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ORIGINAL PAPER

Docking studies suggest ligand-specific δ-opioid receptor conformations

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Abstract An automated docking procedure was used to study binding of a series of δ-selective ligands to three models of the δ-opioid receptor. These models are thought to represent the three ligand-specific receptor conformations. Docking results are in agreement with point mutation studies and suggest that different ligands—agonists and antagonists—may bind to the same binding site under different receptor conformations. Docking to different receptor models (conformations) also suggests that by changing to a receptor-specific conformation, the receptor may open or close different binding sites to other ligands.

Keywords Delta opioid receptor · Docking simulation · Ligand-receptor interactions · Molecular modeling

Introduction

The existence of three types of opioid receptors—μ, δ and κ—in the central nervous system is well documented [1, 2]. Their functions are mediated by the activation of heterotrimeric G-proteins [3]. They are involved in pain regulation through the inhibition of neuronal adenylyl cyclase activity, and in the regulation of multiple other effectors [3]. There are studies indicating that the δ-opioid receptor and/or its specific ligands are also involved in cardioprotection [4, 5]. The δ-opioid receptor is an especially attractive target in the development of new drugs for the control of pain. Compared to other opioid or opioid-like receptors, δ-opioid selective drugs have certain advantages, including: greater relief of neuropathic pain, reduced respiratory depression and constipation, and reduced potential for the development of physical dependence [6]. Based on the results of pharmacological investigations [7], opioid receptors are subdivided into receptor subtypes, but the molecular basis for these subtypes remains to be resolved. There is pharmacological evidence [9] that supports the existence of two subtypes of δ-opioid receptor [8–21]: δ_1 and δ_2. Their existence has also been supported by in vitro binding experiments [10, 16] of some δ-selective ligands, although other studies [12, 13] failed to find any evidence of δ-receptor subtypes. The δ_{cx} and δ_{ncx} subtypes of the δ-receptor were proposed by another research group [14], based on the hypothesis that one subtype of the δ-receptor (δ_{cx}) forms a complex with the μ-receptor while the other (δ_{ncx}) does not. The existence of subtypes of δ_{cx} (δ_{cx-1} and δ_{cx-2}) has been postulated [21]. The two subtypes, δ_{ncx-1} and δ_{ncx-2}, of δ_{ncx} have also been resolved [15]. It has also been suggested [15] that δ_{ncx-1} is equivalent to the δ_1-receptor subtype, and that δ_{ncx-2} is distinct from the cloned δ-opioid receptor [17]. However, the relationship between δ_{ncx}/δ_{nc} and δ_1/δ_2 receptor subtypes remains enigmatic.

Only one δ receptor has been cloned to date [6, 25], and there remains no definitive molecular evidence for distinct subtypes of δ-opioid receptor. It has been suggested that the subtypes mentioned above could result from different posttranslational modifications of the receptor [18], or may represent different binding sites of a single receptor [18], or reflect receptor dimerization [19, 23], or indeed the

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interaction with associated proteins [24]. It has been demonstrated [18] that the δ_1 and δ_2 receptor subtypes are not distinct molecular entities. A hypothesis was proposed [18] that selective ligands would bind the same receptor but at different binding sites. The ability of selective ligands to bind their respective binding site would depend on the conformational state of the δ-opioid receptor [18, 22].

Regions of the δ-opioid receptor involved in ligand binding and mediation of receptor function have been identified: (1) by construction of chimeric receptors containing sequences from μ or κ-opioid receptors [26–30], (2) by site-directed mutagenesis of specific amino acid residues [31–36], and (3) by construction of truncated mutant receptors [37–41].

There is experimental evidence that the interaction of a peptidic ligand with a receptor differs from that of a small ligand [42, 43]. Since small organic molecules, as possible ligands of opioid receptors, are the focus of this work, the study is limited to the non-peptidic ligands of the δ-receptor (see Table 1).

Point-mutation experiments [36] revealed that the Asp95Asn mutation reduced affinity for the δ-receptor of many δ-selective peptidic and non-peptidic (**7** and **8**) agonists. The binding of δ-selective antagonists (**1**,**2** and **4**) and non-selective agonists was unaffected. Studies [6] on chimeric and site-mutagenized δ-opioid receptors established the importance of arginine amino acids in a third extracellular loop (EC3) for the binding of peptidic ligands, while non-peptidic ligands like bremazocine, etorphine and naltrindole (**2**) were not affected. Site-directed mutagenesis experiments showed [31] that Asp128 does not participate in the formation of a salt bridge between the ligand and its receptor, but it does contribute to the stabilization of the binding pocket. The highly selective non-peptidic δ-ligands **2** and **7** were moderately affected [32] by mutations of the following amino acids: Tyr129Phe, Trp274Ala and Tyr308-Phe, indicating that these aromatic residues might form part of an opioid-binding domain. The chimeric receptors and alanine scanning methods were used [27] to show that Val296 and Val297 of the EC3 loop are important for binding of the δ-selective ligand SNC80 (**5**). Leu295 and Ala298 of EC3 were found to be important for binding of **2**. The amino acids Trp284 (TM6) and Ser312 (TM7) were important for both compounds, although to a lesser extent. Binding of ligand **5** was affected more than binding of **2**. The μ/δ chimeric receptor studies [28] confirmed the importance of the EC3 loop for the binding of **2** to the δ-receptor. It was found [28] that modifications of a second extracellular loop (EC2) had no effect on ligand binding.

Other chimeric receptor studies [29] demonstrated that the sixth transmembrane domain (TM6) and the third extracellular loop (EC3) are absolutely critical for δ-opioid receptor selectivity. Point mutations done in that study had a different effect on peptidic and non-peptidic ligands. The δ/μ291–300 receptor mutant (δ-receptor with amino acids 291–300 of a μ receptor) bound [29] non-selective opioid ligands but not δ-selective ligands. Point mutations done on this mutant receptor emphasized the importance of amino acids Leu300, Ala298, Ala299, and the unimportance of Arg291 for ligand binding. Val281 had moderate effect on ligand binding [29]. It was also found [33] that mutations Asp128Asn, Tyr129Phe and Tyr129Ala did not noticeably affect binding of the potent δ-receptor agonist BW373U86 (**7**), while a Tyr308Phe mutation increased binding. It was suggested [33] that these amino acids, together with His278 (TM6), participate in interactions [Asp128(TM3)–Tyr308 (TM7) and Tyr129(TM3)–His278(TM6)] that maintain the δ-receptor in an inactive conformation. Point-mutation experiments [34] confirmed that Trp284 (TM6) is important in the binding of ligands to the δ-receptor, and that amino acids at the extracellular end of TM6 and TM7 are key residues for δ-ligand selectivity. Binding studies [45] on non-peptidic δ-opioid receptor ligands with octahydroisoquinoline structure (**9**, **10**, **11**) revealed the binding site of these ligands to lie between TM5 and TM7 of the δ-receptor. This latter study confirmed the importance of Trp284 (TM6) for ligand binding. On the other hand, in studies on δ/μ chimeric receptors, it was found [30] that the binding site of the highly selective δ-ligand **12** is in the region between the beginning of the first intracellular loop (IC1) and the middle of TM3. A model of the δ-opioid receptor with important amino acids is presented in Fig. 1. The binding pocket for most δ-selective ligands is located close to EC3 and between transmembrane helices TM3, TM6 and TM7.

