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Molecular modeling on solvent effect and interaction mechanism of fentanyl analogs to μ-opioid receptor[1]

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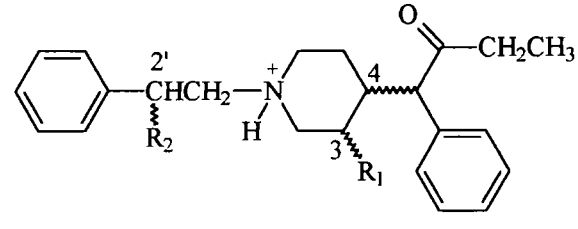
KEY WORDS fentanyl; molecular models; solvents; binding pocket; structure-activity relationship

ABSTRACT

AIM: To do theoretical study about solvation effect and interaction mechanism of fentanyl analogs (FA) to μ opioid receptor (μOR). **METHODS**: Flexible docking (FlexiDock) was performed by using the possible active conformations of FA and optimized 3D structure of μ opioid receptor. Binding energies were calculated. Comparative molecular force field analysis (CoMFA) and quantitative structure activity relationship (QSAR) studies were carried out based on results of flexible docking. Solvation effects were considered by studying interaction of FA with water molecules. Partial least square (PLS) analysis was used to calculate regression equation for analgesic activities using binding energies as descriptive factor. **RESULTS**: 1) Binding conformations of these analogs derived by flexible docking were reasonable. 2) It was most possible for the FA to exist in water solution in the form of binding conformations. 3) Energetic calculation and QSAR analysis showed a good correlation between the calculated binding energies of FA and their analgesic activities. 4) Based on the 3D-model, the possible interaction mechanism of FA with μ opioid receptor can be illustrated reasonably. **CONCLUSION**: The nature of the correlation between the binding affinities and analgesic activities of FA was explained by our modeling result.

INTRODUCTION

The discovery of novel and highly potent analgesic agent with low dependence or habituation is a stimulating and exciting challenge[1]. Among the many potential analgesics, fentanyl[2] (as shown in Scheme 1) and its congeners[3,4] are of great interest not only because of their clinical utilities[5], but also because certain members of this series exhibit unique biological properties[6]. The most promising analogs of fentanyl are methylfentanyl (MF)[3] and ohmefentanyl (OMF)[4], which were synthesized in our laboratory. Structurally, 3-MF contains 2 asymmetric carbon atoms (C3 and C4) and OMF involves 3 chiral carbon atoms (C3, C4 and C2′), so they have 4 and 8 enantiomers, respectively. The stereo-difference in analgesic activities was discovered between *d-cis*-MF and *l-cis*-MF, and it is more tremendous among the 8 stereoisomers of OMF.



Fentanyl	**$R_1 = R_2 = H$**
3-Methylfentanyl	**$R_1 = CH_3$, $R_2 = H$**
Ohmefentanyl	**$R_1 = CH_3$, $R_2 = OH$**

Scheme 1. Structural formulae of FA

Based on our previous 3D-model of μ opioid receptor and possible active conformations of 13 FA[7,8],

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and by using a new program FlexiDock of Tripos[9] which involves flexibility of both ligand and receptor, the present work was to elucidate in detail the interaction mechanism of FA with μ opioid receptor and to demonstrate thermodynamic aspects of the transfer of fantanyl analogs from solution to receptor binding sites.

COMPUTATIONAL METHODS

A series of 13 molecules of fentanyl analogs including fentanyl, 4 enantiomers of 3-methylfentanyl, and 8 enantimers of OMF, were employed in this study. The coordinates of the 3D-structural model of μ-opioid receptor were adopted from our previous modeling results[7,8]. Sybyl 6.4[9] was employed for all the calculations, which were performed on Silicon Graphics Indigo XZR 4000 workstations.

Flexible docking The initial active conformations of FA were docked into the putative binding sites among the 7 helix bundles of the 3D-structural model of μ-opioid receptor. Then the docked ligands were subject to perform flexible docking calculation employing the option of FlexiDock in Sybyl 6.4. During our flexible docking calculation, all the single bonds of ligand and side chains of the amino acid residues around the ligand 8 Å, as well as the orientation of the ligand were taken as variables within the interaction region.

