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Location of the hydrophobic pocket in the binding site of fentanyl analogs in the μ-opioid receptor

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Abstract: Fentanyl is a highly potent and clinically widely used narcotic analgesic. The synthesis of its analogs remains a challenge in an attempt to develop highly selective μ-opioid receptor agonists with specific pharmacological properties. In this paper, the use of flexible molecular docking of several specific fentanyl analogs to the μ-opioid receptor model, in order to test the hypothesis that the hydrophobic pocket accommodates alkyl groups at position 3 of the fentanyl skeleton, is described. The stereoisomers of the following compounds were studied: *cis*- and *trans*-3-methylfentanyl, 3,3-dimethylfentanyl, *cis*- and *trans*-3-ethylfentanyl, *cis*- and *trans*-3-propylfentanyl, *cis*-3-isopropylfentanyl and *cis*-3-benzylfentanyl. The optimal position and orientation of these fentanyl analogs in the binding pocket of the μ-receptor, explaining their enantiospecific potency, were determined. It was found that the 3-alkyl group of *cis*-3*R*,4*S* and *trans*-3*S*,4*S* stereoisomers of all the active compounds occupies the hydrophobic pocket between TM5, TM6 and TM7, made up of the amino acids Trp318 (TM7), Ile322 (TM7), Ile301 (TM6) and Phe237 (TM5). However, the fact that this hydrophobic pocket can also accommodate the bulky 3-alkyl substituents of the two inactive compounds: *cis*-3-isopropylfentanyl, and *cis*-3-benzylfentanyl, indicates that this hydrophobic pocket in the employed receptor model is probably too large.

Keywords: molecular modeling, fentanyl analogs, ligand–receptor interactions, docking simulation

INTRODUCTION

The exceptional opioid analgesic activity of fentanyl (**1**) and its analogs has been well documented in the past forty years.[1] Combined with various anesthetics, they have been employed in surgeries under general anesthesia, to manage postoperative pain and in transdermal patches to control chronic cancer pain.[2,3] Some, such as carfentanil, are in use as veterinary analgesics for the sedation of wild animals. Numerous fentanyl analogs have been synthesized[4] as potential can-

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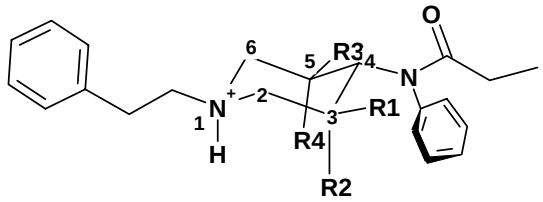
didates for novel drugs but the synthesis of the new analogs remains a challenge because drugs with specific pharmacological properties and with minimal side effects are required.

Molecular modeling of drug–receptor interactions assisted in identifying major functional groups participating in drug–receptor interactions. This may be useful as leading information in a synthesis of new, more specific drugs with increased potency. Modeling may also determine the position of a drug molecule in a receptor and identify the major amino acids participating in the formation of a drug–receptor complex, thus suggesting possible mechanisms of drug action.

In a previous paper,[5] flexible molecular docking was used to study complexes between series of active fentanyl analogs and the μ-opioid receptor. The optimal position and orientation of the fentanyl analogs in the binding pocket were determined. The model explained the high enantiospecific potency and binding of some fentanyl analogs in the binding pocket.

In this paper, flexible molecular docking of the 3-substituted fentanyl analogs **1** to **10** to the model of a μ-opioid receptor is employed in order to test the hypothesis of the hydrophobic pocket in the binding site which accommodates alkyl groups at position 3 of the fentanyl skeleton, Table I.

TABLE I. The studied fentanyl analogs



	Compound	R1	R2	R3	R4
1	Fentanyl	H	H	H	H
2	*cis*-3-Methylfentanyl				
	3*R*,4*S*	H	CH_3	H	H
	3*S*,4*R*	H	H	H	CH_3
3	*trans*-3-Methylfentanyl				
	3*S*,4*S*	CH_3	H	H	H
	3*R*,4*R*	H	H	CH_3	H
4	3,3-Dimethylfentanyl				
	4*S*	CH_3	CH_3	H	H
	4*R*	H	H	CH_3	CH_3
5	*cis*-3-Ethylfentanyl				
	3*R*,4*S*	H	CH_2CH_3	H	H
	3*S*,4*R*	H	H	H	CH_2CH_3