Some experiments [46–49] suggest that important conformational changes in the receptor accompany ligand binding. Receptor states were discovered that can be activated without the effects of an agonist [50], shifting our understanding of receptor activation from a model of inactive and active conformations of a receptor [51] to theories of multiple signaling states where each different agonist could import its own unique active conformation [52].

In this work, we investigate the hypothesis that the three different models of the δ-receptor may represent three different receptor conformations suitable for binding of specific ligands. We also wanted to see if the different binding sites might be available to ligands in different receptor conformations, which might explain the existence of δ-receptor subtypes, as suggested earlier [18].

We report docking of a series of δ-opioid receptor-selective agonists and antagonists to the three different models of the human δ-receptor available from the literature [53–55]. The **R2** receptor was modeled [54] based on the experimental structure [56] of bovine

Table 1 Names and structures of compounds **1–13**

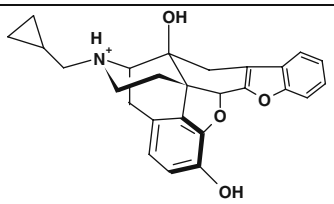
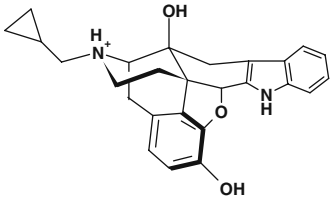
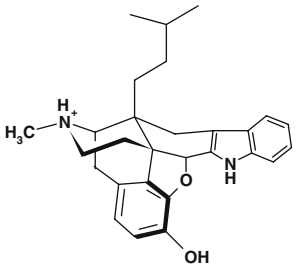
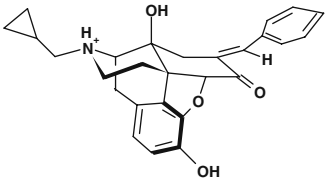
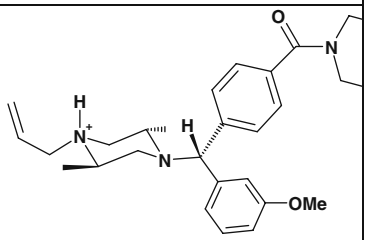
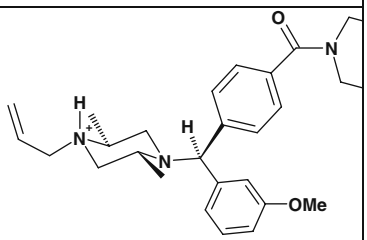
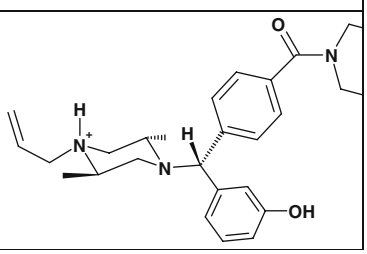
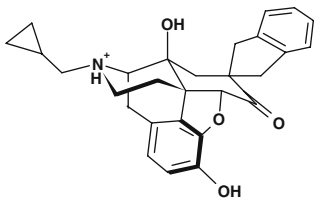
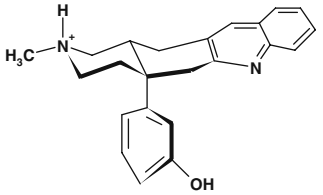
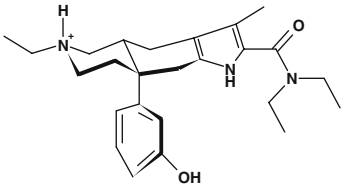
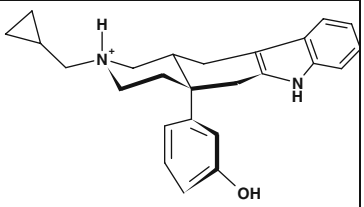
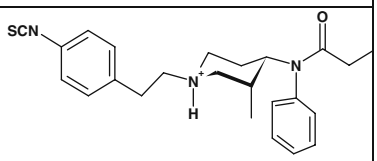
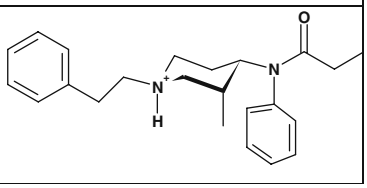
Compound Number	Name		Structure
1	Naltriben	NTB	
2	Naltrindole	NTI	
3	3-Hydroxy-6,7-didehydro-4,5α-epoxy-17-methyl-14β-(3-methyl)butyl-6,7,2',3'-indomorphinan	NTIR	
4	7-Benzylidenenaltrexone	BNTX	
5	(+)-4-[(αR)-α-((2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide	SNC80	
6	(-)-4-[(αS)-α-((2R,5S)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide	SNC67	
7	(+)-4-[(αR)-α-((2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl]-N,N-diethylbenzamide	BW373U86	

Table 1 (continued)

8	7-spiroindanyloxymorphone	SIOM	
9	(-)-3((4aS,12aR)-2-methyl-1,3,4,5,12,12a-hexahydro-2H-2,6-diaza-naphthacen-4a-yl)-phenol	TAN-67	
10	(-)-(4aR,8aS)-6-ethyl-8a-(3-hydroxyphenyl)-3-methyl-4,4a,5,6,7,8,8a,9-octahydro-1H-pyrrolo[2,3-g]isoqinoline-2-carnoxylic acid diethylamine	SB219825	
11	3-((4aS,11aR)-2-cyclopropylmethyl-1,2,3,4,5,6,11,11a-octahydropyrido[4,3-b]carbazol-4a-yl]-phenol	SB206848	
12	*cis*-(+)-3-Methylfentanyl Isothiocyanate	SUPERFIT	
13	*cis*-(+)-3-Methylfentanyl		

rhodopsin (PDB id: 1F88) obtained by X-ray diffraction. The second extracellular loop and the N and C termini were omitted. The **R1** receptor model [53] is a theoretical model of the human δ-receptor calculated using an interactive distance geometry procedure and the system of hydrogen bonds formed by polar transmembrane chains in various proteins of a GPCR family, applied as distance constraints [57]. In this model, the intracellular loops and the N and C termini were omitted. The **R3** model [55] was derived from electron cryomicroscopy data and the Cα coordinate template, followed by computational refinement. Only transmembrane helices were modeled. The difference in backbone conformations of transmembrane helices between **R1** and **R2** is relatively small [58], while **R3** differs in position and orientation of the TM helices, especially TM3 and TM5. However, all three models are 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 possible effects of different media (the difference in rodopsin structure determined in crystal state [56] and in solution [59, 60]), we believe that the receptor models **R1**,**R2**,**R3** may be considered as possible conformations of the δ-receptor, if the docking results obtained using these receptors are in accord with the experimental point mutation studies.