Thus we generated the initial structures of the ligand-receptor complexes. These structures were then successively refined using the minimization option of Sybyl 6.4[9]. The entire complex was minimized using 200 steps of steepest descent, followed by conjugate gradient minimization to a root-mean-square (RMS) energy gradient of 0.07 kcal/mol · Å^2. Amber 4.0 force field and Pullman charges were employed throughout. Calculations were performed with a dielectric constant of 5 to simulate the solvation effect of the ligand in the protein environment. The binding energy (E_{bind}) of the ligand was calculated using following formula:

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

where E_{ligand} is the energy of the ligand corresponding to the overall lowest-energy conformation, and $E_{receptor}$ is the energy of the receptor.

Solvation energy of the ligands Water molecules were used as solvent molecules to simulate the physiological environment of pharmacological systems. The water molecules solvated the FA explicitly with the SOLVENT module of Sybyl 6.4. Then the supermolecule system of a fentanyl analog and a number of water molecules were subject to perform energy minimization using the similar procedure of ligand-receptor complex minimization. The solvation energy, $\triangle E_{sol}$, can be calculated with following formula.

$$\triangle E_{sol} = E_{sup} - E_w - E_{solute} \quad (2)$$

where E_{sup} is the energy of fentanyl-water supermolecule, E_w is the energy of water clust, and E_{solute} is the energy of fentanyl analog.

RESULTS AND DISCUSSION

Binding conformations and CoMFA analysis In our previous study[7,8], we have got the primary binding conformations of FA through systematic search, CoMFA study and BCSPL calculation[10,11]. The active conformation of each FA was used to perform flexible docking calculation with FlexiDock program of Sybyl 6.4 . Fig 1 illustrates the binding conformations for the 13 FA resulted from the FlexiDock program and minimization techniques described above. Tab 1 provides some of the geometric structural parameters data for these conformers. For the sake of comparison, the dihedral angles of the single bonds corresponding to the conformers deduced from BCSPL program[10] are also listed in Tab 1.

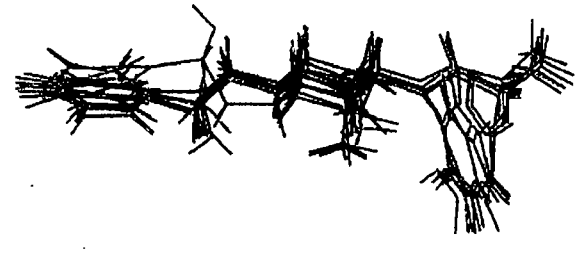

Fig 1. Binding conformations of FA and their aligment in μ opioid receptor binding site predicted by FlexiDock program.

Compared with the binding conformations derived from BCSPL program (Tab 1), small changes have been observed for ω_2, ω_6, and ω_7 of the conformations deduced from FlexiDock program. Great changes, however, have occurred for ω_1, ω_3, ω_4, and ω_5 after flexible docking. This indicates that some adjustments for these dihedral angles have to be made in order to fit

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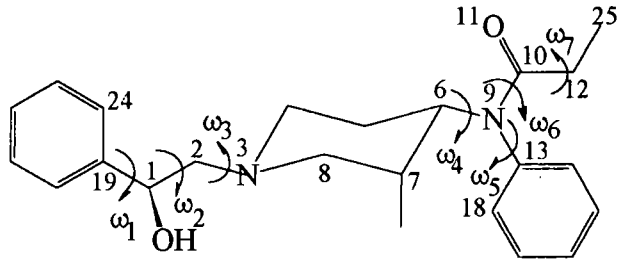
Tab 1. Dihedral angles of the single bonds for the binding conformations of FA deduced from BCSPL (a)* and FlexiDock (b).



