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Development of a Model for the δ-Opioid Receptor Pharmacophore: 3. Comparison of the Cyclic Tetrapeptide, Tyr-c[D-Cys-Phe-D-Pen]OH with Other Conformationally Constrained δ-Receptor Selective Ligands

We have previously proposed a model of the δ-opioid receptor bound conformation for the cyclic tetrapeptide, Tyr-c[D-Cys-Phe-D-Pen]OH (JOM-13) based on its conformational analysis and from conformation–affinity relationships observed for its analogues with modified first and third residues. To further verify the model, it is compared here with results of conformational and structure–activity studies for other known conformationally constrained δ-selective ligands: the cyclic pentapeptide agonist, Tyr-c[D-Pen-Gly-Phe-D-Phe]OH (DPDPE); the peptide antagonist, Tyr-Tic-Phe-PheOH (TIPP); the alkaloid agonist, 7-spiroindanyloxymorphone (SIOM); and the related alkaloid antagonist, oxymorphindole (OMI). A candidate δ-bound conformer is identified for DPDPE that provides spatial overlap of the functionally important N-terminal NH_3^+ and C-terminal COO^- groups and the aromatic rings of the Tyr and Phe residues in both cyclic peptides. It is shown that all δ-selective ligands considered have similar arrangements of their pharmacophoric elements, i.e., the tyramine moiety and a second aromatic ring (i.e., the rings of Phe[3], Phe[4], and Tic[2] residues in JOM-13, DPDPE, and TIPP, respectively; the indole ring system in OMI, and the indanyl ring system in SIOM). The second aromatic rings, while occupying similar regions of space throughout the analogues considered, have different orientations in agonists and antagonists, but identical orientations in peptide and alkaloid ligands with the same agonistic or antagonistic properties. These results agree with the previously proposed binding model for JOM-13, are consistent with the view that δ-opioid agonists and antagonists share the same binding site, and support the hypothesis of a similar mode of binding for opioid peptides and alkaloids. © *1996 John Wiley & Sons, Inc.*

INTRODUCTION

The endogenous opioids Leu- and Met-enkephalin [Tyr-Gly-Gly-Phe-Leu(Met)] are linear pentapeptides that display high affinity to and moderate selectivity for δ-opioid receptors. Early structure–activity studies of Met and Leu enkephalin identified the key structural elements of the peptide opioid pharmacophore as (1) a positively charged N-terminal NH_3^+ group, (2) the aromatic ring and phenolic hydroxyl of Tyr[1], and (3) the aromatic ring of the Phe[4] residue.[1] However, because of the inherent conformational flexibility of short linear peptides, Met- and Leu-enkephalin and related lin-

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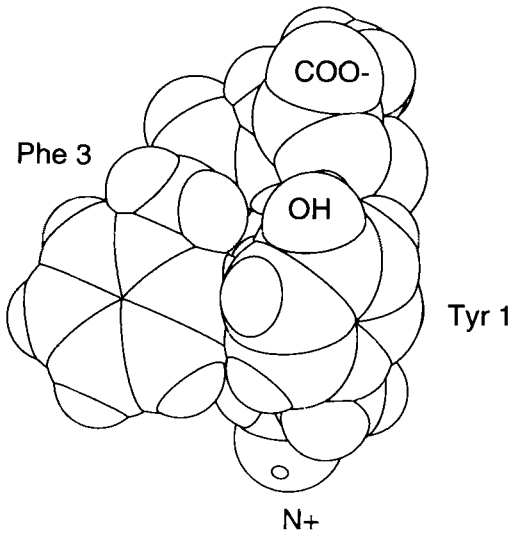


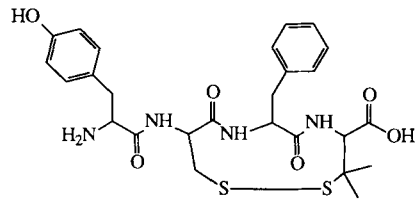
FIGURE 1 Space-filling model of JOM-13, in its proposed δ-bound conformation.

ear analogues are not well suited for the elucidation of the precise spatial arrangement of pharmacophoric elements required for ligand binding to the δ receptor. For such determinations, conformationally constrained analogues are much more appropriate. We have recently investigated one such conformationally constrained δ-selective, cyclic tetrapeptide, Tyr-c[D-Cys-Phe-D-Pen]OH (JOM-13; Pen, penicillamine, is β,β-dimethylcysteine) using a combination of experimental (x-ray crystallography, ^{1}H-nmr spectroscopy) and theoretical (molecular mechanics computations) techniques.[2] This peptide has a single energetically preferred backbone conformation for the cyclic tripeptide portion of the molecule and two major conformers of the disulfide bridge (in the ratio ~ 2:1 in aqueous solution). While the cyclic part of the molecule is conformationally well defined, the key elements of the δ-receptor pharmacophore (exocyclic Tyr^1 residue and Phe^3 side chain) are still very flexible in solution. Therefore, a number of additional constraints were incorporated into the Tyr^1 and Phe^3 side chains of the parent tetrapeptide, and the binding affinities of the resulting analogues were correlated with their conformational propensities, with the underlying assumption that, for such structurally related analogues, the bioactive conformation must lie within the intersection of conformational space available to those analogues that exhibit good binding affinity. This analysis allowed us to propose a precise model for the binding conformation of JOM-13 and its analogues.[3,4] The proposed δ-bound conformation is compact (Figure 1); the Tyr and Phe side chains are close together (distance between the centers of the aromatic rings is 5.7 Å) and have *trans* ($\chi^1 \sim 180°$) and *gauche* ($\chi^1 \sim -60°$) conformers, respectively, while the main-chain fragment between Tyr^1 and the tripeptide cycle is in an extended conformation (ψ of Tyr^1 and ϕ of D-Cys^2 are ~ 160°).

This model of the δ-receptor pharmacophore was developed from the comparison of compounds with the same tripeptide cycle, c[D-Cys-X-D-Pen], where *X* is L- or D-Phe or a structurally related replacement residue.[3,4] The present study further verifies this model by demonstrating that appropriate low energy conformers of other conformationally constrained δ-selective ligands possessing alternative types of rigid "scaffold" connecting their pharmacophoric groups are consistent with it. Analogues from several different structural classes are compared here (Figure 2): Tyr-c[D-Pen-Gly-Phe-D-Pen]OH (DPDPE),[5] a cyclic pentapeptide related to JOM-13 but containing an additional glycine residue interposed between the pharmacophoric Tyr and Phe residues, and that replaces the D-Cys^2 residue of JOM-13 with a second D-Pen residue; the linear δ-selective antagonist Tyr-Tic-Phe-PheOH (TIPP),[6] where Tic is 1,2,3,4-tetrahydroisoquinoline carboxylic acid, a conformationally constrained Phe analogue; the alkaloid δ-selective agonist 7-spiroindanyloxymorphone (SIOM)[7]; and the structurally related δ-selective antagonist oxymorphindole (OMI).[8] While several other δ models, discussed below, have been proposed from comparisons of some of the δ structural classes listed above, our study is the first to attempt to explain observed structure–activity relationships for all these δ-selective peptide and alkaloid agonists and antagonists in the framework of a unified conformational model. Further, previous models encompassing both peptide and alkaloid δ ligands were developed by fitting the more flexible peptide ligand to the rigid alkaloid scaffold, in essence assuming a common binding conformation. Our model, developed from consideration of the tetrapeptide JOM-13 and its analogues, alone, has no such limitation, and consequently provides support for the hypothesis of a similar mode of binding for opioid peptides and alkaloids.