Contrary to some earlier findings [49], recent X-ray studies [61] on rhodopsin have demonstrated that transformation from the ground state to the photoactivated

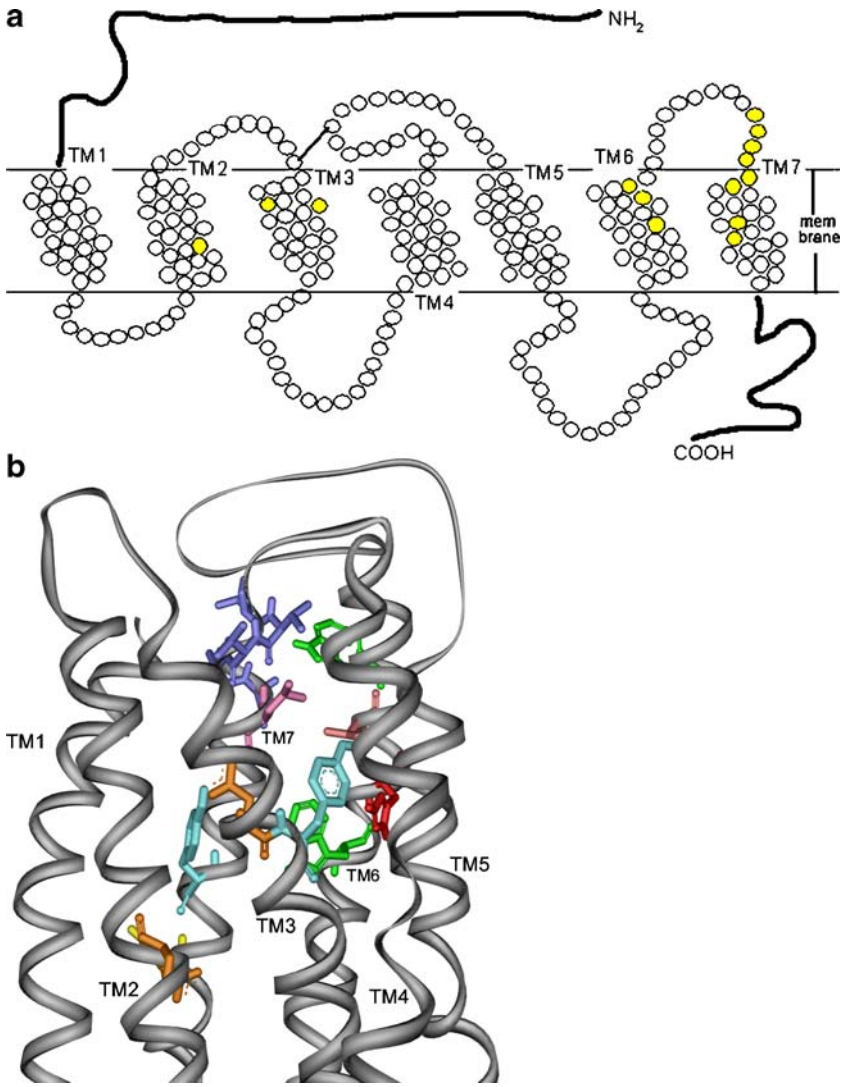


Fig. 1 **a** Serpentine model of the δ-opioid receptor. Amino acids important for ligand binding are in *yellow* (mutagenesis experiments). **b** 3D model of the δ-opioid receptor **R1**, with important amino acids highlighted

intermediate state involved minor changes in receptor structure. It has been suggested [61] that the rigid inactive conformation of the receptor becomes more relaxed upon activation. It has also been suggested [61] that one receptor model may be used in docking calculations of both agonists and antagonists. Bearing this in mind, we hypothesise that all ligands are introduced to the receptor in its undisturbed, ligand-free conformation. Introduction of a ligand molecule, like some point mutations, breaks some of the stabilizing interactions within a receptor, and makes its structure more flexible. The ligand induces a conformation that will be the most favorable for its binding. That conformation is expected to enable optimal receptor–ligand interactions and lead to a stable receptor–ligand complex. In this model, there are no specific active or inactive receptor conformations. Agonists and antagonists may bind to the same or different receptor binding conformations. Whether a receptor will be activated depends on additional interactions that the agonist may have with the receptor.

Although the receptor models used are crude, lacking some or all of the extracellular and intracellular loops, and despite the fact that the conformations of amino acid side chains of the opioid receptors are unknown from experimental studies, we believe that, if supported by the known results from point mutation and chimeric receptor studies, they may offer at least a qualitative picture of the conformational changes undergone by the receptor in order to accommodate different ligands.

Materials and methods

All computations were performed using a P4/Celeron at 1.5 GHz. The δ-receptor models used were taken from the literature: **R1** [53], **R2** [54], **R3** [55]. The only change was made on **R3**, where Leu102 side chain torsional angles were changed to values similar to the corresponding ones in **R1** and **R2**. This change improved the binding geometry

and energy of ligand **12**. Receptor models were treated as rigid. Protonation of the binding site was determined automatically using AutoDockTools 1.4.3 after Kollman charges were assigned to the receptor atoms. The His amino acid was treated as positive (+1).The automated flexible ligand docking experiments were performed with the program AutoDock 3.0.5. [62]. The starting geometries, with protonated ring nitrogen [63], were built using the HyperChem program [64] and subsequently optimized using the semiempirical AM1 method of the same program. The 60×60×60 grid was centered on one of the Asp128 oxygen atoms. In the case of the **R3** receptor model, the grid was centered on one of the Tyr308 aromatic carbon atoms, closest to the center of the receptor. 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 27,000 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 2 kcal/mol), and they were further evaluated based on their vicinity to important amino acids, found experimentally to be located in the binding site of δ-selective non-peptidic ligands. In order to verify the preferred conformation, for some of the ligands, the docking process was done in two steps. After the first step of 250 LGA runs, as described above, the preferred ligand conformation was used as the initial position for the second docking step. No better preferred conformation was found in the second step for any of the ligands studied. 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 important amino acids. The lowest binding energy conformation with maximum number of close contacts to the important amino acids is referred to as the preferred conformation. The distribution of conformational clusters for the selected ligands are represented in Figs. 2b, 3b, and 7c (see below).

Results and discussion

Initially an irreversible δ-receptor ligand, SUPERFIT (**12**), was docked to the **R1** receptor model. The results were compared with those of a previous study [53]. Both studies predict binding of **12** to the binding site located between transmembrane helices TM3, TM5, TM6 and TM7 (binding pocket **BP1**). However, chimeric receptor studies [30] show that the critical region for binding of **12** is from the beginning of the first intracellular loop (IC1) to the middle of TM3, suggesting that the binding site of **12** most likely includes TM2 and TM3.

This discrepancy prompted us to investigate the other available models of the δ-receptor to see if they would produce a ligand(**12**)-receptor complex in agreement with chimeric receptor studies. The hypothesis was that other receptor models may represent different receptor conformations with alternative binding sites for ligand **12**. To test this hypothesis further we performed docking studies with a series of potent and selective δ-receptor ligands (Table 1) to the three receptor models **R1, R2** and **R3**, assuming that they may represent different ligand-selective receptor conformations.

