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Backbone Alignment Modeling of the Structure−Activity Relationships of Opioid Ligands

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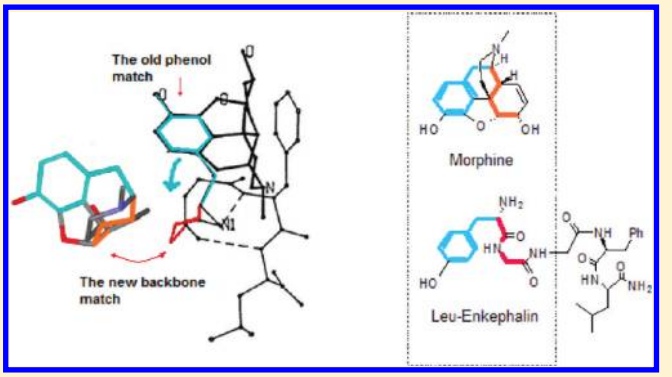
Supporting Information

ABSTRACT: Opioid studies are an important area of modern medicinal chemistry research. In this study we have provided innovative considerations to some long-standing problems in opioid studies, specifically the opioid pharmacophore and the potential binding modes of opioid ligands. Based on a new peptide backbone-alignment concept that we have developed along with this study, we discuss a wide variety of opioid ligands with respect to their structure−activity relationships.



INTRODUCTION

Opioids are highly potent analgesics and have been applied extensively in the clinic for treatment of pains associated with diseases, injuries, and operations. Opioids exert their analgesic activity through interactions with a type of G-protein-coupled receptor, referred to as opioid receptors. There are three different opioid receptors: μ, δ, and κ,[1,2] while corresponding to them are a wide variety of small molecule opioid ligands, derived either naturally or synthetically, agonists or antagonists, or multifunctional or highly selective, each with specific structural features. On the other hand, there are also a large group of natural peptides, called opioid peptides, which are natural endogenous ligands, act at the same receptors, and show the same activities. The discovery of the opioid peptides was only about 35 years ago.[3] Nevertheless, since then there have been numerous endogenous and synthetic opioid peptides discovered. Similar to plant opioids, the native opioid peptides are agonists, multifunctional, or highly selective, depending on their sequence and conformation. An often-encountered common feature of opioid peptides is the four N-terminal residues: Tyr[1]-Gly[2]-Gly[3]-Phe[4], the so-called 'message',[4] which has been generally considered as the pharmacophore of opioid peptides, responsible for their opioid activities. The rest of the sequence of opioid peptides is called the 'address', responsible for their receptor recognition. In contrast, however, it is harder to recognize a common pharmacophoric structure for the wide variety of small molecule opioid ligands.

Because of the remarkable analgesic activities that opioid drugs can produce and also because of the important underlying biology of the endogenous opioid systems that opioid studies have revealed, opioid research has played a significant role in medicinal chemistry research. Even though they have been studied for more than a century, the research on opioids is still very active today.[1,5−9]

Currently opioid research is focused at resolving issues related to the serious side effects associated with opioid drug applications, such as drug tolerance, dependence, and addiction.[5] Yet, in order to solve these issues one of the main strategies is still to focus on the opioid pharmacophore. This information is very important, as it can provide us with the structural basis for understanding the detailed interaction mode of the various opioid ligands with their receptors.[6,10] As an essential problem in this area, we need to know explicitly why small molecular ligands and opioid peptides, as the two classes of apparently different molecules in chemistry, would be able to interact equally effectively with the same receptor system.[7] In this paper, we will focus our discussions on this problem.

RESULTS AND DISCUSSION

Morphine as a Mimic of the Backbone of Opioid Peptides.

Morphine and leucine-enkephalin (Leu-Enk) are two well-known prototypes of opioid ligands. Chemically morphine belongs to the category of alkaloids, possessing a rigid and uniquely three-dimensional (3D)-shaped scaffold along with one basic tertiary amino group and two hydroxyl groups. On the other hand, Leu-Enk is a linear pentapeptide containing the typical message

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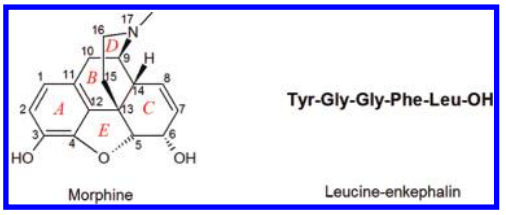
sequence for a peptide opioid ligand, Tyr-Gly-Gly-Phe. Both of the ligands are naturally derived and are agonists at all three opioid receptors.



