February 12, 2010 15:33 WSPC/178-JTCC 00556

Journal of Theoretical and Computational Chemistry
Vol. 9, Supp. 1 (2010) 49–63
© World Scientific Publishing Company
DOI: 10.1142/S0219633610005566

World Scientific
www.worldscientific.com

INTERACTION OF BRIDGED PIPERAZINE DERIVATIVES WITH THE μ-OPIOID RECEPTOR — A THEORETICAL MODEL

VUK MICOVIC

Institute for General and Physical Chemistry
Studentski Trg 12-14, 11000 Belgrade, Serbia
vmicovic@gmail.com

IVAN JURANIC

Faculty of Chemistry
University of Belgrade and Center of Chemistry
IChTM, Studentski Trg 12-14, 11000 Belgrade, Serbia
ijuranic@chem.bg.ac.rs

Received 26 May 2009
Accepted 26 July 2009

The flexible molecular docking was used to study interactions between a series of 3,6-diazabicyclo[3.1.1]heptanes, 9,10-diazatricyclo[4.2.1.1]decanes, and 2,7-diazatricyclo [4.4.0.0]decanes N-substituted by propanoyl and by arylalkenyl groups, and a model of the μ-opioid receptor. It has been found that the optimal position and orientation of the compounds in the ligand–receptor complex resemble that of fentanyl analogs described earlier.[1] This model explains stereochemical effects on binding of the two series of 3,6-diazabicyclo[3.1.1]heptanes, suggesting that the steric interaction of the bridge methylenic group plays the major role in modulating μ-receptor affinity of those molecules. *Ab initio* B3LYP method was used to determine electrostatic potentials of different bridged piperazine derivatives, and to estimate electrostatic contribution to the ligand–receptor complex stability.

Keywords: Molecular modeling; ligand–receptor interactions; docking simulation; bridged piperazine.

1. Introduction

Opioid analgetics, widely used as pain relievers, perform their action through interactions with μ-opioid receptor.[2–4] However the μ-opioid receptor is also responsible for the undesired side effects including development of tolerance and addiction. Therefore, the synthesis of the new opioid analgesics with reduced side effects remains to be of interest.

Bicyclic homologs of piperazine, like 3-substituted 8-propanoyl-3,8-diazabicyclo [3.2.1]octanes,[5] 10 times more potent than morphine, as well as their analogs

with different substituents in the side chain have been synthesized.[6] It has been suggested that the endoethylenic bridge of 3,8-diazabicyclo[3.2.1]octane (DBO) plays an essential role in interacting with the receptor.[7] Derivatives of the 9,10-diazatricyclo[4.2.1.1]decane and of the 2,7-diazatricyclo[4.4.0.0]decane have been made, as well. Some of these derivatives exhibited high μ-receptor affinity and displayed an analgesic potency *in vivo* higher than morphine.

The series of *N*-3-arylpropenyl-*N*-9-propanoyl-3,9-diazabicyclo[3.3.1]nonanes and their reverted *N*-3-propanoyl-*N*-9-arylpropenyl isomers were also synthesized,[8,9] and evaluated for their binding affinity towards opioid receptors μ, δ, and κ. The compounds exhibited a significant affinity towards μ-opioid receptor, in the low nanomolar range, and moderate or negligible affinity towards δ- and κ-receptors. Some of the compounds were tested for *in vivo* analgesic activity[9] and displayed analgesic effect comparable to that of morphine.

Most recently, the series of *N*-3(6)-arylpropenyl-*N*-6(3)-diazabicyclo[3.1.1]heptanes (DBH) were synthesized.[10] It was shown that a number of compounds in these series have high affinity towards μ-opioid receptor (K_i ranging from 3–8 nM), and negligible affinity towards δ- and κ-receptors.

Molecular modeling studies were done[11] on a set of piperazine and 3,8-diazabicyclo[3.2.1]octane derivates in order to determine the main factors modulating their affinity towards the μ-opioid receptor. It was found that the binding to the μ-opioid receptor is promoted by: the presence of hydrocarbon fragments on the nitrogen ring frame, a "correct" orientation of an *N*-propanoyl side chain, and the possibility of accepting a hydrogen bond from the receptor site. The docking studies of some diazatricyclo[4.2.1.1]decanes and 2,7-diazatricyclo[4.4.0.0]decanes have been published.[7] These studies showed the importance of the ionic bond between the protonated nitrogen and Asp147 carboxylate group, and the importance of the hydrogen bond formed between His297 imidazole ring and the carbonyl oxygen of the acyl chain of the ligand.

Prompted by 3D similarity of fentanyl molecule and the molecules of substituted diazabicycloheptanes and diazatricyclodecanes (Figs. 1 and 2), and by the effect of stereochemistry of these molecules to their binding at the μ-receptor, we decide to conduct docking experiments in order to compare their binding to the μ-receptor. Considering the great effect that substituents at the aromatic ring have[10] on the binding constants of DBH, we also investigated the electrostatic potential around these molecules and its compatibility to the μ-receptor.