TABLE I. Continued

6	*trans*-3-Ethylfentanyl				
	3*S*,4*S*	CH_2CH_3	H	H	H
	3*R*,4*R*	H	H	CH_2CH_3	H
7	*cis*-3-Propylfentanyl				
	3*R*,4*S*	H	$CH_2CH_2CH_3$	H	H
	3*S*,4*R*	H	H	H	$CH_2CH_2CH_3$
8	*trans*-3-Propylfentanyl				
	3*S*,4*S*	$CH_2CH_2CH_3$	H	H	H
	3*R*,4*R*	H	H	$CH_2CH_2CH_3$	H
9	*cis*-3-Isopropylfentanyl				
	3*R*,4*S*	H	$CH(CH_3)_2$	H	H
	3*S*,4*R*	H	H	H	$CH(CH_3)_2$
10	*cis*-3-Benzylfentanyl				
	3*R*,4*S*	H	$CH_2C_6H_5$	H	H
	3*S*,4*R*	H	H	H	$CH_2C_6H_5$

The μ-opioid receptor is the primary site of action in the brain for opioid drugs. It is a member of the seven trans-membrane (TM) domains, G protein-coupled (GPCR) receptor superfamily, which are believed to share a common topology and a common mechanism of action. Their 3D structures are at present unknown. One hypothesis [6,7] suggests an agonist binding to G protein-coupled receptors promotes a conformational change which leads to the formation of the activated receptor state. Another hypothesis[8] suggests that a rigid body movement of helices relative to one another is the key step in receptor activation. However, the character of these changes, which link agonist binding and G protein coupling and activation, is not known. It is a subject of intensive modeling[9] and experimental studies, including receptor cloning, site-directed mutagenesis and affinity labeling studies.[10]

Therefore, in order to obtain detailed insight into the key interactions between a ligand and a receptor, molecular models, based on bacteriorhodopsin or rhodopsin templates, of various GPCRs have been developed.[6,9a,9b] Despite inherent difficulties in modeling opioid receptors at the molecular level, several models of μ-opioid receptor are available.[9d,9i,11–13] The earlier studies[9d,9k,11] used "manual docking" to a predefined binding cavity[9k,11] or rigid ligand docking.[9d] The resultant bound conformation and orientation of *cis*-3-methylfentanyl were different in these studies. In our own previous study,[5] flexible molecular docking was used to define the optimal position and orientation of fentanyl analogs in the binding pocket of a μ-opioid receptor and to explain their enantiospecific potency and binding.

In this study, another sequence of fentanyl analogs, Table I, were flexibly docked to a model of the human μ-receptor. The goal was to evaluate the binding orien-

tations and conformations of compounds **1** to **10** and to test the hypothesis of the hydrophobic pocket existing in the receptor which accomodates the 3-alkyl group of a ligand. The results were compared to the available experimental data, Table II.

TABLE II. Experimental potencies (relative to fentanyl) of **1**–**10** for the μ-opioid receptor

Name	Compound	Potency[a]
Fentanyl	**1**	1
cis-3-Methylfentanyl	(±)-**2**	6.1
	(3*R*,4*S*)-**2**	19
	(3*S*,4*R*)-**2**	0.16
trans-3-Methylfentanyl	(±)-**3**	1.17
	(3*S*,4*S*)-**3**	3.3
	(3*R*,4*R*)-**3**	0.8
3,3-Dimethylfentanyl	**4**	nd[b]
cis-3-Ethylfentanyl	(±)-**5**	1.49
trans-3-Ethylfentanyl	(±)-**6**	0.9
cis-3-Propylfentanyl	(±)-**7**	0.55
trans-3-Propylfentanyl	(±)-**8**	0.27
cis-3-Isopropylfentanyl	(±)-**9**	inactive
cis-3-Benzylfentanyl	(±)-**10**	0.0079

[a]Ref. 21, unless otherwise stated; [b]nd–not determined.