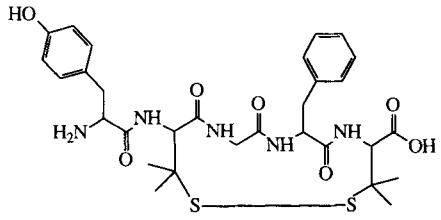
METHODS

Since complete conformational calculations of DPDPE have been reported by others,[9–13] this was not repeated here. Instead, the previously reported theoretically generated[9–13] and x-ray[14] conformers of the D-Pen-Gly-Phe-D-Pen cycle of DPDPE were used in initial struc-



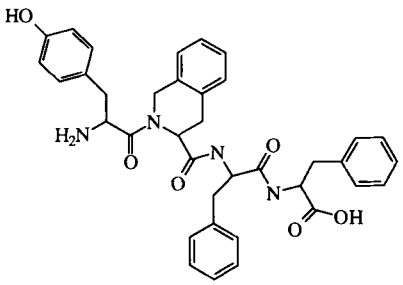
Tyr-c[D-Cys-Phe-D-Pen]OH

(JOM-13)



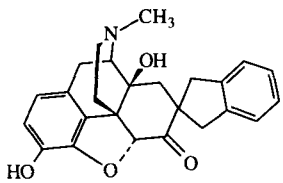
Tyr-c[D-Pen-Gly-Phe-D-Pen]OH

(DPDPE)



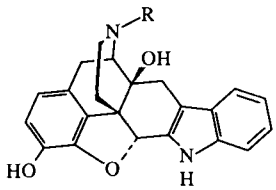
Tyr-Tic-Phe-PheOH

(TIPP)



7-Spiroindanyloxymorphone

(SIOM)



R = Me, Oxymorphindole (OMI)

R = CPM, Naltrindole (NTI)

FIGURE 2 Structures of δ-selective opioid peptides and alkaloids.

tures, and spatial positions of the flexible exocyclic elements (Tyr^1 residue and Phe^3 side chain) were optimized using a conformational search. The initial torsion angles of the D-Pen-Gly-Phe-D-Pen cycle (ψ and χ^1 of D-Pen^2, φ and ψ of Gly^3 and Phe^4, and φ and χ^1 of D-Pen^5) from each published structure of DPDPE were combined with all possible combinations of torsion angles ψ of Tyr^1 and φ of D-Pen^2 (60° increments within allowed areas of the Ramachandran plot) and χ side-chain torsion angles corresponding to rotamers of Phe and Tyr residues ($\chi^1 = -60°$, 60°, and 180°; $\chi^2 = 90°$) for subsequent energy minimization using the CHARMm force field. After energy minimization, every conformation of DPDPE was compared with the initial one to ensure that the conformation of the D-Pen-Gly-Phe-D-Pen cycle was not markedly changed and still corresponded to the same local energy minimum as in the original publication. The differences in all individual torsion angles before and after minimization were < 30°, and rms deviations of all atoms within the disulfide-bridged cycle were < 0.4 Å.

Low energy conformations of the Tyr-Tic fragment (Tyr-Tic-methylamide) of TIPP were calculated using a grid search with subsequent energy minimization in the space of torsion angles ψ and χ^1 of Tyr^1 and ω (*cis* and *trans* configurations) and φ of Tic^2 for two possible conformations of the Tic 6-membered aliphatic ring (all possible combinations of the angles were considered with the same initial values of φ, ψ, and χ^1 angles as for DPDPE). For the alkaloid structures, the energetically preferred equatorial position was chosen for the N$^{\alpha}$-CH$_3$ group. In SIOM, there are two conformers of the 5-membered ring of the 7-indanyl group. The slightly higher energy conformer (ΔE = 1.3 kcal/mol) of SIOM provides better superposition of its 7-indanyl ring with the Phe^3 aromatic ring of JOM-13.

All molecular mechanics calculations of DPDPE, Tyr-Tic-methylamide, and alkaloid opiates were done with the QUANTA 3.2/CHARMm force field.[15,16] A compromise value of the dielectric constant, ε = 10, was used, and the adopted basis Newton–Raphson method of minimization was employed. This intermediate value of ε has previously been found to be appropriate for the conformational analysis of peptides[17] and for computations of electrostatic energy in proteins.[18] Superpositions were done with the QUANTA 3.3 Molecular Similarity system.

RESULTS AND DISCUSSION

The Candidate δ-Bound Conformation of DPDPE

The conformational possibilities of DPDPE have been extensively studied using computational

Table I Comparison of Interproton Distances (Å) Estimated from NOE Cross-Peak Initial Buildup Rates (r_{NOE}) of DPDPE in DMSO[20] and in Aqueous Solution with Calculated Distances from Its Energy-Optimized X-Ray Structure ($r_{x\text{-}ray}$; Conformer I, Table III)

From		To			
Residue	Proton	Residue	Proton	r_{NOE} NMR	$r_{x\text{-}ray}$[a] X-Ray
Tyr	αH	D-Pen[2]	NH	2.8	3.5 (2.3)[d]
Tyr	βH	D-Pen[2]	Me(*pro-S*)	3.2[b]	5.6 (3.5)[d]
Tyr	βH	Tyr	Ar(meta)	2.6	2.4
D-Pen[2]	NH	D-Pen[2]	Me(*pro-S*)	3.6	3.4
D-Pen[2]	αH	D-Pen[2]	Me(*pro-R*)	2.5	2.8
D-Pen[2]	αH	D-Pen[2]	Me(*pro-S*)	2.8	2.4
D-Pen[2]	αH	Gly	NH	2.3	2.2
D-Pen[2]	αH	Gly	αH(*pro-R*)	3.5[b]	4.3
D-Pen[2]	Me(*pro-R*)	Gly	NH	2.7	2.4
Gly	NH	Gly	αH(*pro-R*)	2.8	2.3
Gly	NH	Gly	αH(*pro-S*)	S[c]	2.8
Gly	αH(*pro R*)	Gly	αH(*pro-S*)	1.8	1.8
Gly	αH(*pro-R*)	Phe	NH	3.4	3.4
Gly	αH(*pro-S*)	Phe	NH	2.3	2.1
Phe	NH	Phe	β(*pro-R*)	2.8	2.5
Phe	NH	Phe	β(*pro-S*)	W[c]	3.6
Phe	NH	D-Pen[5]	NH	2.7	2.5
Phe	αH	D-Pen[5]	NH	2.4[b]	3.5
Phe	βH	D-Pen[5]	Me(*pro-S*)	2.9[b]	5.0
D-Pen[5]	NH	D-Pen[5]	Me(*pro-S*)	S[c]	3.0
D-Pen[5]	αH	D-Pen[5]	Me(*pro-R*)	3.0	2.5