2. Results and Discussion

Automated docking of the compounds of Series 1–4 to the TM domain of the μ-opioid receptor resulted in several plausible docking orientations and conformations for each ligand. The resulting ligand orientations and conformations were scored based on the binding energies, and the distance between Asp147 and the

Table 1. Substituents in positions R, R_1, and Ar in the studied compounds.

Compd.	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**
R	H	4-NO_2	2-Cl	3-Cl	4-Cl	H	4-Cl	3,4-Cl_2	H
R_1	H	H	H	H	H	CH_3	CH_3	CH_3	CH_2CH_3
Compd.	**3a**	**3b**	**3c**	**3d**	**3e**	**4a**	**4b**	**4c**	**4d**
Ar	C_6H_5	*p*-NO_2–C_6H_4	*m*-NO_2–C_6H_4	*m*-Cl–C_6H_4	1-Naphthyl	C_6H_5	*p*-NO_2–C_6H_4	*m*-NO_2–C_6H_4	*m*-Cl–C_6H_4

Fig. 1. Structure of the studied compounds.

protonated nitrogen of a ligand. Only a few met these criteria, and they were further evaluated based on experimental results which indicated the important amino acids constituting ligand binding site within the receptor.

The preferred conformation of **2h**, the best binding ligand amongst the compounds studied, positions piperazine ring parallel to the direction of the receptor transmembrane helices, in the region between transmembrane helices TM3 and TM7. The protonated nitrogen is close to the Asp147 of TM3 (+ NH $\cdots$ O− distance is 2.08). The *N*-propanoyl group is oriented towards the extracellular side of the cavity, while the *N*-arylalkyl group (torsional angles 108°, and 180°) positions the phenyl group between TM6 and TM7, Fig. 2. This vertically aligned structure is very similar to the preferred fentanyl conformation in the binding pocket of the μ-opioid receptor. The *N*-arylalkyl group is oriented so that its phenyl group is within 3 Å to the His297 in TM6, in a favorable orientation for a strong interaction with imidazole ring. These two ligand–receptor interactions are very important for the μ-receptor activation. Electron paramagnetic resonance spectra on rhodopsin[2–4] suggested, for instance, rigid body movement of helices TM3 and TM6 relative to each other, upon light activation. On the other side, ethyl group in *N*-propanoyl is 4 Å distant from Trp318 and 6 Å from His319 of TM7, like the *N*-phenylpropanamide group in the case of fentanyl (Fig. 2). The Tyr148 of TM3 is 4 Å apart from the ligand's methylenic bridge. Tyr148 of TM3 and Tyr326 of TM7 are 3 and 4 Å apart from the alkyl chain of the *N*-arylalkyl group, respectively. It has been suggested[12] that Tyr326 together with Asn150 may participate in stabilization

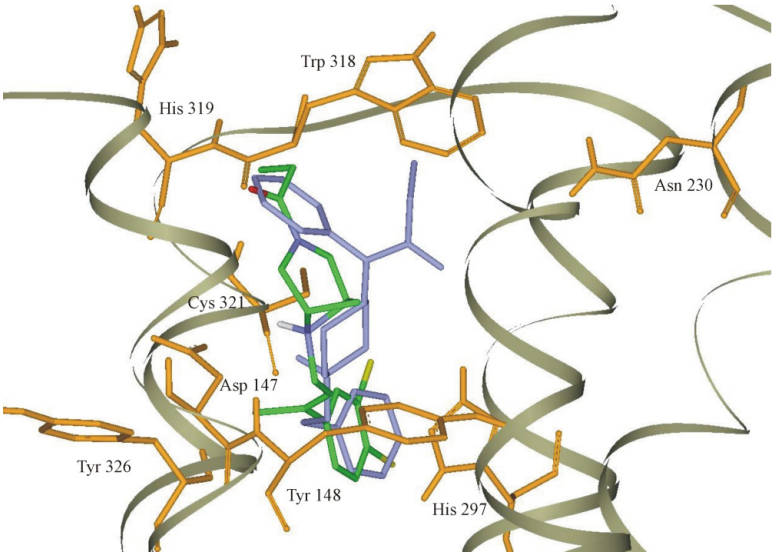

Fig. 2. Superimposed preferred conformations of the compound **2h** (shown with carbons in green color) and fentanyl (shown in light blue color) in the binding pocket of the μ-receptor. All the other compounds of the Series 2 have a very similar conformation. Color online.

of the receptor conformation. The carbonyl group of Cys321 (TM7) is 4 Å apart from the ligand's phenyl group suggesting that any change in phenyl group electron density may affect this interaction. The only interaction that is missing, compared to fentanyl, is the one with the Asn230 (TM5) (distance to **2h** is more than 7 Å). Therefore, the preferred conformation of **2h** is similar to the preferred conformation of fentanyl[1] in the binding pocket of the μ-receptor (Fig. 2).

