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Study on mechanism of interaction of nociceptin and opioids binding with opioid receptor-like 1 receptor[1]

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KEY WORDS nociceptin; lofentanyl; etorphine; ORL1 receptor; molecular docking; binding energy

ABSTRACT

AIM: To study the mechanism of interaction of nociceptin and opioids with ORL1 receptor. **METHODS**: Molecular dynamics study was carried out before nociceptin was manually docked into the binding site of ORL1 receptor; DOCK4.0 program was applied to dock four stereoisomers of lofentanyl and etorphine into the binding pocket of ORL1 receptor; Binding energies were calculated, the relationship between binding energy and binding affinity was studied. **RESULTS**: Nociceptin fits well into the binding pocket, the N-terminal FGGF tetrapeptide is located in the inner region of the binding cavity, the nociceptin (5 - 7) interacts with the conservatively variable residues near the other end of binding pocket, and maybe determines selectivity of ORL1 receptor over dynorphin A, the positively charged core of nociceptin (8 - 13) binds predominantly with negatively charged EL-2 loop, which is thought to be able to mediate receptor activation. The shortest fully active analogue of nociceptin (1 - 13) is also discussed. The main difference between these two opioids and nociceptin exists in the kinds and the number of conserved and variable residues in the binding pocket and thereafter in the strength of their interaction. Prediction for binding affinities of four stereoisomers of lofentanyl has been performed based on their binding energies, the similar pharmacophore of lofentanyl and other fentanyl analogs, and the good correlation between binding energies and their experimental binding affinities ($-\log K_i$ values). **CONCLUSION**: Ligand docking results from this study are helpful in clarifying experimental observations of ligands interaction with opioid receptors, thus furthering biological investigations.

INTRODUCTION

The *opioid receptor-like 1 (ORL1) receptor*[1] is a G protein-coupled receptor belonging to the opioid receptor family, as evidenced by an average shared sequence identity in the putative transmembrane (TM) helices (61 % on average).

Nociceptin[2], named according to its *in vivo* activity, is an endogenous peptidyl ligand of ORL1 receptor. Its 1st and the 17th amino acids are phenylalanine (F) and glutamine (Q), so it is also called orphanin FQ. This peptide binds to ORL1 receptor with K_i value of 0.13 nmol/L, and resembles dynorphin A, an endogenous agonist of κ-opioid receptor, in several respects: both have the same length (17 amino acids), among them 6 are identical and both are highly cationic. Activation of the ORL1 receptor by nociceptin causes inhibition of cAMP synthesis and inward potassium conductance. Unlike dynorphin A and other opioids, sometimes even contrary to the analgesic effects of opioid receptor agonists, nociceptin stimulation of the ORL1 receptor can result in hyperalgesia and/or inhibition of stress and opioid-induced analgesia in the hot plate and tail flick tests. Some structural-activity relationship results[3] have revealed that the functional architectures of nociceptin and dynorphin A are different, the shortest fully active fragment of nociceptin is longer than that of dynorphin A, and the positively charged core of this peptide appears to be more necessary for biological activity than that of dynorphin A. Based on these structural and pharmacological characteristics, we can conclude that different domains of ORL1 and κ-opioid receptor are involved in recognition of nociceptin and dynorphin A, the receptor-ligand interaction mechanism of nociceptin with

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ORL1 must be specific and different from opioids.

In order to get further insight into the interactions between ORL1 receptor and its ligand at molecular level, we decided to construct the 3D-structural models of nociceptin, lofentanyl and etorphine with this special receptor. Our models could provide a basis for further experimental investigation of the interplay of structure and biological functions of opioid receptors.

COMPUTATIONAL METHODS

The theoretical study described here was done in three stages: 1) manually docking nociceptin into the binding site of ORL1 receptor; 2) docking opioids into the binding pocket of ORL1 receptor by using the DOCK 4.0 program[4]; 3) investigating the relationship between the binding energy and binding affinity of ligand-receptor system.

Docking nociceptin into the binding site of ORL1 receptor Construction of the nociceptin-ORL1 receptor complex was started with manually docking lowest-energy conformation of nociceptin into the refined receptor molecule which was developed in our previous study, guided by site-directed mutagenesis and other experimental data[5].

The docking conformation of nociceptin resulted from molecular dynamics, the FGGF residues were assumed to take a conformation of β-turn before the simulated annealing operation. The step size was 0.5 fs, the high temperature was 700 K with the time length of 1500 fs, and the low temperature was 300 K with the time length of 500 fs. After 10 cycles, the 10 minimum conformations of nociceptin were averaged and reminimized with molecular mechanics method.

The initial docking conformation was manually positioned in the binding site in order to keep the two aromatic side chains of Phe1 and Phe4 close to the hydorphobic site formed by side chains of conserved residues such as Phe220, Phe272, Met134, Gly308, Trp276, etc, and to keep the protonated nitrogen atom at amino terminus of nociceptin to form hydrogen bond with the negative charged side chain of conserved Asp130 in helix 3.

The positively charged nociceptin (8 – 13) core, with arginines at positions 8 and 12, was assumed to interact with the acidic extracellular loop 2 (EL-2). This part together with the other 4 residues at the C-terminus of nociceptin were manually docked into the inner face of extracellular domains in order to get most probable interaction between the ligand and receptor. Some adjustments in side chain conformations were made to optimize the interacting mode before energy minimization of the complex.

Docking of opioids into the binding pocket using DOCK4.0 program Primary conformations including 4 steroisomers of lofentanyl were energetically minimized by molecular mechanics method and showed in Fig 1. The ligands were docked into the spheres representing binding sites with anchor-first search algorithm. The best results having the most negative scores were represented as probable interactive conformations of ligands. Every model of ligand-receptor complex was minimized using following parameters: a distance-dependent dielectric constant of 5.0, nonbonded cut-off 8Å, Tripos force field with Kollman-all-atom charges, conjugate gradient minimization till the energy gradient RMS < 0.05 kcal $(mol \cdot Å)^{-1}$. The whole receptor was minimized to convergence.

