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Comparison of cyclic δ-opioid peptides with non-peptide δ-agonist spiroindanyloxymorphone (SIOM) using the message-address concept: A molecular modeling study

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Summary

Based upon the message-address concept, this molecular modeling study used the δ-selective agonist spiroindanyloxymorphone (SIOM) as a molecular template for a conformational search and analysis of δ-selective opioid peptides. It was assumed that the tyramine moiety plays the same role for δ-opioid receptor recognition in both peptide and non-peptide ligands. Using 20 reported low-energy conformations of Tyr-cyclo[D-Cys-D-Pen]-OH (JOM-13) for comparison, the geometrical relationship of the two aromatic rings present in SIOM was used for the identification of potential active conformations of JOM-13, from which two δ-receptor-binding models (I and II) were constructed. Models I and II differ from each other in the arrangement of the peptide backbones. To evaluate the two models, a conformational search of two other known δ-selective ligands, [D-Pen^2,D-Pen^5]enkephalin (DPDPE) and [D-Pen^2,L-Pen^5]enkephalin (DPLPE) was performed, using the geometrical relationship of the two aromatic rings defined in the two receptor-binding models as a molecular template. Among the conformations generated from the molecular simulation, low-energy conformers of DPDPE and DPLPE conforming to models I and II were identified. Unlike model I, conformers of DPDPE and DPLPE that fit model II contain a *cis* amide bond in the Gly^3 residue.

Introduction

Opioid peptides can be analyzed on the basis of the message-address concept [1] for their structure–activity relationship (SAR) [2,3]. One important outcome of this analysis is the observation of a conformational relationship between the Tyr^1 (proposed message) and Phe^4 (proposed address) residues in establishing receptor selectivities. This is particularly relevant for δ-opioid receptor recognition, in that the geometrical relationship of the two aromatic rings has been implicated as a main factor in determining the potency and selectivity of a peptide ligand [4–7].

Enkephalins are the endogenous ligands for δ-opioid receptors [8]. However, their δ-receptor selectivity is low, due to their flexible backbones. By assuming different conformations, this may allow the 'address' motif (Phe^4) to fit into the binding pockets of different receptor types [4,5,9,10]. Previous studies of enkephalin conformations have led to proposals of topographical similarity between peptide and non-peptide opioid ligands [11–14]. However, with the use of nonselective ligands, these models cannot explain the selectivity and potency for a given receptor type. Hence, the question of how peptide and non-peptide opioid agonists interact with the δ-opioid receptors remains unanswered. From the viewpoint of rational drug design, understanding the topographical analogy between peptide and non-peptide opioid ligands is critical for further ligand development.

In the effort of developing potent, selective, and enzymatically stable ligands for δ-opioid receptors, many research groups have employed the strategy of modifying certain amino acid residues and/or introducing conformational constraints to enkephalins [2,3,15–19]. The cyclic peptides [D-Pen^2,D-Pen^5]enkephalin (DPDPE) and [D-Pen^2, L-Pen^5]enkephalin (DPLPE) are successful examples of

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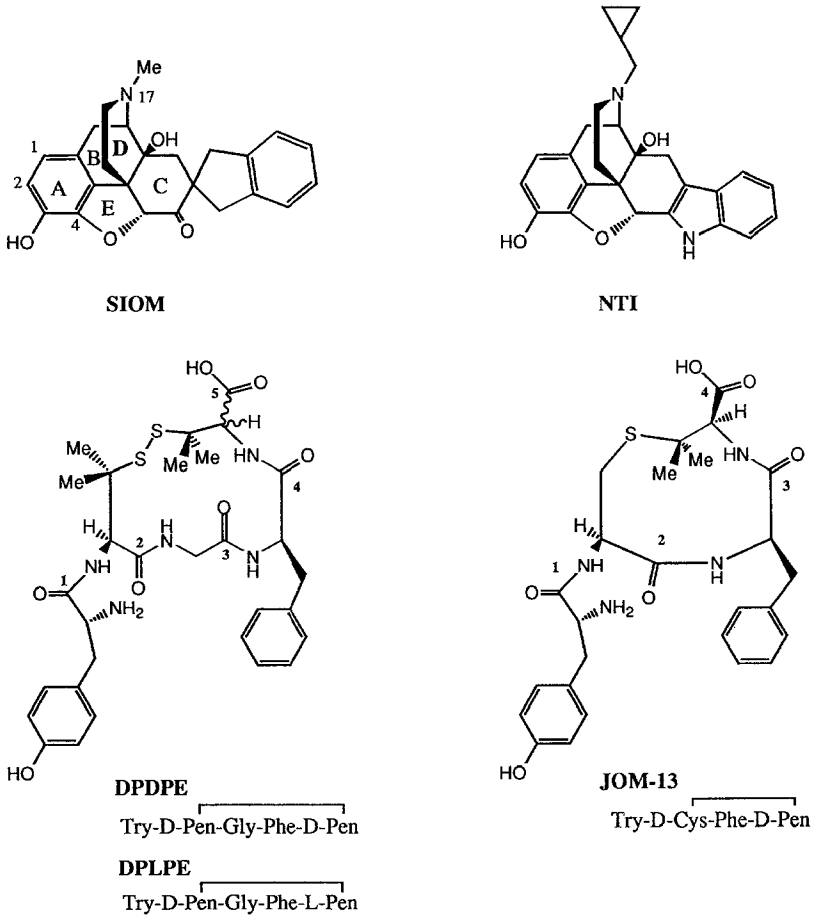

