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Establishment of kappa opioid receptor agonists pharmacophore with molecular modeling method

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KEY WORDS molecular mechanics; kappa opioid receptors; pharmacophore

AIM: To build up nonpeptide κ-opioid receptor agonists pharmacophore. **METHODS:** Five structurally diverse, highly active nonpeptide κ-opioid agonists were retrieved from MDL MDDR database. Molecular mechanics method were used to seek out 50 lower energy conformations for each compound. Taking nitrogen atom of pyrrolidine and acyl acetamide as overlay points, 5 agonists were superimposed to each other with their most favorite conformation. From the overlay map, the structure specificity of nonpeptide κ-opioid receptor agonists were elucidated. **RESULTS:** According to this pharmacophore, the pyrrolidine ring, the carbonyl group of acyl acetamide, and the hydrophobic group attached to acyl acetamide were suggested to be the structure-specific moieties of κ-opioid agonists. Moreover, by comparing κ_1-opioid receptor sequence of mouse with other G-protein-coupled receptors, we determined those conserve residues existing on transmembrane regions which might interact with the suggested groups. The carboxyl of Asp138 might interact with N atom of pyrrolidine by forming a hydrogen bond. The hydroxyl of Ser187 and the carbonyl group of κ-opioid agonists might form another hydrogen bond, which was critical for its kappa selective affinity. The hydrophobic group attached to acyl acetamide might have hydrophobic interaction with aromatic residues of κ-opioid receptors. **CONCLUSION:** These kappa agonists pharmacophore were helpful to select specific positions in the lead compounds to be occupied by hydrophobic moieties to limit their ability to across the blood-brain barrier.

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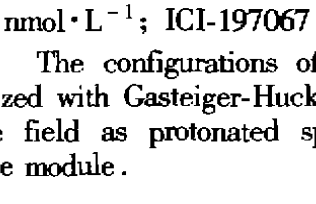
Since the prototype selective κ-opioid agonists, eg U-50488[1], are devoid of respiratory depression, constipation and physical dependence liability side effects associated with morphine-like analgesics, there has been considerable interest in identifying a κ-opioid agonist as an effective and safe pain relieving agent in recent years. The activation of κ-opioid receptors at the oxyntic glands level may modulate gastric secretion[2] suggests that κ-opioid agonists have the potential therapeutic use in the prevention and treatment of pancreatic inflammatory diseases. κ-Opioid receptors are also present in the peripheral terminal of primary afferent neurons and that the activation of these sites reduces hyperalgesia in a rat model of arthritic pain[3]. Therefore, a κ-opioid agonist with a limited capability to penetrate the blood-brain barrier could be effective in treating painful inflammation. These findings expand the gate control theory of pain and suggest new approach such as the development of peripherally-selective opioid analgesics without central side effects.

A binding model for κ-opioid agonists was proposed by Michael Higginbottom[8]. Based on this model, three compounds structurally diverse from U-50488 were designed. Two macrocyclic lactam compounds, PD146884 and PD148282, bind κ-opioid receptor selectively with respect to the μ receptor. However, despite the macrocyclic lactam PD146884 fits their model quite well, it showed no κ-opioid receptor binding affinity. This result promoted us to make further study on nonpeptide κ-opioid agonists.

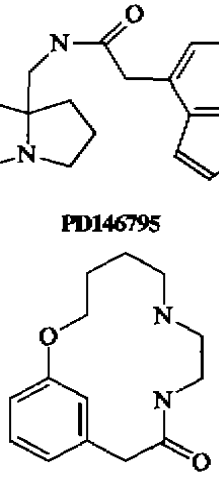
METHODS

This work was performed on Silicon Graphics IRIS R3000 workstation using SYBYL 6.1 and MDL ISIS1.3 software package. First, we retrieved all kappa agonists from MDDR database with search protocol ISIS/BASE. Five agonists were chosen for their high κ-opioid receptor bind affinity: MDL167361, $IC_{50} = 0.90$

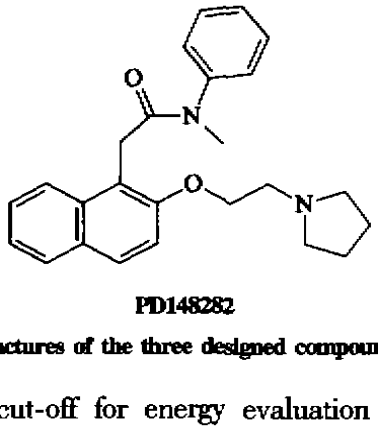
nmol·L^{-1}; GR-89696, IC_{50} = 0.02 nmol·L^{-1}; MDL163725, K_i = 0.24 nmol·L^{-1}; MDL158584, IC_{50} = 0.83 nmol·L^{-1}; ICI-197067, IC_{50} = 6.3 nmol·L^{-1}. The configurations of compounds were minimized with Gasteiger-Huckel charge in Tripos force field as protonated species using SYBYL/Base module.