Compounds		ω_1° (2-1-19-24)	ω_2° (3-2-1-19)	ω_3° (1-2-3-8)	ω_4° (7-6-9-13)	ω_5° (6-9-13-18)	ω_6° (6-9-10-12)	ω_7° (9-10-12-25)
Fentanyl	a	90.1	178.3	−136.5	101.4	92.6	179.0	−179.8
	b	82.4	−158.5	−66.9	173.9	105.8	179.3	−173.1
(3R, 4R)-3-MF	a	79.9	168.0	−163.5	38.9	88.1	−179.0	179.4
	b	104.4	−178.1	−86.9	−9.8	112.2	−177.6	166.1
(3R, 4S)-3-MF	a	82.4	177.0	−138.6	25.0	93.8	−179.4	−177.5
	b	68.9	−167.9	−76.3	3.0	108.1	179.2	−175.4
(3S, 4R)-3-MF	a	85.5	178.4	−139.8	0.8	91.5	−179.7	177.4
	b	107.4	171.6	−96.6	−12.7	101.5	−174.2	−175.5
(3S, 4S)-3-MF	a	87.3	177.9	−138.6	82.6	92.3	179.3	−179.8
	b	66.9	−163.8	−58.8	9.3	81.3	−169.1	178.6
(3R, 4R, 2′R) OMF	a	78.0	170.1	61.6	38.9	87.8	−179.0	178.9
	b	59.1	158.6	161.7	−17.5	106.8	−174.8	160.9
(3R, 4R, 2′S)-OMF	a	59.9	163.4	−141.3	0.7	91.2	−179.5	176.8
	b	87.3	−154.8	−72.4	−12.4	107.1	−174.4	164.8
(3R, 4S, 2′R)-OMF	a	117.6	168.2	−96.5	38.4	87.7	−179.0	179.1
	b	53.7	−156.8	−65.3	7.3	105.3	−179.0	−177.5
(3R, 4S, 2′S)-OMF	a	105.0	164.6	−164.8	0.9	92.3	−179.6	177.8
	b	104.9	−173.2	50.7	19.7	92.1	−174.2	177.5
(3S, 4R, 2′R)-OMF	a	79.9	171.5	62.4	24.8	93.4	−179.5	−178.3
	b	−61.9	166.9	−155.7	−0.3	94.3	−168.4	171.1
(3S, 4R, 2′S)-OMF	a	79.8	163.2	−148.9	83.1	92.4	−178.9	−179.3
	b	82.7	177.8	−89.5	6.1	108.0	−170.0	175.5
(3S, 4S, 2′R)-OMF	a	95.7	−171.2	58.2	27.7	91.4	−179.4	−178.5
	b	128.2	173.9	−150.0	7.7	88.9	−170.3	−178.2
(3S, 4S, 2′S)-OMF	a	90.9	179.2	−132.1	82.7	92.2	179.0	−179.0
	b	85.9	−157.8	−69.9	−16.1	95.1	−172.4	173.9

*See ref 8.

the structural requirement of the μ opioid recepor sites.

In order to test the reasonability of the binding conformations of flexible docking results indirectly, CoMFA analysis was reperformed for these 13 analogs using the FlexiDock conformers and their alignment in the receptor sites (Fig 1). The statistical results of these calculations and the predicted activities for the series of ligands appear in Tab 2 and Tab 3.

CoMFA calculation of 13 FA of Tab 1 employing the binding conformations and their alignment in μ opioid receptor binding site (Fig 1) resulted in a rather good and predictive 3D-QSAR model (Tab 2, 3),

Tab 2. CoMFA results for the FlexiDock conformations.

Cross-validated		Conventional		
r^2_{cross}	optimal Components	r^2	s	F
0.654	3	0.999	0.038	2425.748

Tab 3. Experimental activities and predicted activities with the CoMFA analysis result for FlexiDock conformation.

Compounds	EA[a]	PA[b]	δ[c]
Fentanyl	6.78	6.80	−0.02
(3R, 4R)-3-MF	6.42	6.47	−0.05
(3R, 4S)-3-MF	7.76	7.79	−0.03
(3S, 4R)-3-MF	5.69	5.67	0.02
(3S, 4S)-3-MF	7.01	6.99	0.02
(3R, 4R, 2′R)-OMF	6.71	6.73	−0.02
(3R, 4R, 2′S)-OMF	7.58	7.55	0.03
(3R, 4S, 2′R)-OMF	7.90	7.85	0.05
(3R, 4S, 2′S)-OMF	8.54	8.55	−0.01
(3S, 4R, 2′R)-OMF	4.57	4.58	−0.01
(3S, 4R, 2′S)-OMF	4.57	4.56	0.01
(3S, 4S, 2′R)-OMF	6.69	6.66	0.03
(3S, 4S, 2′S)-OMF	7.41	7.41	0.00

[a] Experimental activities ($-\lg ED_{50}$), [b] Predicted activities, [c] Residual values.

exhibiting a maximum cross-validated r^2 (r^2_{cross}) of 0.654 at three components. As these useful predictive results were derived directly from the conformational alignment of FlexiDock calculation, this indicates that the conformations resulted from FlexiDock are the most possible binding conformations of FA binding to μ opioid receptor. Therefore, CoMFA result tested indirectly the reasonability of the 3D-model of μ opioid receptor and the binding conformations of FA. Accordingly, the interaction mechanism of FA with μ opioid receptor could be explained explicitly with the 3D-models of these ligand-receptor complexes.