Fig. 1. Structure of SIOM, NTI, DPDPE, DPLPE, and JOM-13.

this approach (Fig. 1) [20]. With the restricted conformational mobility of the cyclic structure over the linear peptides, DPDPE has been subjected to NMR, X-ray crystallography, and computer-aided molecular simulation studies in an effort to identify the biologically active conformation(s) for δ-opioid receptor recognition [21–32]. A complication to this approach, however, is that, under different conditions, different sets of low-energy conformations of DPDPE have been reported [22–28,30–32]. Apparently, the conformation of DPDPE, especially of its two aromatic side chains, is environmentally dependent, making the actual receptor-binding conformation(s) difficult to predict [33]. Moreover, it has been proposed that the side-chain conformation may be even more important than the backbone conformation for δ-opioid receptor recognition [34].

In reviewing the strategies used by different groups to systematically search DPDPE conformations [24–26,28,31] the problem of multiple minima hinders a thorough analysis of all possible conformations. To limit the scope of the conformational search, amide bonds were assumed to be all-*trans* in many cases, and the searches were conducted with a 30-degree (or higher) grid over backbone torsion angles. While it is well-known that amide bonds in peptides and proteins prefer the *trans* conformation, the energy difference between *trans* and *cis* amide bond isomers is relatively small (about 2–3 kcal/mol) [35,36]. Theoretically, the less-favored *cis* conformations could be compensated by other favorable interactions. X-ray crystallography studies of some proteins do show *cis* amide bonds [35] and some peptides are known to exist in solution with the *cis* amide conformation as minor isomers [37–41]. Of importance to the current study, Goodman et al. studied some 14-membered cyclic opioid peptides, close analogs of DPDPE and DPLPE, and found 21–28% *cis*-amide-bond-containing isomers in solution [38].

The possibility that *cis* amide bonds can make a significant contribution to peptide-binding affinity seems even more reasonable in ligand–receptor interactions, where the total binding energy could be sufficient to offset the energetic cost of assuming a *cis* amide conformation.* Analyzing their SAR results, Goodman and Schiller et al. constructed a topochemical array of morphiceptin analogs for μ-receptor bioactivity [39]. In that model, a *cis* amide

*$\Delta G = -12$ kcal/mol assuming a K_d of 10 nM; calculated from the relationship $\Delta G = -RT \ln(K_d)$, $T = 298$ K.

bond between Tyr^1 and Pro^2 was required, and was found to be energetically preferred by NMR studies. In studying the structure of β-amyloid plaques, Spencer et al. found that a *cis*-amide-bond-containing conformation of β-amyloid peptide, β34-42, was the best fit to the solid-state NMR results [41]. Inevitably, the assumption of all-*trans* conformations in molecular simulation studies will overlook potential *cis* amide bonds with low-energy conformations. In fact, the apparently low frequency of detecting *cis* amide bonds in crystal structures could be due to the misassignment of amide stereochemistry in structures derived from relatively low-resolution electron-density maps, as argued by Stewart et al [35].

While DPDPE retains a rather flexible ring structure, smaller cyclic enkephalin analogs have also been explored. The highly potent δ agonist, Tyr-cyclo[D-Cys-Phe-D-Pen]-OH (JOM-13, Fig. 1) [42] was a successful outcome of this effort [33]. Solution NMR studies, X-ray crystallography, and computer-aided molecular simulations suggested the backbone of JOM-13 is less sensitive to environmental influences than DPDPE [31,33].

Another approach for developing δ-opioid receptor ligands is based on the modification of morphine structures. Naltrindole (NTI) [43] and spiroindanyloxymorphone (SIOM) [44] were conceived on the basis of the message-address concept (Fig. 1). Their high potency and selectivity were attributed to the benzene moiety near the C-7 position, which may mimic the Phe^4 phenyl ring of enkephalins [43–47]. Pharmacological studies showed that both DPDPE and SIOM are selective ligands for the putative δ_1-receptor subtype [44,48]. Recent molecular biology studies have indicated receptor-binding similarity between the δ-peptides and SIOM toward point mutations of cloned δ-receptor [49]. In this regard, changing Asp^{95} to Asn^{95} in cloned δ-opioid receptors did not affect the binding affinity of δ-antagonists. However, this mutation caused comparable potency shift for SIOM and other δ-peptide agonists, but not δ-agonist BW373U86 [50]. Together with the conformational studies on JOM-13 [17,33,42] these results prompted the current study to compare the conformations of δ-selective peptides with the morphinan-based δ-agonist, SIOM.

