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Original scientific paper

Structural requirements for ligands of the δ-opioid receptor

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Abstract: The δ-opioid receptor is sensitive to ligand geometry. In order to assist the synthesis of new δ-selective opioid ligands, the structure elements of δ-selective opioid ligands necessary for their effective binding were investigated. The automated docking procedure with a flexible ligand was used to simulate the binding of 17 δ-selective ligands to the δ-receptor. It was found that voluminous N-alkyl groups reduce the binding potency of naltrindole derivatives by preventing the ligands from adopting the preferred conformation in the receptor. This was confirmed by enantiospecific binding of chiral compounds where only one enantiomer adopts the naltrindole-like preferred conformation in the binding pocket. Voluminous groups replacing the hydroxyl group in the 3-hydroxybenzyl fragment of naltrindole analogs reduce the binding potency due to unfavorable steric interactions with the receptor. The two diastereoisomers of the potent δ-opioid ligand SNC80 confirmed the preferred binding conformation and the major receptor–ligand interactions.

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

INTRODUCTION

The δ-opioid receptor is an especially attractive target for the development of new drugs for the control of pain. Compared to other opioid or opioid-like receptors, δ-opioid selective drugs have some advantages, including: greater relief of naturopathic pain, reduced respiratory depression and constipation, and reduced potential for the development of physical dependence.[1] Only one δ receptor has been cloned to date;[1,2] but several models of this receptor are available in the literature.[3] These models are consistent with a vast sample of published biophysical and other experimental data[3] but experimental data on the structure of any of the opioid receptors are unavailable. Considering the possible effects of

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different media (the difference in the rhodopsine structure determined in the crystal state[4] and in solution[5]) and the obvious importance of the exact position of the amino acid residues[6] which are different in different proteins, the available models may be considered reliable opioid receptor representatives if they are capable of reproducing point mutation studies and other experimental data.

There is experimental evidence that interaction of peptidic ligands with receptors are different from that of small ligands.[7] Since small organic molecules as possible ligands of the δ-opioid receptor were our target, this study is limited to non-peptidic ligands of the δ-receptor, Table I.

The region of a δ-opioid receptor involved in ligand binding and mediation of receptor function were identified by: a) construction of chimeric receptors containing sequences from μ- or κ-opioid receptors,[8] b) site-directed mutagenesis of specific amino acid residues[9] and c) construction of truncated mutant receptors.[10]

The site-directed mutagenesis experiments[9a] showed that Asp128 does not participate in the formation of a salt bridge between a ligand and the receptor, but it does contribute to the stabilization of the binding pocket. Some highly selective non-peptidic δ-ligands were moderately affected[9b] by mutations of the amino acids: Tyr129Phe, Trp274Ala and Tyr308Phe, indicating that these aromatic residues might be a part of the opioid binding domain. Chimeric receptors and the alanine scan method were used[8b] to show that Val296 and Val297 of the EC3 loop are important for the binding of the δ-selective ligand SNC80. Leu295 and Ala298 of EC3 were important for the binding of naltrindole. The amino acids Trp284 (TM6 – transmembrane helix 6) and Ser312 (TM7 – trans membrane helix 7) were important for both compounds, although to a lesser degree. Point mutations performed on a mutant receptor emphasized the importance of Leu300, Ala298, Ala299 amino acids. Val281 had a moderate effect on ligand binding.[8d] It was found[9c] that Tyr308Phe mutation increased the binding. This amino acid, together with His278 (TM6) was suggested[9c] to participate in interactions [Asp128 (TM3)–Tyr308 (TM7) and Tyr129 (TM3)–His278 (TM6)] that maintain the δ-receptor in an inactive conformation. The model of the δ-opioid receptor showing important amino acids is presented in Fig. 1.

In this study, the docking of a series of δ-opioid-selective ligands to a model of the human δ-receptor available in literature[3a] is reported. It is the model of an active form of the receptor, although the entire concept of active and inactive receptors has recently been questioned. Contrary to some earlier findings,[11] recent X-ray studies[12] on rhodopsine demonstrated that the transformation from the ground state to the photoactivated intermediate state involved minor changes in the receptor structure. It was suggested[12] that the rigid inactive conformation of the receptor becomes more relaxed upon activation. It was also suggested[12] that one receptor model may be used in docking calculations of both agonists and antagonists.