All the other compounds of Series 2 adopted preferred conformations and alignments in the binding pocket very similar to that of **2h**. The structure of compound **2h** is given, as a representative of this group, in Fig. 2, and their binding constants (Table 2) are mainly in the value-range of dissociation constant K_i of fentanyl.[12,13] AutoDock calculated binding energy, E_b, for fentanyl is higher than the binding energies of the compounds in Series 2 with similar binding constant. However, the energy difference is within the reported AutoDock standard error[14] of 2.2 kcal/mol. This error is likely to be smaller for structurally more similar compounds.

Due to this high standard error of calculated binding energies, agreement between the calculated and experimentally determined binding constants, K_i, is qualitative. The general trends in K_i, for Series 2 are reproduced, Fig. 3, but the calculated binding strengths of **2a** and **2f** are overestimated, while that of **2b** is underestimated by AutoDock program. The experimental and the calculated results indicate that **2h** and **2g** are the best binding ligands of Series 2, and **2i** is the least effective in this series.

Table 2. Experimental dissociation constants[a] (K_i), binding energy (E_b), the distance Asp147–ligand (COO–HN+), and dipole moments for the Series 1 and 2.

		R	R_1	K_i^a (nM)	E_b (kcal/mol)	Distance (Å)	Dipole moment (D)
	a	H	H	600 ± 75	−7.69	3.98	4.08
	b	4-NO_2	H	850 ± 70	−7.38	3.22	11.62
	c	2-Cl	H	207 ± 32	−7.92	4.09	5.49
	d	3-Cl	H	270 ± 42	−7.48	4.13	6.80
1	e	4-Cl	H	452 ± 42	−7.62	4.23	7.43
	f	H	CH_3	5% inh. at 1 μM	−7.37	3.06	3.55
	g	4-Cl	CH_3	363 ± 53	−8.13	4.35	7.70
	h	3,4-Cl_2	CH_3	223 ± 15	−8.30	4.35	10.22
	i	H	C_2H_5	237 ± 25	−7.38	3.20	4.04
	a	H	H	208 ± 8	−8.37	2.08	3.73
	b	4-NO_2	H	5.2 ± 0.8	−8.29	2.10	11.42
	c	2-Cl	H	92 ± 4	−8.18	2.04	3.24
	d	3-Cl	H	21 ± 0.7	−8.74	2.22	7.17
2	e	4-Cl	H	16 ± 2	−9.01	2.06	10.26
	f	H	CH_3	178 ± 11	−8.69	2.22	3.67
	g	4-Cl	CH_3	7.9 ± 0.7	−9.47	2.16	6.67
	h	3,4-Cl_2	CH_3	2.7 ± 0.5	−9.79	2.43	10.14
	i	H	C_2H_5	387 ± 12	−7.63	3.93	6.32
Fentanyl				$3.97^b \pm 0.5$	−7.76	2.85	7.62

[a]Reference 10.
[b]Reference 13.

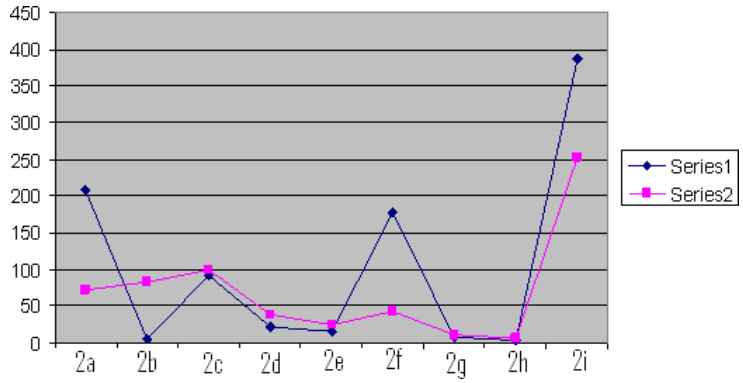

Fig. 3. Experimentally determined (blue) and the calculated (pink) values of K_i ($K_i = 0.1 * e^{E_b/RT}$) for Series 2. Color online.

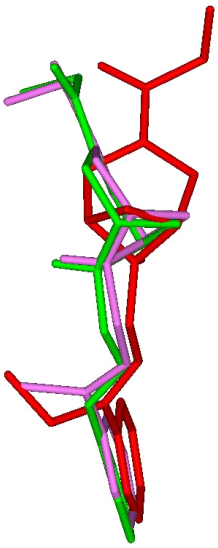

Fig. 4. Superimposed preferred conformations of the compounds **2a**, **2f**, and **2i** (shown in green, magenta, and red colors, respectively). Color online.

This low binding efficacy of **2i** may be explained, at least in part, by the steric interactions of the ethyl group with Tyr326. In the compounds **2a** and **2f**, the less voluminous R_1 groups, H or CH_3, may fit the pocket created by Asp147, Met151, Ile322, and Tyr326, while the ethyl group of **2i** cannot fit that space. Therefore the preferred conformation of **2i** adopts somewhat different orientation, and binds less efficiently, Fig. 4. The experimental curve on Fig. 3 also reveals that the three ligands of the Series 2 which are the least efficient in binding are **2a**, **2f**, and **2i**, i.e. the compounds without the polar substituents at the phenyl ring. This suggests

the importance of electrostatic interactions in ligand binding. This is confirmed by dipole moment calculations which will be discussed later in the text.