TABLE 1
DISTANCE CONSTRAINTS USED FOR DPDPE AND DPLPE CONFORMATIONAL SEARCH

Atom selected		Distance (Å)		Force constant (kcal/mol Å)
Tyr^1	Phe^4	Minimum	Maximum	
C^{γ}	C^{γ}	5.8	6.3	5000
C^{γ}	$C^{\delta 1}$	4.8	5.3	5000
C^{γ}	$C^{\delta 2}$	4.8	5.3	5000
C^{γ}	C^{ζ}	6.3	6.7	5000
C^{ζ}	C^{γ}	4.3	4.7	5000
C^{ζ}	C^{ζ}	4.8	5.3	5000

Recently, G-protein-coupled receptors have been proposed to follow the two-state model of receptor activation [51–53]. This may have implications for the pharmacological activity of the δ-opioid ligands. For example, SIOM was found to be a δ-antagonist at low concentration, but a potent δ-agonist at higher dose when tested in vivo. A similar two-state model was postulated to account for this phenomenon [44]. In addition, the conformational difference of the putative benzene 'address' moiety between SIOM and δ-antagonist NTI was attributed to their distinctive agonist/antagonist activities. It is conceivable that the two putative states (resting or active) of δ-opioid receptors may require two different conformations of their ligands, especially for flexible peptide ligands. In light of this possibility, as well as for comparison, NTI was included in the modeling studies. It should be noted, however, that some evidence indicates that NTI may not interact with δ-opioid receptors in the same way as δ-agonists.

Here we report the conformational analysis of three peptide δ-selective ligands (DPDPE, DPLPE, and JOM-13) in comparison with the non-peptide agonist SIOM. Two models (I and II) of topographical analogy among these ligands are presented.

Methods

Molecular calculations were conducted using Biosym software (InsightII 2.3.0, DISCOVER 2.9, Biosym Technologies, San Diego, CA) operating in an IRIX 4.0.5 environment on a 35/TG personal IRIS workstation. All molecules were in their un-ionized form, and charges were assigned by MOPAC (v. 6.0) [54]. DISCOVER calculations used a Consistent Valence Force-Field (cvff) [55] supplied with the Biosym package. All calculations were conducted at a dielectric constant of 80, unless specified otherwise. Molecules were minimized using steepest descent (200 steps) followed by a quasi-Newton–Raphson algorithm (VA09A, until the maximum derivative was less than 0.001 kcal/Å) [56]. The structures of SIOM and NTI were constructed and minimized as reported [47]. SIOM has two possible conformations due to the flexibility of the spiroindane ring, and the lower-energy conformer was used for this study. It should be noted that the two conformers have their benzene moiety in close approximity to each other. Thus, the selection of one conformer over the other does not contradict the generation of the two receptor-binding models. Coordinates of the 20 conformations of JOM-13 were provided by Lomize et al. [33].

The search for DPDPE and DPLPE conformations was conducted using the following sequence: (i) the two aromatic rings (Tyr^1 and Phe^4) were constrained to the geometry defined in SIOM (Table 1) using the GenericDis Constraint Module (force constant of 5000 kcal/mol Å);

TABLE 2
RMS VALUES OF SUPERIMPOSING DIFFERENT LIGANDS TO SIOM IN MODEL I

	Ligands						
	NTI	JOM-13		DPDPE		DPLPE	
		a	b	ii	iii	B	C
Rms[a]	0.002	0.455	0.452	0.492	0.485	0.490	0.486

[a] The four atom pairs used for superposition are described in the Methods.

TABLE 3
RMS VALUES OF SUPERIMPOSING DIFFERENT LIGANDS TO SIOM IN MODEL II

	Ligands						
	NTI	JOM-13		DPDPE		DPLPE	
		c	d	iv	iv	D	E
Rms[a]	0.002	0.406	0.409	0.431	0.418	0.441	0.434

[a] The four atom pairs used for superposition are described in the Methods.

(ii) the constrained DPDPE/DPLPE was subjected to energy minimization (200 steps of steepest descent), followed by high-temperature dynamics (100 steps of thermoequilibrium to 1200 K, and then 1-ps dynamics at 1200 K) to release internally unfavored interactions; (iii) the total energy was minimized (200 steps of steepest descent followed by VA09A, until the maximum derivative was less than 0.001 kcal/Å); (iv) the constraints were released and the molecule was re-minimized (200 steps of steepest descent followed by VA09A, until the maximum derivative was less than 0.001 kcal/Å); and (v) finally, the minimized conformation was archived and then used in the next cycle (step i). Each of the above cycles provided one energy-minimized conformation of DPDPE/DPLPE, which was collected for comparison with models I and II. This sequence was used to generate a given number of conformations (280 for DPDPE and 200 for DPLPE).

Superposition of δ-ligands was conducted using the Superimpose Module with the consideration of root-mean-square (rms) values of the following atom pairs (peptide: SIOM): ($C^{\varepsilon 1/\varepsilon 2}$:$C^2$, pair 1), ($C^{\zeta}$:$C^3$, pair 2), ($C^{\varepsilon 2/\varepsilon 1}$:$C^4$, pair 3), and ($N^{17}$:$N^1$, pair 4). For these atom pairs the importance of the hydroxy group, the aromatic ring, and the basic nitrogen of the tyramine moiety for δ-opioid receptor recognition were considered. Atom pairs 1 and 3 used either $C^{\varepsilon 1}$ or $C^{\varepsilon 2}$, since rotation of the tyramine aromatic ring caused $C^{\varepsilon 1}$ and $C^{\varepsilon 2}$ to be degenerated. In a given situation, changing atom pair 1 of $C^{\varepsilon 1/\varepsilon 2}$:$C^2$ to $C^{\varepsilon 1/\varepsilon 2}$:$C^4$ and atom pair 3 of $C^{\varepsilon 2/\varepsilon 1}$:$C^4$ to $C^{\varepsilon 2/\varepsilon 1}$:$C^2$ afforded two ways of superposition of the peptide ligands to non-peptide ligands. This operation led to the construction of models I and II in the current study.