TABLE I. Names and structures of the compounds **1–17**

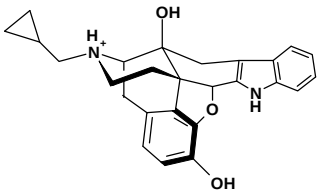
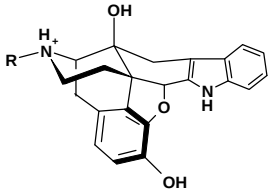
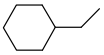
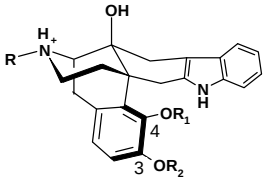
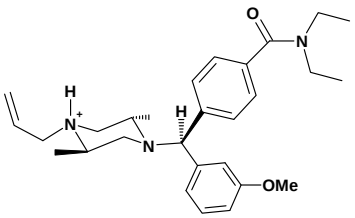
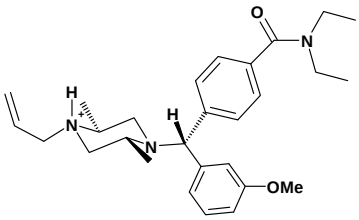
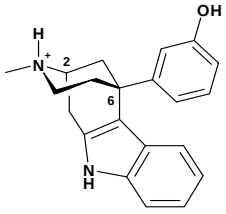
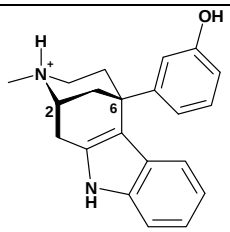
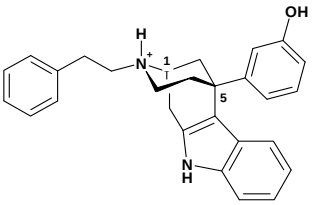
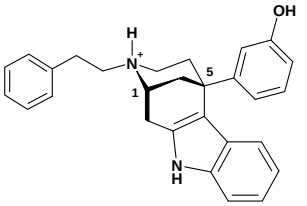
Compound	Name	Structure
1	Naltrindole, NTI	
	Naltrindole derivatives	
2	Oxymorphindole, R: CH_3	
3	R: 	
4	R: 	
5	R: 	
6	R:  ; R_1: CH_3; R_2: H	
7	R:  ; R_1: CH_3; R_2: CH_3	
8	R:  ; R_1: H; R_2: CH_3	
9	R: CH_3; R_1: CH_3; R_2: H	
10	R: CH_3; R_1: CH_3; R_2: CH_3	
11	R: CH_3; R_1: H; R_2: CH_3	
12	(+)-4-[(*αS*)-*α*-((2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide	
13	(–)-4-[(*αR*)-*α*-((2*R*,5*S*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide	
14	(+)-3-[(2*S*,6*R*)-1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-6*H*-azocino[4,5-*b*]indol-6-yl]phenol	

TABLE I. Continued

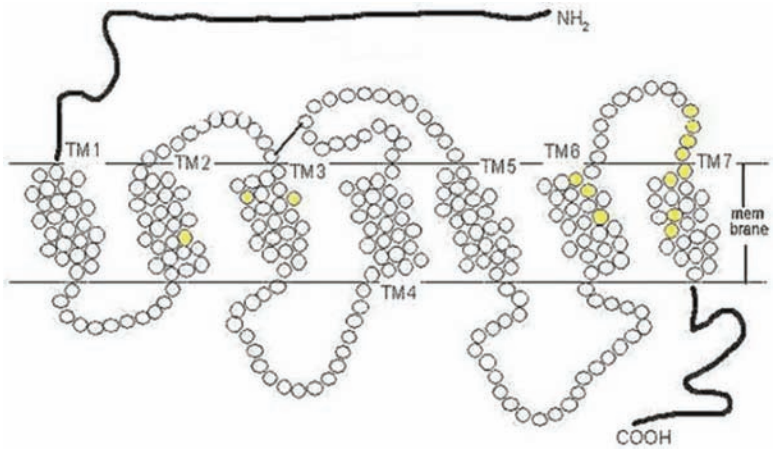
Compound	Name	Structure
15	(–)-3-[(2*R*,6*S*)-1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-6*H*-azocino[4,5-*b*]-indol-6-yl]phenol	
16	(+)-3-[(2S,6R)-1,2,3,4,5,11-Hexahydro-3-(2-phenylethyl)-2,6-methano-6*H*-azocino[4,5-*b*]indol-6-yl]phenol	
17	(–)-3-[(2*R*,6*S*)-1,2,3,4,5,11-Hexahydro-3-(2-phenylethyl)-2,6-methano-6*H*-azocino[4,5-*b*]indol-6-yl]phenol	

