$. In the polar pocket, besides aromatic CH-π interactions between Val $177^{3.28}$ and/or Ser $258^{5.42}$ and the ligand, there are possibilities for hydrogen bond formation between Thr $181^{3.32}$, Ser $262^{5.46}$ and functional groups on the benzene ring.

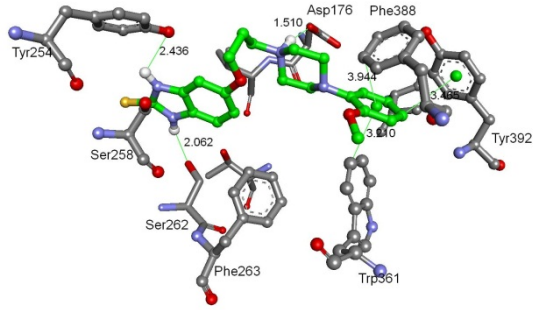


Fig. 3. Simplified representation of the α_{1A} active site with docked compound 13. Only key amino acid residues are shown for clarity.

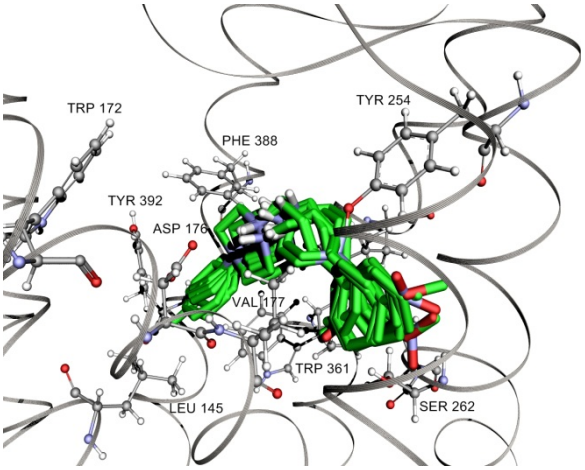


Fig. 4. Overlay of docked compounds 1-10

The trends in binding affinities can be explained if some of the following facts are considered:

Compounds 1-11 [39] tend to form hydrogen bonds with Tyr $254^{5.38}$ and Ser $262^{5.46}$ via nitro or metoxy groups (Table II). The difference in binding affinity between nitro substituted and metoxy substituted aromatic rings originate from their different electronic effects [10]. Since aromatic groups occupy the aromatic binding pocket, aromatic interactions are assumed to play a major role in the binding affinity of these ligands, thus, electron activating groups such as -OMe increase electron density and raise negative values on ESP, overall stabilizing edge-to-face aromatic interactions with partially positive aromatic hydrogens. Binding affinity also depends on steric parameters.

Table II. Summary of results of docked conformations.

Interactions legend:

A: Ser $262^{5.46}$ via MeO-
B: Tyr $254^{5.38}$ via $-NO_2$
C: Ser $262^{5.46}$ via $-NO_2$
D: Tyr $254^{5.38}$ via -O-
E: Tyr $254^{5.38}$ and Ser 262 via NH
F: Ser $262^{5.46}$ via NH
G: Tyr $254^{5.38}$ and Ser $262^{5.46}$ via NH and N=
H: Trp $361^{6.48}$, Phe $388^{7.38}$ edge to face, Ser $258^{5.42}$, Val $177^{3.28}$, CH-π
I: Trp $361^{6.48}$ Phe $388^{7.38}$ edge to face, Val $177^{3.28}$, CH-π
J: Trp $361^{6.48}$ Phe $388^{7.38}$ edge to face
K: Phe $388^{7.38}$ edge to face, Val $177^{3.28}$, CH-π