Analysis of the binding constant data for the two series: Series 1 and 2, reveals systematically lower values of K_i and accordingly better binding of the ligands of the Series 2.

The average binding constant (excluding compounds **1f** and **2f**), for Series 1 and 2, are 400 and 50 nM, respectively. The calculated average binding energies follow this trend qualitatively. The average calculated E_b for Series 1 and 2 are −7.6 and −8.7 kcal/mol, respectively. According to the docking results, this difference is due to the position of the methylenic bridge in the two series of ligands. In Series 1, interactions of the methylenic bridge with Ile322 of TM7 disable ligands of Series 1 to adopt the fentanyl-like orientation in the binding pocket. Mostly, they have a reversed fentanyl-like structure (Fig. 5). The compounds, **1b**, **1f**, and **1i**, also have an alternative structure with the ligand positioned perpendicular to the direction of receptor transmembrane helices, across the region between TM3, TM4, TM5, and TM7. Calculated longer salt bridge and higher binding energies, relative to Series 2, are in the agreement with the experimentally determined K_i values.

Like within Series 1, the compounds of Series 3 (Fig. 6) prefer the reversed fentanyl-like structure, due to the steric interactions of the endoethylenic bridge with Ile322. Again, the protonated hydrogen is not in the best position to form a salt bridge with the Asp147, leading to the binding efficacy in the range between Series 1 and 2. An anomaly was observed for the compound **3e**. This compound

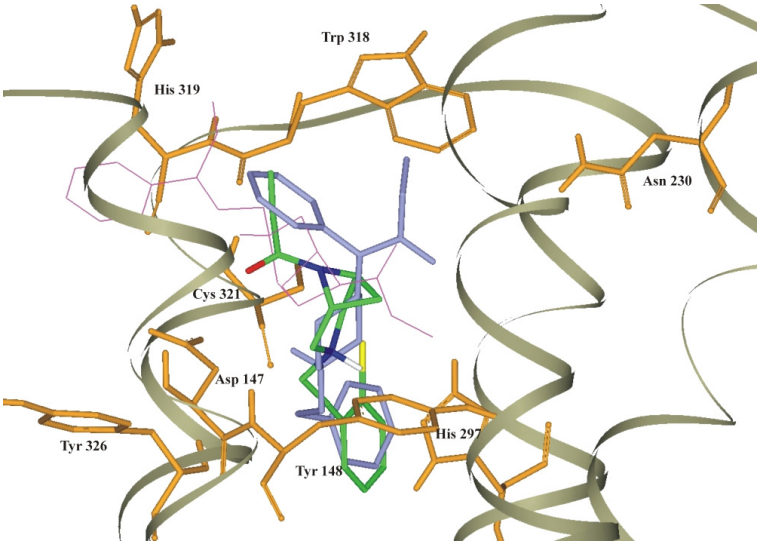

Fig. 5. Superimposed preferred conformations of the compound **1c** (shown with carbons in green color) and **1i** (shown in pink line) and fentanyl (shown in light blue color) in the binding pocket of the μ-receptor. Compounds **1b** and **1f** have a conformation similar to **1i**, while the rest of the compounds of the Series 1 have conformations similar to **1c**. Color online.

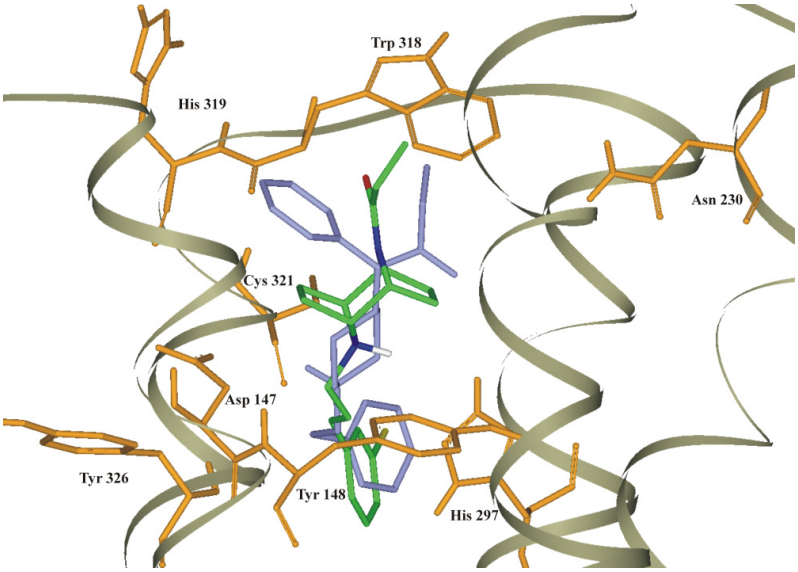

Fig. 6. Superimposed preferred conformations of the compound **3d** (shown with carbons in green color) and fentanyl (shown in light blue color) in the binding pocket of the μ-receptor. All the other compounds of Series 3 have a very similar conformation. Color online.