Solvation effect of FA Solvation energy for both the lowest-energy conformations and binding conformations of FA were calculated employing SOLVENT module of Sybyl 6.4 and formula (2). The result is listed in Tab 4 and graphically shown in Fig 2. For both kinds of conformations, the solvation energies of all analogs were at the same level. For the lowest energy conformations, the solvation energy is about −37 kcal/mol, and for the binding conformations, the solvation energy is about −42 kcal/mol. All the number of water molecules that directly bind to each fentanyl analog (the first sheet of water molecules surrounding the ligand) is 18 (Tab 4). On the other hand, the calculated solvation energy of binding conformation is more negative than that of the lowest-energy conformation of fentanyl analog. This suggests that the solva-

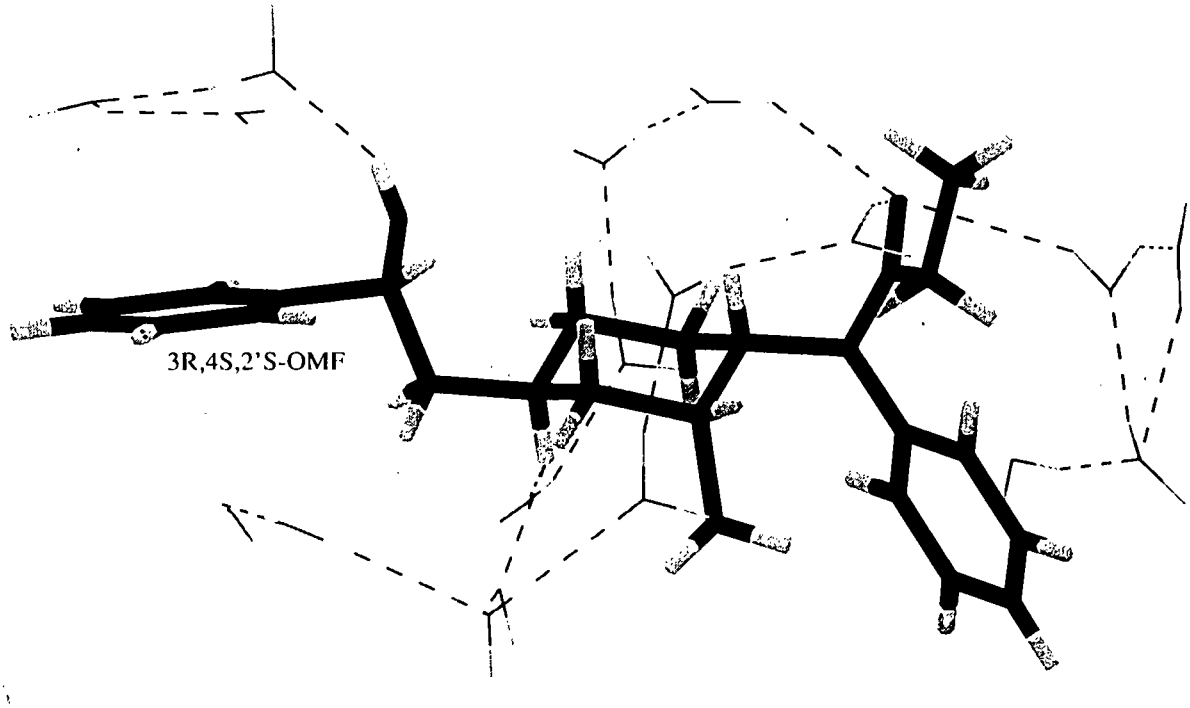


Fig 2. Solvation model of FA with water molecules (only the water molecules of the first sheet have been shown, the dot lines represent the hydrogen bonds).

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Tab 4. Solvation energies (kcal/mol) for the lowest-energy conformations ($\triangle E_{min}^{sol}$) and binding conformations ($\triangle E_{bind}^{sol}$) of FA.