Five conformations of DPDPE (conformers i–v) and DPLPE (conformers A–E) were subjected to energy re-

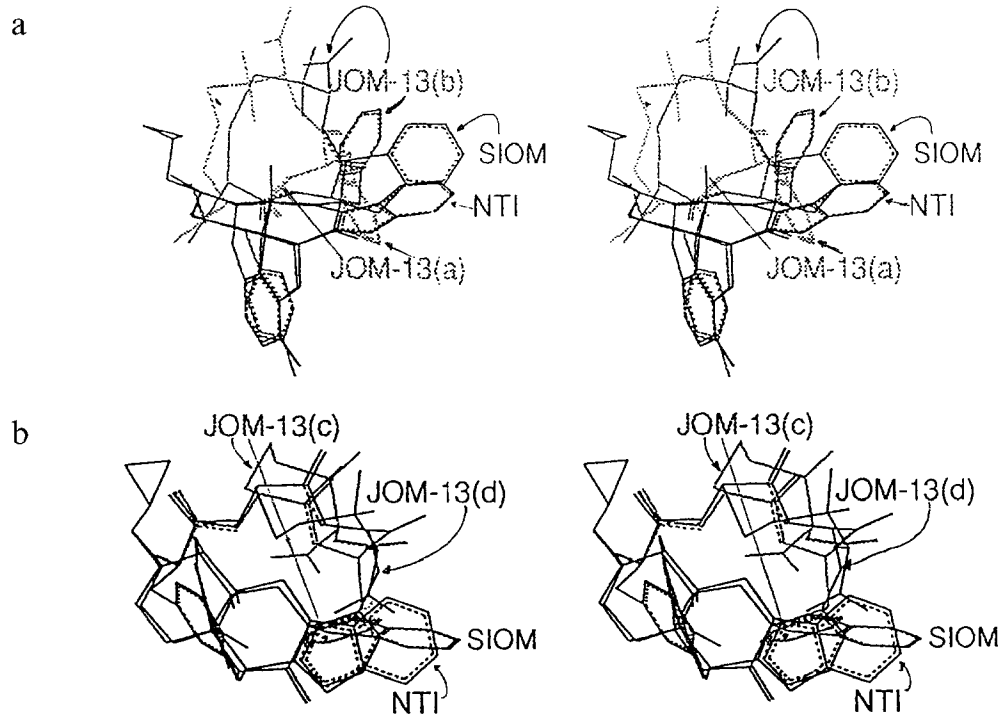

Fig. 2. Construction of the δ-opioid receptor-binding model using JOM-13 and SIOM, model I and model II in stereoview. (a) Model I, conformers JOM-13(a) and JOM-13(b) fit nicely to SIOM; (b) conformers JOM-13(c) and JOM-13(d) were selected to represent model II. NTI was superimposed to SIOM by its tyramine moiety for comparison. Hydrogens are not shown for clarity.

TABLE 4
TORSION ANGLES AND TOTAL ENERGIES OF DPDPE CONFORMERS[a]

Torsion name	Torsion angle (°)				
	i	ii	iii	iv	v
Tyr[1]					
Ψ	127.05	−79.02	−52.69	−76.79	122.75
ω	176.87	−179.63	−163.85	−175.07	167.49
χ_1	−178.06	−172.06	−168.38	−174.92	−175.99
χ_2	−121.35	82.47	−93.95	−103.84	77.95
Pen[2]					
Φ	133.91	85.57	135.49	101.25	−52.40
Ψ	81.36	81.47	−77.27	−71.94	−76.06
ω	−175.66	−175.30	−178.51	−161.36	−144.39
χ_1	−52.31	−57.51	−177.87	−179.26	−177.52
χ_2	133.91	85.57	−74.05	3.63	−137.87
Gly[3]					
Φ	−170.99	−173.05	90.00	−175.45	154.95
Ψ	−70.18	−68.36	72.33	−57.33	−66.19
ω	171.82	170.63	**−6.27**	**14.16**	**13.93**
Phe[4]					
Φ	−64.88	−61.75	−137.62	−81.55	−96.33
Ψ	−47.53	−44.00	84.19	103.67	124.63
ω	−176.65	−179.35	**−7.95**	178.41	161.93
χ_1	66.96	64.52	−68.27	−71.03	−74.78
χ_2	−84.35	89.05	91.04	−80.22	103.63
Pen[5]					
Φ	111.41	104.90	118.82	69.03	95.79
χ_1	−70.78	−70.50	−163.12	−59.16	−69.91
χ_2	74.80	76.53	−90.63	158.24	40.34
C^β-S-S-C^β	109.69	109.07	110.93	−99.45	107.15
ΔE (kcal/mol)	0.00	1.51	6.13	3.97	9.69

[a] Lowest energy conformer and conformers for models I and II; *cis* amide bonds are shown in bold characters.