COMPUTATIONAL METHODS

All computations were performed using a P4/Celeron at 1.5 GHz. The μ-receptor model used in this study was the one built by Ferguson and co-workers,[9d] and kindly made available through www.opiod.umn.edu. The rigid receptor model was used. The automated flexible ligand docking experiments were made with the AutoDock 3.0.5 program.[14] The starting geometries of the neutral ligands were taken from previous studies.[15a,b] The geometries satisfy the suggested fentanyl pharmacophore,[15] by having the piperidine ring in the chair conformation, the *N*-phenethyl and *N*-phenylpropanamide substituents both equatorial and the anilido phenyl α-oriented. The amide bond had the *trans* configuration and the *N*-phenethyl substituent adopted an extended conformation, Table I. Based on the pK_a values of several fentanyl derivatives,[16] the starting geometries were protonated and the protonated geometries of compounds **1**–**10** were optimized using the semi-empirical AM1 method of the HyperChem program.[17] The Gasteiger charges were assigned to the ligand automatically by the AutoDock program. The 60×60×60 grid was centered on one of the Asp147 oxygen atoms and the Lamarckian genetic algorithm (LGA) was used in all docking calculations. The docking process was performed in two steps. In the first short step, consisting of 200 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 limited to 250,000. The best ligand orientation in the first step, based on the score criteria, was used as the input position for the second docking step, where the number of energy evaluations was 2.5×10^6. The second step provided the most probable ligand geometries and orientations in the binding pocket. The resultant ligand orientations and conformations were scored based on the docking and binding energies, and on the distance of Asp147 to the protonated nitrogen of the ligand. The cut-off value for the energies was 8.4 kJ mol^{-1}, and the cutoff value for the distance was 0.45 nm. Site-directed mutagenesis studies[18] have shown that Asp147 to Ala/Asn or Glu point mutations lead to di-

minished binding affinities, presumably due to the loss of a salt bridge or an electrostatic interaction between the negatively charged Asp147 and the protonated nitrogen of the ligand.

RESULTS AND DISCUSSION

As was described in a previous paper,[5] automated docking of some active analogs of fentanyl to the TM domain of the μ-opioid receptor resulted in several plausible docking orientations and conformations for each ligand. The resulting ligand orientations and conformations were scored based on the docking and binding energies and the distance between Asp147 and the protonated nitrogen of the ligand and only a few met the criteria and they were further evaluated based on experimental results indicating the important amino acids constituting the ligand binding site within the receptor.

The best fentanyl (**1**) docking orientation positions the piperidine ring nearly perpendicular to the membrane surface in the region between transmembrane helices TM3, TM5, TM6 and TM7. The protonated nitrogen is close to Asp147 of TM3 (the HN^+–O^- distance is 0.34 nm). The *N*-phenylpropanamide group is oriented toward the extracellular side of the cavity, while the *N*-phenethyl group adopts a *gauche* conformation, placing the phenyl group between TM6 and TM7. This position and orientation of a ligand in the binding pocket of the μ-opioid receptor has been supported by a number of site directed mutagenesis experiments.[5] All the other active analogs of fentanyl studied here adopted very similar conformations and alignments in the binding pocket, Figs. 1 and 2.

It is interesting to note that the results of our studies of the docking of fentanyl analogs to the other available model of the μ-opioid receptor[20] confirmed the location of the binding pocket in the region between the trans-membrane helices TM3, TM5, TM6 and TM7, as well as the position and orientation of the fentanyl ligands within the binding pocket. This adds support to our model of binding of fentanyl analogs and of the activation of the μ-opioid receptor.

*3-Methylfentanyls (**2,3**)*

Substitution at position 3 of the piperidine ring produced some of the most potent fentanyl analogs. The (3*R*,4*S*)-*cis*-3-methylfentanyl, (3*R*,4*S*)-**2**, is about twenty times more potent than fentanyl. However the potency of 3-alkylfentanyls is highly stereosensitive, hence the (3*S*,4*S*)-*trans* isomer, (3*S*,4*S*)-**3**, is only three times more active than fentanyl, while the (3*S*,4*R*)-**2** and (3*R*,4*R*)-**3** are both less active than fentanyl. The potency is known to depend on the size of the alkyl group: 3-propyl- and 3-allyl- substitution leads to diminished activity,[1a] suggesting the existence of a small hydrophobic pocket in the receptor. According to previous docking studies,[5] the geometries of the three isomers of low potency, (3*S*,4*R*)-**2**, (3*S*,4*S*)-**3** and (3*R*,4*R*)-**3**, in the binding pocket are very similar and overlap the "best" fentanyl orientation, while the most potent of the 3-methylfentanyls, (3*R*,4*S*)-**2,** is different.

The molecule of (3*S*,4*S*)-**3** overlaps that of fentanyl and places the equatorial 3-methyl group toward the hydrophobic pocket surrounded by Trp318 (TM7), Ile322 (TM7), Ile301 (TM6) and Phe237 (TM5), *i.e.*, between the transmembrane helices TM5, TM6 and TM7. It was found earlier[5] that Ile 322 in TM7 is the key residue for discrimination among the stereoisomers of 3-methylfentanyl. Its location near the 3-H_{ax} in the "best" fentanyl orientation makes this orientation inaccessible to any analog with a voluminous subsistent at the 3-*ax* position, forcing such a molecule to adopt a different orientation. The increased potency of the (3*S*,4*S*)-**3** isomer, relative to fentanyl, is probably due to the favorable hydrophobic interactions of the methyl group in the pocket.