[a] The closest protons of methyl groups were chosen to calculate distances.
[b] NOE was not detected in aqueous solution.
[c] NOE was not detected in DMSO. S and W (strong or weak) represent qualitative estimation of NOE intensity in aqueous solution.
[d] Distance in alternative conformation (ΔE = 1.3 kcal/mol) of the flexible Tyr[1] residue.

methods,[9-13] nmr spectroscopy,[19,20] and x-ray crystallography.[14] The crystal unit cell contains three independent molecules of DPDPE that have almost identical structure within the conformationally constrained, 14-membered tetrapeptide cycle but differ in the orientation of the flexible exocyclic Tyr[1] residue. This crystal structure of the c[D-Pen-Gly-Phe-D-Pen] cycle was calculated theoretically, prior to its experimental determination (conformers 3a', Table 3 in Ref. 9, and DK11.1, Table 1 in Ref. 10). Together with similar agreement between calculated and experimental conformers obtained for JOM-13,[2] this illustrates the applicability of Assisted Model Building with Energy Refinement (AMBER) and CHARMm force fields, in vacuo, for conformational calculations of short cyclic peptides. The crystal structure of DPDPE is in agreement with NOE cross-peak intensities and vicinal coupling constants $^3J_{H\text{-}NC^\alpha\text{-}H}$ observed in aqueous and DMSO solutions (Tables I and II): most deviations of interproton distances within the c[D-Pen-Gly-Phe-D-Pen] cycle estimated from ^{1}H-nmr spectral data and calculated from the crystal structure of DPDPE (Table III) are < 0.5 Å, and deviations of $^3J_{HNC^\alpha\text{-}H}$ are < 0.5 Hz (Table II). The coupling constants observed for the Gly[3] residue are reproduced well if calculated from the torsion angle φ observed directly in the crystal (98°, 107°, and 99° in molecules 1, 2, and 3, respectively, in the unit cell[14]), but larger differences arise if these coupling constants are calculated from the φ torsion angle of the energy-optimized crystal structure [conformer I, Table III (φ = 66°); Table II]. These discrepancies may be due to flexibility of the Gly φ torsion angle, which in calculations depends on conformations of the conformationally labile exocyclic Tyr[1] residue and the Phe[3] side chain. Additionally, the value of the Gly[3] torsions may be influenced by interactions of main-chain peptide groups with water. A similar situation was observed for JOM-13: the value of the φ angle for the D-Cys[2] residue directly observed in crystal structure "A" of the peptide provides much better agreement with the vicinal coupling constant of

Table II Comparison of $^3J_{H\text{-}NC^\alpha\text{-}H}$ Vicinal Coupling Constants (Hz) Measured for DPDPE in Aqueous Solution ($^3J^{exp}$, from Ref. 20) and Calculated for Crystal Structure[14] and Energetically Optimized (in vacuo) Crystal Structure of DPDPE [$\langle {}^3J^{clc}(\varphi^{x\text{-}ray})\rangle$ and $\langle {}^3J^{clc}(\varphi^{clc})\rangle$, Respectively]

Residue	Coupling Constant	$^3J^{exp}$	$\langle {}^3J^{clc}(\varphi^{x\text{-}ray})\rangle^a$	$\langle {}^3J^{clc}(\varphi^{clc})\rangle^a$
D-Pen^2	H-NC$^\alpha$-H	7.8	9.0–9.8	9.1–9.9
Gly^3	H-NC$^\alpha$-H (*pro-S*)	8.4	8.1–9.5	3.9–4.2
Gly^3	H-NC$^\alpha$-H (*pro-R*)	4.3	3.7–4.9	7.2–7.9
Phe^4	H-NC$^\alpha$-H	6.0	5.2–6.4	5.8–5.9
D-Pen^5	H-NC$^\alpha$-H	8.6	8.7–9.9	8.5–9.2

[a] The $^3J^{clc}_{H\text{-}NC^\alpha\text{-}H}$ constants were calculated using coefficients from Ref. 21. The interval of values for the constants reflects their possible dynamic averaging with equal probability in the interval of φ angle ±30° around the equilibrium value calculated for the energy-minimized structure of DPDPE or directly from crystal coordinates[14] (corresponding values of φ^{clc} and $\varphi^{x\text{-}ray}$ torsion angles are presented in Table III for conformer I). The differences in $\varphi^{x\text{-}ray}$ among molecules 1, 2, and 3 in the crystal unit cell were taken into account calculating the interval of values of $\langle {}^3J^{clc}(\varphi^{x\text{-}ray})\rangle$.

protons H-NC$^\alpha$-H measured in aqueous solution than do the values of φ in energetically optimized conformers.[2] The x-ray structures of both JOM-13 and DPDPE show the presence of numerous water molecules within the crystals which underlies the similarity between the crystal and aqueous conformations.

The overall agreement between the x-ray and nmr data suggests that the conformation of the 14-membered cycle observed in the x-ray structure is also the highest populated conformer in aqueous solution. However, nmr data for DPDPE in DMSO solution reveal some additional nuclear Overhauser effect (NOE) cross peaks (H^α Phe^4/HN D-Pen^5, H^β Phe^4/$C^\gamma H_3$ D-Pen^5, and $C^\alpha H$ D-Pen^2/$C^\alpha H$ Gly^3) that are inconsistent with the crystal structure, suggesting the importance of alternative conformations of the cycle in this solvent. The presence of many such alternative main-chain conformations with similar energies has been clearly demonstrated in theoretical studies of DPDPE.[9-12] This can be contrasted to the observations for JOM-13, in which nmr, x-ray, and computational results all indicate a small set of very similar conformations of the smaller, more rigid 11-membered cycle.

Two alternative conformations of the D-Pen-Gly-Phe-D-Pen cycle, previously identified in computational studies,[9] are stabilized in analogues of DPDPE in which D- and L-Ala are substituted for the Gly^3 residue. These conformations of the cycle with energetically optimized orientations of the flexible exocyclic elements (Tyr^1 residue and Phe side chain) are represented in Table III as conformers II and III. Within the disulfide-bridged cycle, conformer II of DPDPE corresponds to the crystal structure of its D-Ala^3 analogue[22,23] and is similar to the crystal structure of DPDPE itself (Table III), except for torsion angles of the disulfide bridge that assume a different conformer possessing unexpectedly high energy (5.3 kcal/mol), as calculated with the CHARMm force field, in vacuo. This alternate conformer (conformer II, Table III) of the disulfide-bridged cycle has been identified previously in theoretical studies [3e′ in Table 3 of Ref. 9 and 3 in Table 3 in Ref. 13), and its stabilization in the crystal of [D-Ala^3]DPDPE may be due to energetically preferred intermolecular packing and solvation by water, which forms a network of hydrogen bonds with the peptide backbone in the crystal.[22,23] It should be noted that ^{1}H-nmr spectroscopy of [D-Ala^3]DPDPE suggests that this relatively high energy conformer II is not stabilized in aqueous solution since the set of NOEs observed was essentially the same as for the parent peptide, DPDPE.[23]