Binding energy calculation The binding energy ($E_{binding}$) of each ligand with ORL1 receptor was calculated as equation 1:

$$E_{binding} = E_{complex} - (E_{ligand} + E_{receptor}) \quad (1)$$

where E_{ligand} was the energy of the lowest-energy conformation of each ligand, $E_{receptor}$ was the energy of optimized receptor. Solvation energy was not explicitly considered, however, calculations were performed with a dielectric constant of 5 to simulate the solvation effect of the ligands in the receptor environment. The method of calculation and parameters used are set up as described above.

RESULTS AND DISCUSSION

Mechanism of interaction of nociceptin with ORL1 receptor As stated as in our previous paper[6], the constructed ORL1 receptor structure has a deep binding cavity which is situated in the extracellular side of the transmembrane domain between helices 3, 5, 6 and 7. This cavity is partially covered by the extracellular loops, especially by the central part of EL-2 loop which contains disulfide bond and connects with the TMs 4 and 5. The binding pocket consists of conserved residues of Asp130, Tyr131, Met134, Phe220, Phe272, Trp276, Cys304, and Gly308, and conservatively varied residues including Gly212, Pro213, Ala216, Ile219, Val283, Leu301, and Thr305. The majority of residues in the binding pocket have fixed side chain orientations. The 3D model of ligand-receptor complex is shown in Fig 2, and Fig 3

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represents the interaction of nociceptin with ORL1 receptor. Tab 1 is compiled with the intermolecular and intramolecular hydrogen bonds formed by nociceptin and ORL1 receptor. After manual docking, the nociceptin interacted with the receptor perfectly. Its backbone dihedral angle values before and after docking are listed in Tab 2. From Tab 2, we can see that backbone dihedral angle values of nociceptin have changed greatly after the formation of ligand-receptor complex, these changes relate that the lowest-energy conformation of a specific ligand is generally not the active conformation of the same ligand.

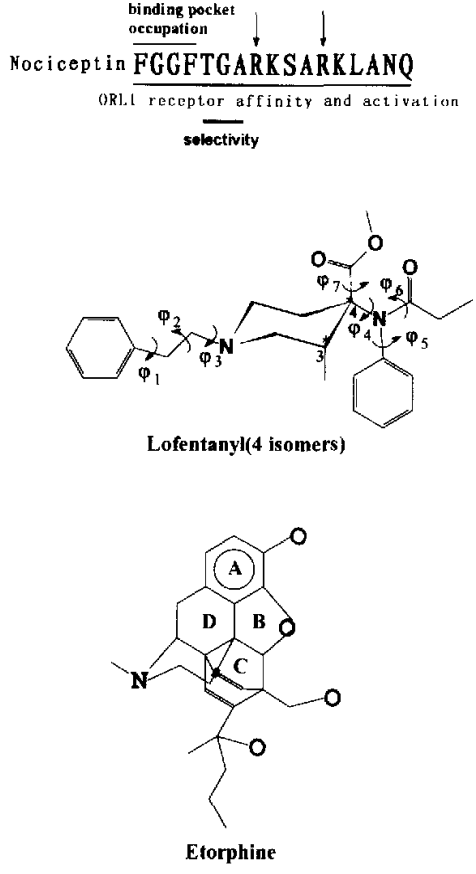

Fig 1. Structural formulae of nociceptin, lofentanyl and etorphine. Amino acids of nociceptin which are critical for biological activity are marked by arrows; Torsional angles of lofentanyl which are measured before and after docking are labeled as in Tab 3, Asterisks represent the chiral carbon atoms of lofentanyl; A, B, C, D represent the center of four rings in etorphine.

Tab 1. Hydrogen bonds formed by nociceptin residuesin an interaction complex with ORL1 receptor.

Molecule	Donor		Acceptor		Molecule
	Residue	Group	Group	Residue	
Nociceptin	Phe1	$-NH_3^{(+)}$	$^{(-)}O^{\delta1}$-	Asp130	ORL1
Nociceptin	Phe1	$-NH_3^{(+)}$	-OH	Thr305	ORL1
ORL1	Tyr131	-OH	O=C<	Gly3	Nociceptin
Nociceptin	Thr5	-NH	O=C<	Gly3	Nociceptin
Nociceptin	Gly6	-NH	O=C<	Phe4	Nociceptin
Nociceptin	Val7	-NH	O=C<	Thr5	Nociceptin
Nociceptin	Arg8	$-N\eta^1H_2$	O=C<	Pro207	ORL1
Nociceptin	Arg8	$-N\eta^1H_2$	O=C<	Gln208	ORL1
Nociceptin	Arg8	$-N\eta^2H_2$	O=C<	Gln208	ORL1
Nociceptin	Arg8	$-N\eta^2H_2$	$^{(-)}O^{\varepsilon1}$-	Asp209	ORL1
Nociceptin	Lys9	$-N\zeta H_3^{(+)}$	O=C<	Gln17	Nociceptin
Nociceptin	Ser10	-OH	O=C<	Ile198	ORL1
Nociceptin	Ser10	-OH	O=C<	Ser10	Nociceptin
Nociceptin	Arg12	$-N^{\delta}H$	$^{(-)}O^{\varepsilon1}$	Glu203	ORL1
Nociceptin	Arg12	$-N^{\varepsilon}H_2$	$^{(-)}O^{\varepsilon1}$	Glu203	ORL1
Nociceptin	Lys13	$-N\zeta H_3^{(+)}$	$^{(-)}O^{\varepsilon2}$	Glu203	ORL1
Nociceptin	Leu14	-NH	O=C<	Arg12	Nociceptin
Nociceptin	Lys13	-NH	O=C<	Val202	ORL1
Nociceptin	Lys13	-NH	$^{(-)}O^{\varepsilon1}$	Glu197	ORL1
Nociceptin	Gln17	$-N^{\varepsilon}H_2$	$^{(-)}O_{\varepsilon1}$	Glu295	ORL1

Tab 2. Backbone dihedral angles of nociceptin before and after docking into the putative binding site of ORL1 receptor.