The goal of the present work was to find and compare the binding conformations of δ-selective ligands and to identify the structural fragments the modifications of which may increase the binding and perhaps activation of a δ-receptor.

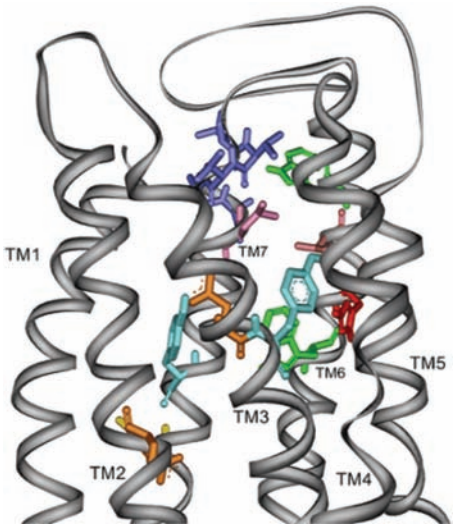
COMPUTATIONAL METHODS

All computations were performed using a P4/Celeron at 1.5 GHz. The employed δ-receptor model was taken from the literature.[3a] The receptor model was treated as rigid. The automated flexible ligand docking experiments were realized with the AutoDock 3.0.5. program.[13] The starting geometries, with a protonated ring nitrogen,[14] were built using the HyperChem program[15] and subsequently optimized using the semi-empirical AM1 method of the same program. The 60×60×60 grid was centered on one of the Asp128 oxygen atoms. The Lamarckian genetic algorithm (LGA) was used in all docking calculations. The docking process was performed in 250 LGA runs; the initial position of the ligand was random. The population was 50, the maximum number of generations was 27000 and the maximum number of energy evaluations was 2.5×10^6. The resultant ligand orientations and conformations were scored based on the binding energies (the cutoff value for the energies was 16.8 kJ/mol), and they were further evaluated based on the vicinity to important amino acids, found experimentally to be located in the binding site of the δ-selective non peptidic ligands. The clusters were ranked in order of increasing binding energy. The lowest binding energy conformations of all the selected clusters were analyzed in terms of their distances to the important amino acids. The

lowest binding energy conformation with the maximal number of close contacts to the important amino acids is referred to as the preferred conformation.



(a)



(b)

Fig. 1. a) Serpentine model of the δ-opioid receptor. Important amino acids are in yellow (mutagenesis experiments); b) 3D model of the δ-opioid receptor with important amino acids.

RESULTS AND DISCUSSION

Despite apparent structural differences, all the studied δ-selective compounds have similar 3D geometries (Table I), consisting of a protonated piperidine (or piperazine) ring and two aromatic rings. These three structural elements may adopt similar spatial positions in different ligand-receptor complexes, but may, as well, occupy different positions and orientations within the receptor.

Automated docking of compound **1** to the δ-opioid receptor model resulted in several plausible docking orientations and conformations for the ligand. The resulting ligand orientations and conformations were scored based on the values of the binding energies and by the number of close contacts to the receptor amino acids known to form the binding pocket within the δ-receptor, Fig. 1. Based on these criteria, the preferred conformation of compound **1** in the binding pocket of the δ-receptor model has a binding energy (E_b = –40.3 kJ/mol) 6.3 kJ/mol above the global minimum conformation. Its orientation is very similar to the one proposed earlier, and follows the "message–address concept",[16] Figs. 2 and 3.