PD146795



PD146884



PD148282

Structures of the three designed compounds

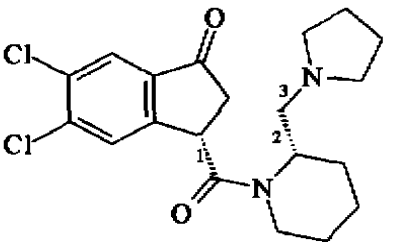
The cut-off for energy evaluation was 0.8 nm, the dielectric constant was set to be 7.0, and the dielectric function depended on the distance. Through systematic search on all rotatable bonds by the increment of 5 degree and further optimization, the ground-state conformer of 5 agonists were achieved. With the ground-state conformer as the starting conformation, we researched the critical rotatable bonds of each compound by the increment of 5 degree systematically. In this way, we got 50 conformations (including the ground-state conformer) whose energy were lower than other conformations for each agonist and put them into 5 databases, respectively. Taking these conformations as the overlay choices, we employed DISCO module in SYBYL software to detect the possible common binding sites automatically and overlaid the 5 agonists each other by their favorite conformation.

For understanding the interaction of κ-opioid receptors agonists with κ-opioid receptors, κ_1-opioid receptor subtype in mouse was taken into account. Since there was already study on the nature and putative function of some conserved residues of 39 G-protein-coupled, receptors (GPCR)[5], we compared the sequence similarity of κ_1-opioid receptor with other GPCR to determine the sequence regions putatively included in the 7 transmembrane domains and the conserved residues existing in the TMs which might have interaction with the deduced κ-opioid pharmacophore.

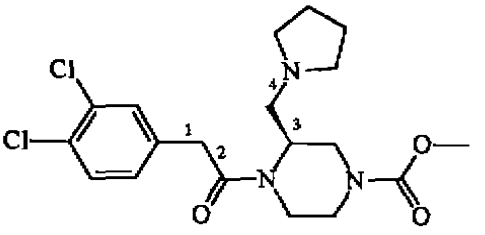
RESULTS AND DISCUSSION

Pharmacophore The 5 selected κ-opioid receptor agonists were represented (Fig 1). GR-89696 was the most effective agonist of all these compounds, while ICI-197067 was the least effective. The binding affinity of ICI-197067 with κ-opioid receptor was about one-tenth of MDL167361 and MDL158584. Inspecting their structures, we found that the main difference between them was that the pyrrolidine ring was linked with different groups. The pyrrolidine linked to ring groups which were hydrophilic (GR-89696) or conjugated system (MDL-167361) except ICI-197067. Such kind of hydrophilic interaction between ligand and κ-opioid receptors was beneficial for the improvement of its antinociceptive potency. After energy minimization, the lowest energy conformers of 5 κ-opioid agonists were obtained. The ground-state of these compounds and the energy of their overlay conformation were listed in Tab 1.

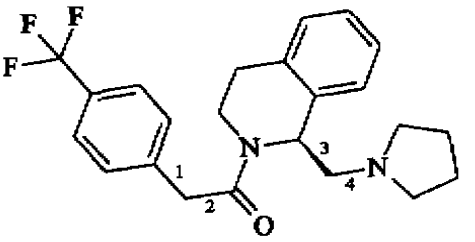
Because the energy difference between the ground-state conformation and the overlay conformation were so small that it could be overcome in the environment of organisms, we believed that the overlay conformation were also stable *in vivo*. Using compound GR89696 as the fitting template structure, other 4 agonists were fitted onto it to produce the pharmacophore map. The root mean square (RMS) fit values



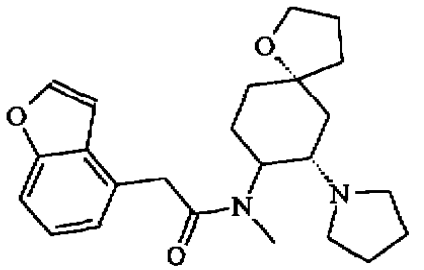
MDL167361



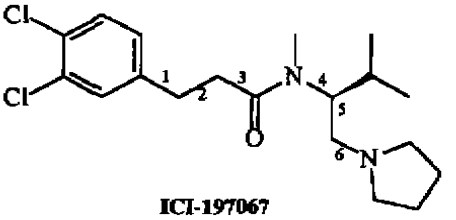
GR-89696



MDL163725



MDL158584



ICI-197067

Fig 1. Structures of the five selected non-peptide kappa agonists (the labeled bonds were critical rotatable bonds).

over the 4 defined points (atom N10 of pyrrolidine, atom N2, C11, and O13 of acyl acetamide) were listed in Tab 1. The deduced κ-opioid receptor agonist pharmacophore map was presented in Fig 2.