Residues		φ°	Ψ°	Residues		φ°	Ψ°
Phe1	b		-56.52	Ser10	b	-90.71	150.14
	a		47.54		a	-70.36	-21.44
Gly2	b	70.37	-16.96	Ala11	b	-163.42	-176.96
	a	178.88	53.27		a	-63.51	172.11
Gly3	b	-172.60	63.40	Arg12	b	-70.99	71.38
	a	-154.92	44.26		a	-54.03	153.32
Phe4	b	-50.29	163.72	Lys13	b	-85.06	67.19
	a	-96.35	57.31		a	94.14	-34.39
Thr5	b	-44.04	-39.25	Leu14	b	-135.95	-54.04
	a	-82.31	52.05		a	-59.58	124.77
Gly6	b	-165.34	54.68	Ala15	b	66.38	-70.67
	a	-96.44	57.68		a	-53.69	-70.26
Ala7	b	-156.40	48.19	Asn16	b	-56.08	-54.54
	a	-72.98	177.65		a	-150.34	159.57
Arg8	b	-172.34	-71.34	Gln17	b	53.29	
	a	-129.49	-63.40		a	-51.80	
Lys9	b	-65.05	-44.80				
	a	-64.92	-42.57				

b: before docking; a: after docking.

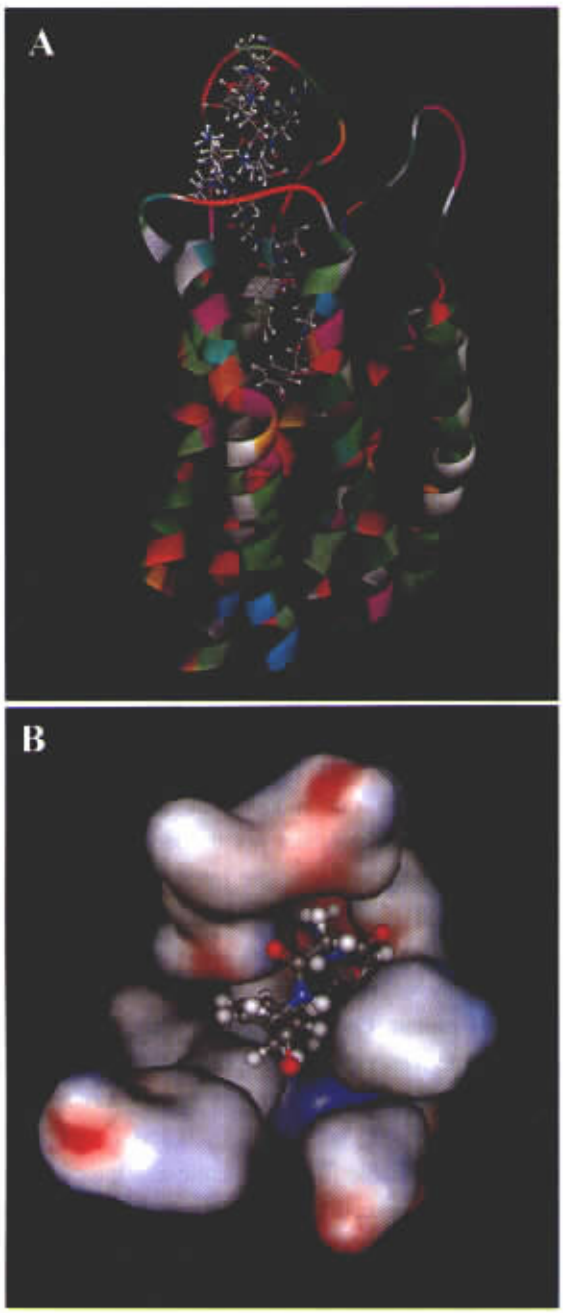

Fig 2. Molecular model of nociceptin-ORL1 receptor complex. (A): The nociceptin is shown in ball-and-stick style, the ORL1 receptor is represented as flat ribbon. (B): molecular surface showing N-terminus FGGF of nociceptin in the binding pocket and some residues near the TM-EL loops interface.

The N-terminal tetrapeptide FGGF binds in a highly conserved region, which is the inner part of the binding pocket. The topology of the FGGF binding site is in good consistance with the shape of other opioids binding pockets existing in μ- or δ-receptors, which is mapped by site-directed mutagenetic approaches[5]. The protonated nitrogen at the N-terminus of nociceptin interacts with negatively charged Asp130 through electrostatic and hydrogen bonding. This interacting mode is general and has been proved in the opioid receptor family with their specific ligands[7]. Furthermore, as shown in Fig 3, the hydroxyl oxygen at the side chain of Thr305 also forms hydrogen bond with the protonated N-terminus of nociceptin, and therefore, makes the ligand interacting with ORL1 receptor more potent than other ligands, such as lofentanyl as discussed below. The side chain of Phe1 is

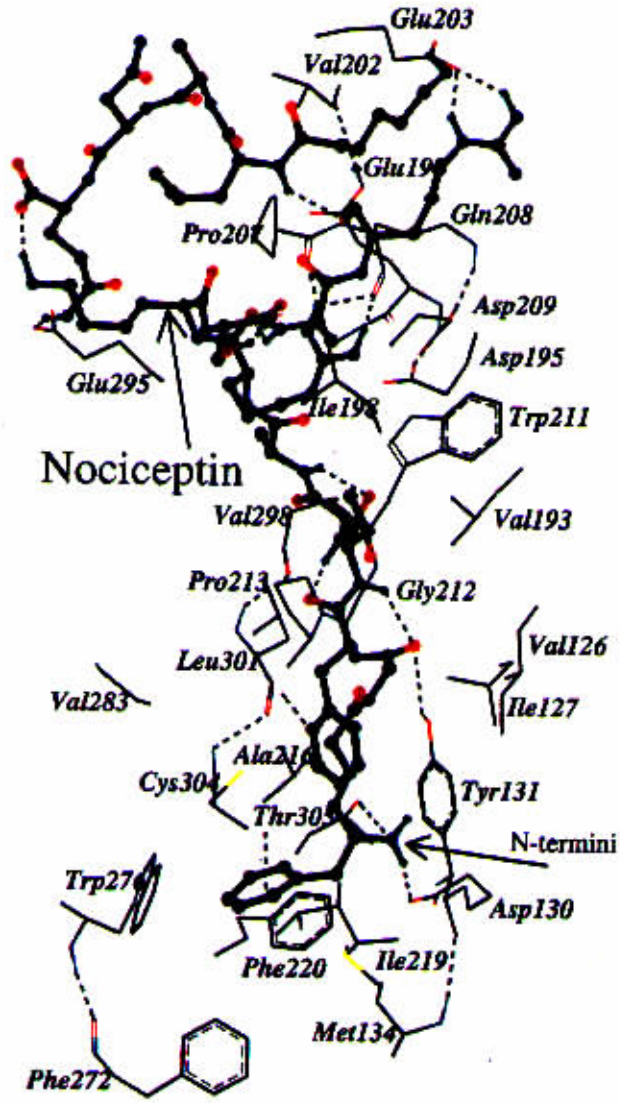

Fig 3. Binding mode of nociceptin with ORL1 receptor, the intramolecular and intermolecular hydrogen bonds are represented by dashed lines. Only the side chains of residues of ORL1 receptor (within 5 Å around the ligand) interacting with nociceptin are represented as atoms, the nociceptin is shown in ball-and-stick style, the side chains of residues of ORL1 receptor interacting with nociceptin are shown in stick style, all the hydrogens except those forming the hydrogen bonds are hidden.