is the least efficient according to the experimental data, but it has the strongest binding energy (E_b) according to our calculations. This is probably due to the overestimation, by AutoDock program, of the hydrophobic stabilization due to the second aromatic ring. Overestimated stability of the 3e-receptor complex may, as well, be the consequence of the receptor model used and its deficiencies. The cavity between helices TM6 and TM7, where the aromatic group of the ligand is located may have been oversized according to the recent work[17] on molecular dynamics simulations of the μ-opioid receptor in the membrane aqueous systems, where the arrangement of the α-helices of the transmembrane receptor domain became more compact relative to an isolated receptor model. This is probably not important for the less voluminous groups but the naphthyl group may face some important steric interactions in the receptor.

Due to the twist conformation of the piperazine ring in the ligands of Series 4 (Fig. 7), the endoethylenic bridge may avoid steric clash with Ile322. These compounds have a fentanyl-like positioning in the μ-opioid receptor, with short salt bridge to the Asp147. In Series 3 and 4, both experimental and calculated binding affinities are uniform along the series, excluding compounds **3e** and **4b**. This probably reflects different orientation of the phenyl ring, or perhaps different ligand specific receptor conformation,[16] relative to Series 1 and 2. The low experimentally determined binding efficacy of **4b** may even be an error in experimental determination.

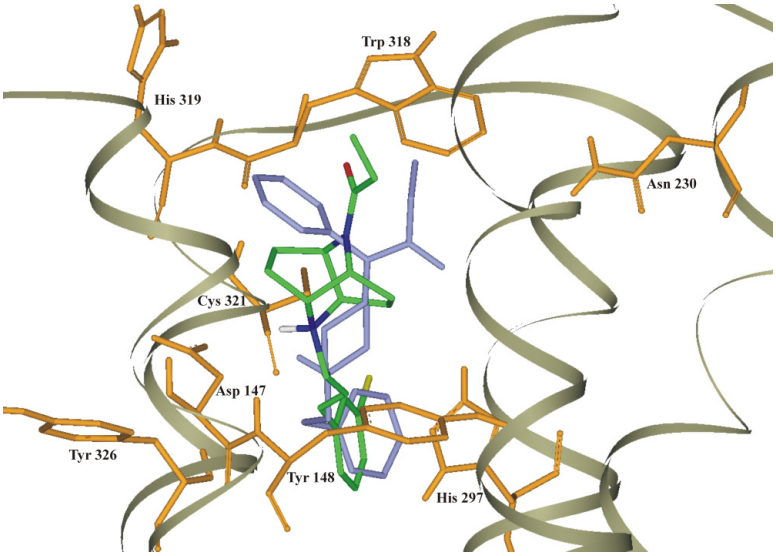

Fig. 7. Superimposed preferred conformations of the compound **4d** (shown with carbons in green color) and fentanyl (shown in light blue color) in the binding pocket of the μ-receptor. All the other compounds of Series 4 have a very similar conformation. Color online.

Discrepancies of the calculated E_b and the experimentally determined binding constants for some of the compounds (**2b** and **4b** for instance), and the variation of K_i with substitution at the aromatic ring, prompted us to look for more sophisticated method for the evaluation of electrostatic ligand–receptor interactions. The electrostatic potentials around the ligands were calculated with *ab initio* B3LYP method, and the Molekel program[15] was used for representation of the potentials at the van der Waals surfaces of the ligands.

It was found that the electrostatic potentials around different ligands in all series are similar (Fig. 8).

Only the compounds bearing halogen or nitro group substituent showed noticeable difference in electrostatic potential around the phenyl ring. The *ab initio* calculated electrostatic potential is consistent with the shift of electron density from the phenyl ring towards $-NO_2$ and $-Cl$ substituents, increasing a molecular dipole moment, Tables 2 and 3, and strengthening the electrostatic ion–dipole and dipole–dipole interactions.

The regression analysis of the experimentally determined binding constants, K_i, and the calculated binding energies, E_b, was done for the 26 compounds studied (**1f** was omitted), using the following correlation equation:

$$\text{Log}\, K_i = A * E_b + B * f + C,$$

where f is the correction constant and the coefficients A, B, and C are 0.94, 0.47, and 9.43, respectively. The analysis of the regression analysis results revealed that

