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Modeling the ligand specific μ- and δ-opioid receptor conformations

MILAN SENĆANSKI[1#], MILOVAN D. IVANOVIĆ[2], SONJA VUČKOVIĆ[3] and LJILJANA DOŠEN-MIĆOVIĆ[2*]

[1]Innovation Center of the Faculty of Chemistry, University of Belgrade, [2]Faculty of Chemistry, University of Belgrade and Center for Chemistry, ICTM, Studentski trg 12–16, P. O. Box 51, 11158 Belgrade and [3]Department of Pharmacology, Clinical Pharmacology and Toxicology, School of Medicine, University of Belgrade, Belgrade, Serbia

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Abstract: An automated docking procedure was applied to study the binding of a series of μ- and δ-selective ligands to ligand-specific μ- and δ-opioid receptor models. Short-time molecular dynamic simulations were used to obtain ligand-specific μ- and δ-opioid receptors from arbitrarily chosen models of the active form of these receptors. The quality of receptor model depended on the molecular volume of the ligand in the receptor–ligand complex used in the molecular dynamic simulations. Within a series of ligands of similar size (volume), the results of ligand docking to the obtained ligand–specific receptor conformation were in agreement with point mutation studies. The correlation of the calculated and the experimentally determined binding energies was improved in relation to the initial receptor conformation.

Keywords: molecular modeling; opioid receptor; ligand–receptor interactions; docking simulation.

INTRODUCTION

Three types of opioid receptors: μ, δ and κ (or MOR, DOR and KOR, respectively), are involved in pain regulation by inhibiting neuronal adenylyl cyclase activity, but they also participate in the regulation of multiple other effectors.[1] There are also studies indicating that the δ-opioid receptor and/or its specific ligands are involved in cardioprotection.[2] Based on the results of pharmacological investigations,[3] opioid receptors have been subdivided into receptor subtypes, but the molecular basis of these subtypes remains to be resolved. Both, MOR and DOR have been cloned.[4–6] A hypothesis was made[7] that selective ligands might bind the same receptor but at different binding sites. The ability of

* Corresponding author. E-mail: lmicovic@chem.bg.ac.rs
# Serbian Chemical Society member.
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the selective ligands to bind their respective binding site would depend on the conformational state of the opioid receptor.[7,8]

The regions of MOR and DOR involved in ligand binding and mediation of receptor function have been identified: a) by the construction of chimeric receptors containing sequences of MOR or DOR receptors,[9–14] b) by site-directed mutagenesis of specific amino acid residues[15–21] and c) by the construction of truncated mutant receptors.[22–26]

In the case of DOR, it was found by point-mutation experiments[21] that Asp95Asn mutation reduced the affinity of many DOR-selective peptidic and non-peptidic agonists for DOR. Studies[5] on chimeric and site-mutagenized DOR established the importance of the third extracellular loop (EC3) arginine amino acids for the binding of peptidic ligands while non-peptidic ligands were unaffected. Site-directed mutagenesis experiments showed[16] that Asp128 contributes to the stabilization of the binding pocket. Mutations of amino acids Tyr129Phe, Trp274Ala and Tyr308Phe suggested that these aromatic residues might be a part of an opioid binding domain. Chimeric receptors and the alanine scan method were used[11] to show that Leu295, Val296, Val297 and Ala298 of the EC3 loop are important for the binding of DOR-selective ligands. The amino acids Trp284 (TM6) and Ser312 (TM7) are important but to a lesser degree. It was also found[12] that modifications of the second extracellular loop (EC2) had no effect on ligand binding. Other chimeric receptor studies[13] demonstrated that the sixth transmembrane domain (TM6) and the third extracellular loop (EC3) are absolutely critical for δ-opioid receptor selectivity. Point mutations emphasized the importance of Leu300, Ala298, Ala299 amino acids, and the unimportance of Arg291 for ligand binding. Val281 had moderate effect on ligand binding.[13] It was suggested[18] that interactions [Asp128(TM3)–Tyr308 (TM7) and Tyr129 (TM3)–His278(TM6)] maintain the δ-receptor in an inactive conformation. Point-mutation experiments[19] confirmed that Trp284 (TM6) is important for the binding of ligands to the DOR, and that amino acids at the extracellular end of TM6 and TM7 are key residues for δ-ligand selectivity. Binding studies[27] on a series of non-peptidic DOR ligands revealed that the binding site of these ligands is between TM5 and TM7 of the DOR. The binding pocket for most DOR-selective ligands is located close to the EC3 loop and between the transmembrane helices TM3, TM6 and TM7.