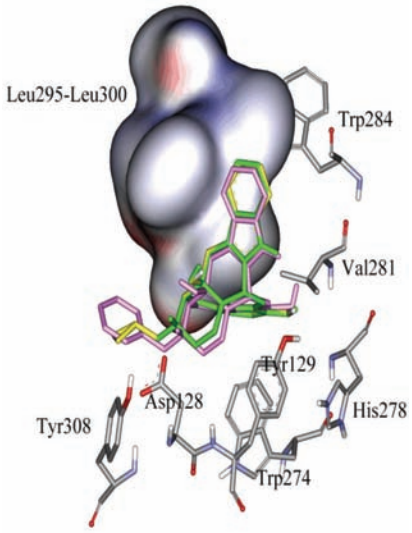


Fig. 2. Ligands: **1** (yellow), **2** (green) and **3** (pink) in the binding pocket.

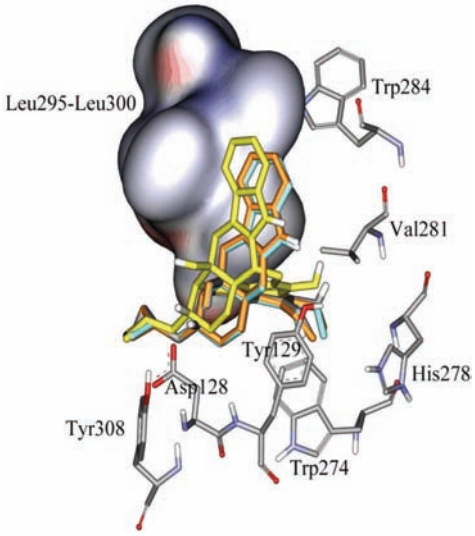


Fig. 3. Ligands: **1** (yellow), **6** (gray), **7** (orange) and **8** (blue) in the binding pocket.

The protonated piperidine and the phenolic component form the "message" moiety related to ligand binding and the indolic component represents the "address" moiety and determines ligand selectivity besides binding. According to the docking results, the "message" moiety interacts (within 0.4 nm) with Asp128, through salt bridge formation ($^{+}$NH···O^{-} distance 0.27 nm), with Tyr129 of TM3 and Tyr308 of TM7 (all known from point mutation studied[9b]), and with Gln105 and Leu102 of TM2. The major interaction with Gln105 is hydrogen bond formation to the 14-hydroxy group of **1** (NH···O distance is 0.22 nm). It was found earlier[17a] that the 14-hydroxy group plays an important role in δ-selectivity and the binding potency of some δ-selective ligands. On the other hand, it is generally believed[16] that the residues at the top of TM6 and TM7 form a hydrophobic pocket to accommodate the indolic moiety of **1**. According to the docking results

presented here, this hydrophobic pocket is formed by Val297, Val296 (EC3) and Leu300 (TM7) (known from point-mutation studies) and by the Ala195 and Val196 of EC2.

The four derivatives of **1**, ligands **2**–**5**, differ in their experimentally determined binding constants, Table II. While the *N*-methyl derivative, **2**, has a binding affinity towards the δ-receptor comparable to that of **1**, the *N*-benzyl, *N*-cyclohexylmethyl and *N*-cyclohexylethyl derivatives (**3**, **4** and **5**, respectively) are far less efficient. The docking results are in agreement with the experimental findings. The preferred conformations of **2** and **3** overlap in the binding pocket with the preferred conformation of **1**, Fig. 2, but their binding energies (–38.4 and –35.4 kJ/mol, respectively) are high relative to that of **1**. The other two derivatives, **4** and **5**, cannot even adopt the preferred conformation characteristic for naltrindoles. It seems that the size of the pocket, surrounded by the amino-acid residues Leu102 and Gln105 of TM2 and Ile304 and Tyr308 of TM7 is too small to accommodate voluminous alkyl groups. Therefore any modification of the *N*-alkyl part of the ligand will be limited by the size of the modified group.