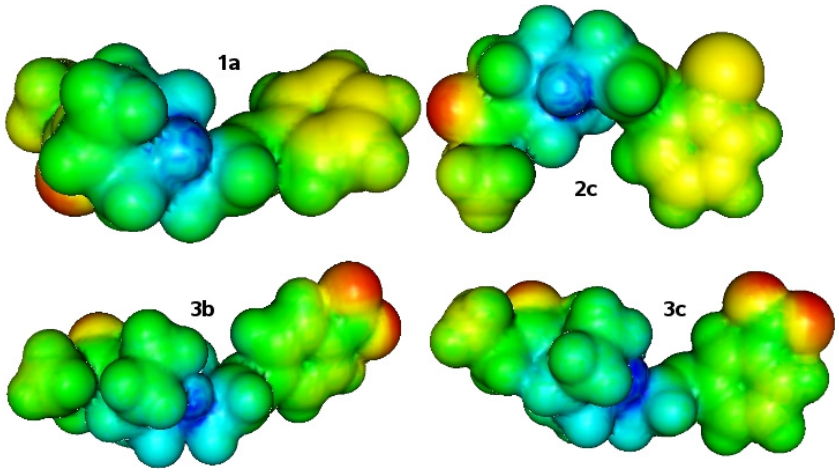

Fig. 8. Electrostatic potential maps of **1a**, **2c**, **3b**, and **3c**. Color online.

Table 3. Experimental dissociation constants[a] K_i, binding energy (E_b), the distance Asp147–ligand (COO–HN+) and dipole moments for the Series 3 and 4.

		Ar	K_i^a (nM)	E_b (kcal/mol)	Distance (Å)	Dipole moment (D)
	a	C_6H_5	10 ± 2	−8.25	4.29	5.21
	b	4-NO_2–C_6H_4	23 ± 0.6	−8.29	4.54	13.66
3	c	3-NO_2–C_6H_4	40 ± 2.9	−8.71	4.18	11.98
	d	3-Cl–C_6H_4	36.6 ± 2	−8.74	4.33	8.92
	e	1-Naphthyl	180 ± 117	−10.0	4.44	7.63
	a	C_6H_5	7 ± 0.6	−8.72	2.33	4.36
	b	4-NO_2–C_6H_4	475 ± 14	−8.75	2.50	11.24
4	c	3-NO_2–C_6H_4	20 ± 1.2	−9.05	2.34	13.01
	d	3-Cl–C_6H_4	13 ± 0.6	−9.37	2.42	9.58
	Fentanyl		$3.97^b \pm 0.5$	−7.76	2.85	7.62

[a]Reference 7.
[b]Reference 13.

the correction constant was needed only for six of the compounds studied. Binding energy of the compounds **1i**, **2b**, **3b**, **3a**, and **4a** is underestimated by AutoDock energy calculations, and binding energy of the compounds **3e** and **4b** is overestimated by these calculations.

The correction constant f does not take the same values for all the abnormal cases. Its magnitude actually reflects the extent of deviation, it has the highest values for **3e** and **4b**, for instance.

The orientations of molecular dipole moments of **1i**, **3b**, **3a**, and **4a** are different. Actually the dipole moment orientation depends considerably on the orientation of *N*-propanoyl group. The compounds with underestimated or overestimated binding energies have disordered structures in this region.

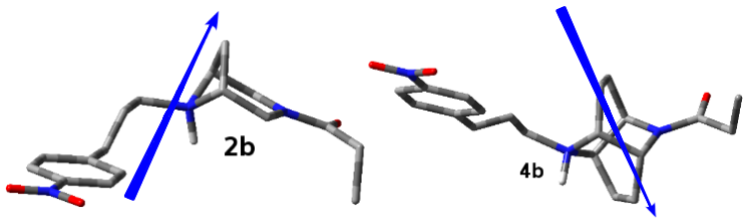

Fig. 9. Dipole moment of compounds **2b** and **4b**. Color online.

Rationalization for these discrepancies may be found in the analysis of the magnitude and direction of the molecular dipole moments. It was discussed, earlier in this paper, that the compounds of Series 2, bearing polar substituent on the phenyl ring, bind better to the receptor than the non-substituted compounds. According to the above regression analysis, binding of **2b** is underestimated by AutoDock calculations, but the binding of **4b** is overestimated. This difference may be explained by different direction of their dipole moments, Fig. 9, indicating different electron distribution around the molecules which may affect ligand–receptor interactions.

This difference in dipole moment orientation for **2b** and **4b** is due to different orientation of their *N*-propanoyl group in their preferred conformations in the binding pocket. *N*-propanoyl group in **2b** is oriented towards the region between TM3 and TM7 while *N*-propanoyl group in **4b** is between TM4 and TM7. Therefore important interactions of *N*-propanoyl group with receptor might have been overestimated for **4b** and underestimated for **2b** by AutoDock program. The other possibility might be the overestimated stabilization of fentanyl-like conformation of **4b**. The alternative conformation, positioned perpendicular to the direction of receptor transmembrane helices, is the most abundant in the cluster of **4b** conformations and it is only 0.6 kcal/mol higher in energy than the fentanyl-like conformation.

Additionally, perhaps the major reason for the weak binding of **2i** is its dipole moment, Fig. 10. While **2a** and **2f** have dipole moments differing in magnitude, but not considerably in direction relative to the best binding ligand, **2h**, of Series 2, the dipole moment of **2i** differs considerably in magnitude and in direction from **2h**.