δ-Opioid receptor antagonists (NTI, NTB, NTBR, BNTX)

Automated docking of compounds **1–4** to the three δ-opioid receptor models resulted in several plausible docking orientations and conformations for each ligand. The preferred conformations of compounds **1** and **2** in the binding pocket of the **R1** receptor model have a binding energy (E_b=−9 kcal/mol) 1.5 kcal/mol above the global minimum for these compounds. Their orientation is very similar to one proposed earlier [65], and follows the "message-address concept". The protonated piperidine and the phenolic component form the "message" moiety related to ligand binding, and an indolic (benzofuryl) component represents the "address" moiety and determines ligand selectivity and binding. According to the docking results, the "message" moiety interacts (within 4.0 Å) with Asp128, through salt bridge formation (+NH...O^- distance = 2.67

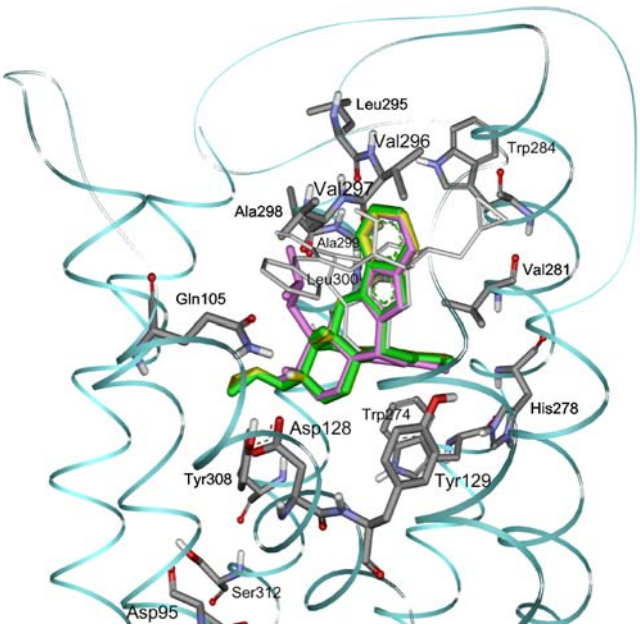
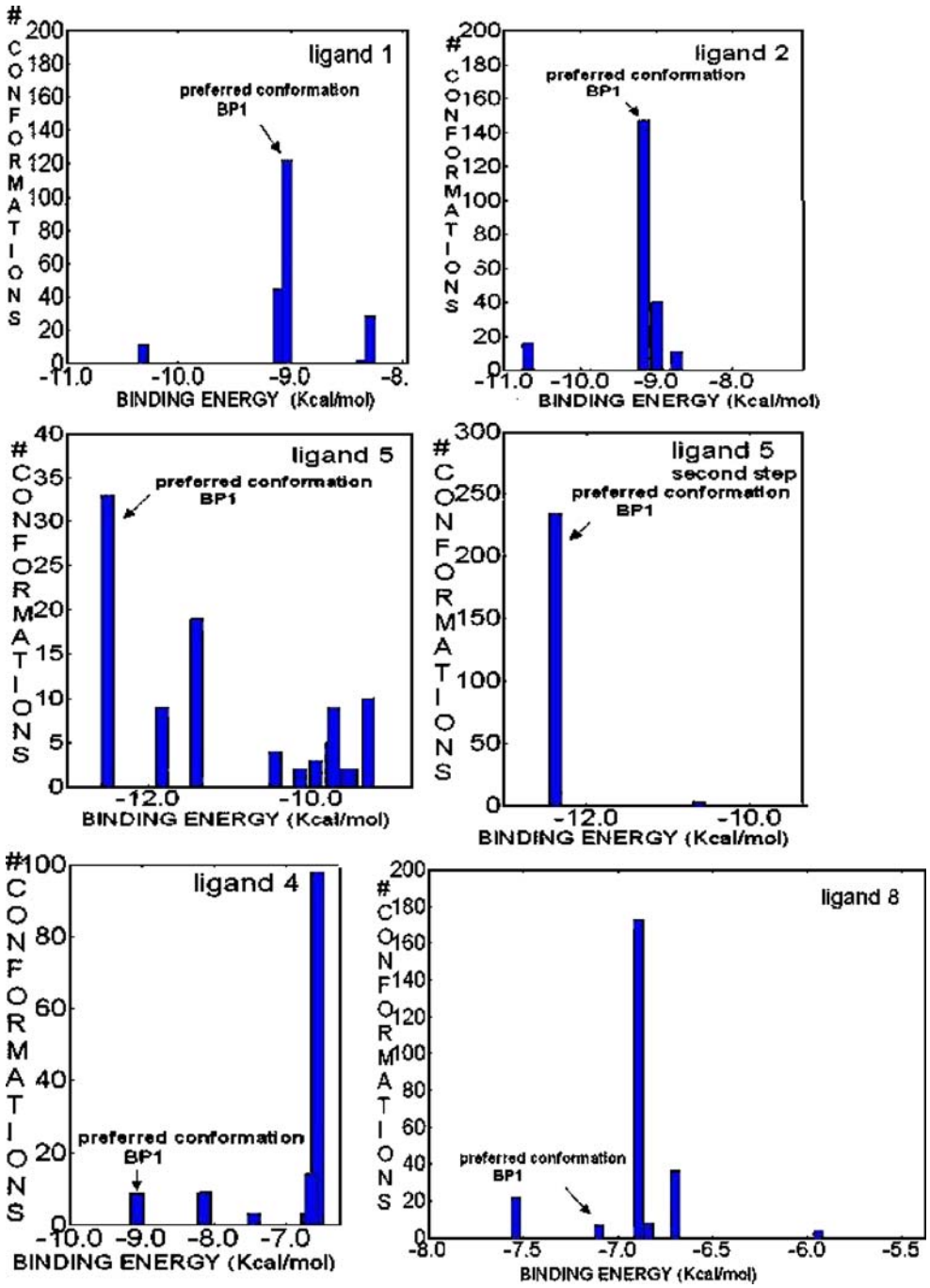


Fig. 2 **a** Ligands **1** (*yellow*), **2** (*green*), **3** (*pink*) and **4** (*white*) in the binding pocket *BP1* of the **R1** δ-opioid receptor model. **b** Distribution of the conformation clusters in the **R1** δ-receptor, for the **R1**-receptor ligands **1**, **2**, **5**, and the **R2**-receptor ligands **4** and **8**

Fig. 2 (continued)



Å), and with Tyr129 of TM3 and Tyr308 of TM7 (all known from point mutation studies [32], as well as with Gln105 and Leu102 of TM2. The major interaction with Gln105 is via hydrogen bond formation to the 14-hydroxy group of **1** or **2** (NH···O distance = 2.21 Å). It was found earlier [66] that the 14-hydroxy group plays an important role in δ-selectivity and binding potency of compounds **2** and **8**. On the other hand, it is generally believed [65] that residues at the top of TM6 and TM7 form a hydrophobic pocket to accommodate the indolic moiety of **2**. 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 Ala195 and Val196 of EC2.

In the best complex made by ligand **3** and the **R1** receptor model, the ligand is aligned so that it overlaps the preferred conformations of **1** and **2**. The reduced binding potency of this compound relative to **1** and **2** found in experimental studies (Table 2) seems to stress the importance of the 14-hydroxy group. Although the 14β-(3-methyl)-butyl chain of **3** is comfortably positioned within the hydrophobic pocket created by Cys121, Leu125 (TM3)

and Val297 (EC3), it lacks the hydrogen bond associated with 14-hydroxy group of compounds **1** and **2** (Fig. 2). Therefore, the **R1** receptor model seems to correspond to a receptor conformation specific for binding of selective δ antagonists, i.e., **1**, **2** and **3**. At the same time, the cloned δ-receptor has been reported [68] to bind **2** better than **5**, DPDPE or TIPP(ψ).