It was found by chimeric receptor studies[9] that the major determinant for the binding of MOR-selective alkaloids exists in the region spanning the transmembrane segments TM5 to TM7 of the MOR. Segments TM6 and TM7, as well as the third extra-cellular (E3) loop of the μ-receptor, were important[28] for the binding of agonists, such as morphine and fentanyl analogs, while the first extra-cellular loop (E1) was not important for MOR-selective non-peptide ligands. Site directed mutagenesis studies[15] indicated that Asp147 is the primary binding site in the MOR, as the counter ion for the protonated nitrogen of opioid ligands. The

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importance of charged residues in TM2 (Asp 114), TM3 (Asp147) and TM6 (His297) was evidenced,[15] as well as the modest involvement of N- and C-terminal domains in the ligand–receptor interactions. Site directed mutagenesis studies[29–31] established the importance of the TM7 residues Cys321, Tyr326, Trp318 and His319 and the TM3 residue Tyr148 for the activation of MOP. It was also established by mutation experiments,[32] that Asn230 of TM5 is involved in the binding of morphine.

Some experiments[33–36] suggested that important conformational changes in the receptor accompany ligand binding. Receptor states were discovered that can be activated without the effects of an agonist,[37] shifting thus the understanding of receptor activation from a model of inactive and active conformations of a receptor[38] to theories of multiple signaling states whereby each agonist could import its own unique active conformation.[39]

In this work, molecular dynamics were used to model ligand–specific receptor conformation from an arbitrary model of an active receptor. This ligand–specific receptor conformation is expected to provide more realistic geometry of a receptor–ligand complex obtained by ligand docking, and to give a better correlation between the calculated and experimentally determined binding energies within a series of closely related ligands. Molecular volume was the molecular descriptor which defined the closely related group of ligands.

COMPUTATIONAL METHODS

All computations were realized on an Intel(R) Core(TM)2 Quad CPU Q9650 at 3.00 GHz or Silicon Graphics® Octane2 workstation. The initial active forms of the MOR and DOR models were obtained from Prof. H. I. Mosberg. The receptor models were based on the structure of the β2-adrenergic receptor (PDB code 2rh1) and were modeled in a receptor–ligand complex with cyclic pentapeptide agonist ligands.[40] The series of agonists (Tables I, II and III) were at first docked to the obtained original models of the active forms of the MOR or DOR. The receptor models were initially treated as rigid. In the second docking experiment, the flexibility of the binding pocket amino acids was taken into account. The automated flexible ligand docking was performed by the Auto Docking program.[41]

TABLE I. Compounds **1**–**11**, ligands of the MOR

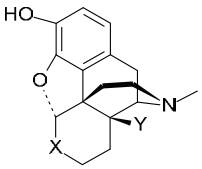
Structure	Compound	X	Y
	**1**	C=O	H
	2	CH–OH	H
	3	C=O	H

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TABLE I. Continued

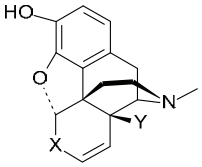
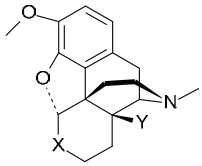
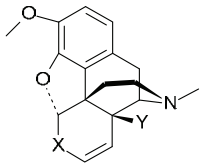
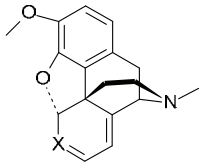
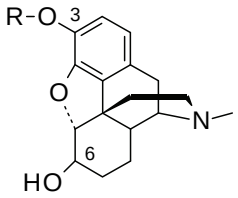
Structure	Compound	X	Y
	**4**	CH–OH	H
	**5**	C=O	OH
	6	C=O	H
	7	CH OH	OH
	8	CH–OH	OH
	**9**	C=O	H
	10	CH–OH	H
	**11**	–C···OMe	–

TABLE II. Morphine 3-esters, ligands of the MOR



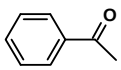
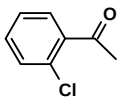
Compound	R	Name
4	H–	Morphine
12		3-Benzoylmorphine
13		3-(2-Chlorbenzoyl)-morphine

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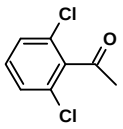
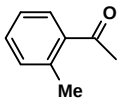
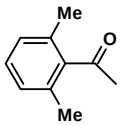
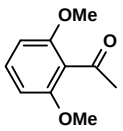
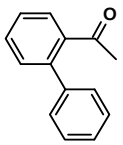
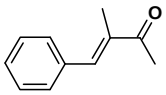
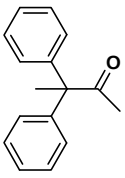
TABLE II. Continued