Closer inspection of the structures and distribution of the electron density hints that there are several contributions that influence binding of ligands. Beside the size and orientation of dipole moment, the availability of protonated nitrogen for donation of hydrogen bonding and availability of amide oxygen as a hydrogen bond acceptor (both could be compromised by steric hindrance) are equally important.

Overlapping of the preferred conformations for the compounds reveals that the 20 compounds with good correlation in regression analysis overlap well in the binding pocket, and the oxygen atom of their *N*-propanoyl group is positioned in the same direction, towards the region between TM3 and TM7. On the other hand, the compound with underestimated and overestimated binding energies have more disordered structures, particularly in the region of the *N*-propanoyl group, indicating

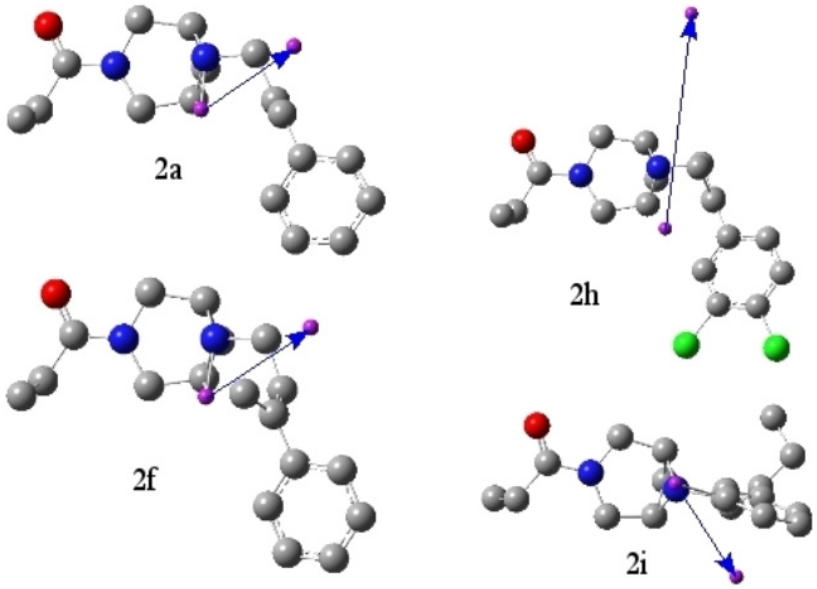

Fig. 10. Dipole moment orientations for the compounds **2a**, **2f**, **2i**, and **2h**. Color online.

that the reasons for the discrepancies may be different for each compound. One of the reasons may be the position of the amide oxygen relative to the potential proton donor of the receptor.

3. Experimental Section

All computations were performed using a Pentium IV computer, with AMD Athlon^{tm} 64 3000+ processor, on Linux Suse 10, and/or Windows XP, operating systems. The μ-receptor model used in this study was equal to the one used earlier[1] and described.[18] This receptor model is consistent with a vast sample of published biophysical and other experimental data. Without experimental data on structures of any of the opioid receptors, and considering the difference in rhodopsin structure determined in crystal state[19] and in solution,[20,21] we think that some receptor model may be considered as valid if the docking results obtained using this receptor, are in accordance with the experimental point mutation studies. The rigid receptor model was used. The automated flexible ligand docking experiments were made with AutoDock 3.0.5. program.[14] The most stable starting geometries of the ligands were obtained by systematic conformational search done with the semiempirical AM1 method of the Hyperchem program.[22] These geometries werefurtheroptimized using Gaussian03 program package[23] at the B3LYP level with the 6-311++G** basis set. Electrostatic potentials and the molecular dipole moments were calculated by the same method on the preferred docked conformation of the ligand molecules. Based on pK_a values of similar compounds[24] the arylalkyl substituted nitrogen of the

ligand was protonated before optimization. The $60 \times 60 \times 60$ grid was centered on one of the Asp147 oxygen atoms and the Lamarckian genetic algorithm (LGA) was used in all docking calculations. The docking process was performed with 250 LGA runs, the initial position of the ligand was random. The population was 50, the maximum number of generations was 27,000 and the maximum number of energy evaluations was 2.5106. The docking procedure provide the most probable ligand conformations and orientations in the binding pocket. The resultant ligand orientations and conformations were scored by binding energies (the cutoff value for the energies was 2 kcal/mol) and on the distance of Asp147 to the protonated nitrogen of a ligand (the cutoff value for the distance was 4.5 Å). Site-directed mutagenesis studies on μ-opioid receptor have shown[25] that Asp147 to Ala/Asn or Glu point mutations lead to diminished binding affinities of μ-opioid selective ligands, presumably due to the loss of a salt bridge, or an electrostatic interaction between the negatively charged Asp147 and the protonated nitrogen of the ligand.