	Interactions					
Compound	Calculated salt bridge distance, R (Å)	Hydrogen bonds	Aromatic interactions	Experimental Ki, nM	Experimental ΔG, kcal/mol	1/R, $Å^{-1}$
1	4.206	A	H	47.1	-9.99	0.238
2	3.375	A	H	48.6	-9.97	0.296
3	3.656	B	I	43.1	-10.04	0.274
4	2.179	C	H	158	-9.27	0.459
5	3.337	A	H	33.2	-10.19	0.300
6	3.324	-	H	667	-8.42	0.301
7	2.286	-	H	80	-9.67	0.437
8	3.595	-	H	300	-8.89	0.278
9	3.592	-	H	>1000	-	0.278
10	4.704	A	H	>1000	-	0.213
11	2.184	-	H	7.8	-11.05	0.458
12	3.366	D	I	29.15	-10.27	0.297
13	1.510	E	H	1.95	-11.87	0.662
14	3.945	-	J	429	-8.68	0.253
15	3.011	-	J	493	-8.60	0.332
16	3.925	D	J	26.35	-10.33	0.255
17	3.547	D	J	5.95	-11.21	0.282
18	3.928	D	J	62.5	-9.82	0.255
19	4.050	D	J	121	-9.43	0.247
20	3.248	-	I	93.3	-9.58	0.308
21	3.211	-	I	67.6	-9.77	0.311
22	2.500	-	I	11.4	-10.83	0.400
23	2.652	-	I	19.3	-10.51	0.377
24	2.504	-	I	9.8	-10.92	0.399
25	3.588	-	J	42.7	-10.04	0.279
26	2.618	-	K	15.5	-10.64	0.381
27	3.287	-	I	5.13	-11.30	0.304
28	3.076	-	I	4.7	-11.35	0.325
29	3.118	-	I	3.24	-11.57	0.321
30	2.992	-	I	0.42	-12.78	0.334
31	3.066	-	I	15.5	-10.64	0.326
32	3.114	-	I	3.02	-11.61	0.321
33	2.901	-	I	1.1	-12.21	0.345
34	3.538	-	I	204.1	-9.12	0.283
35	2.413	F	H	154.9	-9.28	0.414
36	3.457	-	I	123	-9.42	0.289
37	3.555	-	-	676.1	-8.41	0.281
38	2.338	G	H	0.13	-13.47	0.428
39	2.224	-	K	22.4	-10.42	0.428

Different substitutions in the benzene ring produce large differences in the binding constants, as in triades 2-4, 5-7, 8-10. Nitro- and metoxy- substituted benzene rings form hydrogen bonds with Ser $262^{5.46}$, where its efficiency decreases along positions 2-,3-,4-. The large difference in triade 8-10 is probably due to bumps of Cl with Val $177^{3.28}$, Ser $262^{5.46}$ and Ser $258^{5.42}$, so compounds 9 and 10 have experimentally unmeasurable large Ki values. Compound series 12-15 and 16-19 [40]: the difference in binding affinity of 12 and 13 can be explained by taking into account the electron activating effect of the -OMe group on the benzene ring , while -CF_3 substituted aromatic systems (compounds 15 and 19), with a electron deactivating effect are reported to have much larger Ki values. Also, the repulsion between the aromatic electron system and fluorine atoms should be taken into account. The same can be explained for series 16-19. We should also mention the effect of sulphur[e] atom: its presence in the benzimidazole ring raises ESP values, again making stronger CH-π interactions with Val $177^{3.28}$, as seen from the difference in Ki between compounds 13 and 17. On the other hand, aromatic groups such as naphtyl and 3-CF_3Phe experimentally show much lower binding affinity, which can be explained as achieving a boundary longitude of the ligand in the binding pocket. Compounds 20-37 [41,42]: there are seven triade series of compounds alternating in the substituent or structure of the aromatic system. In their docked conformations the substituted benzene rings are oriented towards Trp $361^{6.48}$, forming an edge-to-face interaction. Electronic effects of ring subtituents are dominating for binding affinity of these compounds, as seen in Table I, by comparing binding energies and electronic properties of the substituent. The differences in binding energies for different R substituents in condensed aromatic fragments originate from different inductive effects and polarizability of Cl and Br, involving CH-π interactions with Val $177^{3.28}$, as shown form ESP surfaces (Figure 5).

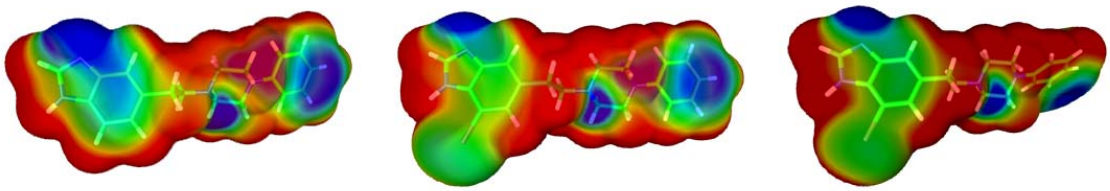


Fig. 5. ESP surfaces of compounds 34-36, respectively. Red colour stands for positive ESP, while blue colour is negative.