Compound	R	Name
14		3-(2,6-Chlorbenzoyl)-morphine
15		3-(2-Methylbenzoyl)-morphine
16		3-(2,6-Dimethylbenzoyl)-morphine
17		3-(2,6-Dimethoxybenzoyl)-morphine
18		3-(2-Phenylbenzoyl)-morphine
19		3-(α-Methylcinnamoyl)-morphine
20		3-Pivaloylmorphine
21		3-(2,2-Diphenylpropionyl)-morphine

In the subsequent docking experiments, the receptor models were optimized as follows. The selected ligands were manually docked to the original (obtained) receptor models using Accelrys Discovery Studio® (DS 2.5.5) visualizer. The orientation of the ligand inside the binding pocket was such as to: 1) provide interactions between the protonated nitrogen of the ligand and the important receptor residues (Asp 147 of TM3) in the MOR, or the corresponding Asp128 in the DOR; 2) form a hydrogen bond between the OH group of the ligand and the His297 residue of TM6 in the MOR (or His 278 in the DOR) and 3) provide close interactions with Trp318 of TM7 in the MOR (or Leu300 in the DOR). The resulting complex structure was refined by molecular mechanics with a conjugated gradient algorithm in vacuum

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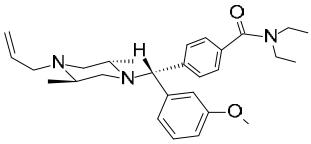
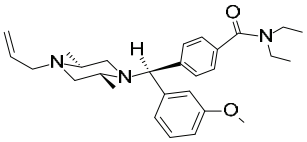
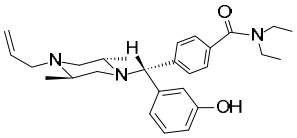
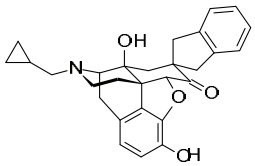
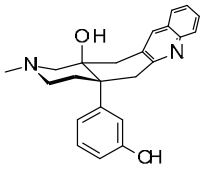
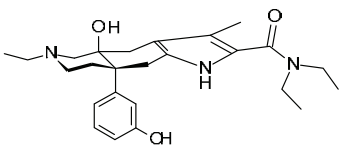
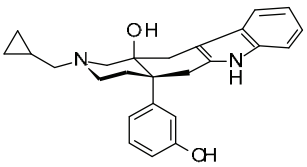
to a *RMS* gradient below 0.4 kJ mol^{-1} nm^{-1}, using CHARMm force field[42] with Momany–Rone charges.[43] A short molecular dynamics simulation (5 ps) with 0.1 fs time steps at 300 K was then applied. The lowest energy structure of the complex was selected and re-optimized providing a ligand–specific receptor conformation. Docking with Auto Dock Vina was repeated using the ligand–specific receptor conformation as a rigid target. Calculated values

TABLE III. Compounds **22**–**28**, ligands of the DOR

Compound name	Structure	Number
SNC80		**22**
SNC67		**23**
BW373U86		**24**
SIOM		**25**
TAN-67		**26**
SB219825		**27**
SB206848		**28**

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of the ligand binding energies were collected, and the structures of the resultant molecular complexes were analyzed.

The initial ligand geometries, with protonated ring nitrogen,[44] were built by application of HyperChem program[45] and subsequently optimized by the semi-empirical PM3 method of the same program. The protein receptor and the ligands were prepared with MGL tools.[46] A 30×30×30 grid was applied, with the grid center positioned between Asp147 and His297 in the MOR (Asp128 and His278 in the DOR). The docking process was performed with exhaustiveness equal to 100. The resultant ligand orientations and conformations were scored based on binding energies (the cut-off value for the energies was 8.4 kJ mol^{-1}), and by the formation of the salt bridge (shorter than 0.25 nm). The lowest binding energy conformations were further evaluated based on their distances to the important amino acids. The conformation with the lowest binding energy, with a salt bridge shorter than 0.25 nm and with maximum number of close contacts to the important amino acids is referred to as the preferred conformation.