Compounds	n^*	$\triangle E_{min}^{sol}$	$\triangle E_{bind}^{sol}$
fentanyl	18	-37.757	-44.119
(3R, 4R)-3-MF	18	-37.647	-44.100
(3R, 4S)-3-MF	18	-35.466	-41.204
(3S, 4R)-3-MF	18	-36.531	-42.968
(3S, 4S)-3-MF	18	-37.404	-42.298
(3R, 4R, 2′R)-OMF	18	-37.566	-44.064
(3R, 4R, 2′S)-OMF	18	-39.529	-45.051
(3R, 4S, 2′R)-OMF	18	-36.644	-42.646
(3R, 4S, 2′S)-OMF	18	-36.552	-40.972
(3S, 4R, 2′R)-OMF	18	-35.728	-41.419
(3S, 4R, 2′S)-OMF	18	-35.722	-41.012
(3S, 4S, 2′R)-OMF	18	-36.200	-41.350
(3S, 4S, 2′S)-OMF	18	-37.443	-43.423

*The number of water (H_2O) molecules that directly bind to FA.

tion effect of the binding conformations of FA is stronger than that of the lowest-energy conformations. Therefore, in the environment of physiological liquid, it is most possible for FA to exist in the state of binding conformations.

Two features contribute the binding process of a ligand to a receptor: the first one is that the conformation change of the ligand from its lowest-energy conformation (L_{lowest}) to the binding conformation (L_{bind}); and the second is that the ligand binds to the receptor in the form of binding conformation (Equation 3). Energetically, we can, therefore, consider the overall free energy of binding to have two components: the free energy cost $\triangle G_{lowest}$ ($\triangle G_{lowest} > 0$), of fixing the flexible ligand in the binding conformation, and the free energy, $\triangle G_{bind}$, of receptor binding from the binding conformation. Accordingly, solvation effect makes it favorable for FA to exist in aqueous solution in the state of binding conformations. This means that FA should not overcome the energy barrier ($\triangle G_{Lowest}$) before they bind to μ opioid receptor, which is beneficial for their binding. This might be one of the reasons that why a lot of FA have high affinities to μ opioid receptor and thus have potent analgesic activities.

$$L_{lowest} + R \xrightarrow{\triangle G_{lowest}} L_{bind} + R \xrightarrow{\triangle G_{bind}} LR \quad (3)$$

In aqueous solution, hydrogen bonds are formed between FA and water molecules (Fig 2): the hydrogen atom connecting to the nitrogen atom of piperidine ring hydrogen bonds to the oxygen atom of water, carbonyl oxygen hydrogen bonds to two water molecules, and 2′-hydroxy hydrogen bonds to the oxygen atom of one water molecule.

Interaction mechanism Although some extracellular loops of μ opioid receptor are involved in the discriminatory binding of selective ligands, these loops play their roles either by exerting a positive or negative control on ligands entry into the receptor binding sites or by their containing specific contact points[12]. The functional roles of some residues positioned within the binding region are very intriguing. Exploring the binding pocket in the 7 helix bundles has been extended by various experimental methods such as site-directed mutagenesis and receptor chimeric test. As a simplified and idealised description method used in this work, molecular modeling of ligand-receptor interaction could offer us both model of FA interacting with μ opioid receptor and fundamentally or may be realistically binding pocket of this system.

Fig 3 illustrates the predicted interaction pattern. In general, the binding pocket of FA with μ opioid receptor is principally formed between helices 3, 4, 5, 6, and 7. Residues in close proximity to the binding pocket are Ile144, Asp147, Tyr148, Met151, Trp192, Leu200, Ile238, Ile242, His297, Val300, Tyr326, and Ser329. All of these residues take part in interactions between FA and μ opioid receptor.

As shown in Fig 3, the FA are bound in an extended conformations, allowing the methyl group to fit into a small hydrophobic "pocket" formed by the side chains of Ile144, Tyr148, Met151, and Leu200. The 4-*N*-phenyl ring penetrates into a hydrophobic "pocket" nearing above small hydrophobic "pocket" formed by the side chains of residues of Tyr148 and Trp192, and this phenyl ring runs parallel to the two aromatic rings of Tyr148 and Trp192 with π-π stacking interaction. The carbonyl oxygen atom interacts with the imidazole ring of His297 through electrostatic interaction. The hydrogen atom connecting to the nitrogen atom of the piperidine ring hydrogen bonds to the $O^{\delta 1}$ and $O^{\delta 2}$ atoms of Asp147 on one hand, and this protonated nitrogen (bearing + 1 net atomic charge) interacts with Asp147 (bearing − 1 net atomic charge) through electrostatic interaction on the other hand. 2′-hydroxyl of FA hydrogen bonds to the side chains of Ser329, and 1-β-phenyl ring interacts with the aromatic ring of Tyr326 also