Tab 1. The internal energy of 5 compounds in their overlay conformation.

Agonist	E_{ground}/ kcal·mol^{-1}	$E_{overlay}$/ kcal·mol^{-1}	ΔE/ kcal·mol^{-1}	RMS fit/ nm
MDL167361	25.404	29.039	3.635	0.0369
GR89696	19.913	19.913	0.000	0.0000
MDL163725	15.421	15.421	0.000	0.0250
MDL158584	37.055	38.825	1.770	0.0260
ICI-197067	19.895	20.923	1.028	0.0477

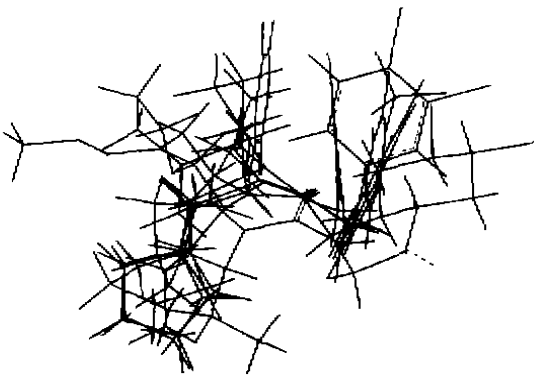


Fig 2. The deduced κ-opioid receptor agonist pharmacophore map.

There were at least three binding sites which were specific to κ-opioid receptor (Fig 2). One was the pyrrolidine ring, the others were the carbonyl of acyl acetamide and the hydrophobic group connected with acyl acetamide. Some pharmacophore parameters were summarized in Tab 2, of which DistN-N expressed the distance between N atom of pyrrolidine and N atom of acyl acetamide, DistN-O expressed the distance between N atom of pyrrolidine and O atom of acyl acetamide, DistN-C was the distance between N atom of pyrrolidine and C atom of acyl

Tab 2. The measured distances and torsion angle according to the pharmacophoremap.

Agonists	Dist N-N/ nm	Dist N-O/ nm	Dist N-C/ nm	Torsion (°)
MDL167361	0.3065	0.4319	0.3472	118.6
GR89696	0.2450	0.4478	0.3484	133.3
MDL163725	0.2977	0.4664	0.3853	109.4
MDL158584	0.3000	0.4630	0.3819	111.1
ICI-197067	0.2490	0.4621	0.3637	-142.3

acetamide. Torsion was the dihedral angle formed by O13, C11, N2 and N10 atoms. The mean values of DistN-N and DistN-O were 0.28 nm and 0.45 nm, respectively.

The coupling constants between H3 and H4 (Fig 3) of compound MDL163725 were consistent with a pseudoaxial and pseudoequatorial position of the 4 protons, indicating that the piperidine ring adopted a half chair conformation[6]. This was in line with our molecular modeling result. The angle of C11 – N2 – C3, C11 – N2 – C1, and C1 – N2 – C3 are 118.1°, 121.9°, and 118.7°, respectively. This meant that C1 – N2 – C3 – C11 constitutes a planar system, which was also in agreement with experimental result[6].

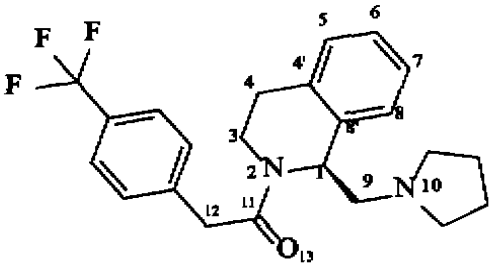


Fig 3. Atom ID number of compound MDL163725.

Interaction mechanism In the previous study, primary structure alignment of GPCRs clearly defines 7 highly conserved hydrophobic sequences corresponding to transmembrane regions connected by hydrophilic sequences with only very low conservation[5]. However, it was not possible to localize precisely the starting and ending amino acids of the transmembrane regions from hydropathicity profiles. Because there existed specific conserved residues in TM regions of all GPCRs, the way that we used to determine the starting and ending amino acids is to align κ_1-opioid receptor sequence with other GPCRs allowing the conserved residues at the same positions (Fig 4).