However, the δ-selective antagonist **4**, does not have a ligand orientation similar to that adopted by compounds **1**–**3**. Although it forms an equally stable complex (E_b= −9.1 kcal/mol), its preferred conformation is distinct (Fig. 2), and the ligand has fewer interactions with the amino acids of the binding pocket defined by experimental studies. Experimental studies have also shown [67] that the cloned δ-receptor has a higher affinity for **1** than for **4**. Different binding of **1** and **4** to the δ_{ncx} receptor has been reported [17]. The two compounds have also been found to be subtype selective by other authors, with **4** binding selectively to the δ_1 receptor subtype and **1** binding selectively to the δ_2 subtype [69, 70]. All these data suggest that **R1** might be the receptor-specific conformation for ligands **1**, **2** and **3**.

The docking experiment using the **R2** receptor model revealed better agreement in ligand orientation of **1**, **2** and **4**. Their preferred conformations overlap in the binding pocket BP1, located between transmembrane helixes TM3 and TM7. All three compounds have stable ligand–receptor complexes (binding energies, E_b=−8.4, −8.5, −9.6 kcal/mol, respectively) and have close contacts (<6 Å) with all the important amino acids in binding pocket BP1 (Fig. 3) from the bottom of the binding pocket (Asp128, Tyr129, Tyr308) to the top of TM6 and TM7 [Val281 (TM6), Trp284 (TM6), Leu300 (TM7)]. Amino acids Val296 and Val297 are not in contact with the ligands due to the conformation of EC3, which is different from that in the **R1** receptor model. The binding energy of **4** in BP1 is lower than the binding energies of other ligands, suggesting that **R2** might be closer than **R1** to the compound **4**-specific conformation of the δ-opioid receptor.

The **R2** receptor model opens an additional binding pocket, BP2, at the top of the transmembrane region between helixes TM1,TM2,TM3 and TM7 for the δ-receptor ligands. BP2 is the only binding site for compound **3** and the major binding site for the other antagonists: **1**, **2**, and **4** (E_b=−9.7, −9.5, −10.0 kcal/mol, respectively). However, in this binding pocket, the ligands would be far from the important amino acids. Therefore, despite lower calculated binding energies, BP2 cannot be the binding site for any of the compounds **1**–**4**.

The **R3** receptor model binds all the antagonists to the BP2 pocket (binding energy ranging from −8.0 to −9.6 kcal/mol), where they are far from any amino acids known to affect their binding and potency. Therefore, **R3** cannot be the ligand-specific receptor conformation for any of the antagonists studied.

δ-opioid receptor agonists (SNC80, BW373U86, SIOM, TAN67, SB206848, SB219925)

The convulsive and antinociceptive activities of compounds **5** and **7** are mediated through the same type of δ-receptor and may be antagonized by **4** (the putative δ_1 antagonist) and by **1** (the putative δ_2 antagonist) [71]. On the other hand, **5** induces dose-dependent hypothermia that is blocked, or decreased, by **2** and **1** (an δ_2 antagonist), while **4** (an δ_1 antagonist) has no effect [72]. The effects of **2** and **1** on the regulation of anxiety-related behavior were antagonized by **5** [73]. It was found that **4** and **1** antagonized DPDPE and deltorpin II-induced inhibition of adenyl cyclase, respectively, but they could not discriminate between [^{3}H]DPDPE and [^{3}H]deltorpin II in binding experiments [11]. Most of these effects suggest that **R1**, the postulated receptor specific conformation for ligands **1**, **2** and **3**, might also be the ligand-specific receptor conformation for compounds **5** and **7**.

The docking studies reported here reveal that the preferred conformations of **5** and **7** (E_b=−12.5 kcal/mol and −11.9 kcal/mol, respectively) in binding pocket BP1 are similar to that of **2** (Fig. 4a). All three compounds have a protonated nitrogen close to Asp128 (TM3). The $NH^+\cdots O^-$ distances are 3.43, 3.44 and 2.67 Å for **7**, **5** and **2**, respectively. The NEt_2 groups of **5** and **7** overlap the benzene ring of the indolic group of compound **2**. The phenolic groups of **5** and **7** are positioned in the direction of the phenolic group of **2**, but extend further toward Trp274

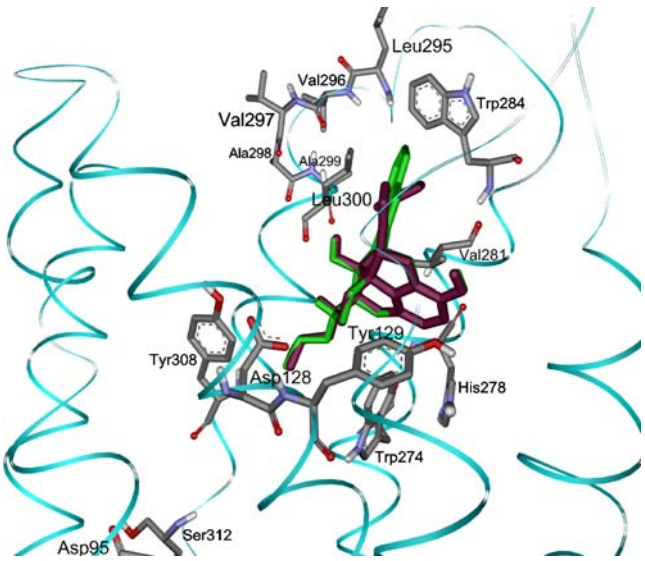
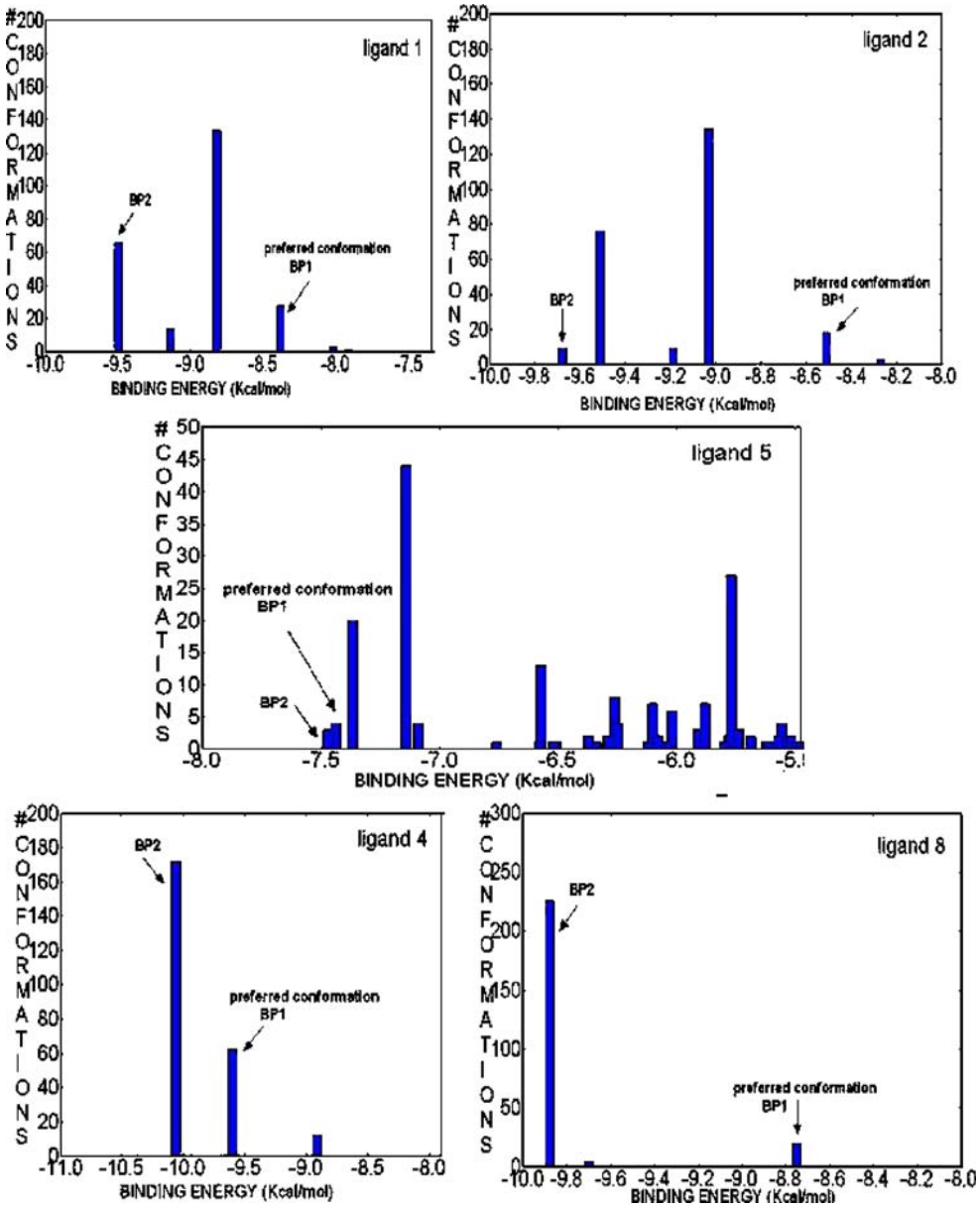