TABLE 5
TORSION ANGLES AND TOTAL ENERGIES OF DPLPE CONFORMERS[a]

Torsion name	Torsion angle (°)				
	A	B	C	D	E
Tyr[1]					
Ψ	−79.47	−74.87	−79.16	−75.94	122.39
ω	−169.98	−178.30	−179.57	−176.80	−175.09
χ_1	178.79	−169.83	−170.32	−175.18	−161.84
χ_2	−111.95	−90.16	87.75	73.45	−88.48
Pen[2]					
Φ	87.11	96.37	90.13	105.30	−52.19
Ψ	−112.51	86.92	94.01	−66.48	−75.11
ω	170.08	−164.33	−161.84	−161.07	**8.60**
χ_1	175.71	−70.16	−58.33	178.72	50.60
χ_2	71.81	27.42	176.91	−61.48	134.54
Gly[3]					
Φ	66.10	141.58	159.71	177.70	−101.80
Ψ	−106.96	−96.62	−73.22	−56.62	121.00
ω	161.88	166.70	161.73	**11.65**	**−7.87**
Phe[4]					
Φ	−82.12	−104.38	−84.06	−76.08	−131.22
Ψ	110.86	103.10	97.38	129.84	74.42
ω	−166.71	−160.03	173.22	175.30	−178.78
χ_1	57.58	−174.62	53.53	−71.18	−171.08
χ_2	88.65	−97.86	88.82	−80.13	−110.73
Pen[5]					
Φ	−108.31	−112.14	−133.60	−103.44	47.34
χ_1	−53.17	−44.06	−79.44	65.62	−69.18
χ_2	−123.76	−179.82	82.53	−132.34	68.32
C^β-S-S-C^β	103.29	115.37	108.06	124.37	−111.00
ΔE (kcal/mol)	0.00	3.13	5.34	2.91	12.27

[a] Lowest energy conformer and conformers for models I and II; *cis* amide bonds are shown in bold characters.

evaluation at three dielectric constants (ε = 1, 10, and 80), and compared to the calculation results from reported conformations. Reported conformations of DPDPE and DPLPE were constructed according to the published torsion angles, and subjected to energy evaluation at ε values of 1, 10, and 80 (200 steps of steepest descent followed by VA09A, until the maximum derivative was less than 0.001 kcal/Å).

Results and Discussion

Based upon SAR studies, it was assumed that the tyramine moiety of both non-peptide and peptide opioid ligands plays a similar role in δ-opioid receptor recognition. This assumption has been applied in previous molecular modeling studies. This moiety in SIOM was, therefore, selected for superposition with the tyramine moiety of JOM-13. The comparison (rms value ~ 0.45, Table 2) of SIOM with the 20 reported conformations [33] of JOM-13 revealed that two of the low-energy conformers, JOM-13(a) and JOM-13(b), have the phenyl group of the Phe^3 residue located in a conformational space occupied by the spirobenzene ring in SIOM (Fig. 2a). In this case (model I), it can be noted that the aromatic rings of tyramine residues in JOM-13 and SIOM are nearly coplanar with each other and the peptide backbone is located above (up front of) the C and D rings of SIOM.

In an alternative superposition (Fig. 2b), conformers of JOM-13(c) and JOM-13(d) were identified to have their phenyl groups located in the vicinity of the spiroindanyl ring of SIOM (rms value ~ 0.4, Table 3). In this case (model II), in which the basic nitrogen was kept in nearly the same position, the tyramine aromatic groups are not coplanar, as compared to the nearly coplanar alignment in model I. In addition, model II differs from model I in that it has the backbone of JOM-13 located behind rings