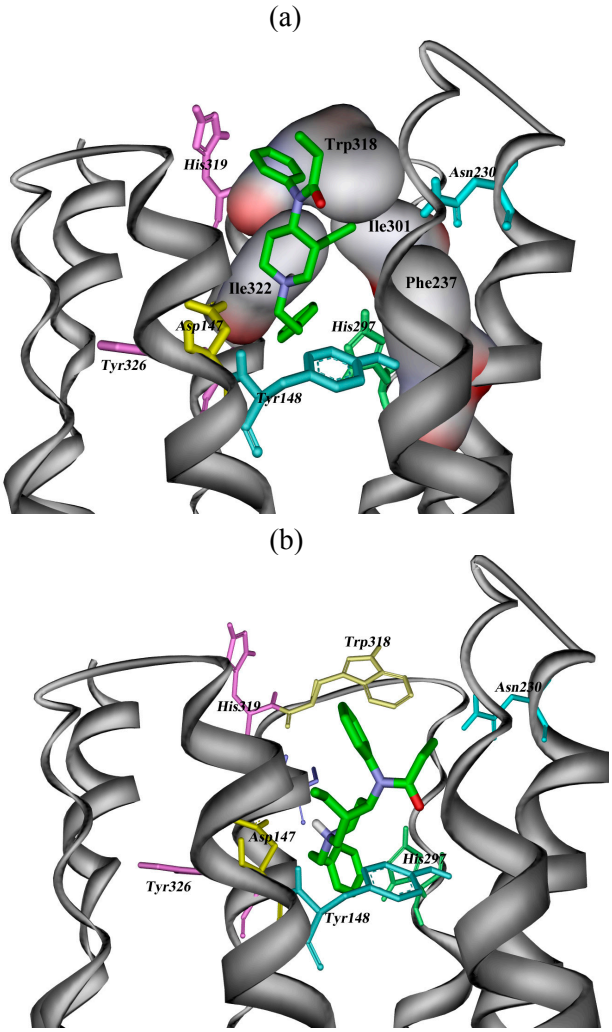


Fig. 1. The two enantiomers of *cis*-3-ethylfentanyl (**5**) in the binding pocket: a) (3*R*,4*S*)-**5**; b) (3*S*,4*R*)-**5**.

The two least active 3-methylfentanyls, the (3*S*,4*R*)-**2** and the (3*R*,4*R*)-**3**, overlap the "best" fentanyl orientation but the 3-methyl group is oriented away from the hydrophobic pocket and towards Asp147, with which it has unfavorable steric interactions.

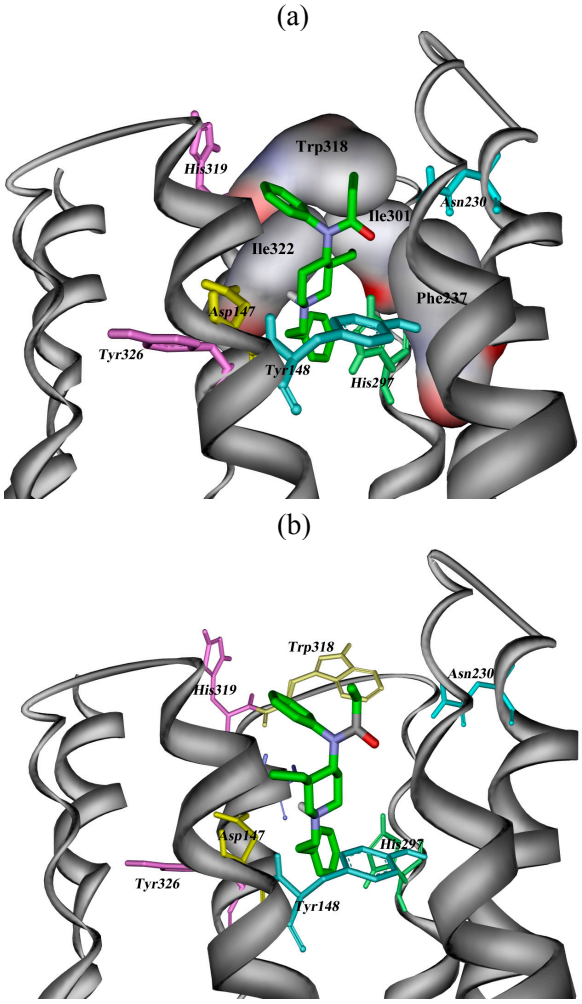


Fig. 2. The two enantiomers of *trans*--3-ethylfentanyl (**6**) in the binding pocket: a) (3*S*,4*S*)-**6**; b) (3*R*,4*R*)-**6**.