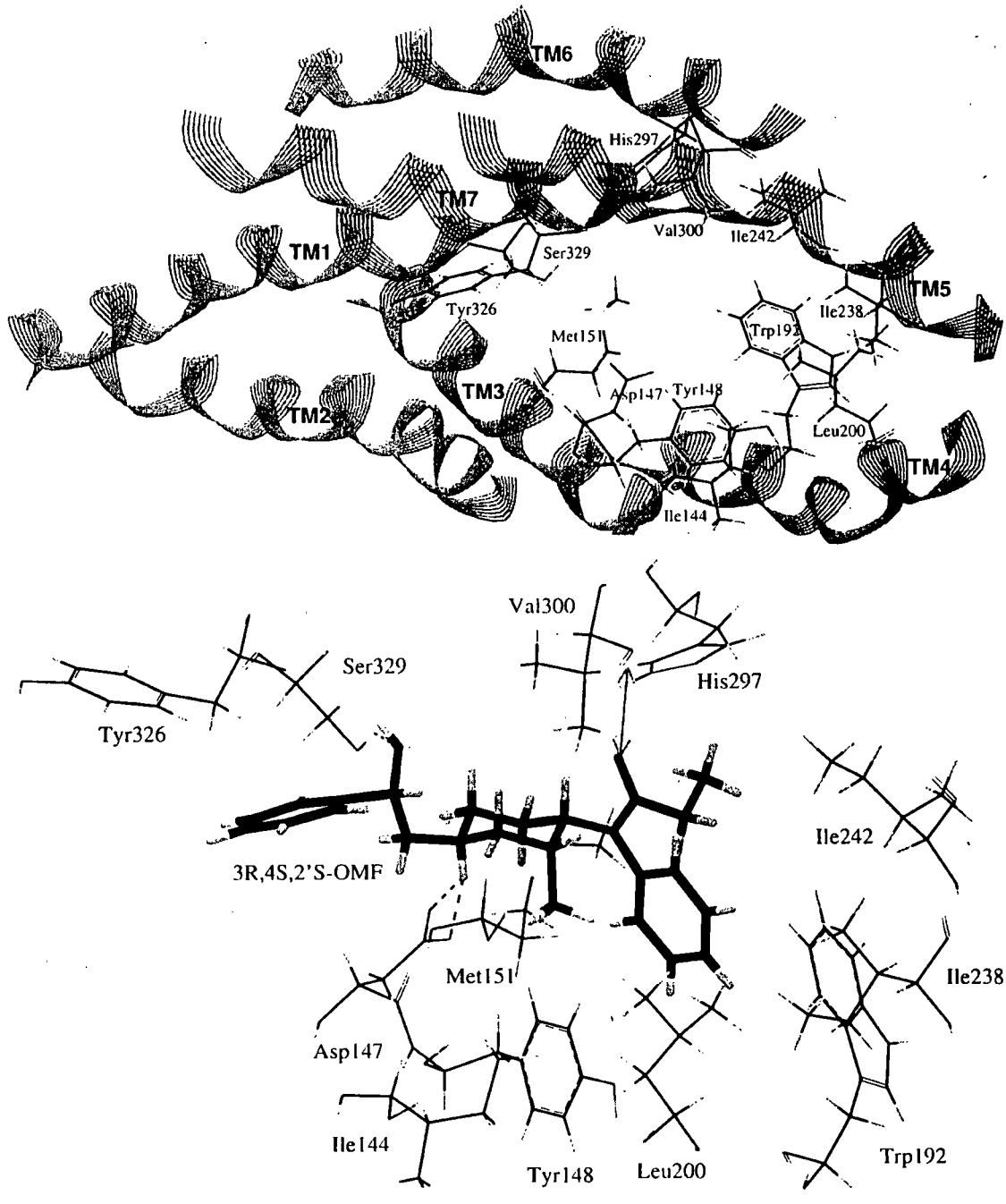

Fig 3. Structural represent of FA complexing with μ opioid receptor.
a) Putative binding pocket of μ opioid receptor, the ribbons represent the structures of 7 transmembrane helices, and the principle amino acid residues that compose the binding pocket are labeled.
b) The interaction model bewteen FA and μ opioid receptor, the dot lines represent the hydrogen bonds, and the double-arrow line represents the electrostatic interaction.

through π-π stacking interaction. This interaction model is consistant with the pharmacophore of FA derived from theoretical calculations and QSAR analysis.