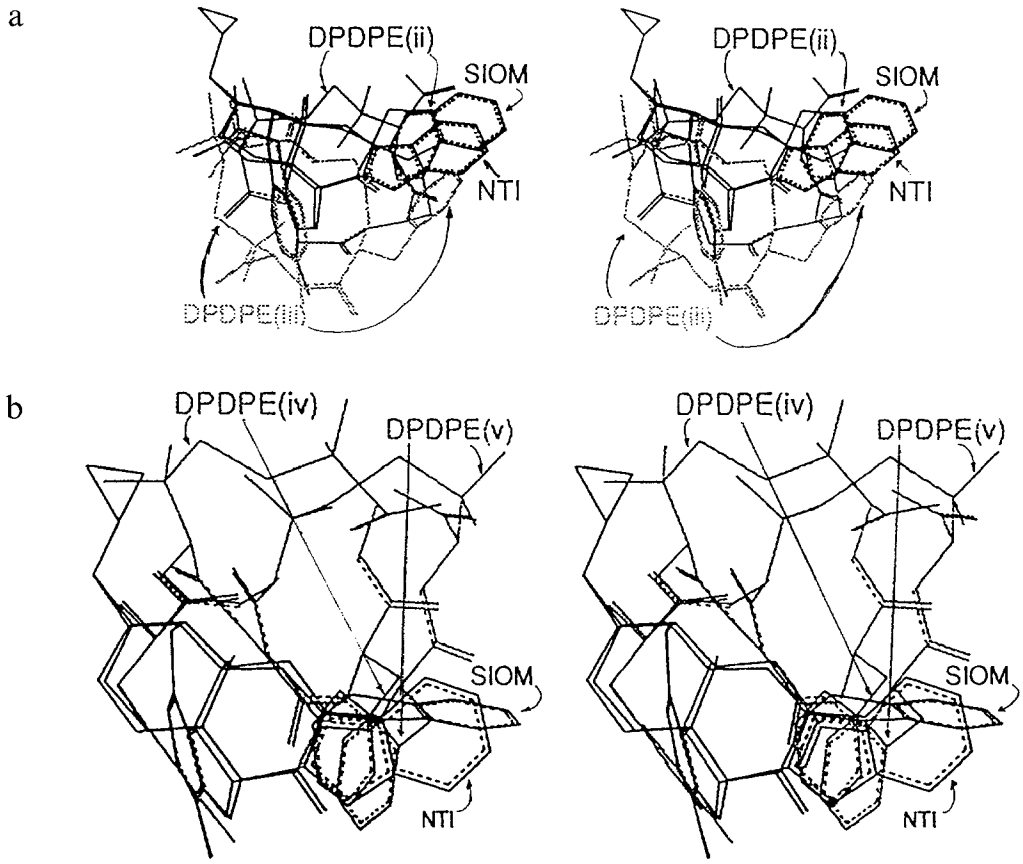

Fig. 3. Superposition of four conformers of DPDPE to SIOM in stereoview. (a) Conformers DPDPE(ii) and DPDPE(iii) conform model I when superpositioned to SIOM; (b) conformers DPDPE(iv) and DPDPE(v) conform model II when superpositioned to SIOM. NTI was superimposed to SIOM by its tyramine moiety for comparison. Hydrogens are not shown for clarity.

A, B, and E (down behind the C and D rings) of the morphinan nucleus (Fig. 2b). In convergence to our findings, the conformations of JOM-13 that fit model II were recently proposed by Mosberg et al. to be the δ-receptor pharmacophore based upon the analysis of conformationally more restricted analogs of JOM-13 [18,19].

To further evaluate these two models, a conformational search of two cyclic δ-selective peptides, DPDPE and DPLPE, was performed. Computing methods previously employed for these ligands either went through systematic conformational searches [25,26,28,31,32] or through dynamic conformation sampling methods [24]. Although these peptides are conformationally constrained relative to a linear enkephalin, their residues still possess considerable conformational freedom. Hence, a thorough evaluation of all possible combinations of all torsion angles is not practical. Therefore, all-*trans* amide bonds were assumed, or limited molecular dynamic sampling were used to access manageable numbers of conformations in the reported studies [25,26,28,31,32]. Thus, even though the reported low-energy conformations of DPDPE and DPLPE do not conform to model I and/or model II, it is

TABLE 6
ENERGY COMPARISON OF CONFORMERS DPDPE (i)–(v) WITH REPORTED CONFORMATIONS

Dielectric constant	Energy Δ E (kcal/mol)[a]										
	Conformers					Reported conformations					
	i	ii	iii	iv	v	L[b]	W[c]	F[d]	M[e]	T_1[f]	T_2[g]
1	0.00	2.82	12.73	2.53	15.64	1.18	5.74	6.36	6.36	6.74	10.69
10	0.00	1.55	6.76	3.91	10.32	2.58	7.40	5.05	5.05	7.40	10.96
80	0.00	1.51	6.13	3.97	9.69	2.67	7.35	4.97	4.97	7.35	10.92

Conformers (i)–(v) are the same as in Table 2.
[a] Total energy relative to conformer DPDPE(i).
[b] Chew et al. [26], Table 2, conformer 4.
[c] Wilkes et al. [28], Table 1, conformer DK11.85.
[d] Froimowitz [25], Table VII, conformer 1.
[e] Mosberg et al. [29], Table VIII, conformer III'.
[f] Flippen-Anderson et al. [23], Table 5, molecule 1.
[g] Flippen-Anderson et al. [23], Table 5, molecule 2.

TABLE 7
ENERGY COMPARISON OF CONFORMERS DPLPE (A)–(E) WITH REPORTED CONFORMATIONS

Dielectric constant	Energy Δ E (kcal/mol)[a]										
	Conformers					Reported conformations					
	A	B	C	D	E	1[b]	2[c]	3[d]	4[e]	5[f]	6[g]
1	0.00	2.93	–2.60	0.27	16.09	4.43	4.49	1.04	6.64	3.46	10.75
10	0.00	2.89	4.48	2.52	12.43	4.72	7.24	2.74	7.27	4.63	10.20
80	0.00	3.13	5.34	2.91	12.27	4.84	7.71	3.11	7.33	4.76	10.14

Conformers (A)–(E) are the same as in Table 3.
[a] Total energy relative to conformer DPLPE(A).
[b] Chew et al. [26], Table 1, conformer 1.
[c] Chew et al. [26], Table 1, conformer 2.
[d] Chew et al. [26], Table 1, conformer 3.
[e] Chew et al. [26], Table 1, conformer 5.
[f] Chew et al. [26], Table 1, conformer 11.
[g] Chew et al. [26], Table 1, conformer 13.

possible that previous methods used for the conformational search of DPDPE and DPLPE may have missed important conformers.*