Unlike [D-Ala^3]DPDPE, which exhibits greatly reduced δ affinity compared with DPDPE but has a similar crystal structure, Gly to L-Ala^3 replacement, to yield [L-Ala^3]DPDPE, does not significantly affect δ binding[24] but leads to stabilization of a rather different main-chain structure of the disulfide-bridged cycle.[22,23] In the crystal structure of DPDPE, the combination of Gly^3 main-chain φ and ψ torsion angles (φ = +98°, ψ = −141°, Table III) corresponds to the area of the Ramachandran plot that is forbidden for L residues but is allowed for Gly and D residues. Therefore, the Gly to L-Ala^3 replacement stabilizes an alternative conformer of the cycle (III in Table III), also previously calculated for DPDPE ("1" in Table 7 in Ref. 9), with relative energy 0.3 kcal/mol and φ and ψ torsion angles of residue 3 (φ = −98°, ψ = −87°) more appropriate for L residues. Stabilization of the 14-membered cycle of this conformer of [L-Ala^3]DPDPE in aqueous solution is suggested by the appearance of new NOE cross peaks between

Table III Comparison of DPDPE Conformers (I–IV) Calculated with CHARMm Force Field, the Proposed δ-Bound Conformation of JOM-13,[3,4] and the Micelle-Bound Structure of Met-Enkephalin Determined by ^{1}H-nmr Spectroscopy[37,a]

Peptide Conformer	DPDPE I[b] "crystal"	DPDPE II "*D*-Ala-like"	DPDPE III "*L*-Ala-like"	DPDPE IV δ-Bound	JOM-13 δ-Bound[c]	Met-enkephalin SDS Bound[d]
ΔE (kcal/mol)	0.0	5.3	0.3	0.0	—	—
Tyr^1						
ψ	−37 (−157)	−36	−41	136	138 (159)	167
χ^1	59 (−68)	70	68	−159	−167 (70)	
χ^2	−80 (118)	−78	67	−99	80 (84)	
D-Pen^2						
φ	127 (110)	138	75	138	165 (136)	144
ψ	**−147 (−147)**	**−125**	**43**	**44**	**41 (18)**	51
χ^1	**−40 (−58)**	**−167**	**−57**	**−49**	**−58 (−51)**	
χ^2	**−39 (−73)**	**173**	**171**	**−179**	**−148 (−141)**	
χ^3 (S-S)	**−107 (−105)**	**−107**	**112**	**111**	**94 (89)**	
Gly^3						
φ	**66 (98)**	**115**	**−98**	**−165**	—	164
ψ	**−110 (−141)**	**−55**	**−87**	**36**	—	56
Phe^4						
φ	**−78 (−74)**	**−160**	**−84**	**−143**	**−85 (−84)**	−159
ψ	**−46 (−36)**	**−61**	**−41**	**−56**	**−40 (−15)**	−70
χ^1	−59 (−67)	−179	−55	−58	−59 (−83)	
χ^2	99 (−85)	78	108	96	93 (76)	
D-Pen^5						
φ	**137 (126)**	**127**	**139**	**103**	**141 (133)**	−106
χ^1	**−69 (−51)**	**70**	**−80**	**−66**	**−71 (−76)**	
χ^2	**174 (174)**	**−79**	**71**	**77**	**52 (50)**	

[a] Calculated conformers I, II, and III of DPDPE correspond closely to the crystal structures of DPDPE, itself, and its D-Ala^3 and L-Ala^3 analogues, respectively, while IV is the proposed δ-bound conformer of DPDPE. Measured torsion angles of crystal structures of DPDPE (I), [D-Ala^3]DPDPE (II), [L-Ala^3]DPDPE (III), and JOM-13 are indicated in parentheses. The torsion angles of the disulfide-bridged cycle are indicated in bold.

[b] Torsion angles for "molecule 1," one of three molecules in the unit cell, are indicated in parentheses. Relative energy ΔE refers to energy-optimized crystal conformer.

[c] From Refs. 2–4. The D-Pen^2 residue of DPDPE is replaced by D-Cys^2 in JOM-13. Torsion angles of "conformer A," one of two conformers of JOM-13 observed in the unit cell (and the major conformer in aqueous solution[2]), are indicated in parentheses.

[d] Structure "3" of Met-enkephalin from Table 7 in Ref. 37. The D-Pen^2 and D-Pen^5 residues of DPDPE are replaced by Gly^2 and Met^5, respectively.

NH protons (NH Pen^2/NH Ala^3 and NH Ala^3/NH Phe^4) and the significant decrease in intensity for the H$^\alpha$ Pen^2/NH Ala^3 cross peak in [L-Ala^3]DPDPE compared to the parent peptide, DPDPE.[23] These NOE cross peaks are indicative of correlated changes of adjacent ψ (D-Pen^2) and φ (Xxx^3) torsion angles (also coordinated with a change in structure of the disulfide bridge) in this conformer (Table III).

The energy-minimized crystal structure of the smaller (des-Gly^3), disulfide-bridged cycle in JOM-13 (Table III) is very different from that of DPDPE, but is remarkably similar to the crystal structure of the more constrained, high affinity [L-Ala^3]DPDPE and the corresponding low energy conformer (conformer III, Table III) of DPDPE: deletion of the Gly residue produces only minor (<40°) changes for all individual torsion angles of conformer III of DPDPE within the cycle (Table III). However, the inserted Gly residue affects the size of the cycle, preventing good superposition of the key δ-opioid pharmacophore residues, Tyr and Phe, of conformer III of DPDPE with JOM-13. Similarly, these critical Tyr and Phe residues are widely separated in conformer I of the D-Pen-Gly-Phe-D-Pen cycle of DPDPE and remain so for all low energy (<3 kcal/mol) combinations of exocyclic torsion angles (ψ and χ^1 for Tyr^1, φ for D-Pen^2,