RESULTS AND DISCUSSION

Automated docking of compounds **1**–**21** to the original MOR model,[40] and compounds **22**–**28** to the original DOR model[40] resulted in a similar docking pattern. The ligands **1**–**11** in the MOR, in the best orientation for possible salt bridge formation with Asp147, are presented in Fig. 1. However, all the ligands are located in the wide cavity near the extracellular ends of the transmembrane helices TM3, TM6 and TM7. The distance between Asp147 (Asp128 in the DOR) and the protonated nitrogen of the ligand ($O^{-}\cdots H–N^{+}$) was at least 0.6 nm, Fig. 1. Therefore, the key interaction for ligand binding and activation, which is be-

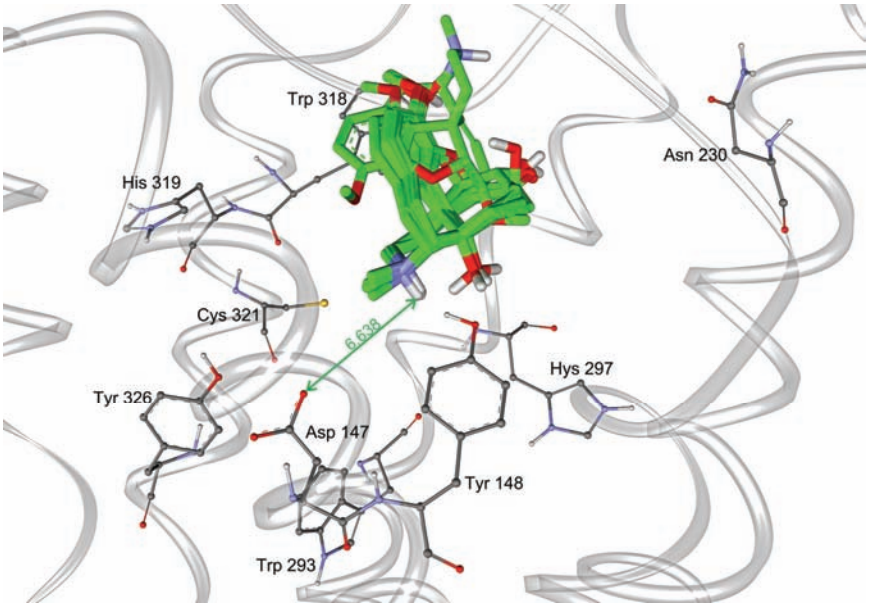

Fig. 1. Typical positions of the compounds **1**–**11** in the binding pocket of the initial MOR.

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lieved to be the salt bridge formation, was not predicted by the model. The same was true for MOR ligands **12**–**21** and DOR ligands **22**–**28**. The area in the original receptors, around Asp147 (Asp128 in the DOR) was too narrow to accommodate even small ligands and to allow the formation of the salt bridge. The use of receptors with flexible amino acids within the binding pocket did not improve the model. Moreover, the correlation of the calculated binding energies to the experimentally determined ones was poor, the correlation coefficient being –0.36 for the compounds **1**–**11,** Table IV. The experimentally determined and the calculated binding energies had opposite trends, Fig. 2.

This was expected since the original active receptors used in this work were modeled with voluminous molecules, *i.e.*, naphthylalanine-substituted cyclic pentapeptide[40] ligands. This is also in agreement with earlier results[47] which suggested that ligands bind to their ligand–specific receptor conformations.

TABLE IV. The studied MOR receptor agonist: molecular volume, experimentally determined K_i, binding energies, E_b(exp), and the binding energies calculated by Vina, E_b(calcd), for the initial MOR receptor, and the correlation coefficient between experimentally determined and calculated E_b values

No.	Ligand name	Molecular volume nm^3	K_i(exp) nM	E_b(exp)[a] kJ mol^{-1}	E_b(calcd) kJ mol^{-1}
1	Hydromorphone	0.754	0.26[b]	–54.7	–34.3
2	Dihydromorphine	0.758	0.39[b]	–53.7	–34.3
3	Oxymorphone	0.765	0.36[c]	–53.9	–33.9
4	Morphine	0.765	1.8[d]	–49.9	–33.5
5	Oxycodone	0.811	16[e]	–44.5	–34.7
6	Hydrocodone	0.801	19.8[c]	–44.0	–34.7
7	β-Oxycodol	0.829	33.7[c]	–42.6	–33.1
8	α-Oxycodol	0.814	187[c]	–38.4	–35.2
9	Codeinone	0.790	459[f]	–36.2	–33.9
10	Codeine	0.811	6300[f]	–29.7	–34.7
11	Thebaine	0.847	7400[f]	–29.3	–34.7
Correlation coefficient R (R^2)		–	–	–0.36 (0.13)	–

[a]E_b(exp) = 2.48 ln ($10^{-9}K_i$); [b]ref. 48; [c]ref. 49; [d]ref. 50; [e]ref. 51; [f]ref. 52

An effort was made to find the ligand specific receptor conformations of the MOR and DOR for ligands **1**–**28** by short molecular dynamics simulation of the complex obtained by manual docking of a specific ligand to the original receptor model.[40] Since Auto Dock Vina does not take into account receptor flexibility, a unique receptor had to be modeled for a set of ligands of similar size. Therefore the MOR ligands were divided into two groups (group **1**–**11**, Table I, and group **12**–**21,** Table II) based on their molecular volume, and the DOR ligands formed the third group, Table III. At least one representative molecule of small volume in each group was manually docked to the original receptor in such an orientation that a salt bridge was formed between the protonated nitrogen of the ligand and