Attempts to correlate these two molecules have been a long-time interest. There are several models in the literature regarding the matching of morphine with opioid peptides, e.g., see refs 11−14. Most of the models have focused on the 3D relationships of the side chain groups, (especially the tyramine moiety and the phenyl group of Phe[4]) but not on the peptide and alkaloid backbones. The tyramine was chosen because it is the most obvious common feature in morphine and opioid peptides and was believed to be a necessary pharmacophore. In addition, there are difficulties in recognizing a single pattern from the highly variable conformations of the peptides. Therefore even at this time, we still need an exact definition of the structural correlation between morphine and opioid peptides. Indeed, morphine's 3D architecture appears to contain the critical information that can be useful in identification of the important bioactive conformations of various opioid peptides.[7]

In this regard, we have reinvestigated the literature and propose here a new model, which features a match between the skeleton of morphine and the backbone of Leu-Enk.

Match of Morphine to the Backbone of [Leu^5*]-Enkephalin.* In 1988 Aubry et al. reported on the X-ray crystal structure of

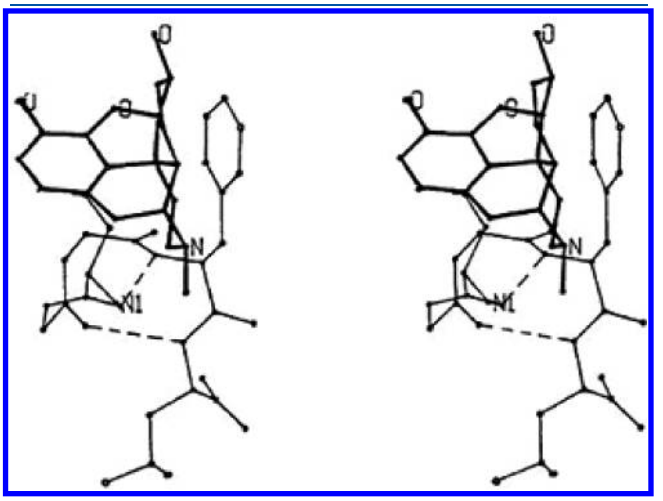


Figure 1. Aubry's match of morphine with the crystal structure of Leu-Enk. Reproduced from ref 15 with permission of The Royal Society of Chemistry.

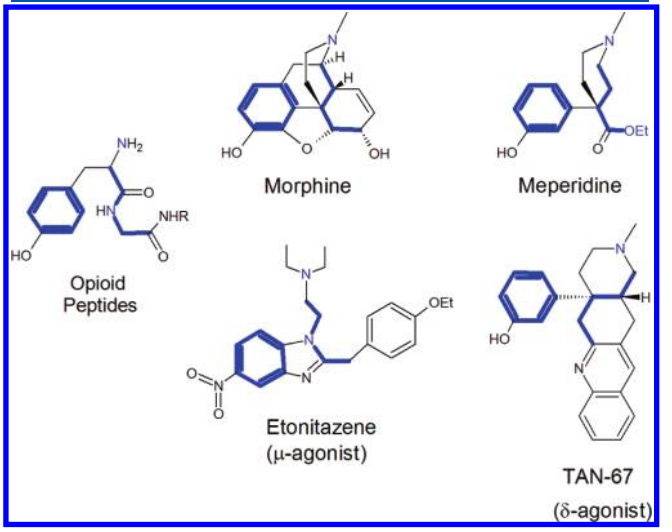


Figure 3. The backbone match model derived segments as the key elements of opioid pharmacophore.