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surrounded by several hydrophobic and aromatic side chains of residues Ile219, Phe220, Phe272, Trp276, Met134, and Gly308. Interestingly, the phenyl ring of Phe1 runs parallel with the phenyl ring of Phe220 and interacts with each other via aromatic π-π stacking. Residue Gln280 (which is not shown) lies almost nearly to the outside of the binding site of Phe1 but still contacts weakly with the binding pocket. This is in agreement with the unchanged binding property of the Q280H mutant receptor with respect to nociceptin. Gly2 and Gly3 of nociceptin are closely surrounded by several conserved residues as Tyr131, Ala216, Ile127, Val283, Leu301 and Cys304. .The carbonyl oxygen of Gly3 forms hydrogen bonds with the side chain of Tyr131 and the -NH group of Thr5. This additional intramolecular hydrogen bond (Thr305-NH...O=C<Gly3) further stabilizes the conformation of FGGF sequence of nociceptin in its binding site. The side chain of Phe4 runs almost perpendicular to the phenyl ring of Phe1 and parallel to the side chain of Tyr131 of ORL1 receptor, forming favourable face-face and face-edge stacking interactions.

As shown in Fig 3, Thr5, Gly6, Ala7 bind with side chains of residues Gly212, Pro213, Val283, Leu301, Val193 and Val298 of ORL1 receptor that are near the end of the binding cavity and towards the EL-2 loop. These residues are located at the interface between EL-2 loop and transmembrane helices and are somewhat variable among the opioid receptor family. Lapalu *et al*[8] have demonstrated that the positions 5 and 6 of nociceptin are the major determinants of ORL1 and κ-opioid receptor selectivity, which was identified by structure-activity studies of dynorphin A/nociceptin hybrid peptides. In accordance with these experimental results, the side chain of Thr5 of nociceptin is located near a partially polar environment provided by Pro213 and Gln286 (which is not shown in Fig 3). Furthermore, the hydrogen bonds formed by Thr5 and Gly3 (as described above), carbonyl oxygen of Thr5 and -NH group of Val7, which are shown in Tab 1 and Fig 3, clearly show that any change in this part of nociceptin results in potentially unfavourable interaction decreasing to some extent of the binding affinity of nociceptin with ORL1 receptor.

The positively charged core of nociceptin (8 – 13) interacts predominantly with the negatively charged EL-2 loop. From Fig 3 and Tab 1, we can see that this interaction has the electrostatic and hydrogen bonding characteristics. Some intramolecular hydrogen bonds are formed, which make the residues Lys9 and Gln17 less important for activation of nociceptin than other residues namely Arg8, Arg12 and Lys13, but they are not dispensable in the total complementarity of nociceptin with ORL1 receptor. Intermolecular hydrogen bonds, which are mainly formed between positively charged residues Arg8, Arg12, and Lys13 of nociceptin and negatively charged residues Asp209, Glu203, and Glu197 of ORL1 receptor, fundamentally account for electrostatic interaction of nociceptin with EL-2 loop of ORL1 receptor. The other hydrogen bonds between residues of nociceptin and EL-2 loop namely Arg8 and Pro207, Pro208; Lys13 and Val202; Gln17 and Glu295, contribute to the formation of hydrophobic and hydrogen bonding interaction between nociceptin and ORL1 receptor.

Based on the interaction model of nociceptin with ORL1 receptor as described above, some important experimental results can be explained reasonably by our constructed complex model, which in turn offers a validation of our model. Butour *et al*[9] declared that binding test, according to the pseudopeptide analogue of nociceptin, formulated as [Phe1Ψ(CH_2-NH)Gly2] nociceptin (1 – 13)-NH_2 had high affinity with ORL1 receptor. It could be demonstrated by our model that the change in the width at two Gly residues but not the length of FGGF sequence will not greatly affect the interaction of nociceptin in the conserved binding pocket, because two aromatic Phe residues have more important contribution towards binding affinity of nociceptin than do Gly2 and Gly3. The Q280H mutant ORL1 receptor[7] can still retain the affinity and activation of nociceptin because the mutated residue has little influence on the conservativity of the binding pocket among helices 3, 5, 6 and 7. The Phe1 residue is indispensable in the aromatic π-π interaction described above, so excision of Phe1 from nociceptin results in an inactive peptide because it destroys simultaneously the electrostatic and hydrogen bonding between Phe1, Phe4 of nociceptin and Asp130, Tyr131 of ORL1 receptor. Replacing Gly6 with Arg residue in the nociceptin[8] greatly adds steric hindrance and electrostatic repellence of Arg302 at the interface of TM helices and extracellular loops, thereby resulting in an unacceptable interaction, and lowering the binding affinity to the receptor. Residues 5 – 7 of nociceptin bind in a nonconserved region composed of variable amino acids, together with information from structure-activity study[3,5], thus a conclusion can be drawn that positions 5 and 6 are the important determinants of nociceptin binding selectivity between ORL1 and κ-receptors. On the other hand, the N-terminal tetrapeptide FGGF has been shown to be essential for receptor occupation and activation. This is well in ac-

cordance with our binding model of nociceptin represented in Fig 2 and 3. The positively charged core of nociceptin (8 – 13) forms a number of hydrogen bonds and electrostatic interactions with the receptor. Together with the interactions in the binding pocket, these factors demonstrate that the minimal fragment sequence needed to fully activate the ORL1 receptor must be nociceptin (1 – 13)[10] or its analogues as shown in Fig 1.