Fig. 3 **a** Ligands **2** (*green*), and **4** (*brown*) in the binding pocket *BP1* of the **R2** δ- opioid receptor model. **b** Distribution of the conformation clusters in the **R2** δ-receptor, for the **R2**- ligands **4**, **8**, and the **R1**-receptor ligands **1**,**2** and **5**

Fig. 3 (continued)



and His278 of TM6. These ligand interactions with TM6 may be the major difference between antagonists and agonists, and the key process in receptor activation. It has been suggested earlier that movement of helices TM3, TM6 and TM7 is essential for the activation of rhodopsin [74] and the human δ-receptor [75]. However, association of the phenolic part of the ligand and TM6 is not strong enough to initiate noticeable movement of TM3, TM6 and TM7, therefore **5** and **7** are relatively weak analgesics in mice [76], although more potent than an analog with an unsubstituted phenyl group [77]. The analgesic activity is probably partially exerted through the hydroxyl group.

Compounds **5** and **7** were found to have similar activity [77], because **5** is believed [77] to be metabolized to **7**. The enantiomer of **5**, compound **6**, has lower affinity for the cloned human δ-receptor than **5** [77]. According to the docking results, this may be explained by classical "three point theory". Compound **6**, in its preferred conformation (Fig. 4b) binds to the same binding pocket as **5**, but in a different orientation. It lacks two of the three interactions that stabilize the complex of **5** and the δ-receptor. There is no salt bridge to the Asp128, or the phenolic–His278 close interaction. In agreement with this model of binding is the reduced difference in the experimentally measured binding energies [76] of the two enantiomers of the 3-hydroxybenzyl analog of **6**, possibly because the phenolic group of this analog allows hydrogen bond formation with Asp128.

Receptor model **R2** opens two binding sites, BP1 and BP2, to ligands **5** and **7**, where they bind with equal probabilities. This indicates nonspecificity of **R2** for either **5** or **7**.

Docking to the **R3** receptor model situated ligands **5** and **7** exclusively in binding pocket BP2. Positioning of these ligands to BP1, where most of the important amino acids are, requires >3 kcal/mol more energy. This indicates that **R3** cannot be the ligand-specific receptor conformation for **5** and **7**.

Compound **8** is a putative δ_1-selective agonist [78]. Docking to the **R1** receptor model revealed only two possible orientations of the ligand, both in BP1. However, their relatively high energies (−7.5 and −7.1 kcal/mol), and the lack of interaction with important EC3 amino acids suggest that **R1** may not be the ligand-specific receptor conformation for **8**. The **R3** receptor model binds **8** exclusively to the BP2 region, where it is far from the amino acids known to form the binding pocket for nonpeptidic ligands. This suggests that **R3** cannot be the ligand-specific receptor conformation for **8**. The **R2** receptor model binds **8** to both binding sites, BP1 (E_b=−8.8 kcal/mole) and BP2 (E_b=−9.9 kcal/mol; Fig. 5). In BP1, **8** overlaps **4**, and has close contacts with the majority of the important amino acids. This suggests that the **R2** receptor model may be close to the preferred receptor conformation for **8**. However, the fact that an antagonist, **4**, and an agonist, **8**, completely overlap in the binding pocket BP1 of **R2** offers no explanation for receptor activation.

The second group of studied agonists (**9** –**11**) belongs to the novel class of δ-receptor ligands with an octahydroisoquinoline structure, including **9**, a δ_1-specific agonist. Experiments with μ/δ receptor chimera [45] determined that a critical site for receptor/ligand interactions was located between TM5 and TM7. The Trp284 located at

Table 2 Experimental binding constants, K_i (nM) and IC_{50} (nM)

Compound	Type	δ− receptor		μ− receptor	
		K_i experimental	IC_{50} experimental	K_i experimental	IC_{50} experimental
1	δ-selective antagonist	0.39±0.21[a]	0.04[f]	48[a]	
		0.36[s]		12.4[s]	
2	δ-selective antagonist	0.30±0.13[a]	2.1[f]	29[a]	
		0.19±0.07[b]		34.4±0.6[b]	
		0.04±0.02[d]			
		0.42[e]			
		1.5±6[h]		19.9±0.6[h]	
		0.15±0.01[j]		27.5±7.7[j]	
3	δ-selective antagonist	1.4[s]		186[s]	
4	δ-selective antagonist		37[f]		
		0.1[l]		13.3[l]	
		6.8[s]		9.1[s]	
5	δ-selective agonist	0.818[p]		3900[p]	
			2.88±0.35[k]		2,467±200[k]
		7.0±0.7[c]			
		2.19±0.29[d]			
6	δ-selective agonist	218[p]	430±41[k]	7450[p]	9,366±798 [k]
7	δ-selective agonist	0.086[p]			
		0.16±0.02[a]			
			0.3[f]		
		1.63[e]			
		1.8[i]		15[i]	
			1.49±0.33[k]		9.71±0.37[k]
8	δ-selective agonist	4.1±1.0[b]		88.4±11.7[b]	
		1.4[m]	40[f]	10.6[m]	
9	δ-selective agonist	0.647[g]		775[g]	
		1.3±0.3[h]		240±20[h]	
10	δ-selective agonist	0.6±0.2[h]		340±50[h]	
		0.9±0.2[r]	26 [r]	129±30[r]	
11	δ-selective agonist	1.7±0.4[h]		290±110[h]	
12	irreversible δ-selective agonist	3.66±1.03[n]		7.65±1.76[n]	
13	μ-selective agonist	56.20±11.34[n]		0.49±0.23[n]	