The most potent of the four stereoisomers is (3*R*,4*S*)-**2**. Although it occupies the same binding pocket as the other three isomers, it is rotated and shifted[5] relative to the other three isomers, in order to relieve the steric interactions of the axial 3-methyl group and Ile322 (TM7) in the hydrophobic pocket between helices TM5, TM6 and TM7. While maintaining a good salt bridge (HN^+–O^- distance 0.373 nm), a molecule in this orientation connects TM3 and TM6 through the *N*-phenethyl group, which has favorable edge-to-face interactions with the imidazole ring

in His297 (TM6). Simultaneously, the *N*-phenylpropanamide phenyl group is much closer to Trp318 (TM7) and His319 (TM7) than in the case of fentanyl itself. Considering the importance of the boundary region between TM6, TM7 and the third extracellular loop for both activity and selectivity of opioid ligands, this proximity explains the exceptional potency of this isomer of 3-methylfentanyl.

*3,3-Dimethylfentanyl (**4**)*

Although 3,3-dimethylfentanyl was synthesized,[19] its pharmacological activity and binding constant have never been determined. Both enantiomers 4*S* and 4*R* of 3,3-dimethylfentanyl were considered in the docking studies reported here. The position and orientation of (4*S*)-**4** in the binding pocket is similar to the most potent of the 3-methylfentanyl isomers, (3*R*,4*S*)-**2**. (4*R*)-**4** is similar to the other three, less potent 3-methylfentanyl isomers and to fentanyl itself. Therefore, it is to be expected that (4*R*)-**4** would have a potency comparable to that of fentanyl, and that (4*S*)-**4** would be even more active.

However, the present major goal was to investigate the binding profile of ligands with voluminous 3-alkyl substituents, in order to confirm the position and to determine the size of the hydrophobic pocket in the binding site of the fentanyl analogs in the μ-opioid receptor.

*3-Ethylfentanyl (**5,6**)*

As with the 3-methylfentanyls, the *cis* isomer (**5**) of the 3-ethylfentanyl is more active than the *trans* isomer (**6**), Table II. One of the two enantiomers of **5**, (3*R*,4*S*)-**5,** Fig. 1a, has the same position and orientation in the binding pocket as the most potent of the stereoisomers of 3-methylfentanyl, (3*R*,4*S*)-**2,** with the 3-ethyl group extending deep into the hydrophobic pocket and toward Asn230. The position and orientation of (3*S*,4*R*)-**5** resembles that of fentanyl (**1**), Fig. 1b. This means that, as in the case of 3-methylfentanyl, the (3*R*,4*S*)-**5** isomer is expected to be the more potent of the two *cis* stereoisomers of 3-ethylfentanyl. The position and orientation of the two *trans* isomers of 3-ethylfentanyl (**6**) in the binding pocket are close to that of fentanyl (**1**), Fig. 2. Therefore they are expected to have activities comparable to that of fentanyl.

*3-Propylfentanyl (**7,8**)*

The molecule of (3*R*,4*S*)-**7** has two orientations in the binding pocket with nearly equal energies. One is similar to the position of the highly potent (3*R*,4*S*)-**2** and the other one to the position of fentanyl. This may be the reason for the reduced potency of **7** relative to **2** and **5**. The other reason for the low potency of **7** is inability of (3*S*,4*R*)-**7** to adopt a fentanyl-like orientation, *i.e.*, an active orientation because of the steric bulk of the *n*-propyl group and its steric interactions with Asp147. Yet another reason for the diminished potency of **7** may be the size of the *n*-propyl group, which is probably too big compared to the size of the hy-

drophobic pocket. However, this was not obvious from our calculations where the *n*-propyl group, adopting *gauche* conformation, fits well to the relatively big hydrophobic pocket surrounded by the trans-membrane helices TM5, TM6 and TM7.

In the case of the *trans* isomer of 3-propylfentanyl (**8**), the (3*S*,4*S*)-**8** enantiomer adopts a fentanyl-like orientation. However, the complete inability of the other enantiomer (3*R*,4*R*)-**8** to adopt any fentanyl-like orientation in the binding pocket may be the reason for the measured low activity of **8**, which is about three times less active than fentanyl.