Analyzing summary interactions in Table II, there is a good explanation for binding energies, and also confirmation of the statement suggested above. The interactions are divided into three terms: salt bridge distance, presence of hydrogen bonds and presence of aromatic interactions. It can be seen from Table II that the lowest Ki values belong to ligands with shorter salt bridge distances, and hydrogen bonds with appropriate amino-acid residues. Some quantitative correlation can be extracted by plotting 1/R vs binding energy, where R represents hydrogen bond distance between donor-acceptor atoms in ligand and aminoacid residue. The b.e. are calculated via the relation $\Delta G=RT\ln(Ki)$, where R=8.314 J/molK, and T = 298 K. For instance, the compound 13 has 1.51 Å short salt bridge and two hydrogen bonds, with Tyr $254^{5.38}$ and Ser $262^{5.46}$ via NH, showing the Ki value of 1.95 nM. Also, electron activating groups such as MeO, as well as steric effects have influence on ligand interactions. In comparison, compound 15 has 3.011 Å long salt bridge, no hydrogen bonds and $–CF_3$ group in the aromatic system, and a Ki value of 493 nM.

Having those facts in mind, we now discuss the influence of ligand structure on binding affinity. We can generally divide ligands into three parts: head, body and tail. The head consists of a substituted aromatic system, benzene or indene type, connected to the body via alkane, ether, or alkene chain. The body consists of a piperazine ring, with protonated aliphatic nitrogen (constant structure), and the tail consists of a substituted benzene system. The crucial structure properties of the head leading to high binding affinity are groups that:

1) can form hydrogen bonds, such as -NO_2 and -O-CH_3 groups (compounds 1-10). Different electronic effects of these two groups and their positions on the benzene ring should be taken into account (see above in text).

2) have electronic activating substituents (triades 22-37)

The difference in binding affinity between compounds 1 and 11 is also noticed, thus leading to the conclusion that (in)availability for C-C bond rotation is crucial. The reason for this probably lies in the moving ability of ligands to enter into the binding pocket. Also, the presence of oxygen in the alkane chain raises binding affinity, again making it possible for the ligand to form additional hydrogen bonds. For example, the distance between oxygen atoms in the ether chain of [the] ligand and of Tyr $254^{5.38}$ is 2.693 Å. Influence of the tail structure on binding affinity is due to electronic effects of substituents. The highest affinity is shown in the compound with the ligand with the -OMe group, then the -Cl (activating groups) while the lowest is associated with deactivated pyrimidine system (compounds 35-37), again explained via ESP surfaces (Figure 6).

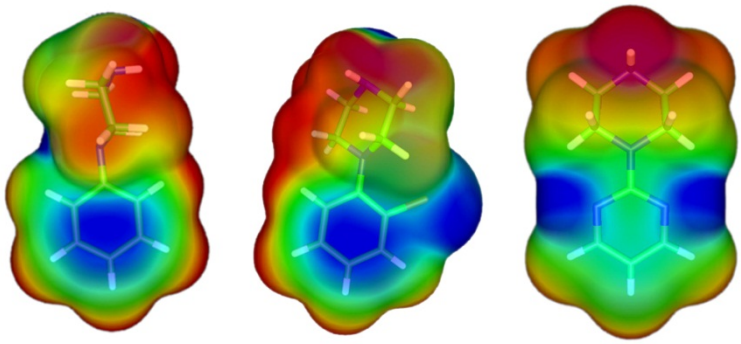


Fig. 6. ESP surfaces of tails of ligands 25-27, 31-33, and 37, respectively. Red colour stands for positive ESP, while blue colour is negative.

It can be seen that the aromatic ring alters ESP value when nitrogen is in the ring, i.e when the system is electronically deactivated, electron density is lower and edge-to-face interactions, responsable for ligand affinities are weaker (see Table II).

4. Conclusions

In this paper we performed molecular dynamics optimization of α_{1A} adrenergic receptor followed by docking of arylpiperazine ligands that have low to high affinity to the α_{1A} receptor. The docking results show high overlying of ligand structures with stabilizing aromatic interactions and hydrogen bonds. Protein-ligand key interactions are, as proposed, an anchor salt bridge between the protonated nitrogen of the ligand and Asp $176^{3.27}$, aromatic interactions (edge-to-face and CH-π), and hydrogen bonds. We do not report here the calculated binding energies for reason of experimental correlation inconsistency and the incapability of docking forcefields to take in account aromatic interactions, but *ab initio* calculations of ESP surfaces give qualitative explanations of the differences in binding constants. Also, molecular dynamics simulation is found to be helpful for total realistic optimization of receptor-ligand modeled system as in this case. The results presented here may be interesting from the synthetic point of view as they suggest structural elements that may lead to ligands of high affinities for α_{1A} adrenergic receptors.

Acknowledgements

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