Besides the interactions described above, hydrophobic interactions could also take place between following pairs: 3-methyl group and side chain of

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Met151; phenyl ring in 4-phenylpropanamide and side chains of Ile238 and Ile242; and piperidyl ring and side chain of Val300. Residues such as Ile144, Tyr148, and Met151 have the function not only to form a hydrophobic pocket in which the methyl group just can be fitted, but also to stabilize the trans rotamer for the side chain of Asp147, which is in agreement with the molecular modeling resulted from Mosberg's group[13]. It is interesting that the length of the major axis of binding pocket of μ opioid receptor is about 17 Å, which is just longer than the distance between two "ends" of FA (16 Å) (Fig 3). This shows that FA could properly fit into the pocket. Outside the binding pocket, two pair hydrogen bonds are formed respectively by side chains of Thr118 and Thr153, and by side chains of Trp293 and Tyr336. These pairs of hydrogen bonds play an important role in the formation of binding pocket. Aromatic interaction among residues in the 7 helix bundles is another feature of these ligand-receptor systems. This kind of interaction exists mainly between following pairs of residues of aromatic side chains: Tyr148 and Phe152, Trp192 and Phe152, Phe152 and Phe156, Trp192 and Phe241, Phe289 and Trp293, Trp293 and Tyr336, and Phe289 and Tyr336. Many experimental phenomena[14,15] can be explained by above results. Besides the conserved residue Asp147 has already been tested as the main interaction point with a wild variety of ligands including FA[14], the His297 has also been verified as another anchor point for many times in experiments such as site-directed mutagenesis or molecular modeling[13-15]. Tyr326 was thought as an universal interaction point[14]. All these facts indicate that the 3D-model of ligand-receptor complexes for FA with μ opioid and binding pocket deduced from modeling results in this paper are perfectly reasonable and have important significance in practice.

Correlation between activities and binding energies Tab 5 is compiled with the binding energy data of fentanyl analog-receptor complexes. Satisfied that the 3D-structures of ligand-receptor complexes were indeed reasonable, we then performed a classical QSAR to explore whether the analgesic activities of these analogs could be correlated with the energetic information. Employing partial least squares (PLS) method of Sybyl 6.4/QSAR, we calculated regression equation for the analgesic activities ($-\lg ED_{50}$), using the total binding energies E_{bind} as the sole descriptor variable. A perfect correlation was found between the analgesic activities and binding energies (Equation 4), and this relationship is shown graphically in Fig 4. This relationship suggested that those potential analgesic agents which would exhibit stronger binding energies using this paradigm would therefore be expected to have greater efficacy toward analgesic action.

$$-\lg ED_{50} = -0.708 - 0.094\ E_{bind}$$
$$r^2 = 0.898,\ F = 96.374,\ s = 0.403 \qquad (4)$$

Tab 5. Calculated energies of FA (kcal/mol).

Compounds	E_{lcf}^{l} [a]	E_{bcf}^{l} [b]	$E_{complex}$ [c]	E_{bind} [d]
Fentanyl	11.100	24.979	-1388.927	-75.148
(3R, 4R)-3-MF	12.828	31.965	-1388.172	-73.121
(3R, 4S)-3-MF	13.037	28.950	-1402.023	-90.190
(3S, 4R)-3-MF	13.601	29.381	-1377.445	-66.167
(3S, 4S)-3-MF	10.584	25.147	-1391.463	-77.168
(3R, 4R, 2'R)-OMF	14.856	35.853	-1389.291	-79.268
(3R, 4R, 2'S)-OMF	10.109	30.414	-1401.177	-86.407
(3R, 4S, 2'R)-OMF	11.579	28.838	-1406.729	-93.429
(3R, 4S, 2'S)-OMF	13.392	29.196	-1413.418	-101.931
(3S, 4R, 2'R)-OMF	12.393	25.411	-1373.522	-61.036
(3S, 4R, 2'S)-OMF	13.739	22.920	-1374.584	-63.444
(3S, 4S, 2'R)-OMF	10120	33.557	-1385.989	-71.230
(3S, 4S, 2'S)-OMF	12.346	30.396	-1397.044	-84.511

[a] The energy of the lowest-energy conformation of ligand. [b] The energy of the binding conformation of ligand. [c] The total energy of ligand-receptor complex. [d] The binding energy.