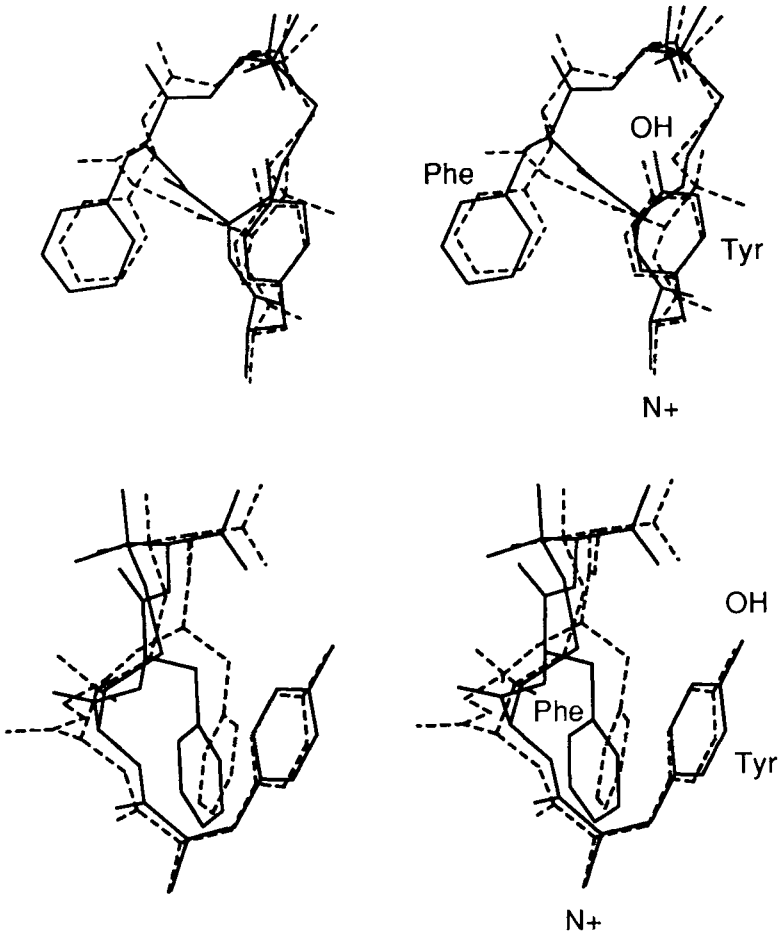


FIGURE 3 Superposition (stereoview) of δ-bound conformers of JOM-13 (solid line) and DPDPE (dashed line) shown from two perspectives. N^{α} and OH groups of the tyramine fragment are indicated.

and χ^1 for Phe^4). Consequently, the pharmacophore elements of these residues (i.e., their aromatic rings and N^{α} of Tyr^1) cannot be superimposed with those of Tyr^1 and Phe^3 residues of JOM-13 in its proposed δ-bound conformation.

Although conformer III of DPDPE does not provide good superposition of the key δ-opioid pharmacophore residues Tyr and Phe with those of JOM-13, excellent superposition with the proposed δ-bound model for JOM-13 can be achieved with a low energy conformer of DPDPE previously calculated by Froimowitz (conformer 3b in Ref. 9), which differs from conformer III of Table III only in the φ and ψ angles of the flexible Gly residue. This conformer (IV in Table III) is isoenergetic with the minimized crystal conformer of DPDPE and is proposed here to be the probable δ-bound conformer of DPDPE. Superposition (Figure 3) of the proposed δ-bound conformers of JOM-13 and DPDPE provides a good fit of their respective Tyr^1 residues, Phe aromatic rings, and C-terminal COO^- groups (rms deviation for the 19 corresponding nonhydrogen atoms is 0.56 Å). This carboxyl group is important for δ selectivity and affinity in both cyclic peptides and corresponds to the similarly important side-chain COO^- group of Asp or Glu residues in deltorphins.[25] The aromatic rings of the Phe residues are overlapped in JOM-13 and DPDPE but differ slightly in orientation; the aromatic rings of the Phe and Tyr residues are closer to a parallel arrangement in the latter. The χ^1 angle of the Phe side chain is $\sim -60°$ in the δ-bound conformations of both peptides, as was determined for JOM-13 from our studies of its β-methyl substituted Phe^3 analogues.[4] It is this rotamer of the Phe side chain that was observed in all crystal conformers of JOM-13, DPDPE, L-Ala^3[DPDPE], and D-Ala^3[DPDPE],[2,14,22] and that is preferred for DPDPE in aqueous solution (rotamer population $\sim$ 60%) and in DMSO

(rotamer population ~ 70%),[20] as well as for the (2S, 3S)-β-methyl 2′,6′ dimethyl-Tyr[1] (TMT[1]) analogue of DPDPE in DMSO (rotamer population ~ 75%).[26]

A common feature of the proposed δ-bound conformations of JOM-13 and DPDPE is the *trans* conformer of the Tyr[1] side chain ($\chi^1 \sim 180°$) and compact packing of its aromatic ring with the disulfide-bridged cycles, stabilized by hydrophobic and van der Waals interactions and by a hydrogen bond between $O^{\eta}H$ of tyrosine and the C-terminal COO^- groups in both peptides. This feature may be important for stabilizing the bound conformation. The free energy stabilizing contribution of a hydrogen bond between two flexible side chains in aqueous solution is relatively small, ~ −0.3 kcal/mol,[27] but can reach −1.6 to −2.0 kcal/mol when a hydrogen bond is embedded in a rigid nonpolar environment in a protein.[28,29] The nmr data provide indications that, in solution, the Tyr[1] aromatic ring interacts with the rest of the molecule. For example, depending on experimental conditions, NOE cross peaks between the Tyr[1] aromatic ring and the $C^{\gamma}H_3$ and NH protons of D-Pen[2],[19,30] and from H^{β} of Tyr[1] to $C^{\gamma}H_3$ of D-Pen[2],[20] have been observed. Moreover, the vicinal coupling constants H-$C^{\alpha}C^{\beta_2}$-H and H-$C^{\alpha}C^{\beta_3}$-H of Tyr[1] in DPDPE (9.5 and 6.5 Hz) and JOM-13 (9.5 and 6.0 Hz) suggest that conformers with $\chi^1 \sim 180°$ are preferred (rotamer population > 60%).[20,31] As a result, nmr-derived structures of DPDPE are rather compact, with Tyr[1] and Phe[3] side chains pointed toward each other and interacting with the disulfide-bridged cycle.[19,20] Such intramolecular close packing of the Tyr[1] side chain was not observed in crystal structures of either peptide; instead, the side chain adopts spatial positions providing optimal intermolecular packing of peptide and water molecules in the crystal.

Similar "local folding"[32] features may also be present in linear opioid peptides. ^{1}H-nmr studies of deltorphin-I,[33] its TMT[1] analogue,[26] and dermenkephalin[34] in DMSO solution; of deltorphin-II in dodecylphosphocholine micelles[35] and aqueous solution[36]; and of Met-enkephalin in SDS micelles[37] demonstrate the formation of relatively compact, though still flexible, structures with several medium-range NOE contacts. In all these peptides except [TMT[1]]deltorphin-I, medium-range NOEs involving the Tyr[1] aromatic ring indicate its interaction with the rest of the molecule. In all three DMSO studies, the χ^1 angle of the Phe[3] residue in deltorphins and dermenkephalin was determined unequivocally as −60°, in agreement with the crystal, solution, and proposed bound conformers of JOM-13 and DPDPE. This side-chain conformer of the Phe residue was also observed in all "β-turn" crystal structures of linear enkephalins[38] and in crystal structures of the δ-selective peptide agonist DTLET (Tyr-D-Thr-Gly-Phe-Leu-Thr)[39] and the antagonist *N*,*N*-diallyl-(O-*t*-butyl)-Tyr-Aib-Aib-Phe-Leu-OMe.[40] This side-chain conformer may be stabilized by local interactions of the aromatic side-chain and main-chain peptide groups of the adjacent small (Gly, Ala, or Aib) residue, as has been detected by nmr spectroscopy, for short fragments of bovine pancreatic trypsin inhibitor in aqueous solution.[41] It is especially interesting that, in complex with SDS micelles, Met-enkephalin forms a well-defined structure, very similar to the δ-bound conformation of DPDPE proposed here (Table III), which is stabilized by a hydrophobic cluster at the water/detergent interface involving the Tyr[1], Phe[4], and Met[5] side chains. In the set of Met-enkephalin conformers calculated from nmr data in micelles,[37] the χ^1 torsion angle of the Tyr[1] side-chain is ~ 180° and χ^1 of Phe[3] may fluctuate between the +60° and −60° positions, again agreeing with the δ-receptor bound model of DPDPE presented here. Taken together, these results suggest that receptor bound conformations of opioid peptides may be partially prearranged in solution, especially when the peptides interact nonspecifically with hydrophobic surfaces.