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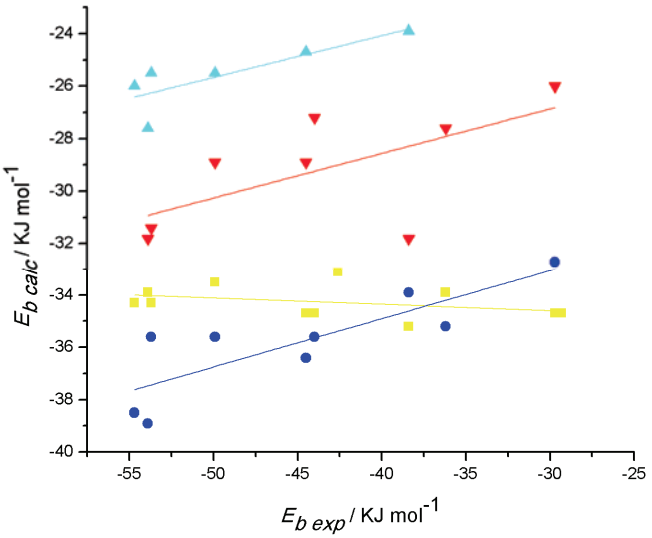

Fig. 2. Trends in the binding energies, E_b, of ligands **1–11** with different receptor models. Original MOR – ■, MOR optimized with ligand **4** – ●, MOR optimized with ligand **2** – ▼ and MOR optimized with ligand **3** – ▲.

the Asp147 (Asp 128) of the receptor, and hydrogen bond formation was enabled between the phenolic hydroxyl group and the His297 (His278) of the receptor. The structure of a complex was optimized and subsequently submitted to the short molecular dynamics run. The lowest energy conformation of the complex found by molecular dynamics was isolated and re-optimized. The ligand was removed and the optimized receptor model was used for docking. The collected binding energies E_b, and the resulting docking structures are presented in Table V, and Fig. 3, for the series **1**–**11**. In this group of ligands, compounds **1**–**6** and **10** were used for receptor optimization and docking in order to investigate the effect of ligand size on the quality of the receptor model. It can be seen in Table V and Fig. 2 that all ligands (**1**–**4**) with a small molecular volume improved the receptor model. This is illustrated by the increase of binding energy correlation coefficient from –0.36, for the initial MOR, to 0.83 for the receptor optimized with morphine (**4**) as the ligand. Only compound **1** insignificantly improved the correlation coefficient, just to a value of 0.22. The correlation coefficient for the larger molecules **5, 6** and **10** were either close to zero or had negative value. The best correlation of E_b was achieved with compounds **3** and **4** which, besides low molecular volume, had maximum number of hydrogen bond donors and acceptors. In addition, in the morphine (**4**) optimized receptor, the preferred ligand conformation was always the lowest energy conformation for ligands **1–10**, Fig. 3. Only thebaine (**11**) had no conformation with a salt bridge; it was in fact too big to enter the binding site created by the receptor optimization, which might be

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the reason for its weak binding to the MOR, K_i = 7400. Therefore, although the original binding site at the top of the receptor was still present and accommodated the ligands, complex optimization provided the receptor model with a new binding site where the ligands bind with higher affinity.

TABLE V. MOR receptor agonists: molecular volume, experimentally determined binding energies, E_b(exp), binding energies calculated by Vina, E_b(calcd), for the ligand specific MOR receptor

No.	Ligand name	Molecular volume nm^3	E_b(exp)[a] kJ mol^{-1}	E_b(calcd) / kJ mol^{-1}						
				Ligand used in complex optimization						
				4	**3**	**2**	**1**	**5**	**10**	**6**
1	Hydromorphone	0.754	–54.7	–38.5	–26.0	–	–31.4	–	–26.0	–36.8
2	Dihydromorphine	0.758	–53.7	–35.6	–25.5	–31.4	–31.4	–35.2	–26.0	–33.9
3	Oxymorphone	0.765	–53.9	–38.9	–27.6	–31.8	–33.9	–36.8	–26.8	–27.2
4	Morphine	0.765	–49.9	–35.6	–25.5	–28.9	–33.5	–36.4	–26.4	–34.7
5	Oxycodone	0.811	–44.5	–36.4	–24.7	–28.9	–33.5	–	–28.0	–28.5
6	Hydrocodone	0.801	–44.0	–35.6	–	–27.2	–32.2	–	–26.8	–37.3
7	*β*-Oxycodol	0.829	–42.6	–	–	–	–	–	–	—
8	*α*-Oxycodol	0.814	–38.4	–33.9	–23.9	–31.8	–32.2	–35.2	–27.6	–25.5
9	Codeinone	0.790	–36.2	–35.2	–	–27.6		–38.5	–28.5	
10	Codeine	0.811	–29.7	–32.7	–	–26.0	–32.2	–36.0	–29.3	–36.4
11	Thebaine	0.847	–29.3	–	–	–	–33.9	–40.2	–	–
Correlation coefficient *R* (R^2)		–	–	0.83 (0.69)	0.825 (0.68)	0.65 (0.43)	–	–	–	–