*3-Isopropylfentanyl (**9**) and 3-benzylfentanyl (**10**)*

As the *cis* isomers are the more potent stereoisomers of the known 3-alkylfentanyls, the pharmacological activities of only *cis*-3-isopropylfentanyl (**9**) and *cis*-3-benzylfentanyl (**10**) were determined.[19] Both were inactive or had very low activity, Table II.

According to the docking studies, (3*S*,4*R*)-**9** and (3*S*,4*R*)-**10** cannot adopt any fentanyl-like orientation due to steric crowding. Thus, they cannot be active according to our model. However, (3*R*,4*S*)-**9** and (3*R*,4*S*)-**10** adopt a position and orientation in the binding pocket similar to those of a molecule of the very potent (3*R*,4*S*)-**2**. Therefore their experimentally determined lack of activity was unexpected. The reason for this discrepancy might be the high values of log*P* for these compounds, especially for compound **10**, which may affect the bioavailability of a compound. However, compounds **7** and **9** have similar values of log*P* and yet different potencies. Another reason might be the oversized hydrophobic pocket in the employed receptor model, which would enable the accommodation of even bulky substituents, such as an isopropyl or a benzyl group. The oversized hydrophobic pocket may be the consequence of *in vacuo* modeling of the receptor structure. According to recent work[22] on simulations of the molecular dynamics of the μ-opioid receptor in a membrane–aqueous system, the arrangement of the α-helices of the transmembrane receptor domain became more compact relative to an isolated receptor. The atoms in the upper portion of TM3, TM5 and TM6 shifted inward from 0.02 to 0.38 nm, yielding a more compact binding pocket.

CONCLUSION

In the present study an automated docking procedure was applied in order to determine the optimal position and orientation of the ten fentanyl analogs in the binding pocket of the μ-opioid receptor, and to confirm the existence of the hydrophobic pocket accommodating the non-polar substituents at position 3 of the fentanyl skeleton. The quality of the model of the receptor–ligand complexes was estimated on the basis of their binding and docking energies, the distance between Asp147 (TM3) and the protonated amine nitrogen of the ligand, and the agreement with point-mutation experimental data, as described earlier.[5]

It was shown that all the active compounds occupy the same binding pocket in the receptor, located near the extracellular region and between the transmembrane helices TM3 to TM7. The ligand molecule is parallel to the transmembrane helices, with the 4-phenylpropanamide group pointing to the extracellular region and the *N*-phenethyl group placed deep in the pocket in the region between TM6 and TM7.

3-Alkyl substituted fentanyl stereoisomers with the 4*R* configuration and the alkyl substituent bulkier than the ethyl group cannot adopt a fentanyl-like orientation due to steric crowding, and, according to our model, cannot be active.

3-Alkyl substituted fentanyl stereoisomers with the 4*S* configuration can adopt a fentanyl-like, active orientation. All the 3-alkyl substituents of the stereoisomers with 4*S* configuration occupy the same hydrophobic pocket located between TM5, TM6 and TM7, and surrounded by the amino acids Trp318 (TM7), Ile322 (TM7), Ile301 (TM6) and Phe237 (TM5). However, in the employed receptor model, this hydrophobic pocket seems to be too large, accommodating even bulky groups such as isopropyl and benzyl and suggested that 3-isopropyl and 3--benzylfentanyl could be moderately active.