Comparison of JOM-13 with δ-Selective Alkaloids

The results described above strongly suggest a similar δ-receptor binding mode for JOM-13 and DPDPE, as well as for linear enkephalin and deltorphin analogues. The development, by Portoghese and co-workers,[7,8,42-46] of a class of high δ-affinity and moderate δ-selectivity alkaloids, which includes both agonists and antagonists, provides a structurally distinct set of compounds for comparison with this δ model. In this alkaloid series a second aromatic moiety, proposed to be a critical δ-pharmacophore element corresponding to the enkephalin Phe side chain, was introduced, linked to the morphinan structure of naltrexone or oxymorphone. The structures of SIOM, an example of a δ-selective agonist in this series, and of OMI, an antagonist member of the series, are shown in Figure 2. The superposition of JOM-13 in its proposed δ-bound conformation with these agonist and antagonist members of this alkaloid series is shown in Figures 4 and 5, respectively. As can be seen from

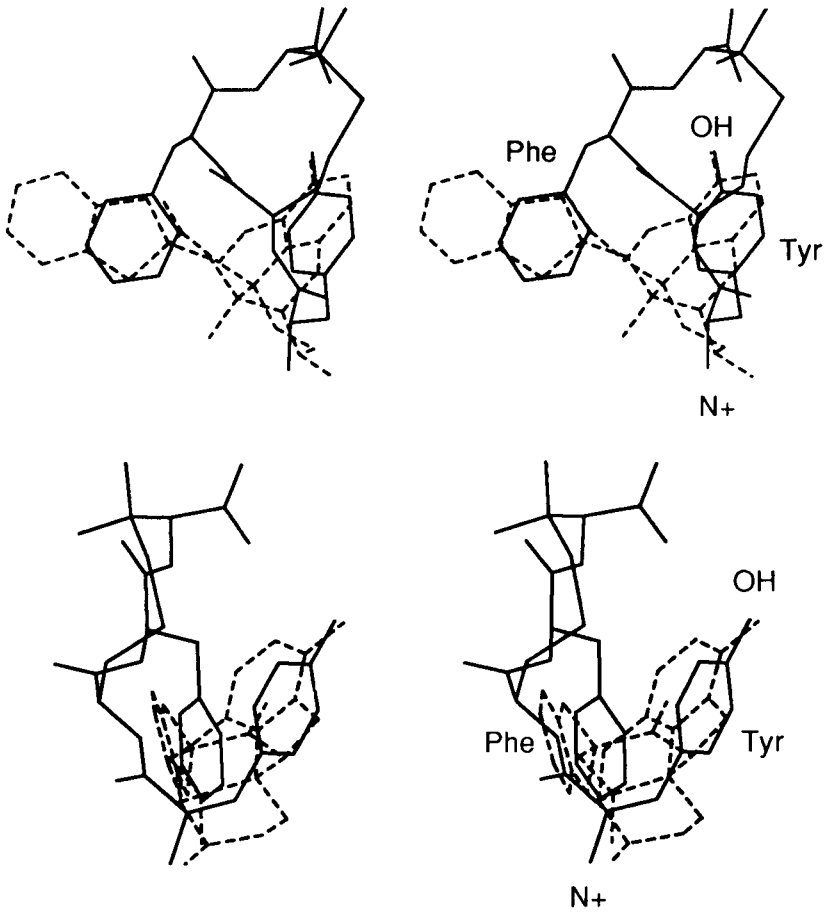


FIGURE 4 Superposition (stereoview) of JOM-13 (solid line) and δ-selective nonpeptide agonist SIOM (dashed line, conformer of 7-indanyl ring with ΔE = 1.3 kcal/mol) shown from two perspectives.

these superpositions, JOM-13, in its proposed δ-bound conformation,[3,4] and the δ-selective alkaloid ligands have similar arrangements of their tyramine fragments and "second" aromatic rings (the phenyl ring of the Phe residue in peptides and the benzene moiety of both the indanyl system of SIOM and the indole system of OMI). The orientations of these "second" rings are identical in peptide and alkaloid agonists (Figure 4), but differ in the antagonist, OMI (Figure 5). The superposition presented in Figure 4 provides overlap of key tyramine atoms, N^{α}, $C^{\epsilon 1}$, $C^{\epsilon 2}$, C^{η}, and O^{η} of the Tyr^1 residue of JOM-13 with the corresponding atoms of SIOM (rms deviation < 0.5 Å) and coplanarity and spatial proximity of the peptide Phe^3 aromatic ring with the benzene ring of the alkaloid indanyl group. In the tyramine fragment, the functionally important O^{η} atoms[1,47] almost coincide (the distance between them is 0.3 Å), while the aromatic ring of Tyr^1 and its counterpart in the alkaloid are situated in slightly different planes and shifted relative to each other by rotation around the common O^{η} point. This type of superposition is consistent with structure–activity results observed for δ-selective opioid peptides; in this arrangement, the 2′- and 6′-methyl groups attached to the Tyr^1 aromatic ring in high affinity analogues of DPDPE[48,49] and additional aliphatic rings incorporated into residue 1 analogues of JOM-13, which also display high affinity,[3] would be overlapped with aliphatic rings of the alkaloid. The most obvious deviation from ideal overlap of corresponding pharmacophore elements in the peptide and alkaloid structures shown in Figure 4 involves the benzene moieties of the Phe^3 residue of JOM-13 and of the indanyl group of SIOM. In the superposition these rings are coplanar but are shifted such that the Phe^3 aromatic ring coincides with the indanyl cyclopentyl ring. Structure–activity relations for JOM-13 indicate that such a deviation can be accommodated without adversely affecting δ-binding affinity. The structural requirements for this "benzene"