[a]E_b(exp) = 2.4775 ln ($10^{-9}K_i$); K_i from Table IV

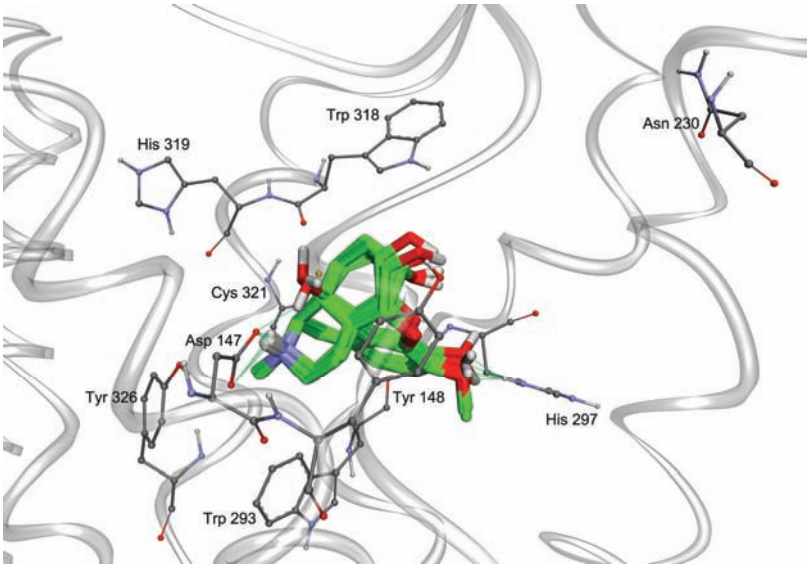

Fig. 3. Preferred conformations of compounds **1**–**10** in the binding pocket of the MOR optimized in the complex with morphine (**4**).

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Based on these data, it was concluded that morphine 3-esters, Table II, are too large to use in the morphine-specific receptor model; hence, they must have their own, ligand–specific receptor model derived from a complex optimized with a representative ligand for the series **12**–**21**. Docking of compounds **12**–**21** to the original MOR resulted in complex structures with ligands positioned high in the receptor, between helices TM2–TM7, similar to compounds **1**–**11**. None of the ligands had a salt bridge with Asp147. Ligand **15** was selected to be the representative ligand for receptor optimization because of its lowest molecular volume. The calculated and experimentally determined binding energies for compounds **12**–**21** are reported in Table VI. The correlation of E_b(calcd) and E_b(exp) was poor, the correlation coefficient being close to zero. However, lower binding energies were obtained and the qualitative picture was improved, Fig. 4. The low energy conformations of the ligands have a salt bridge with Asp147 and close interactions with other important amino acids of the binding pocket, such as a hydrogen bond to Tyr148 and electrostatic interaction with His297. Only for compounds **14**, **18** and **21,** the experimentally determined K_i values of which were high and thus indicated weak binding, were conformations with a salt bridge not among the low energy ligand conformations.

TABLE VI. MOR receptor ligands **12**–**21**: molecular volumes, experimentally determined K_i, experimentally determined binding energies, E_b(exp), binding energies calculated by Vina, E_b(calcd), for the ligand specific MOR receptor (optimized with ligand **15**)

Ligand	Molecular volume, nm^3	K_i(exp)[a] / nM	E_b(exp)[b] kJ mol^{-1}	E_b(calcd) / kJ mol^{-1}	
				Initial receptor	Receptor optimized with ligand **15**
12	1.065	29	–43.1	–	–46.0
13	1.106	160	–38.9	–	–45.2
14	1.132	8200	–28.9	–	–42.3
15	1.102	230	–37.7	–44.0	–47.3
16	1.139	320	–36.8	–	–45.6
17	1.209	360	–36.8	–	–40.6
18	1.238	2600	–31.8	–	–45.6
19	1.197	69	–41.0	–	–46.0
20	1.038	52	–41.4	–	–38.1
21	1.350	790	–34.7	–	–46.9
Correlation coefficient R (R^2)	–	–	–	–	0.02 (0.0004)