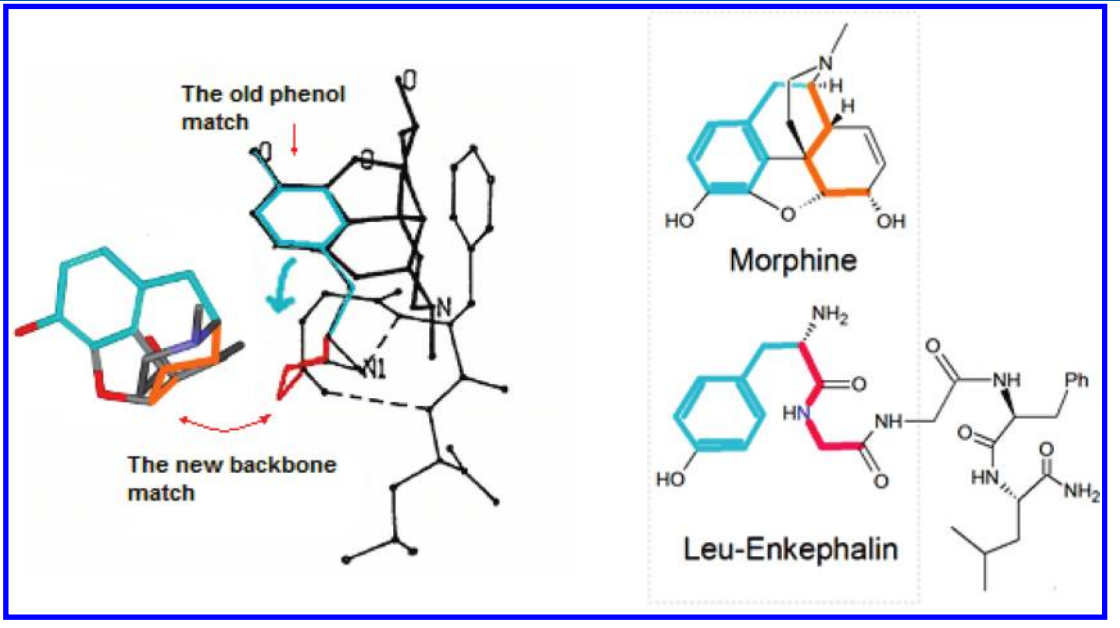


Figure 2. New match of morphine to the crystal structure of Leu-Enk.

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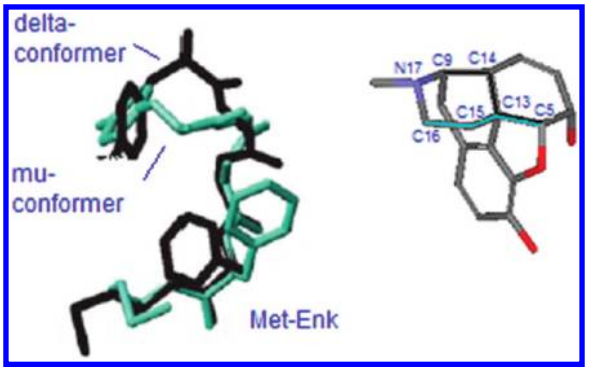


Figure 4. Comparison of morphine with the μ- and δ-conformers (cyan and black, respectively) of Met-Enk. The image of Met-Enk is reprinted from ref 22 with permission from Elsevier; copyright (2004).

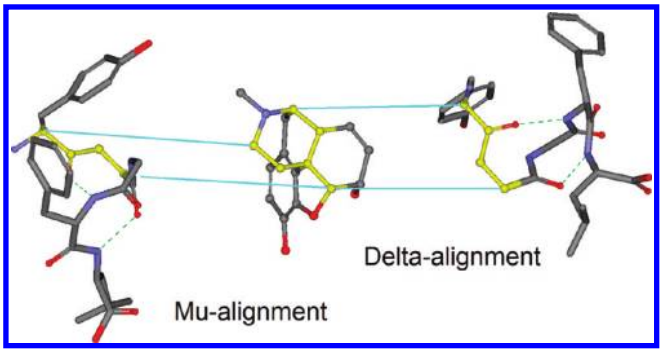


Figure 5. Two ways for Leu-Enk to align with morphine.

Leu-Enk that shows the backbone with two turns and two H-bonds (4→1 and 5→2).[15,16] This structure is favored as the active conformation over the other known crystal structures of Leu-Enk.[17]

Aubry et al. subsequently compared the crystal structure of Leu-Enk with that of morphine by superimposing their common phenol moieties (Figure 1). Their model showed that "the Tyr^1 and Phe^4 aromatic rings have a close orthogonal arrangement analogous to the tyramine and cyclohexenyl rings in morphine",[15] a feature that had been well documented in previous studies.

However, by re-examining the crystal structures of Leu-Enk, we have identified a different way in matching the two molecules, that is, by fitting part of the scaffold of morphine into the backbone instead of the phenol moiety of Tyr^1 of Leu-Enk, (see the orange- and red-highlighted structural moieties in Figure 2). As shown in the figure, the match demonstrates excellent overlap, including the four related bonds and their 3D-orientations, a fine indication that the scaffold of morphine could resemble the backbone of Leu-Enk (refer to the structures at the right panel).

It can be argued that a backbone match is more meaningful than a match by side chains because the side chain groups in a peptide are more flexible and will adopt different conformations in different situations. In contrast, the backbone is relatively more rigid and more stable and hence more reliable in elucidating the low-energy associated bioactive conformations of the peptide. As a matter of fact, Leu-Enk's crystal structure would become more morphine-like if we would rotate the phenol moiety to match that of morphine (see the blue arrow in Figure 2), while keeping the backbone conformation of the peptide unchanged.