Comparing our model of nociceptin-ORL1 receptor complex with the results of the molecular simulation of dynorphin A binding to κ-opioid receptor[11], the main difference exists in the binding region of EL-2 loop and the interface of EL-2 loop-helical bundles. It is certain that different domains of these two receptors are involved in the recognition of nociceptin and dynorphin A and subsequent activation of these two receptors, although the conserved binding pockets are similar. So the opioid binding site in the ORL1 receptor can be restored by site-directed mutagenic or chimeric experiments, just as reported by Meng *et al*[12]. It has already been reported that the conserved His residue in the TM6 is very important for the binding of opioids and peptides to μ, δ, and κ opioid receptors. Thus the mutation of Val279, Gln280, and Val281 to the counterpart conserved residues Ile, His, and Ile of μ, δ, and κ opioid receptors can restore, or even increase the binding affinity of endogenous peptides with ORL1 receptor [dynorphin (1 – 17) by 3 times, dynorphin (1 – 13) by 3 times, dynorphin (1 – 8) from very low K_i value to 0.57 μmol/L, and α-neoendorphin by one numerical order of K_i value[12]]. The positive charge brought about by the mutation of Ala216 to Lys can also increase the binding affinity of the peptides mentioned above to ORL1 receptor because this mutation can increase the strength of electrostatic interaction between negatively charged side chains of these peptides and the side chain of residue Lys. Simultaneous mutations of VQV (residues of 279-281) to IHI, TVIA (residues of 125-128) to TVIS, and Ala216 to Lys (or the mutation of residue Thr305 to Ile in alternative combination) brought about the loss of binding affinity of nociceptin to ORL1 receptor can be explained. These mutations not only made conformational alteration in the binding pocket of nociceptin in ORL1 receptor, especially the conformational and hydrophobic charge in properties change (Ala128 to Ser) near the conserved residue Asp130, but also destroyed the direct interaction between the nociceptin and ORL1 receptor. As the residue Ala216 could contribute to the hydrophobic interaction between nociceptin and ORL1 receptor, Thr305 could form hydrogen bonds with nociceptin as shown in Fig 3 and Tab 1, although these residues did not have the same significance as Asp130 in the binding process of nociceptin to ORL1 receptor. From these mutational experiments, one can see that the binding affinity of nociceptin with ORL1 receptor, and the selectivities of ORL1 receptor among noceceptin, dynorphin derivatives come from some subtype conserved residues shown in Fig 3, especially the conformational restriction of residues near the most important binding site of Asp130. In Meng's study[12], the EL-2 loop of κ-opioid receptor is not absolutely essential for activation of that receptor and mainly acts as filter for or controlling access of μ/δ-selective opioids to the transmembrane binding site of κ-receptor. But the EL-2 loop together with several variable residues in the EL-2 loop-helices interface of ORL1 receptor have an important role in the selectivity and activation of nociceptin-ORL1 receptor system. Hybrid peptide study of nociceptin-dynorphin A[5] has shown that TGARKSAR sequence (Fig 1) could activate nociceptin towards the ORL1 receptor, and the Arg8 residue in this sequence is of paticular importance. These results could be clarified by interaction mechanism described above and as shown in Fig 3 in that more abundant hydrogen bonds are formed by Arg8 with residues from receptor than with other residues in nociceptin.

Interaction of opioids with ORL1 receptor

Based on the sequence and structural homology of ORL1 with μ, δ, κ-opioid receptors, it was natural that a lot of opioids were used to test their binding affinities and other pharmacological properties with ORL1 receptor to gain more understanding of opioid receptors at molecular level. Unfortunately, only few opioids (lofentanyl and etorphine) were found to have high to moderate affinities for ORL1 receptor[5]. Lofentanyl is a selective agonist of μ-opioid receptor, etorphine is a non-selective universal agonist of opioid receptors. In order to explore in detail the vestigial opioid binding site and compare it with that of nociceptin in ORL1 receptor, and furthermore, to test the reliability of above docking results, these ligands including four steroisomers of lofentanyl were docked into the putative transmembrane binding pocket of ORL1 receptor. An advanced program DOCK4.0 was used to tackle the interaction mode of these small organic molecules with the ORL1 receptor. After a tree-like conformation search for receptor site matching, the ligand-receptor matching quality was scored principally based on their mutual van der Waals and electrostatic in-

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teractions. During the docking operation, the side chains of the residues in the possible binding pocket were represented as spheres which were generated by sphegen program, then the scoring grids were created to score the matching process of ligand-receptor interaction. Every model of ligand-receptor complex was minimized after the docking was completed. The docking results are represented in Fig 4 and 5. The conformational changes of these ligands before and after docking are listed in Tab 3. Comparing the binding conformations of lofentanyl before and after docking, small changes have been observed for φ_4, φ_5, φ_6, and φ_7 of the conformations deduced from DOCK4.0 program. Significant changes, however, are observed for φ_1, φ_2, and φ_3 after docking. This indicates that some adjustments for these dihedral angles have to be made in order to fit the structural requirement of the ORL1 recepor binding sites. As shown in Fig 4, the phenylethyl group connecting the piperidine cycle of lofentanyl is packed closely with several aromatic side chains of conserved residues of Phe220, Phe272, Phe276, Phe224, with which the π-π stacking interaction takes place; and nonpolar residues of Met134 and Gly308, with which the hydrophobic interaction is generated. The hydrogen atom connecting to the nitrogen atom of the piperidine ring forms a hydrogen bond to the $O^{\delta 1}$ atom of Asp130 on one hand, and this protonated nitrogen (bearing + 1 net atomic charge) interacts with Asp130 (bearing − 1 net atomic charge) through electrostatic interaction on the other hand. This interacting mode is in consistence with that of fentanyl analogs interaction with μ-opioid receptor[13], and also in accordance to nociceptin interaction with its parent receptor. The piperidine cycle and its methyl group may interact with side chains of Tyr131, Cys304 and Thr305 through hydrophobic interaction. Interestingly, hydroxyl group at the side chain of Thr305 forms a hydrogen bond with the carbonyl oxygen of the $-CH_2COOCH_3$ group connecting the central piperidine ring. The other phenyl ring of lofentanyl is surrounded by hydrophobic side chains of Ile127 and Val126, and there is somewhat weak π-π stacking interaction between this phenyl ring and side chain of Tyr131. Ile111 and Val298, which are situated at the interface of EL loops-transmembrane helices, can interact with the propionyl group of lofentanyl, although they are loosely packed with each other. From the model of lofentanyl interacting with ORL1 receptor and nociceptin-ORL1 complex (Fig 4), we can conclude that there is indeed a very conservative binding pocket existing in the ORL1 receptor in which the N-terminal FGGF residues of nociceptin are well packed. In other words, this binding pocket is very essential for the binding affinity of nociceptin with ORL1 receptor, which is identical to some extent to the binding site in the fentanyl analogues-μ opioid receptor system.