TABLE II. Experimental binding constants, K_i and IC_{50}

Compound	δ-Receptor	
	K_i / nM	IC_{50} / nM
1	0.22±0.13[a], 0.04[b]	–
2	0.9±0.2[a]	–
3	115±32[b]	–
4	94.5±13.6[b]	–
5	181±35[b]	–
6	0.40±0.3[a]	–
7	19.0±2.0[a]	–
8	21.8±7.0[a]	–
9	4.5±0.5[a]	–
10	41.0±4.0[a]	–
11	218±33[a]	–
12	15.2[c]	56.5±3.10[d]
13	4.12[c]	3.50±0.39[d]
14	–	660.1±160.2[e]
15	–	5.6±1.5[e]
16	–	>1000[e]
17	–	6.2±0.6[e]

[a]Ref.17; [b]Ref.18; [c]Ref.19; [d]Ref.20; [e]Ref.21

The other group of studied compounds was the ligands obtained by the opening of the 4,5-bridge in either **1** or **2**. The in this way created 3-hydroxy-4--methoxyindomorphinans (**6** and **9**), 3,4-dimethoxyindomorphinans (**7** and **10**) and 3-methoxy-4-hydroxyindomorphinans (**8** and **11**) show reduced binding potency towards the δ-receptor compared to **1** and **2**. This reduction in binding po-

tency is small for **6** and **9** but increased in the case of the 3-methoxy compounds. Differences in affinity at a δ-receptor were earlier assigned[17b] to a shift in the relative position of the indole. The present docking study showed that this shift is small, less than 0.15 nm, Fig. 3. The reduced binding affinity may also be due to the presence of the 4-hydroxyl and the lack of a 3-hydroxyl group.[17b] However, the preferred conformations of compounds **6**–**11** do not allow a 3-hydroxy group to form any hydrogen bond or other important electrostatic interaction. When the 3-hydroxyl group is replaced by a methoxy group, a steric clash occurs with Ile215 of TM5. Therefore, the reduced affinity of the ligands **7**, **8**, **10** and **11** for the δ-receptor may be due to steric interactions. This suggests that voluminous groups at the position 3 are unlikely to improve binding properties of indolomorphinans.

The study of stereoisomers and their interactions with a particular receptor may be very useful. Two pairs of enantiomers were studied: the (+) and (–) forms of 3-[(2*S*,6*R*)-1,2,3,4,5,11-hexahydro-3-methyl-2,6-methano-6*H*-azocino[4,5-*b*]indol-6-yl]phenol (compounds **14** and **15**), and their 2-phenylethyl analogs **16** and **17**. The two enantiomers in both compounds have noticeably different binding affinities towards the δ-receptor.[21] The (–) form (**15** and **17)** in both compounds binds better to the receptor. According to docking studies, this is the consequence of different preferred conformations of the (+) and (–) ligands in the binding pocket of the receptor. Compound **15** overlaps **1**, Fig. 4, and has most of the major interactions with the receptor: the protonated nitrogen forms a salt bridge with Asp128, the phenolic group interacts with the amino acids at the beginning of the EC3 loop, while the aromatic (indolic) group interacts with Tyr129 of TM3 and with Thr211 and Ile215 of TM5. Compound **14** cannot adopt this preferred conformation. The picture is less clear for compounds **16** and **17**, where the flexible molecule can adopt a number of different conformations. The only explanation for the higher binding potency of **17** over **16** is the conformation of **17** which partially overlaps the preferred conformation of compound **13**, known to have high binding potency, Fig. 5, Table II. Compound **16** cannot adopt a similar conformation. The noticeable difference in binding affinity towards the opioid δ-receptor of SNC80, (+)-4-[(αR)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide and its enantiomer[19] was explained earlier[22] by the inability of the enantiomer to achieve the three important interactions with the receptor. SNC80 (K_i = 0.181 nM) has a higher affinity for the cloned human δ-receptor than its enantiomer (K_i = 218 nM).[19] On the contrary, the α*S*,2*S*,5*R* diastereoisomer of SNC80, compound **12**, has lower affinity for the cloned human δ-receptor than the enantiomer, **13**. According to the docking results, this may also be explained by the classic "three point theory". Compound **13** in its preferred conformation has a protonated nitrogen close to Asp128 (TM3) and forms a salt bridge. The NEt_2 groups are located close in

the hydrophobic pocket at the beginning of EC3. The 3-methoxybenzyl group points toward Trp274 and His278 of TM6, Fig. 5. These ligand interactions with TM6 may be the major difference between antagonists and agonists and the key process in receptor activation. It was suggested earlier that movement of helices TM3, TM6 and TM7 is essential for activation of rhodopsin[23] and the human δ-receptor.[3b] It was also suggested[24] that the 3-methoxy group was metabolized to a 3-hydroxyl group and that the analgesic activity was performed partially through this hydroxyl group.[24] Compound **12**, on the contrary, cannot adopt this favorable conformation and has only weak interactions with the receptor, about 100 times weaker that SNC80.