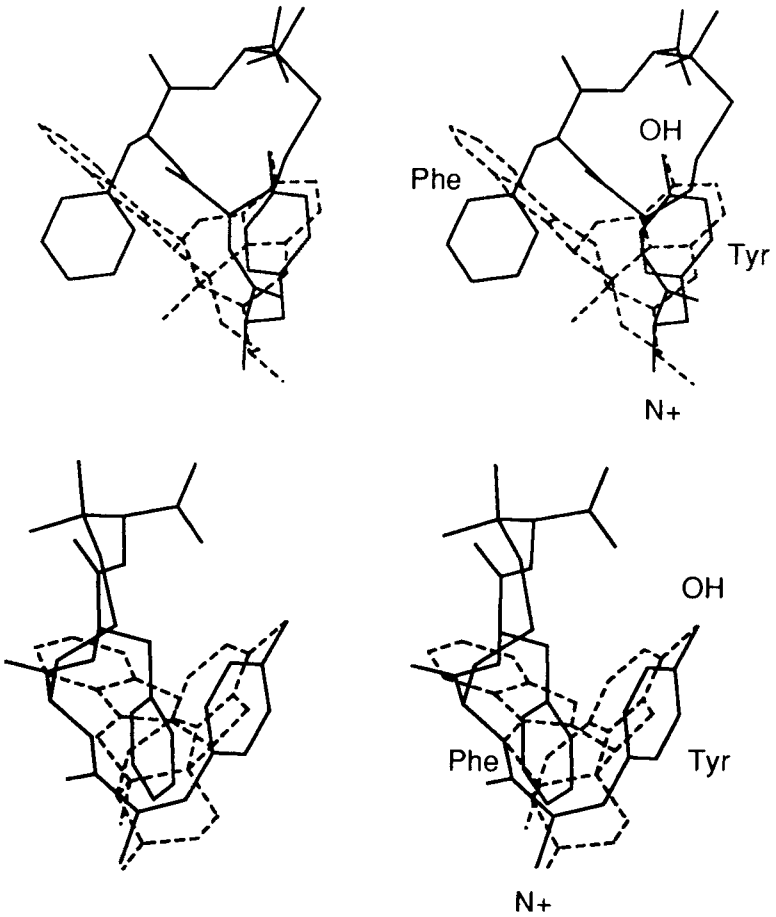


FIGURE 5 Superposition (stereoview) of JOM-13 (solid line) and δ-selective alkaloid antagonist OMI (dashed line) shown from two perspectives.

δ-binding site have been extensively studied using many different modifications of the Phe^3 side chain in JOM-13.[50] The most important features of this pharmacophoric group are hydrophobicity and a "flat", preferably aromatic structure. Of special note, replacement of the Phe residue by the larger Trp, 1-naphthylalanine, or 2-naphthylalanine (1-Nal; 2-Nal) residues does not affect δ binding.[50] The 7-indanyl benzene ring of SIOM is overlapped completely with the larger aromatic groups of Trp^3 and Nal^3 in corresponding high affinity analogues of JOM-13. It should be noted that all the mentioned modifications of the residue 3 side chain have only minor influences on conformations of the Tyr^1 residue and the disulfide-bridged cycle of JOM-13 (Lomize and Mosberg, unpublished results).

The superposition shown in Figure 4 is particularly noteworthy since it depicts the fit of a rigid alkaloid structure to an independently derived peptide model. It has often been assumed that the tyramine moiety of morphine and related alkaloids corresponds to the tyrosine residue of opioid peptides and that these corresponding structures interact with the same receptor binding sites. Consequently, previous superpositions of alkaloid and peptide opioids, from the earliest attempts at matching potent oripavine structures to Met-enkephalin[51] to more recent superpositions of the OMI-related antagonist, naltrindole (NTI) with DPDPE[9] and with the amino terminal tripeptide fragment, Tyr-Tic-PheOH, of TIPP,[52] have mapped the more flexible peptide structure onto the more rigid alkaloid structure. The limitations of such approaches are clear since they can provide no confirmation of the initial assumption of similar binding modes. The results presented here, by contrast, strongly support this assumption.

Comparison of JOM-13 with δ Antagonists

NTI, the initial moderately δ-receptor selective alkaloid in the series developed by Portoghese and co-workers, was derived from the alkaloid antago-

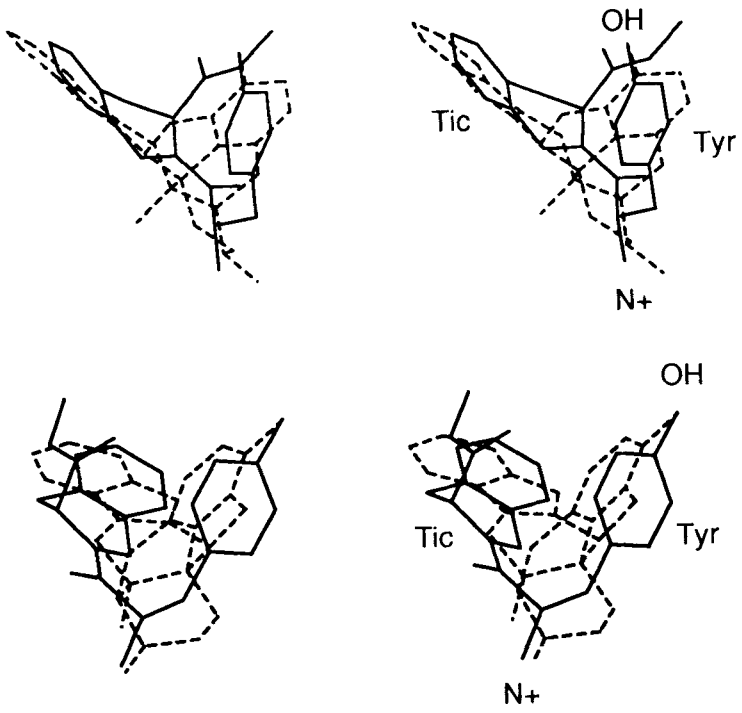


FIGURE 6 Superposition (stereoview) of Tyr-Tic methylamide fragment of δ-selective antagonist TIPP (solid line, torsion angles: Tyr^1 − ψ = 131°, χ^1 = −177°, χ^2 = −92°; Tic^2 − ω = 177°, ϕ = −85°, ψ = 120°, χ^1 = 52°, χ^2 = −37°) with δ-selective alkaloid OMI (dashed line) shown from two perspectives.

nist naltrexone. The resulting antagonist behavior of NTI, therefore, was not surprising and could be attributed to the presence of the N-cyclopropylmethyl substituent, long associated with antagonist actions in morphinan alkaloids. However, it soon became clear that, for δ ligands, antagonism results not only from this cyclopropylmethyl group, since its replacement by a methyl substituent (i.e., transformation of NTI to the oxymorphone derivative, OMI), associated in morphinan structures with agonist behavior, does not alter its δ-receptor antagonistic properties in antinociceptive assays.[53] Comparison of superpositions of SIOM and OMI, agonist and antagonist structures, respectively, both derived from oxymorphone, with the model for JOM-13 (Figures 4 and 5) suggests that the orientation of the benzene moieties, the critical second aromatic function in the peptide and alkaloid δ ligands, plays the decisive role in determining efficacy. As noted above, superposition of the agonists JOM-13 and SIOM results in a coplanar arrangement of these aromatic rings (Figure 4), while superpositioning of JOM-13 with the antagonist OMI leads to a tilted arrangement of these rings. The importance of the orientation of the benzene moiety for antagonism in the alkaloid series, which includes OMI, SIOM, and NTI, has also been suggested by Portoghese and co-workers.[46]