Backbone-Derived Fragments Are the Key Elements of the Classical Opioid Pharmacophore. Figure 3 shows several examples of structurally different opioids.[1,6] Although there is a large variation in their structures, the essential elements as illustrated in the above morphine-Leu-Enk match model are present in all of them, including the phenolic (or phenyl) moiety, the basic amino group, and the backbone-derived bonds (shown in thick blue lines), which are also in good agreement with the classical three-point pharmacophore model of opioids featuring a phenolic site, a cationic site, and a hydrophobic region.[10]

Compared to the classical model, however, our model emphasizes key backbone segments as the core structure, so as to give a more defined picture of the pharmacophore of opioids and a better correlation with the structure of opioid peptides.

However, it should be noted that considerations of matching the backbone of opioid peptides and the morphine skeleton are not novel and have already been examined in the literature.[18,19] But these older models only considered matching the 2D-structures of the molecules without comprehensive consideration of the experimental data. Our current model agrees with both the 2D and the 3D structures of the related bonds and atoms (see the detailed discussion in below sections) and is supported by X-ray crystal structures.

Does Morphine Mimic the μ-/δ-Conformers Simultaneously? Interaction of an opioid peptide with opioid receptors has been suggested through two steps: First it has to be transferred into the biomembrane (a lipid phase), where the binding sites of opioid receptors are located. Within the lipid phase it then adopts the bioactive conformation suitable for the recognition by the receptors.[20,21]

In an NMR study of the bioactive conformations of methionine-enkephalin (Met-Enk, Tyr-Gly-Gly-Phe-Met) in biomembrane-mimicking micelles, two types of major conformers (μ and δ) were found, which were suggested to be responsible for μ- and δ-receptor binding, respectively.[22] This result is of interest because it provides inspiration for us to consider the role of morphine's N17−C16−C15−C13, a seemingly unimportant moiety in the classical model. By comparing morphine with this NMR model, we can see that the C16−C15−C13 fragment corresponds to the μ-conformer, while C9−C14−C13 to the δ-conformer (Figure 4).

Therefore the structure of morphine appears to contain key elements that can mimic the two active backbone conformers of opioid peptides, μ and δ. Thus we now can see two ways for the match, either by aligning the peptide backbone to C9−C14−C13−C5 (the δ-alignment) or by aligning the peptide backbone to C16−C15−C13−C5 of morphine (the μ-alignment) (Figure 5). (Note that we use three bonds here for the match instead of the four-bond match in the previous section, and this is necessary for differentiating the three types of opioid ligands as will be discussed in the following sections.) In contrast, the previous one-way match only matched to the C9−C14−C13−C5 fragment. This two-way alignment for Leu-Enk's crystal structure into two binding modes may account for the dual δ and μ activities of Leu-Enk.[23]

Moreover the two-way alignments may help in understanding the unique structure−activity relationships (SARs) of some opioids. For example, both norbuprenorphine[24] and 7-spiroindanooxymorphone (SIOM)[25] are semisynthetic opioid agonists, with norbuprenorphine being μ-selective and SIOM δ-selective. By the μ or the δ alignment of the crystal structure of Leu-Enk (on the right panel of Figure 6), we can see that the F-rings of both of the ligands appear to mimic the Gly^3-Phe^4 of Leu-Enk,

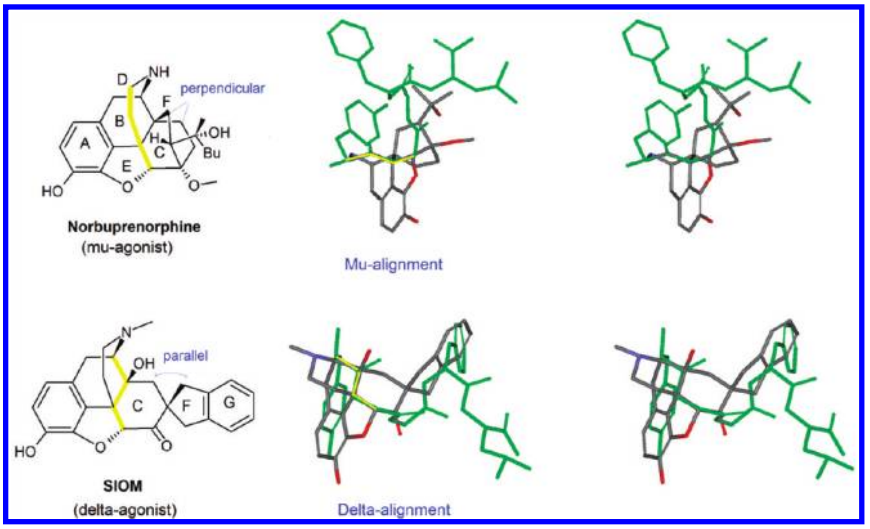


Figure 6. The μ-/δ-alignment of Leu-Enk with μ-/δ-selective ligands. (The aligned sections are highlighted in yellow.)