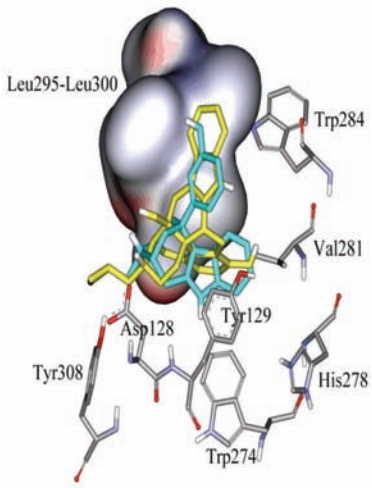


Fig. 4. Ligands **1** (yellow) and **15** (blue) in the binding pocket.

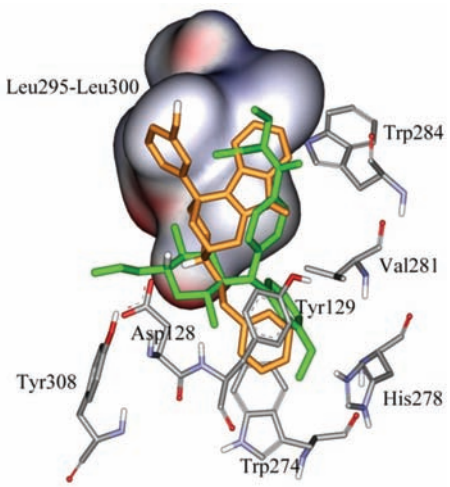


Fig. 5 Ligands **13** (green) and **17** (orange) in the binding pocket.

CONCLUSIONS

An automated docking procedure was applied in order to determine the preferred conformations of a series of δ-opioid receptor selective ligands in the binding pocket of the receptor. The quality of the receptor-ligand complexes was estimated based on their binding energies and the ability to reproduce point mutation experimental data. The following results are believed to assist in synthesis of new δ-selective ligands with a high binding potency. The preferred naltrindole conformation was found to be similar to the one suggested earlier,[16] Fig. 2. The voluminous N-alkyl groups in compounds **1**–**5** are expected to reduce the binding potency by preventing the ligand from adopting the preferred conformation in the binding pocket. Voluminous groups replacing the hydroxyl group in the 3-hydroxybenzyl fragment of naltrindole analogs, **6**–**11**, reduce the binding potency due to unfavorable steric interactions with the receptor. Ligand interactions with

the amino acids at the beginning of EC3 are essential for ligand binding. The docking of two diastereoisomers, **12** and **13**, of the potent *δ*-opioid ligand SNC80 confirmed the preferred binding conformation of this compound proposed earlier,[22] and the major ligand–receptor interactions: protonated nitrogen interacting with Asp128 of TM3, hydrophobic groups interacting with EC3 and the phenolic group interacting with TM6 and TM5.

Acknowledgments. This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia.

ИЗВОД

НЕОПХОДНИ ЕЛЕМЕНТИ СТРУКТУРЕ ЗА ЛИГАНДЕ *δ*-ОПИОИДНОГ РЕЦЕПТОРА

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δ-Опиоидни рецептор је осетљив на геометрију лиганада. Да би се олакшала синтеза нових *δ*-селективних опиоидних лиганада, у раду су испитивани елементи њихове структуре који су неопходни за ефикасно везивање. Коришћен је аутоматизовани докинг поступак са флексибилним лигандом да би се симулирало везивање 17 *δ*-селективних једињења за *δ*-рецептор. Нађено је да волуминозне N-алкил групе смањују ефикасност везивања деривата налтриндола тако што спречавају ова једињења да заузму конформацију погодну за везивање. Ово је потврђено енантиоспецифичним везивањем хиралних једињења код којих само један енантиомер има оријентацију сличну налтриндолу у везујућем месту. Волуминозне групе које замењују 3-хидрокси групу код аналога налтриндола смањују ефикасност везивања због неповољних стерних интеракција са рецептором. Начин везивања два дијастереоизомера ефикасног *δ*-опиоидног лиганда SNC80, потврђује најбољу конформацију за везивање и најважније рецептор–лиганд интеракције.

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