[a] [29] [b], [34] [c], [26] [d], [27] [e], [32] [f], [36] [g], [44] [h], [45] [i], [81] [j], [82] [k], [83] [l], [84] [m], [78] [n], [80] [p], [76] [r], [85] [s], [86]

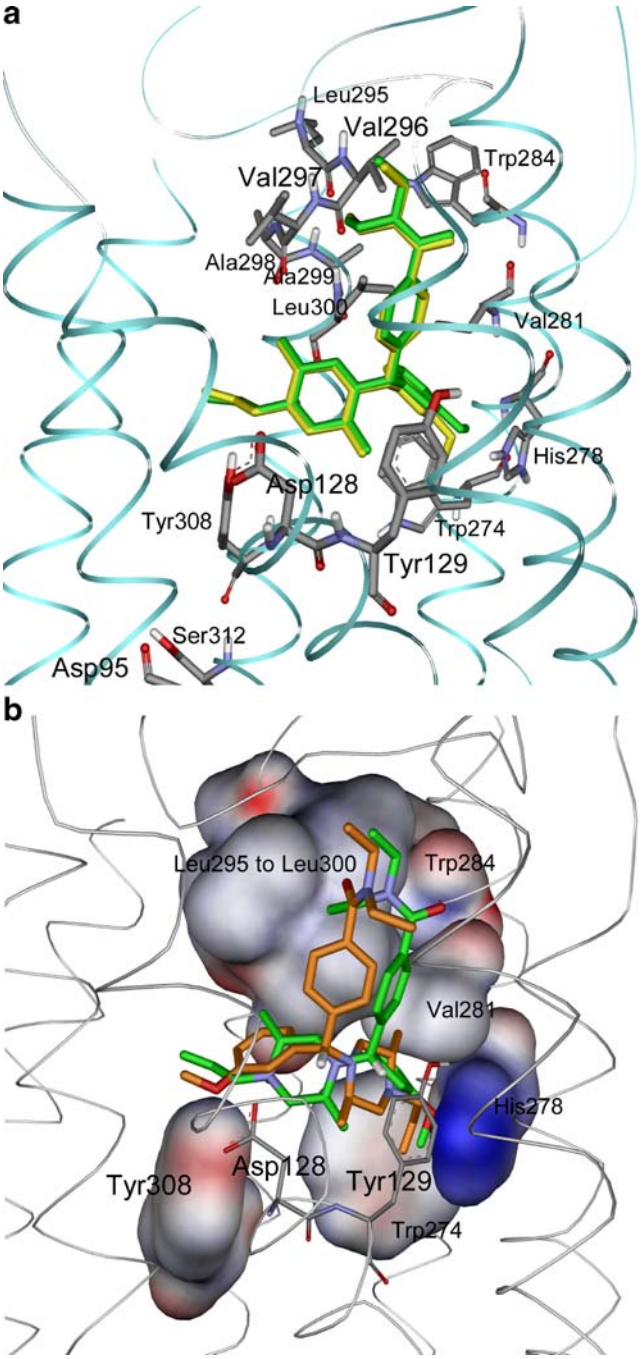


Fig. 4 **a** Ligands **5** (*yellow*), and **7** (*green*) in binding pocket BP1 of the **R1** δ- opioid receptor model. **b** Ligands **5** (*green*), and **6** (*orange*) in binding pocket BP1 of the **R1** δ-opioid receptor model

the top of TM6 was particularly important for the binding [45] of this group of ligands, but affecting **10** more than **9** or **11**. According to the docking results with the **R1** receptor model, **9** binds to the binding pocket between TM3 and TM7 (BP1) but is not close to TM6, nor could it be affected by the Trp284 mutation. The same is true for both **10** and **11**. Therefore, the **R1** model can hardly be the ligand specific conformation for any of these three molecules. The **R3** receptor model binds all three ligands, **9–11**, to both binding sites BP1 and BP2. The complex where the ligand is in BP2 is more stable. Therefore, **R3** is unlikely to be their ligand-specific receptor conformation. The receptor model **R2** binds **9** and **10** mainly in BP2, so it is also not their ligand specific receptor conformation. However, **11** binds to BP1 and BP2 of **R2** with comparable affinity (E_b=−8.0 kcal/mol and −8.5 kcal/mol, respectively). Besides, in **R2, 11** has close contact with Trp284 (Fig. 6), and may be affected by the Trp284 mutation. The **R2** receptor model may be close to the ligand-specific δ-receptor conformation of **11**.

Contrary to the other compounds studied, the experimental data for SUPERFIT (**12**) suggest [79] that the segment from the beginning of the first intracellular loop to the middle of the TM3 of the δ receptor is essential for the selective binding of this molecule. This would correspond to ligand binding to the BP2 region of the δ-receptor. According to the docking studies reported here, only the **R3** model binds **12** to BP2 (E_b=−10.1 kcal/mol; Fig. 7). The **R3** receptor model binds **12** to the BP1 binding pocket as well, but the energy of binding is more than 2 kcal/mol higher. The other two δ-receptor models, **R1** and **R2**, bind **12** exclusively to the BP1 region, suggesting that the **R3** receptor model is the ligand-specific receptor conformation for **12**. Ligand **12** is known [14] as the δ_1 selective ligand. Pretreatment of membranes with **12** depleted membranes of the δ_1 binding site [14]. The other δ_1 ligands bind to BP1, which lies between helices TM3 and TM7. It seems that, while binding to the δ-receptor, ligand **12** induces receptor conformation **R3**, thus blocking binding pocket BP1 and preventing other ligands from binding. The structurally closely related (3R,4S)-3-methylfentanyl (**13**), binds to

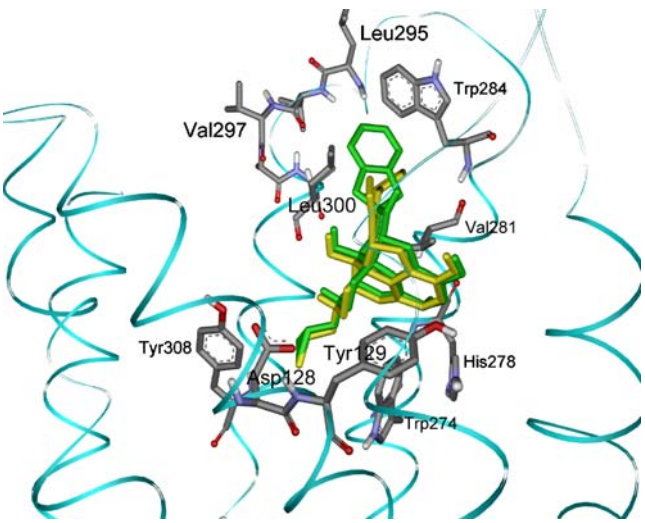


Fig. 5 Ligands **8** (*yellow*), and **4** (*green*) in binding pocket BP1 of the **R2** δ- opioid receptor model