In the case of etorphine binding with ORL1 receptor as shown in Fig 5, the interaction between this ligand and receptor is much less pronounced compared with that of lofentanyl and nociceptin. From Fig 5, we can see that the etorphine molecule is bulkier than lofentanyl and the N-terminus of nociceptin, so it can not fit well into the binding pocket. Also the conformation of etorphine does not change very much before and after docking, as some important distances and torsional angles in etorphine keep almost the same as is evident in Tab 3. One part of etorphine including cycles C, D (Fig 1), piperidine cycle and its protonated nitrogen group are packed through hydrophobic interaction with several conserved residues of Phe220, Met134, Tyr131, and Asp130 of ORL1 receptor in the binding pocket. The protonated nitrogen at the piperidine ring interacts with the negatively charged side chain of Asp130 through electrostatic and hydrogen bonding, but this kind of interaction is somewhat weak because the distance between the positively and negatively charged atoms is of 2.8 Å, which is longer than the normal length of a hydrogen bond. This may be the main reason that the binding affinity of etorphine is much less potent (K_i = 540 nmol/L) than that of lofentanyl (K_i = 24 nmol/L). The side chain at the double bond of ring C (Fig 1) is surrounded by hydrophobic residues of Val126, Ile127, Ala128, and Ile129. The other part of etorphine including the phenyl ring A, the hexabasic cycle, the pentabasic cycle B interact with residues of ORL1 receptor of Ile54, Leu104, Leu301, Arg302, Cys304, and Thr305 of ORL1 receptor. The interacting mode in this region is mainly hydrophobic, besides, a hydrogen is formed by the hydoxyl oxygen connecting phenyl ring A and the $-N^{\varepsilon}H_2$ group at the side chain of Arg302.

On the whole, etorphine and lofentanyl pack with the ORL1 receptor through electrostatic, hydrophobic and hydrogen bonding, although the interaction is not as pronounced as that of nociceptin-ORL1 receptor system. Part of etorphine, near to its piperidine ring, does not fit fully into the conserved binding pocket of ORL1 receptor. Only some conserved residues in the binding site are involved in the interacting process of opioids like lofentanyl (discussed above) and etorphine. Amino acid residues namely Asp130, Tyr131, Met134, Phe220,

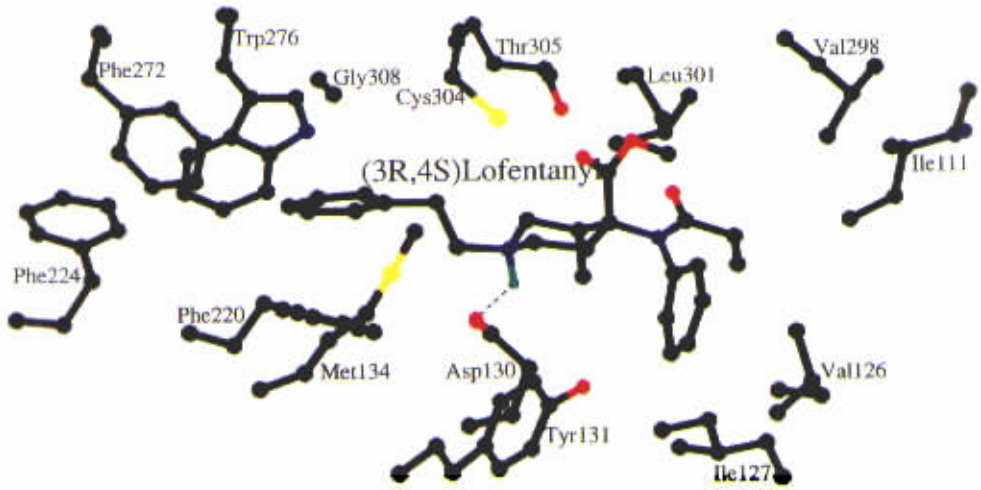

Fig 4. Interacting model of lofentanyl with ORL1 receptor. The dashed line represents the hydrogen bond formed between protonated nitrogen at piperidine cycle of lofentanyl and side chain of conserved Asp130 of ORL1 receptor, the style shown is as in Fig 3.

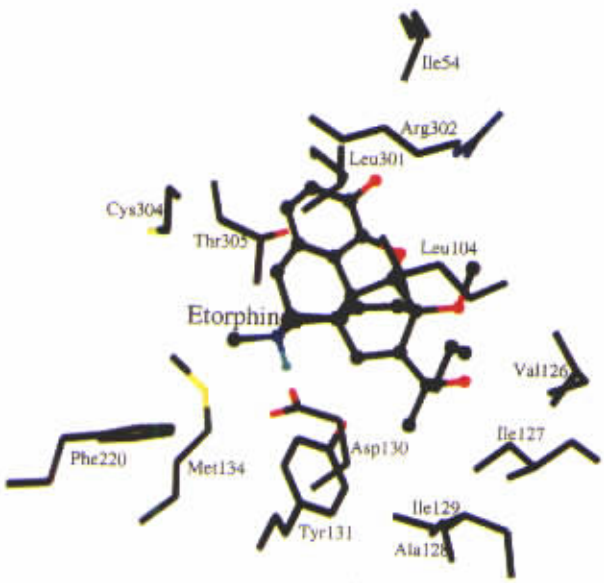

Fig 5. Interaction model of etorphine with ORL1 receptor, the representative style is as in Fig 3.