The site-directed mutagenesis results of the conserved aspartate suggested that the Asp residue on TM3 bound the cationic headgroup of the neurotransmitter[7,8], which implied that the protoned nitrogen atom of pyrrolidine of κ-opioid receptor agonists forms a hydrogen bond with the carboxylic acid of Asp138.

In TM4, the shadowed Ser residue was conserved in the catechol and indolamine receptors but exchanged for a Trp in the muscarinic receptors. It might be important for the ligand affinity[5]. Therefore, we speculated that the carbonyl oxygen of acyl acetamide of κ-opioid receptor agonists might form a hydrogen bond with Ser187. Besides, introducing carboxyl group at the piperazine ring as compound GR-89696 would improve its κ-opioid receptor binding affinity significantly. However, only from the sequence alignment, we could not decide precisely which residues had interaction with this carboxyl group. But we inferred that it will form electrostatic interactions with those polar residues of κ-opioid receptors (Fig 5).

The presence of an electron-withdrawing and lipophilic substituent in para and/or meta positions was required for potent antinociceptive activity. Ortho substitution was not well tolerated. This perhaps related to the different influence for the electron distribution over phenyl ring by substitute at para, meta, and ortho position. As the result, the interaction of agonist with aromatic residues of κ-opioid receptors through π – π interaction would also be different.

Fig 6 lists 3 compounds whose structure were similar with the κ-opioid receptor agonist pharmacophore building compounds. IC_{50} for μ- and κ-opioid receptor of compound (1) were 0.01 nmol · L^{-1} and 11 nmol · L^{-1}[9]. Compound (3) was useful for the treatment of psychotic disorders, cerebral ischemia, convulsions and parkinsonism, as well as in the prevention of neurotoxic damage and neurodegenerative diseases. It showed high affinity for σ receptor (K_i = 0.34 nmol · L^{-1}) while being devoid of binding affinity for κ-opioid receptor and with only very low affinity for dopamine D2 receptor (K_i = 1112 nmol · L^{-1})[10]. Compound (2) is one of the most potent and selective σ-receptor agonists but little or no affinity for dopamine D_2, κ-opioid or phencyclidine receptors[11]. The main structure difference between compound (2), (3) and our deduced κ-opioid receptor agonist pharmacophore was that compound (2) and (3) did not have the carbonyl group attached to acyl acetamide for all κ-opioid receptor agonists which might interact with residue Ser187 of κ-opioid receptor. It was

TM1	mOPRK1	60	V I I T A V Y S V V F V V G L V G N S L V M F 82
	5hta	37	V I T S L L L G T L I F C A V L G N A C V V A 59
	Dldr	25	V L T G C F L S V L I L S T L L G N T L V C A 47
	Alar	46	I S V G L V L G A F I L F A I V G N I L V I L 68
TM2	mOPRK1	99	F N L A L A D A L V T T T M P F Q S A V Y 119
	5hta	76	G S L A V T D L M V S V L V L P M A A L Y 96
	Dldr	65	I S L A V S D L L V A V L V M P W K A V T 85
	Alar	85	V N L A I A D L L L S F T V L P F S A T L 105
TM3	mOPRK1	131	C K I V I S I D Y Y N M F T S I F T 148
	5hta	119	C D L F I A L D V L C C T S S I L H 126
	Dldr	97	C D I W V A F D I M C S T A S I L N 114
	Alar	118	C D I W A A V D V L C C T A S I L S 135
TM4	mOPRK1	183	W L L A S S V G I S A I V L G G 198
	5hta	159	L T W L I G F L I S I P P M L G 174
	Dldr	147	G A W T L S V L I S F I P V Q L 162
	Alar	168	S V W V L S T V I S I G P L L G 183
TM5	mOPRK1	228	I C V F V F A F V I P V L I I I V C Y T 247
	5hta	197	I Y S T F G A F Y I P L L L M L V L Y G 216
	Dldr	197	I S S S L I S F Y I P V A I M I V T Y T 216
	Alar	205	L F S S L G S F Y I P L A V I L V M Y C 224
TM6	mOPRK1	275	L V L V V V A V F I I C W T P I H L F I L V 296
	5hta	346	T L G I I M G T F I L C W L P F F I V A L V 367
	Dldr	271	T L S V I M G V F V C C W L P F F I L N C M 292
	Alar	295	T L G I V V G M F I L C W L P F F I A L P L 316
TM7	mOPRK1	309	L S S Y Y F C I A L G Y T N S S L N P V L 329
	5hta	379	T L L G A I I N W L G Y S N S L L N P V I 399
	Dldr	308	T N T F D V F V W F G W A N S S L N P I I 328
	Alar	327	D A V F K V V F W L G Y F N S C L N P I I 347

Fig 4. Sequence alignment of the TM regions between mOPRK1, 5-HT$_{1A}$, D$_1$ and α_1 receptor (shadowed residues are conserved in GPCRs).