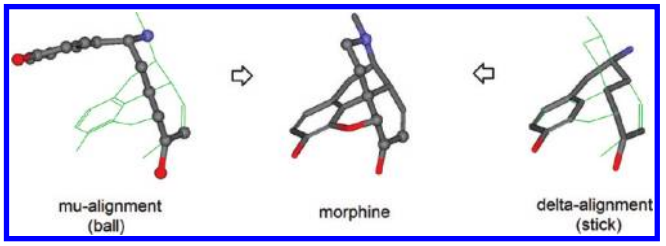


Figure 7. Morphine as a backbone-derived peptidomimetic.

being either perpendicular (norbuprenorphine, in μ alignment) or parallel (SIOM, in δ alignment) to their C-rings, (in Figure 6, Leu-Enk is green colored, the ligands are in gray, and the aligned sections are highlighted in yellow). Similar steric relationships (either perpendicular or parallel) can be seen more clearly by comparison of the F- and the C-rings of the ligands as shown in the left panel of Figure 6. This assessment may help to explain the distinct receptor binding selectivity of these two ligands.

Furthermore, our studies indicate that the morphine scaffold can be evolved from Tyr^1-Gly^2, the first two residues of the message sequence of opioid peptides, in the two different alignments (Figure 7).

In the μ-alignment, not only do the backbone of Tyr^1-Gly^2 and the fragment C16−C15−C13−C5−C6 of morphine overlap perfectly but the absolute stereochemistry of the amino group of the peptide (in the L-configuration) also fits well with that of morphine. Thus this alignment also can explain a perplexing problem for the previous types of matching,[18,19] that is, how the amino group of morphine would appear not to be from a L- but from a D-Tyr^1. From our studies it now is apparent that the classical model was only related to the δ-alignment.

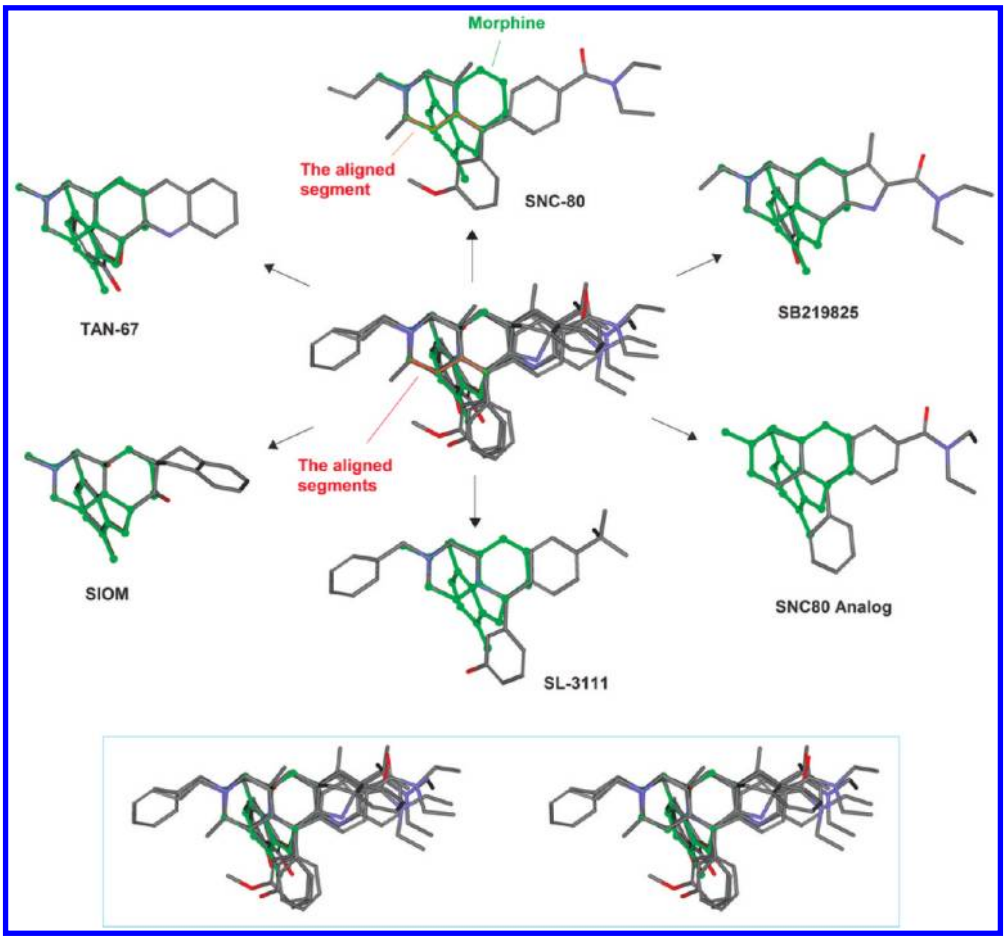
However not everything is for the μ-alignment, and it seems that the *p*-hydroxyphenylmethyl group of morphine is from the δ-alignment (see Figure 7), which implies that morphine does not completely mimic the peptide. Presumably, nature designed this and some other subtle alterations in morphine's structure, which allows for the special binding properties of this molecule. Morphine does not have the address sequence that a peptide ligand has, so it must have certain other structural features that can account for its binding profile. (Although an agonist at all the three receptors, morphine shows μ-affinity as the major).[26] Thereafter, these subtle alternations in morphine's structure can be considered to be the structural feature for its special binding properties.