[a]Ref. 53; [b]E_b(exp) = 2.4775 ln ($10^{-9}K_i$)

The selected DOR agonists are presented in Table III and the resultant binding energies and molecular properties are reported in Table VII. Docking to the original DOR followed the same trends as the docking of the MOR ligands to the MOR. The ligands were positioned high in the receptor and none of the ligand conformations had a salt bridge with Asp 128. The correlation of the ex-

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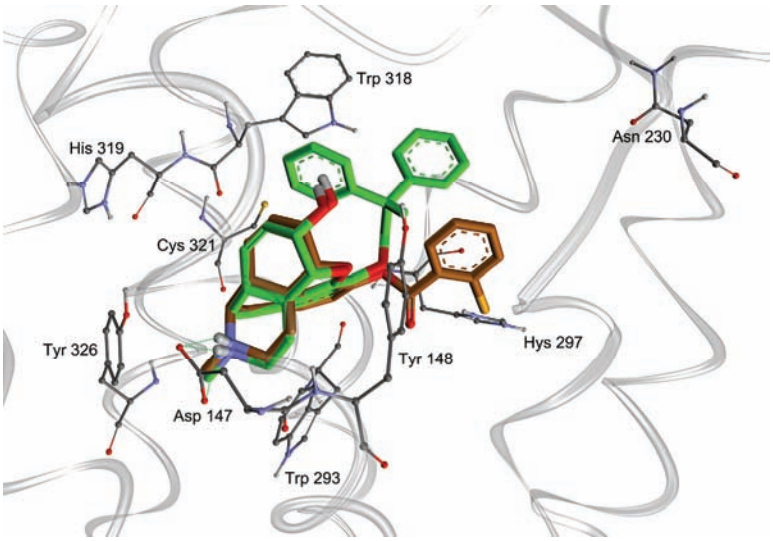

Fig. 4. Preferred conformations of compounds **13** and **21** in the binding pocket of the MOR optimized with ligand **15**.

TABLE VII. DOR receptor ligands **22**–**28**: molecular volumes, experimentally determined K_i, experimentally determined binding energies, E_b(exp), binding energies calculated by Vina, E_b(calcd), for the initial receptor, and for the ligand specific DOR (optimized with ligand **26**)

Ligand	Molecular volume, nm^3	K_i(exp)[a] / nM	E_b(exp)[b] kJ mol^{-1}	E_b(calcd) / kJ mol^{-1}	
				Initial receptor	Receptor optimized with ligand **26**
22	1.377	0.818	–51.9	–33.1	–38.9
23	1.378	218	–38.1	–35.6	–33.1
24	1.319	0.086	–57.3	–34.3	–38.1
25	1.155	4.1	–47.7	–49.4	–38.1
26	1.000	0.649	–52.3	–36.8	–39.3
27	1.213	0.6	–52.7	–34.3	–33.5
28	1.096	1.7	–49.8	–39.3	–36.8
Correlation coefficient R (R^2)	–	–	–	0.23 (0.053)	0.56 0.32)

[a]Ref. 47 and references therein; [b]E_b(exp) = 2.4775 ln ($10^{-9}K_i$)

perimentally determined E_b values and the calculated ones was low, close to zero, Fig. 5. Ligand **26** with its low molecular volume was used for optimization of the receptor to its ligand specific conformation. The docking results with the optimized receptor are also reported in Table VII. The ligand preferred conformations whose binding energies are reported, are the ones with low binding energies (within 8.4 kJ mol^{-1} above the global minimum), with a salt bridge shorter than 0.25 nm and the maximum number of interactions with other important amino acids of the binding pocket, Fig. 6. The trend of the binding energies for

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the DOR ligands **22**–**28** showed improvement. While the correlation coefficient was close to zero, as in the case of the original receptor, it was corrected to 0.57 (R^2 = 0.33) for the optimized receptor, which confirms the necessity of using ligand specific receptor conformations for docking.

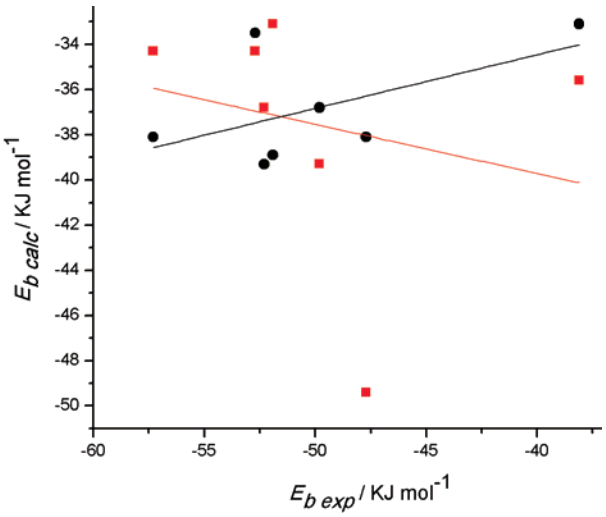

Fig. 5. Trends in the binding energies, E_b, of ligands **22**–**28** to the DOR model optimized with ligand **15** – ● and original receptor – ■.