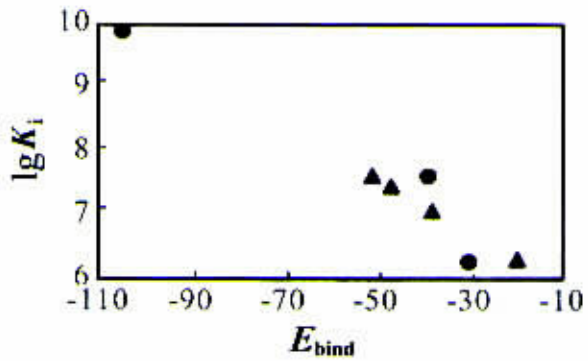

Fig 6. Correlation between binding energies (kcal/mol) and binding affinities ($-\lg K_i$) of nociceptin, lofentanyl, etorphine binding with ORL1 receptor. The circular points (●) show the experimental K_i values of nociceptin, lofentanyl and etorphine, the angular points (▲) represent the predicted $-\lg K_i$ values of four steroisomers of lofentanyl.

Cys304, and Thr305 in the binding pocket are commonly related to the interaction between nociceptin, opioids and ORL1 receptor. The main difference of interaction between nociceptin and opioids with ORL1 receptor exists in the kinds and number of residues which are comprising the binding pocket, the top portion of binding cavity and the interface of EL-2 loop-helical bundles. The vestigial opioids binding pocket on ORL1 recptor is similar to that in other opioid receptors. This has been demonstrated by our constructed models of opioids interacting with ORL1 receptor, and therefore binding affinities of opioids with ORL1 receptor can be heightened by site-directed mutagenesis or other experimental methods.

Correlation between binding affinities and binding energies As stated in our previous studies, lofentanyl is very similar in molecular structure to fentanyl analogs especially 3-methylfentanyl. They have the same number of chiral carbon atoms (C3 and C4) and steroisomers. The substitution groups at the central piperidine cycle are almost the same between 3-methylfentanyl and lofentanyl except that there is an additional $-CH_2COOCH_3$ group connecting the chiral C_4 atom

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Tab 3. Conformational changes of lofentanyl (including four steroisomers) and etorphine before and after docking.

Compounds		φ_1°	φ_2°	φ_3°	φ_4°	φ_5°	φ_6°	φ_7°
(3R,4R)-Lofentanyl	b	-93.2	171.8	-173.9	-153.4	90.2	179.9	66.1
	a	113.3	-166.0	70.3	61.7	80.2	175.1	44.8
(3R,4S)-Lofentanyl	b	-92.5	171.9	-176.6	-168.5	97.8	179.0	65.5
	a	79.4	-168.5	65.6	-164.3	81.6	-176.6	78.1
(3S,4R)-Lofentanyl	b	-91.8	171.8	-174.2	-177.4	91.4	179.8	59.1
	a	-75.3	60.8	-89.6	-172.9	96.6	172.9	82.3
(3S,4S)-Lofentanyl	b	-95.2	171.4	-175.6	-170.7	72.5	-176.0	80.1
	a	76.8	134.2	62.6	0.9	79.4	178.1	-109.5
		d_1(Å)	d_2(Å)	d_3(Å)	d_4(Å)	ω_1°	ω_2°	ω_3°
Etorphine	b	4.239	3.943	3.519	2.306	-38.7	41.6	7.0
	a	4.209	3.940	3.500	2.308	-36.6	42.2	4.5

φ_1, φ_2, φ_3, φ_4, φ_5, φ_6 and φ_7 are labeled as shown in Fig 1 of lofentanyl; d_1, d_2, d_3 and d_4 are the distances between the center of the ring A, B, C, D (the ring name is labeled in Fig 1) to the protonated nitrogen at the piperidine cycle of etorphine respectively; ω_1, ω_2 and ω_3 are the torsional angles between the rings and protonated nitrogen (PN) at the piperidine cycle named as A, B, C, PN; B, C, PN, D; D, A, B, C respectively.

Tab 4. Biding energies of nociceptin, lofentanyl, etorphine complexing with ORL1 receptor (the energy of ORL1 receptor is -294.112 kcal/mol).

Compounds	K_i(nmol/L)	$-\lg K_i$	PA	E_{mini}	E_{active}	$E_{complex}$	$E_{binding}$
Nociceptin	0.13[a]	9.89	9.913	22.572	78.479	-376.414	-104.874
Lofentanyl	24[a,b]	7.62	7.129				-38.872[c]
(3*R*,4*R*)-Lofentanyl			7.46	14.635	19.762	-326.342	-46.865
(3*R*,4*S*)-Lofentanyl			7.63	18.674	31.707	-326.326	-50.888
(3*S*,4*R*)-Lofentanyl			6.31	14.887	21.944	-298.957	-19.732
(3*S*,4*S*)-Lofentanyl			7.08	16.306	24.923	-315.809	-38.003
Etorphine	530[a]	6.27	6.758	67.392	72.808	-256.817	-30.097

[a]: see ref 9, [b]: this value is of the raceme of lofentanyl, [c]: the averaged binding energies of four steroisomers of lofentanyl. PA: the predicted binding affinities of these ligands; E_{mini}: energy of the lowest-energy conformation; E_{active}: energy of the active conformation; $E_{complex}$: energy of the ligand-receptor complex; $E_{binding}$: the calculated binding energy.

in lofentanyl (Fig 1). *Lofentanyl can also act as an agonist of* μ-opioid receptor with a high value of binding affinity ($K_i = 0.14$ nmol/L)[14]. Furthermore, there is an identical ligand-binding pocket among ORL1 and other opioid receptors which is discussed in this paper and our previous studies[6]. *Taking all these information together*, we can say that there is a common pharmacophore group existing in lofentanyl and other fentanyl analogues. The binding mode of lofentanyl and 3-methylfentanyl to μ-opioid receptor must be identical to each other, the binding style of lofentanyl to μ-opioid receptor and ORL1 receptor may be similar to a high extent. In our previous studies, we have explored some relationships between binding energies of fentanyl analogues with μ-opioid receptor and their experimental binding affinities ($-\log K_i$). The rationale is that the potency of these agonists complexing with opioid receptors is in direct proportion to their binding energies, and the results have proved to be reliable.