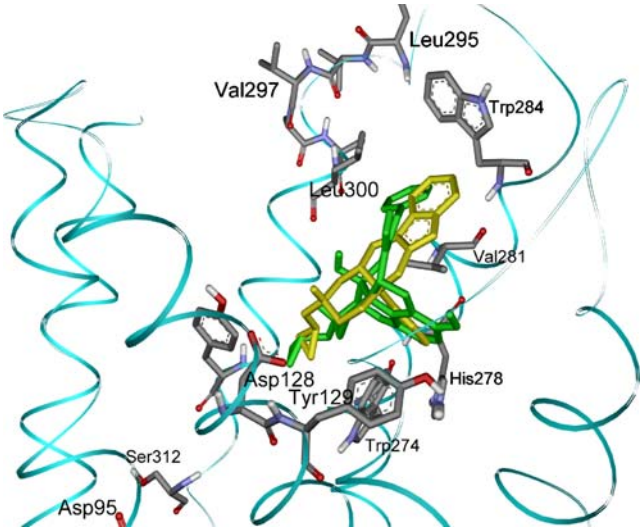


Fig. 6 Ligands **11** (*yellow*), and **4** (*green*) in binding pocket BP1 of the **R2** δ- opioid receptor model

BP2, but less efficiently (E_b=−8.4 kcal/mol), which is consistent with experimental data [80] (see Table 2).

Conclusions

An automated docking procedure was applied in order to determine preferred binding pocket for a series of δ-opioid receptor selective ligands in three models of the δ-opioid receptor. These models were assumed to represent the three ligand-specific receptor conformations. The quality of the receptor–ligand complexes formed was estimated on the basis of the binding energies, and their ability to reproduce experimental point mutation data.

It was found that different ligands, both agonists and antagonists, and both δ_1- and δ_2-selective, may occupy the same binding pocket, defined by point mutation experimental data, under different receptor conformations. The results are supported by an earlier study [16] suggesting the existence of the two binding sites, δ_1 and δ_2, in a single δ-receptor. The ability of the selective ligands to bind the specific binding sites would depend on the conformational state of the receptor [16]. Antagonists **1**–**3** and agonists **5** and **7**, all δ_2-selective ligands, share the same δ-receptor conformation, **R1**, with the binding site between helices TM3 and TM7. Unlike antagonists, the agonists related to the **R1** receptor model (conformation) have close interactions with amino acids in both TM3 and TM6, initiating their relative movement and receptor activation [54, 74]. The **R2** receptor model (conformation) binds the δ_1 selective antagonist **4**, and agonists **8** and **11**, to the same binding pocket between helices TM3 and TM7, but not some of the other δ_1 selective ligands studied.

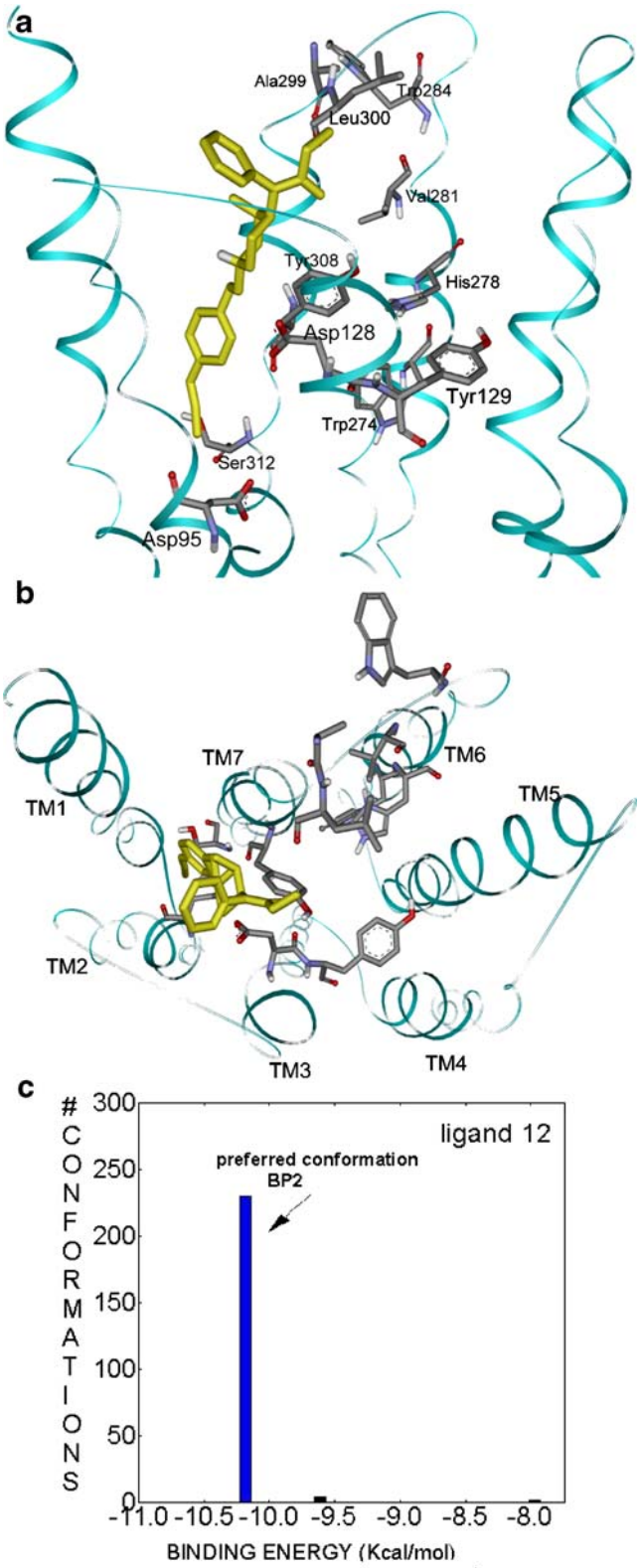


Fig. 7 SUPERFIT, **12**, (*yellow*) in binding pocket BP2 of the **R3** δ-opioid receptor model: **a** side view, **b** view from the extracellular side. **c** Distribution of the conformation clusters for **R3**-receptor ligand **12**

By changing to a ligand-specific conformation, the receptor may open or close other binding sites to other ligands. Going from the **R1** receptor model, where BP1 is an exclusive binding pocket, to the **R2** receptor model, a new binding pocket BP2 starts to open for ligands. In the **R3** receptor model, BP2 becomes the major binding site. The **R3** receptor model is the ligand-specific receptor conformation for compounds **12** and **13**, binding **12** to the binding pocket between helices TM1, TM2, TM3 and TM7, in agreement with experimental data. Ligand **12** may prevent other δ_1-selective ligands from binding in different binding sites by inducing receptor conformation **R3**, where their binding sites would be inaccessible.

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