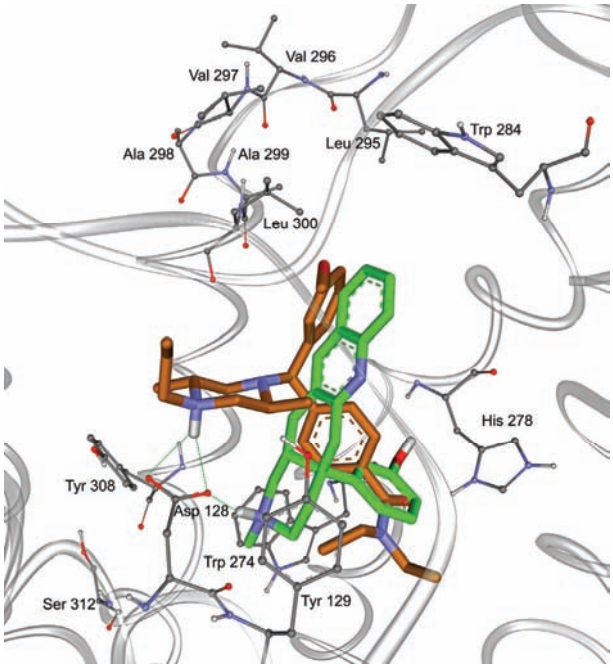

Fig. 6. The preferred conformations of ligands **22** and **26** in the DOR model optimized with compound **26**.

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CONCLUSIONS

An automated docking procedure, combined with a short molecular dynamics simulation, was applied in order to determine ligand specific receptor conformations for MOR and DOR receptors. It was established that one receptor conformation cannot reproduce correctly the binding of different ligands. Instead, a method is proposed to model a ligand specific receptor conformation for a series of ligands of similar size. The method consists of using an arbitrary model of an active receptor, manually docking a ligand representative of a series of ligands of similar size to the receptor, following the existing knowledge of key ligand–receptor interactions; optimizing the receptor–ligand complex; allowing the receptor and the ligand to adjust their conformations in a short molecular dynamics run; extracting the most stable receptor–ligand complex structure and re-optimizing its geometry. The receptor models obtained in this way were used for docking of different MOR and DOR ligands. They improved the resulting receptor ligand complexes both qualitatively (increased number of close interactions with amino acids important for binding, as found by point mutation studies) and quantitatively (improved correlation between the experimentally measured and calculated binding energies).

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ИЗВОД

МОДЕЛОВАЊЕ КОНФОРМАЦИЈА μ- И δ-ОПИОИДНИХ РЕЦЕПТОРА СПЕЦИФИЧНИХ ЗА ПОЈЕДИНЕ ЛИГАНДЕ

МИЛАН СЕНЋАНСКИ[1], МИЛОВАН Д. ИВАНОВИЋ[2], СОЊА ВУЧКОВИЋ[3] и ЉИЉАНА ДОШЕН-МИЋОВИЋ[2]

[1]Иновациони центар Хемијског факултета, Универзитет у Београду, [2]Хемијски факултет, Универзитет у Београду и Центар за хемију, ИХТМ, Студентски трг 12–16, п. пр. 51, 11158 Београд и [3]Институт за фармакологију, клиничку фармакологију и токсикологију, Медицински факултет, Универзитет у Београду

Рачунска метода аутоматизованог докирања примењена је на везивање серије лиганада, специфичних за μ- и δ-рецепторе, за моделе ових рецептора. Краткотрајна молекулско динамичка симулација је коришћена за добијање конформација ових рецептора које су специфичне за поједине лиганде, полазећи од случајно изабраног модела активираног рецептора. Квалитет овако добијеног модела рецептора зависи од молекулске запремине лиганда у лиганд–рецептор комплексу коришћеног у молекулско-динамичкој симулацији. За серију лиганда сличне запремине резултати докирања су у складу са експерименталним резлтатима мутација аминокиселина у рецептору. Корелација израчунатих и мерених енергија везивања је побољшана у односу на резултате добијене са полазном конформацијом рецептора.

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