SAR studies of opioid peptides showed the Tyr^1 residue to be the most important element of the opioid 'message', and the Phe^4 residue to be the putative 'address' according to the message-address concept. In addition, the importance of the geometrical relationship of the two aromatic rings for δ-opioid receptor recognition has been postulated [2,10,15,16]. Since models I and II clearly established the geometrical relationship of the two aromatic rings, this information was employed to limit the conformational search for DPDPE and DPLPE. Accordingly, the two aromatic rings were locked into the geometry defined in SIOM (Table 1), and the rest of each molecule was allowed to relax to assume energetically favorable arrangements by running high-temperature molecular dynamics. The resulting conformations were minimized. Finally, the geometrical constraints were removed, and the conformations were minimized again. These distance constraints brought the conformations of DPDPE and DPLPE close to the geometry of the two aromatic rings in SIOM. Since we are interested in conformations with a particular geometry (of the two aromatic rings), this strategy highly reduced the number of possible conformations and a tremendous amount of computing time was saved.

The conformers of DPDPE generated from the search span an energy range of over 27 kcal/mol. Among these, conformer DPDPE(i) has the lowest energy. Listed in Table 4 are the torsion angles of conformers DPDPE (i)–(v). Note that some of the low-energy conformations have *cis* amide bonds that were overlooked in prior studies because all amide bonds were forced to be *trans*.

According to model I (Fig. 4a), DPDPE(ii) and DPDPE(iii) have a close similarity (rms value ~ 0.45, Table 2) with SIOM, conformer JOM-13(a), and conformer JOM-13(b). This similarity is also revealed by the spatial arrangement of the backbones of JOM-13(a), JOM-13(b), DPDPE(ii), and DPDPE(iii). On the other hand, DPDPE(iv) and DPDPE(v) fit nicely to model II (Fig. 4b, Table 3), while their backbones occupy a similar space as JOM-13(c) and JOM-13(d). Interestingly, both DPDPE(iv) and DPDPE(v) have a *cis* amide bond in the Gly^3 residue.

All five conformers of DPDPE (i–v) were subjected to energy re-evaluation at three dielectric constants (ε = 1, 10, 80) and compared with the conformations of DPDPE reported by other research groups. As shown in Table 6, DPDPE conformers (i)–(v) are within the low-energy range at different dielectric constants. Note that *cis*-amide-bond-containing conformers do have a little higher energy than the all-*trans* conformers, but the differences are not substantial.

DPLPE is diastereomeric with respect to DPDPE only at the fifth amino acid (L- instead of D-form), and it has similar δ-opioid-receptor selectivity and potency as DPDPE [20]. DPLPE(A) was the lowest-energy conformer found in the search, while the highest-energy conformation was 21 kcal/mol higher. Listed in Table 5 are the torsion angles of conformers DPLPE (A)–(E). As indicated in bold, *cis*-amide-bond conformations are revealed again. Conformers DPLPE(B) and DPLPE(C) are consistent with model I (rms value ~ 0.49, Table 2, Fig. 4a), while conformers DPLPE(D) and DPLPE(E) fit to model II (rms value ~ 0.44, Table 3, Fig. 4b). Beside DPLPE(E), energy re-evaluation indicated these conformations to be within the low-energy range, compared to previously reported conformations (Table 7).

Thus, as shown in models I and II (Fig. 5 for an overview), it seems feasible that opioid peptides can have the two aromatic rings oriented to resemble SIOM. This finding justified the application of the message-address concept in the SAR study of opioid ligands. In addition, the finding that there are two different ways (models I and II) to topographically compare δ-opioid peptides with the

*Reconstructing the reported low-energy conformations of DPDPE and DPLPE failed to conform model I or model II with regard to the spatial relationship of the two aromatic rings. These negative results prompted us to the search for potential δ-peptide conformations which may have been previously overlooked.