Development of Backbone Alignment Models to Assess the SARs of Small Molecular Opioid Ligands. As discussed above, there are two ways for Leu-Enk to align with morphine, the μ- and the δ-alignments. But we have mostly used the μ-alignment for our modeling. This is because the one-model two-way alignments used above were mainly for differentiating the orientations of Leu-Enk at residues 3−4 but not residues 1−2. However, we have realized that this type of differentiation can actually be accomplished as well if we take a two-model one-way approach, that is, to use two models instead of one where each takes one alignment. The two models are generated by placing the 2−4 peptide units differently, (a peptide unit is defined as the amide functional group of two adjacent amino acid residues along with the two C^α atoms, namely, C^α−CO−NH−C^α), while maintaining the first one at the same position so as to account for the different selectivity. This consideration appears to be more realistic, as Leu-Enk is a flexible peptide ligand, and it can and should adopt different conformations at the distinct binding sites of the three opioid receptors.

In the two-model approach, it appears that we can use either of the two alignments of Leu-Enk for our modeling, as we can always adjust the orientation of peptide unit 2 as long as the related bonds are flexible, no matter which orientation unit 1 takes. However, it seems that the μ-alignment is preferred, because this orientation appears to be more suitable for most of the ligands with different structures. Only in occasional cases does the δ-alignment appear to be adequate, such as in the case with etonitazene (vide infra). In fact, in many other cases, there is no need to differentiate between the ways of alignment because many of the opioid ligands have a six-membered ring in their structures that can account for both the μ- and the δ-alignment simultaneously.

Additionally, in the following modeling we will use morphine as the template instead of Leu-Enk. Morphine appears to be a good ligand for all three types of opioid receptors, and its molecular architecture is a close mimic of the 3D structure of

Scheme 1. Alignment of a Group of δ-Agonists and Stereoview of the Assembly



Leu-Enk. Thus its scaffold can work as a universal core template for all the opioid ligands, while more importantly some of its key structural features, such as the fused ring systems, and the related chiral centers can be used as an additional guide to facilitate the alignments of many of the opioid ligands (the details will be given in the following discussions).

Construction of the Agonist-Assembly Models. For the continued development of the backbone alignment concept, we have chosen a number of known small molecular opioid agonists[1,6,8,27] to align with the key elements of morphine. Most of the chosen ligands possess the apparent structural elements suitable for the alignment, while others the structural features are not so apparent. For the latter case, we have applied a trial-and-error process in order to get the best alignment mode as possible. Thus, three agonist-assembly models have been built up, corresponding to the three types of opioid ligands (see Schemes 1−3).

Alignment of the δ-Agonists. It is relatively easy to align the nonpeptide δ agonists,[6] TAN-67, SIOM, and SB219825, since all of the three ligands have similar structural scaffolds that correspond to that of morphine. The other three ligands, SNC-80,[28] SNC-80 analog, and SL-3111, do not so closely resemble each other, but each have a six-membered piperidine/piperazine ring, which appears to be promising as the equivalent of morphine's D-ring. Their six-membered rings have similar substitution patterns as well as similar steric settings (the relative ring conformation and/or the related chiral centers configurations), which correspond well to that of morphine and morphine analogs.