The recently discovered high affinity, highly δ-selective peptide antagonist TIPP,[6] which has no substituent on the terminal amine nitrogen, is consistent with the concept of a second locus affecting efficacy. In TIPP and related peptides, the aromatic ring corresponding to the Phe side chain of JOM-13 and DPDPE, which is responsible for the δ selectivity, affinity, and antagonism in this peptide series, belongs to the Tic^2 residue. Evidence in support of this include the observed moderate δ-affinity and -antagonist properties of the Tyr-Tic dipeptide,[54] the high δ-binding affinity of Tyr-Tic-LeuOH tripeptide in which only the side chain of the Tic^2 residue can correspond to the second aromatic pharmacophore element,[55] and the dramatically reduced δ-binding and -antagonistic properties accompanying removal of the Tic^2 aromatic ring via replacement of Tic^2 by L-pipecolic acid.[56] To further analyze the possibility that the Tic^2 aromatic ring of TIPP and the indole ring of OMI bind with the same subsite of the δ-opioid receptor, we calculated the set of low energy conformers of the crucial Tyr-Tic dipeptide (as the C-terminal methylamide; see Methods) and superimposed them

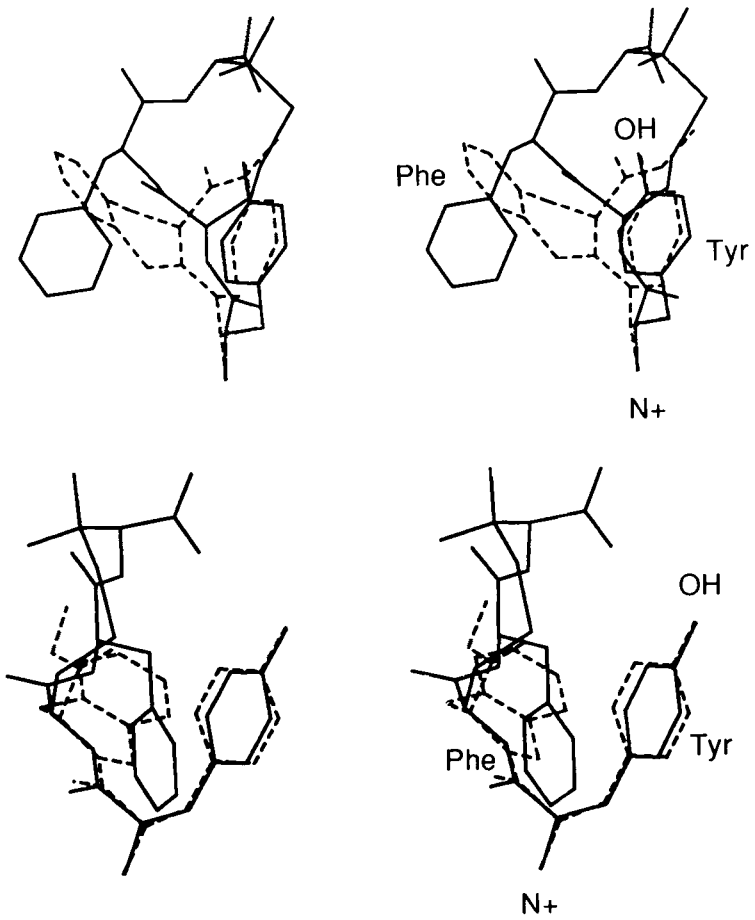


FIGURE 7 Superposition (stereoview) of proposed δ-bound conformer of peptide agonist JOM-13 (solid line) and the lowest energy conformer of Tyr^1-Tic^2 methylamide (dashed line, torsion angles as in Figure 6) shown from two perspectives.

with OMI. The dipeptide is very rigid with only 9 conformers within the energy interval 0–3 kcal/mol. Superposition of the lowest energy conformer of Tyr-Tic-methylamide with OMI (Figure 6) provides a good overlap of all pharmacophore elements in the peptide and alkaloid antagonists and supports the hypothesis of a similar binding mode. The same type of superposition of the δ-selective tripeptide Tyr-Tic-PheOH with OMI was recently considered by Wilkes and Schiller[52] as one of two possible alternatives. The superposition of Tyr-Tic methylamide with the proposed δ-bound conformer of JOM-13 is shown in Figure 7. As with the superposition of the antagonist OMI with JOM-13, depicted in Figure 5, the superposition shown in Figure 7 indicates excellent overlap of the respective tyramine portions of the two molecules, with the second aromatic rings of the structures (Tic side chain of Tyr-Tic methylamide, Phe side chain of JOM-13) occupying similar regions of space but tilted with respect to each other. The superposition of Tyr-Tic methylamide with the antagonist OMI and the agonist JOM-13 thus further supports the hypothesis that agonist vs antagonist activity within the structurally diverse set of analogues considered here is dependent on the orientation of the second benzene-like aromatic moiety, with both peptide and alkaloid antagonists sharing similar orientations and with peptide and alkaloid agonists sharing a different orientation.

CONCLUSIONS

We have previously developed a model for the binding conformation of ligands at the δ-opioid receptor based on conformational analysis of the cyclic tetrapeptide, JOM-13, and its analogues with conformationally constrained first or third residues.[2–4] In the present report, a similar δ-bound conformer has been identified for the structurally related δ-selective pentapeptide, DPDPE, using lit-

erature data and auxiliary conformational calculations. This conformer of DPDPE differs from, but is isoenergetic with, the crystal structure of the peptide, and has a conformation of the D-Pen-Gly-Phe-D-Pen cycle that is very similar to those in the crystal structures of the high δ-affinity peptides, [L-Ala^3]DPDPE and JOM-13. The δ-bound model of JOM-13 is also consistent with the structures of the more rigid δ-selective alkaloid opiates, having spatially equivalent arrangements of its pharmacophore elements (i.e., Tyr^1 residue and Phe aromatic ring) with the corresponding elements of the alkaloids SIOM and OMI (tyramine moiety and benzene ring, respectively), and fits well the lowest energy conformer of the Tyr-Tic fragment (containing the key pharmacophore elements) of the peptide antagonist TIPP. The model clearly distinguishes between agonist and antagonist conformations; the second aromatic rings (the benzene moieties of Phe^3, Phe^4, and Tic^2 residues in JOM-13, DPDPE, and TIPP, respectively, and of the indole and 7-indanyl ring systems in OMI and SIOM, respectively), while overlapped, have different orientations in agonists and antagonists. This leads to the suggestion that there are two spatially separated regions of the δ-receptor binding site at which transduction can be blocked: the first region interacts with substituents (allyl, cyclopropylmethyl) of the opioid cationic amino function, while the second one interacts with the aromatic benzene moiety of δ-selective ligands. Further, the results presented here strongly support the view that opioid peptides and alkaloids interact with the δ receptor in a similar fashion, with identical orientations in peptide and alkaloid ligands with the same agonistic or antagonistic properties.

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