4. Conclusions

The automated docking procedure was applied in order to determine the optimal position and orientation of the four series of bridged piperazine derivatives in the binding pocket of the μ-opioid receptor, and to compare these to the preferred conformation of fentanyl analogs in the same receptor. It was found that the preferred conformations of the bridged piperazine derivatives are similar to those of fentanyl. The major interactions with receptor are: the salt bridge of the protonated nitrogen to Asp147, the aromatic ring interactions with His297 (electrostatic in the case of polar substituents on a phenyl ring), and the propanoyl group interactions with His319 and Trp318. Like in the case of fentanyl analogs, steric interactions in the binding pocket have major impact on the binding of these compounds. Steric interactions of the methylenic bridge with Ile322 of TM7 are responsible for the difference in binding affinity of Series 1 and 2. Twisted conformation of the piperazine ring in Series 4 enables ethylenic bridge to avoid these interactions and to maintain binding affinity comparable to that of Series 2. Electrostatic potentials around all the ligands in Series 1–4 are similar, except in the aromatic region. Correlation of the experimentally determined binding constant to the calculated binding energies is good for 20 ligands studied. The deviation, for some of the ligands, from this correlation is due to the variations of the electron distribution around the aromatic ring upon substitution. These variations, described by *ab initio* quantum chemistry calculations are not reproduced by AutoDock program resulting in deviations in the calculated binding energies for some of the compounds.

Acknowledgments

The authors gratefully acknowledge the financial support of Serbian Ministry of Science; Grants 142010 and 142074.

References

1. Dosen-Micovic Lj, Ivanovic M and Micovic V, *Bioorg Med Chem* **14**: 2887, 2006.
2. WR Martin, *Pharmacol Rev* **35**:283, 1983.
3. Cometta-Morini C, Maguire PA and Loewe GD, *Mol Pharmacol* **41**:185, 1992.
4. Raynor K, Kong H, Chen Y, Yasuda K, Yu L, Bell GI and Reisine T, *Mol Pharmacol* **45**:330, 1994.
5. Cignarella G, Occelli E, Cristiani G, Paduano L and Testa E, *J Med Chem* **6**:764, 1963.
6. Cignarella G, Occelli E and Testa E, *J Med Chem* **8**:326, 1965.
7. Vianello P, Albinati A, Pinna GA, Lavecchia A, Marinelli L, Borea PA, Gessi S, Fadda P, Tronci S and Cignarella G, *J Med Chem* **43**:2115, 2000.
8. Pinna GA, Murineddu G, Curzu MM, Villa S, Vianello P, Borea PA, Gessi S, Toma L, Colombo D and Cignarella G, *Il Farmaco* **55**:553, 2000.
9. Pinna GA, Cignarella G, Loriga G, Murineddu G, Mussinu JM, Ruiu S, Fadda P and Fratta W, *Bioorg Med Chem* **10**:1929, 2002.
10. Loriga G, Manca I, Murineddu G, Chelucci G, Villa S, Gessi S, Toma L, Cignarella G and Pinna GA, *Bioorg Med Chem* **14**:676, 2006.
11. Barlocco D, Cignarella G, Greco G and Novellino E, *J Comput Aid Mol Des* **7**:557, 1993.
12. Mansour A, Taylor LP, Fine JL, Thompson RC, Hoversten MT, Mosberg HI, Watson SJ and Akil H, *J Neurochem* **68**:344, 1997.
13. Brine GA, Stark PA, Liu Y, Carroll FI, Singh P, Xu H and Rothman RB, *J Med Chem* **38**:1547, 1995.
14. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK and Olson AJ, *J Comput Chem* **19**:1639, 1998.
15. Molekel 5.3.0.6, Swiss National Supercomputing Centre, Manno, Switzerland.
16. Micovic V, Ivanovic MD and Dosen-Micovic Lj, *J Mol Mod* **15**:267, 2009.
17. Zhang Y, Sham YY, Rajamani R, Gao J and Portoghese PS, *Chem Bio Chem* **6**:853, 2005.
18. Subramanian G, Paterlini MG, Larson DL, Portoghese PS and Ferguson DM, *J Med Chem* **43**:381, 2000.
19. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M and Miyano M, *Science* **289**:739, 2000.
20. Yeagle PL, Denis C, Choi G, Alderfer JL, Albert AD, *Mol Vis* **6**:125, 2000.
21. Yeagle PL, Choi G and Albert AD, *Biochemistry-US* **40**:11932, 2001.
22. Hypercybe, 419 Phillip St, Waterloo, ON N2L 3X2, Canada.
23. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA, Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M,

Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, and Pople JA; *Gaussian 03, Revision C02*, Gaussian, Inc, Wallingford, CT, 2004.
24. Gilson MK, Gilson HSR and Potter MJ, *J Chem Inf Comput Sci*, **43**:1982, 2003.
25. Metzger TG, Paterlini MG, Ferguson DM and Portoghese PS, *J Med Chem*, **44**:857, 2001.

February 12, 2010 15:33 WSPC/178-JTCC 00556