Supporting insights have emerged from the alignments. For example, for SNC-80 in the orientation as shown, the exoring chiral center presents SARs that well supports this alignment, where the stereoisomers with higher binding affinities (in the *R*-configuration)[28] place the smaller *m*-methoxyl phenyl group at one side, while the larger and rigid *p*-amidophenyl moiety is at the other side, which corresponds to the δ-specific area (see Figure 8), so as to give rise to the δ-selective nature of this ligand.

Assembly of All the Aligned Ligands. When all of the aligned δ-selective ligands are placed together as an assembly (see Figure 8), we can see several interesting features. For example, (i) the key segments of all the ligands are in good alignment with that of morphine, which speaks for them being the essential structural elements of opioid ligands; (ii) all the ligands have moieties that extend into the δ-specific area, which would account for their δ-selectivity; (iii) TAN-67, SIOM, and SB219825 are the three most rigid ligands, and they all have a phenol moiety relative to that of morphine that can serve as the major structural feature for their receptor recognition and binding. But SNC-80, SNC-80 analog, and SL-3111 do not have a similar phenol moiety. So in order for adequate recognition and binding, presumably they must have some other structural features for compensation. It can be suggested that the additional aromatic substituents (SL-3111's N-*m*-hydroxyphenyl[29,30] and

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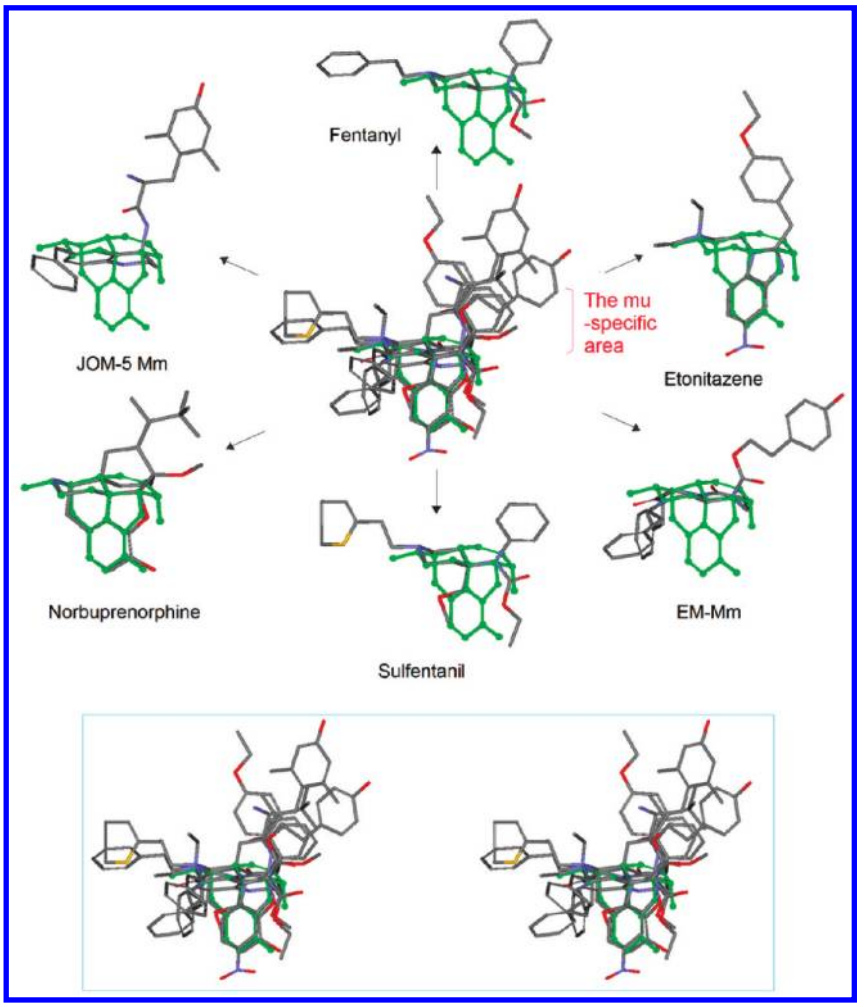
Scheme 2. Alignment of a Group of μ-Agonists and Stereoview of the Assembly



SNC-80 and SNC-80 analog's phenyl rings) as shown in the lower position of the structures may be this type of auxiliary moieties, whose presence may contribute to the receptor binding affinity of these ligands. This viewpoint is of interest as it might be a useful concept to assist in the assessment of many other related opioid ligands for their SAR's.