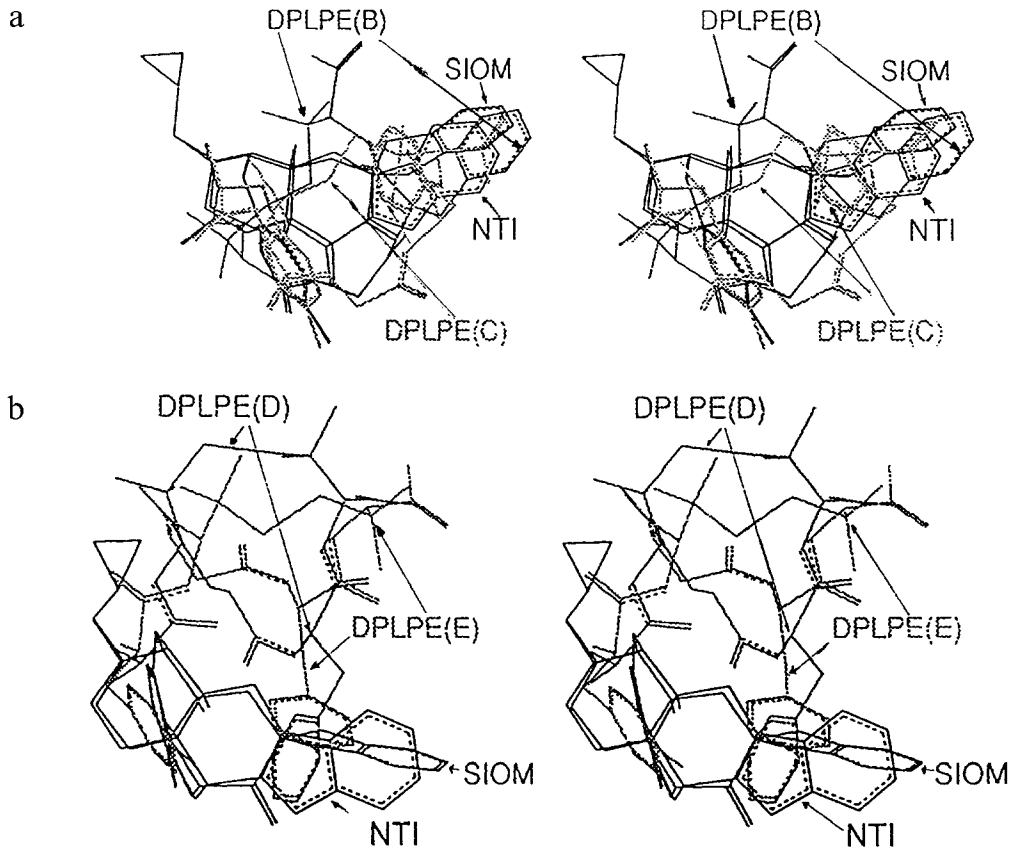

Fig. 4. Superposition of four conformers of DPLPE to SIOM in stereoview. (a) Conformers DPLPE(B) and DPLPE(C) conform model I when superpositioned to SIOM; (b) conformers DPLPE(D) and DPLPE(E) conform model II when superpositioned to SIOM. NTI was superimposed to SIOM by its tyramine moiety for comparison. Hydrogens are not shown for clarity.

non-peptide agonist SIOM provides an opportunity for the development of new δ-selective opioid ligands. SAR studies of opioid ligands based upon models I and II will be able to test the validity of the current study. Since model II differs from model I by requiring DPDPE and DPLPE to have at least one *cis* amide bond, the synthesis of conformationally constrained enkephalin analogs bearing a *cis* amide bond in the third residue should be of primary interest. Considering the situation of receptor binding, only one model (I or II) may be sterically suitable to one receptor binding site. It is also possible that these two models may represent two different binding states (such as the rest state versus the active state [53]) of a given ligand, or it may represent the active conformations of different ligands (such as agonist versus antagonist). Models I and II are derived from the study of δ-agonists. It should be noted that the δ-antagonist NTI fits to both model I and model II. Although evidence has

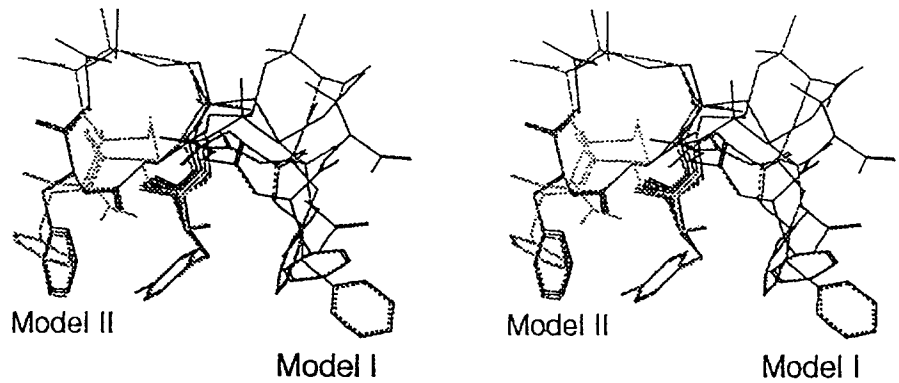

Fig. 5. Comparison of models I and II. For an overview, conformers JOM-13(a), JOM-13(c), DPDPE(ii), DPDPE(iv), DPLPE(B), and DPLPE(D) are not identified individually. Models I and II were superimposed by the tyramine residues. Model I is displayed at the right while model II is shown at the left. Hydrogens are not presented for clarity.

been presented that NTI may not interact with δ-opioid receptors in the same way as δ-agonists, the molecular details of the differences still remain to be addressed.

Conclusions

Within a low-energy range, the δ-selective peptide ligands JOM-13, DPDPE, and DPLPE can have their 'address' phenyl group (Phe^4) adopt a similar spatial arrangement as the non-peptide δ-ligand SIOM. These results support the message-address concept for designing non-peptide opioid ligands.

The current molecular modeling studies provided two possible δ-receptor-binding models, model I and model II. While both have the putative 'address' phenyl group located in the same region as that in SIOM, the two models differ in the arrangement of their peptide backbones. Model I has the peptide backbones located above C and D rings of the morphinan nucleus, but model II has the backbones of δ-peptides arranged behind the A, B, and E rings of the morphinan nucleus.

The strategy employed in the current study, i.e., using conformationally constrained ligands to direct the search for a possible receptor-binding model, appears to be successful, and is waiting for further experimental validation. Efforts to apply models I and II for the rational design of δ-selective ligands are in progress.

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