As in our previous studies, Tab 4 is compiled with the binding energy data of nociceptin, lofentanyl (including 4 steroisomers), and etorphine interacting with ORL1 receptor, calculated according to equation (1). Satisfied with the 3D-structural models of ligand-receptor complexes we then performed a linear regression analysis[15] using binding energies as a sole determinant. In this analysis, the average value of binding energies of lofentanyl isomers is used based on the fact that the K_i value of lofentanyl is of its raceme. The correlation of the binding energies with binding affinities is graphically shown in Fig 6. Compared with 3-MF, the binding energies of the four steroisomers of lofentanyl are in the same order regarding their numerical value and have similar correlation regarding steroisomeric phenomenon. Based on the

present data and the method used in our previous study, the binding affinities of 4 isomers of lofentanyl to ORL1 receptor were calculated in order to confirm that the (3*R*, 4*S*)-lofentanyl has higher binding affinity than other isomers. The predicted K_i values of four steroisomers of lofentanyl are also compiled in Tab 4 and shown in Fig 6. Although the binding process of agonists to opioid receptors is complex regarding their thermodynamic aspects, especially the entropic effect, the binding free energy should correlate with their binding energies. It could be concluded from this study that binding energies of these ligands correlate well with their binding affinities, and in turn this relationship could be used to verify the reliability of constructed molecular models. Accordingly, the high correlation between binding energies and binding affinities of these ligands is an additional validation of 3D-structural models of the ligand-receptor complexes predicted by our modeling methods, and the predicted K_i values of four steroisomers of lofentanyl could be used as a reference in further experimental studies reganding opioid receptors.

In conclusion, we have constructed three-dimensional structures of nociceptin, lofentanyl, and etorphine-ORL1 receptor complexes, our models match well with the information of interaction characteristics derived from several experimental strategies, and offer another verification of ORL1 receptor model built in our previous work. The binding affinities of four stereoisomers of lofentanyl are reasonably predicted by our models. These results carry some significance in the theoretical studies about biological interactions of opioid receptors with their ligands.

REFERENCES

1 Mollereau C, Parmentier M, Mailleux P, Meunier JC. ORL1, a novel member of the opioid receptor family, cloning, functional expression and localization. FEBS Lett 1994; 341: 33-8.
2 Meunier JC, Mollereau C, Toll L, Parmentier M, Costentin J. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature 1995; 377: 532-5.
3 Lapalu S, Moisand C, Butour JC, Mollereau C, Meunier JC. Different domains of the ORL1 and κ-opioid receptors are involved in recognition of nociceptin and dynorphin A. FEBS Lett 1998; 427: 296-300.
4 Ewing TJA, Kuntz ID. Dock: Version 4.0. California: University of California in San Diego (UCSD); 1998.
5 Reinscheid RK, Higelin J, Henningsen RA, Monsma JFJ, Civelli O. Structures that delineate orphanin FQ and dynorphin A pharmacological selectivities. J Biol Chem 1998; 273: 1490-5.
6 Huang XQ, Jiang HL, Luo XM, Chen KX, Zhu YC, Ji RY, *et al*. Comparative molecular modeling on 3D-structure of opioid receptor-like 1 receptor. Acta Pharmacol Sin 2000; 21: 529-535.
7 Huang XQ, Jiang HL, Luo XM, Zhu YC, Chen KX, Ji RY, *et al*. Molecular modeling on the interaction of fentanyl analogs: from solvation to μ-opioid receptor. Acta Pharmacol Sin 2000; 21: 46-54.
8 Lapalu S, Moisand C, Mazarguil H, Cambois G, Mollereau C, Meunier JC. Comparison of the structure-activity relationships of nociceptin and dynorphin A using chimeric peptides. FEBS Lett 1997; 417: 333-6.
9 Carpenter KJ, Dickenson AH. Evidence that [Phe1Ψ (CH_2-NH) Gly2] nociceptin-(1-13)-NH_2, a periphery ORL1 receptor antagonist, act as an agonist in the rat spinal cord. Br J Pharmacol 1998; 125: 949-51.
10 Guerrini R, Calo G, Bigoni R, Rizzi A, Regoli D, Salvadori S, *et al*. Structure-activity study of the nociceptin (1-13)-NH_2 N-terminal tetrapeptide and discovery of nociceptin receptor antagonist. J Med Chem 1998; 41: 3360-6.
11 Aterlini G, Portoghese PS, Ferguson DM. Molecular simulation of dynorphin A-(1-10) binding to extracellular loop 2 of the κ-opioid receptor. A model for receptor activation. J Med Chem 1997; 40; 3254-62.
12 Meng F, Taylor LP, Hoverstem MT, Ueda Y, Civelli O, Akil H. Moving from the orphanin FQ receptor to an opioid receptor using four point mutation. J Biol Chem 1996; 271: 32016-20.
13 Rong SB, Zhu YC, Jiang HL, Chi ZQ, Chen KX, Ji RY, *et al*. Molecular modeling of μ opioid receptor and receptor-ligand and interaction. Acta Pharmacol Sin 1997; 18: 317-22.
14 Bot G, Blake AD, Li SX, Reisine T. Fentanyl and its analogs desensitize the cloned mu opioid receptor. J Pharmacol Exp Ther 1998; 285: 1207-18.
15 Stahle L, Wold S. Multivariate analysis and experimental design in biochemical research. Prog Med Chem 1988; 25: 292-37.

孤啡肽和阿片类配体与阿片孤儿受体相互作用的机制研究[1]

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关键词 孤啡肽；洛芬太尼；埃托啡；阿片孤儿受体；分子对接；结合能

目的：研究孤啡肽和阿片类配体与阿片孤儿受体相

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互作用的分子机制. **方法**: 用分子动力学方法计算孤啡肽的最低能构象; 通过分子对接程序将孤啡肽、阿片类配体对接到阿片孤儿受体的结合口袋中; 通过结合能的计算研究配体对受体的亲和力与它们的结合能之间的关系. **结果**: 孤啡肽(1－4)残基位于结合口袋的底部, 孤啡肽(5－7)残基位于结合口袋的顶部, 孤啡肽(8－17)残基与孤儿受体的第二膜外环区结合; 阿片类配体和孤儿受体的结合方式与孤啡肽的情况类似, 区别在于孤儿受体参与配体结合的残基种类和数量不同, 因而亲和力不同; 配体－受体的结合能与配体的亲和力之间有很好的相关性; 预测了洛芬太尼四个异构体与阿片孤儿受体的亲和力. **结论**: 该研究能够解释许多实验事实, 有助于进一步理解阿片受体与配体相互作用的分子机制并设计新的分子生物学实验.

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