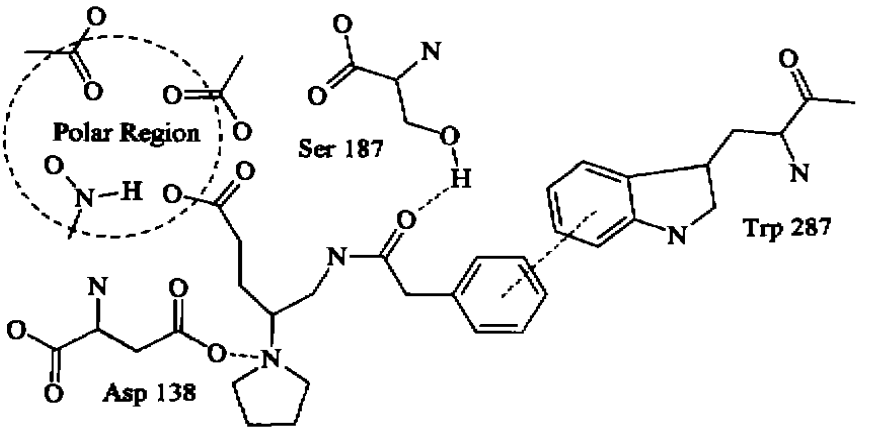


Fig 5. Schematic representation of hypothetical interaction sites of nonpeptide kappa receptor agonists with kappa opioid receptor.

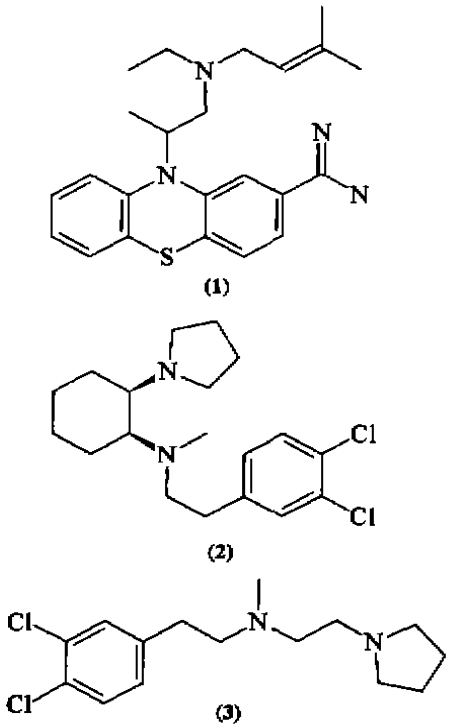


Fig 6. Chemical structures of selective centrally-acting κ-opioid agonist and some sigma receptor agonists.

interesting that such carbonyl oxygen atom was replaced by atom S in compound (1), while it still pocessed relative lower analgesic activity with 11 nmol · L^{-1} binding affinity for κ-opioid receptor. Therefore, though the other structure specificity was well kept in compound (2) and (3) except the carbonyl of acyl acetamide, the disappearence of κ-opioid receptor binding ability demonstrated the importance of the carbonyl group to its selective κ-opioid binding affinity.

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445~450 (13)

用分子模构法建立 κ 阿片受体激动剂的药效基团

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关键词 分子力学；κ 阿片受体；药效基团 R971-2

目的：建立非肽类 κ 阿片受体激动剂的药效基团. **方法**：从 MDL MDDR 数据库中选出五个高活性非肽类 κ 阿片受体激动剂，以四氢吡咯环 N 原子和乙酰胺基团为叠加点，用分子模构法建立非肽类 κ 阿片受体激动剂的药效基团. **结果**：四氢吡咯环、乙酰胺的羰基和与乙酰胺相连的疏水基团为非肽类 κ 阿片受体激动剂共同结构特征. 推测受体 Asp138 与四氢吡咯环的 N 原子构成氢键，Ser187 可能与激动剂的乙酰胺羰基以氢键形式相作用. 与乙酰胺相连接的疏水性基团可能与受体有疏水作用. **结论**：建立的药效基团有助于设计新型高效 κ 阿片受体激动剂.