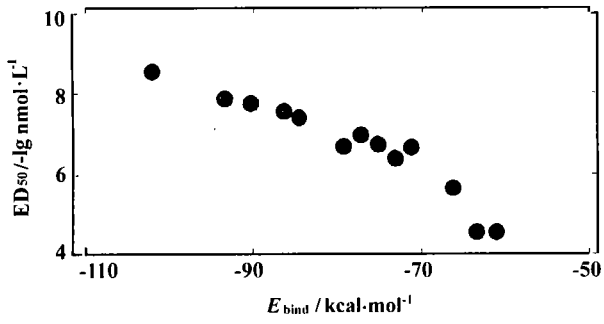

Fig 4. Correlation between binding energies (E_{bind}) and analgesic activities ($-\lg ED_{50}$).

Inspection of the structures of the 13 FA in aqueous solution and in μ opioid receptor binding site, they almost have the same rotational single bonds and have similar hydrogen bond sites with both water molecules and receptor, therefore, they should have the same rotational and transitional entropy loss and entropy gain due to new vibrational modes associated with the ligand-receptor nonconvalent interaction, as well as have the same increase in entropy due to release of bound water molecules when ligands bind with receptor from the physiological liquid. So the overall entropy loss for all these FA is at the same level. According to thermodynamic principle, the binding free energies ($\triangle G_{bind}$) of these analogs should correlate with their binding enthalpies $\triangle H$. In addition,

$$\triangle H = \triangle\triangle U + \triangle(PV) = \triangle\triangle U + \triangle nRT$$
$$= (E_{bind} - \triangle E_{sol}) + \triangle nRT \quad (5)$$

where E_{bind} is the binding energy of ligand with receptor, $\triangle E_{sol}$ is the solvation energy of the ligand, and $\triangle n$ is the molar unite difference of after and before ligand binding to receptor. Because all the FA bind with the same number of water molecules before they bind to the receptor ($n = 18$, Tab 4), they should have the same $\triangle n$. On the other hand, as mentioned above, the solvation energies for all FA are at the same level (Tab 4).

Thereby we can deduce that the binding free energies (E_{bind}) of these analogs to μ receptor should correlating with their binding affinities.

According to above argument, we can see that the binding energies of FA with μ opioid receptor correlate with their binding affinities is a natural matter. In fact, we have known that the binding affinities of FA correlate with their analgesic activities. Therefore, the good correlation between our calculated binding energies, E_{binds}, and analgesic activities, $\lg ED_{50}$s, of FA (Equation 4 and Fig 4) is an indirect proof for the reasonability of the 3D-structures of ligand-receptor complexes predicted by our modeling methods.

In summary, molecular models in this study are satisfactory in explaining the interaction mechanism of FA with μ opioid receptor. This kind of study is probably helpful for designing novel analgesic compounds.

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芬太尼类似物溶剂化效应和与μ阿片受体作用机制的分子模拟[1]

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关键词　芬太尼；分子模型；溶剂；结合口袋；构效关系

目的：研究芬太尼类配体(FA)的溶剂化效应以及和μ阿片受体的相互作用机制．**方法**：将芬太尼类配体进行溶剂化，柔性对接到μOR的七个α螺旋束之内，计算结合能并进行CoMFA和QSAR研究．**结果**：(1)得到FA的溶剂化模型．(2)得到FA与μOR相互作用的模型，FA通过静电作用、氢键和疏水作用与μOR结合．(3)描述了μOR中适合于FA的结合口袋，主要位于μOR的第3，4，5，6，7跨膜螺旋中，形成口袋的主要残基为Ile144，Asp147，Tyr148，Met151，Trp192，Leu200，Ile238，Ile242，His297，Val300，Tyr326，Ser329．(4)FA-μOR的结合能与FA的镇痛活性之间有很好的相关性．**结论**：该研究有助于全面了解FA与μOR的相互作用机理和设计新的镇痛化合物．

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