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И З В О Д

ПОЛОЖАЈ ХИДРОФОБНОГ МЕСТА ЗА ВЕЗИВАЊЕ АНАЛОГА ФЕНТАНИЛА ЗА μ-ОПИОИДНИ РЕЦЕПТОР

ЉИЉАНА ДОШЕН–МИЋОВИЋ, МИЛОВАН ИВАНОВИЋ и ВУК МИЋОВИЋ

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Фентанил је наркотички аналгетик високе активности и широке клиничке примене. Добијање његових аналога, у смислу да се развију високо селективни агонисти μ-опиоидног рецептора одређених фармаколошких својстава, и даље је изазов, како за експерименталну тако и за теоријску хемију. У овом раду описано је флексибилно уклапање молекула неколико аналога фентанила у μ-опиоидни рецептор, у циљу провере претпоставке да у скелету рецептора постоји хидрофобно место које прихвата алкил групе у положају 3 код аналога фентанила. Испитивани су стереоизомери следећих једињења: *cis*- и *trans*-3-метилфентанил, 3,3-диметилфентанил, *cis*- и *trans*-3-етилфентанил, *cis*- и *trans*-3-пропилфентанил, *cis*-3-изопропилфентанил и *cis*-3-бензилфентанил. Одређени су оптимални положај и оријентација ових аналога фентанила у месту везивања у μ-рецептору који објашњавају њихову енантиоспецифичну активност. Нађено је да 3-алкил група *cis*-3*R*,4*S* и *trans*-3*S*,4*S* стереоизомера свих активних једињења заузима хидрофобно место између TM5, TM6 и TM7, које чине аминокиселине Trp318 (TM7), Ile322 (TM7), Ile301 (TM6) и Phe237 (TM5). Међутим, чињеница да ово хидрофобно место може да прихати и волуминозне 3-алкил супституенте два неактивна једињења, *cis*-3-изопропилфентанила и *cis*-3-бензилфентанила, указује на то да је оно у случају коришћеног модела рецептора вероватно веће него у природном рецептору.

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REFERENCES

1. a) A. F. Casy, R. T. Parfitt, *Opioid Analgesics*, Plenum Press, New York, 1986, pp. 287–301; b) A. F. Casy, "Opioid Receptors and Their Ligands"in *Advances in Drug Research*, B. Testa, Ed., Vol 18, Academic Press, London, 1980, pp. 178–272; c) M. Williams, E. A. Kowaluk, S. P. Americ, *J. Med. Chem*. **42** (1999) 1481
2. G. Capogna, M. Camorcia, M. O. Columg, *Anesth. Analg*. **96** (2003) 1178
3. a) W. Jeal, P. Benfield, *Drugs* **53** (1997) 109; b) http://www.duragesic.com
4. V. D. Kiricojević, M. D. Ivanović, I. V. Mićović, J. B. Djordjević, G. M. Roglić, Lj. Došen–Mićović, *J. Serb. Chem. Soc.* **67** (2002) 793; b) I. V. Mićović, M. D. Ivanović, S. M. Vučković, M. Š. Prostran, Lj. Došen–Mićović, V. D. Kiricojević, *Bioorg. Med. Chem. Lett*. **10** (2000) 2011; c) I. V. Mićović, M. D. Ivanović, S. M. Vučković, D. Jovanović–Mićić, D. Beleslin, Lj. Došen–Mićović, V. D. Kiricojević, *Heterocyclic Commun*. **4** (1998) 171 and references cited therein; d) I. V. Mićović, G. M. Roglić, M. D. Ivanović, Lj. Došen–Mićović, V. D. Kiricojević, J. B. Djordjević, *J. Chem. Soc., Perkin Trans. 1* (1996) 2041; e) U.S. Patent 5,489,689 (1996); f) U.S. Patent 4,179,569 (1979); g) Van Daele, M. F. L. DeBruyn, J. M. Boey, S. Sanczuk, J. T. M. Agten, P. A. J. Janssen, *Arzneim-Forsch. (Drug. Res.)* **26** (1976) 1521; h) L. V. Kudzma, S. A. Sevemak, M. J. Benvenga, E. F. Ezell, M. H. Ossipov, V. V. Knight, F. G. Rudo, H. K. Spencer, T. C. Spaulding, *J. Med. Chem*. **32** (1989) 2534; i) M. D. Ivanović, *Ph.D. Thesis*, Chemistry Dept., University of Belgrade, 1998 (in Serbian)
5. Lj. Došen–Mićović, M. Ivanović, V. Mićović, *Bioorg. Med. Chem.* **14** (2006) 2887, and references therein.
6. J. A. Bikker, S. Trumpp–Kallmeyer, C. Humblet, *J. Med. Chem*. **41** (1998) 2911, and references therein.
7. a) Z. X. Wang, Y. C. Zhu, W. Q. Jin, X. J. Chen, C. Jie, R.Y. Ji, Z. Q. Chi, *J. Med. Chem.* **38** (1995) 3652; b) Y. F. Lu, H. Xu, L. Y. Liu–Chen, C. Chen, J. Partilla, G. A. Brine, F. I. Carroll, K. C. Rice, J. Lai, F. Porreca, W. Sadee, R. B. Rothman, *Synapse* **28** (1998) 117
8. D. L. Farrens, C. Altenbach, K. Yang, W. L. Habbell, H. G. Khorana, *Science* **274** (1996) 768
9. a) A.G. Beck–Sickinger, *Drug Discovery Today* **1** (1996) 502; b) D. R. Flowe, *Biochim. Biophys. Acta* **1422** (1999) 207; c) G. Subramanian, M. G. Paterlini, P. S. Portoghese, D. M. Ferguson, *J. Med. Chem*. **41** (1998) 4777; d) G. Subramanian, M. G. Paterlini, D. L. Larson, P. S. Portoghese, D. M. Ferguson, *J. Med. Chem*. **43** (2000) 381; e) A. Lavecchia, G. Greco, E. Novellino, F. Vittorio, G. Ronsisvalle, *J. Med. Chem*. **43** (2000) 2124; f) H. I. Mosberg, C. B. Fowler, *J. Peptide Res*. **60** (2002) 329; g) M. Aburi, P. E. Smith, *Protein Sci.* **13** (2004) 1997; h) I. D. Pogozheva, A. L. Lomize, H. I. Mosberg, *Biophys. J.* **72** (1997) 1963; i) M. Filizola, M. Carteni-Farina, J. J. Perez, *J. Comput.-Aided Mol. Design* **12** (1998) 111; j) J. Li, P. Huang, C. Chen, J. K. de Riel, H. Weinstein, L.-Y. Liu-Chen, *Biochemistry* **40** (2001) 12039; k) H. L. Jiang, X. Q. Huang, S. B. Rong, X. M. Luo, J. Z. Chen, Z. Tang, K. X. Chen, Z. C. Zhu, W.Q. Jin, Z. Q. Chi, Y. J. Ru, Y. Cao, *Int. J. Quantum Chem.* **78** (2000) 285
10. a) H. Kong, K. Raynor, K. Yasuda, S. T. Moe, P. S. Portoghese, *J. Biol. Chem.* **286** (1993) 23055; b) C. K. Surratt, P. S. Johnson, A. Moriwaki, B. K. Seidleck, C. J. Blaschak, J. B. Wang, G. R. Uhl, *J. Biol. Chem*. **269** (1994) 20548; c) J. Zhu, J.–C. Xue, P.–Y. Law, P. A. Claude, L.–Y. Luo, J.–L. Yin, C.–G. Chen, L.–Y. Liu–Chen, *FEBS Lett.* **384** (1996) 198; d) J. Heerding, K. Raynor, H. Kong, L. Yu, T. Reisine, *Reg. Pept.* **54** (1994) 119
11. I. D. Pogozheva, A. L. Lomize, H. I. Mosberg, *Biophys. J*. **75** (1998) 612
12. J. McFadyen, T. Metzger, G. Subramanian, G. Poda, E. Jorvig, D. M. Ferguson, *Prog. Med. Chem.* **40** (2002) 107
13. H. I. Mosberg, C. B. Fowler, *J. Peptide Res*. **60** (2002) 329

14. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comput. Chem*. **19** (1998) 1639
15. a) Lj. Došen–Mićović, I. V. Mićović, *J. Serb. Chem. Soc*., **61** (1996) 1117; b) Lj. Došen–Mićović, M. D. Ivanović, G. Roglić, I. V. Mićović, *Electron. J. Theor. Chem. (EJTC)* **1** (1996) 199
16. a) P. S. A. Glass, *J. Clin. Anes.* **7** (1995) 558; b) J. P. Tollenaere, H. Moereels, M. van Loon, *Prog. Drug Res.* **30** (1986) 91
17. Hypercybe, Inc., 419 Phillip St., Waterloo, ON N2L 3X2, Canada
18. a) J. Heerding, K. Raynor, H. Kong, L. Yu, T. Reisine, *Reg. Pept.* **54** (1994) 119; b) K. Befort, L. Tabbara, S. Bausch, C. Chavkin, C. Evans, B. Kieffer, *Mol. Pharmacol.* **49** (1996) 216
19. I. V. Mićović, G. M. Roglić, M. D. Ivanović, Lj. Došen–Mićović, V. D. Kiricojević, J. B. Popović, *J. Serb. Chem. Soc*. **61** (1996) 849
20. A. L. Lomize, I. D. Pogozheva, I. H. Mosberg, *J. Comput. Aided Mol. Des.* **13** (1999) 325
21. M. D. Ivanović, I. V. Mićović, S. Vučković, M. Prostran, Z. Todorović, V. D. Kiricojević, J. B. Djordjević, Lj. Došen–Mićović, *J. Serb. Chem. Soc***. 69** (2004) 511
22. Y. Zhang, Y. Y. Sham, R. Rajamani, J. Gao, P. S. Portoghese, *Chem. Biochem*. **6** (2005) 1.