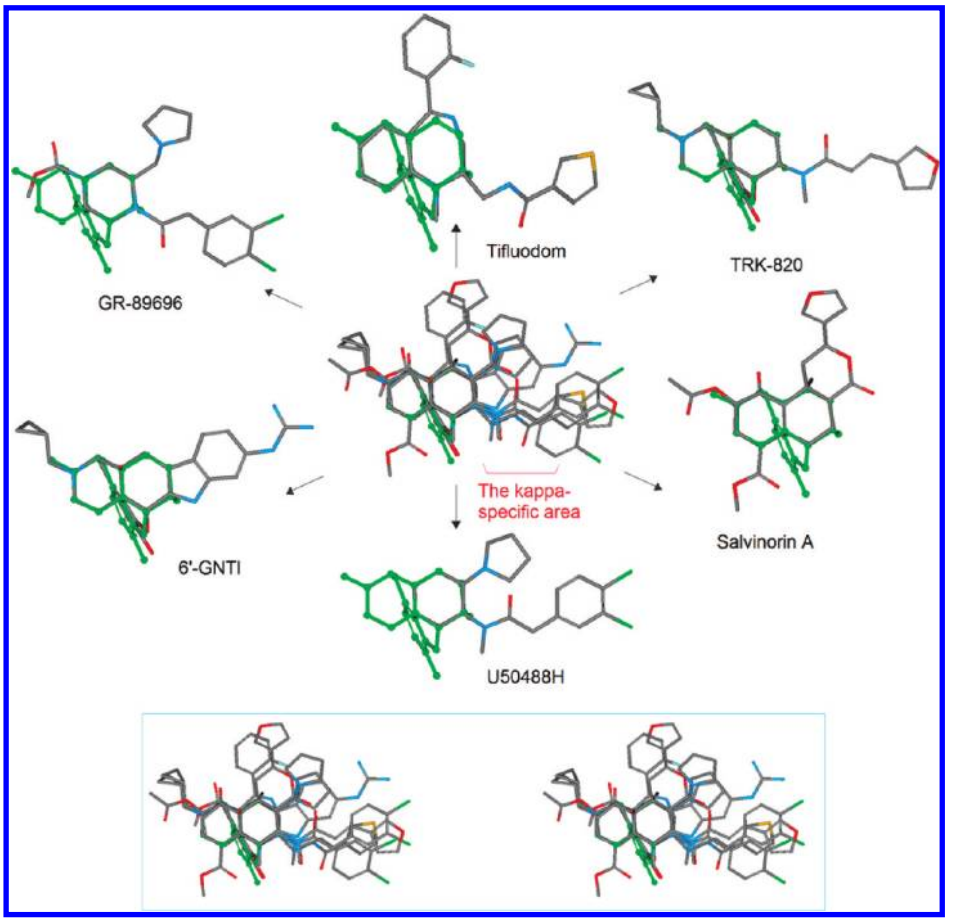
Alignment of μ- and κ-Agonists. Using the same approach, the ligand alignment models for both μ- and κ-agonists have been assembled (see Schemes 2 and 3). For the alignments of both μ- and κ-agonists, the key segments were also smoothly aligned to that of morphine. And the alignment assemblies also are able to inform us of several interesting SAR insights. The following are a few examples to show the details: endomorphin mimetic (EM-mimic), etonitazene, and salvinorin A.

EM-mimic is a synthetic μ-agonist, which is equipotent to morphine.[31] This compound does not have a cationic amino group but still presents high affinity for the μ-receptor. There appears to be several ways for this molecule to be aligned with morphine. In the current alignment, the two fused rings of EM-mimic are nicely superimposed with that of morphine, and the chiral centers of both molecules are well matched too, which would justify this alignment. Also, it appears that this alignment can place the three substituents of the EM-mimic in proper positions that are consistent with some of the other μ-agonists. Especially, the relatively rigid *N*-arylethoxylcarbonyl group is placed at the μ-specific area to account for this molecule's μ-selectivity (see Figure 9).

Etonitazene[32] shows very high μ-selectivity and -affinity.[1] This compound has a rigid benzoimidazole framework, which when its *N*-diethylaminoethyl group is placed in the 'δ-alignment mode' can well match with the A- and E-rings of morphine, while the bulky and rigid *p*-ethoxylphenylmethyl group can orient directly into the μ-specific area (see Figure 10). These unique structural features may account for this molecule's high μ-selectivity and -affinity.[32]

Salvinorin A is a diterpenoid-type natural product with highly selective and potent κ-activity.[33] It has a unique scaffold that is quite different from many other opioids. To identify its key segment, this compound was managed to align with morphine in the way that the fused cyclohexyl rings of this ligand are superimposed with that of morphine, while the central chiral centers of both ligands are well matched (Figure 11). Thus, this setting appears to be a good one. In the current mode, salivinorin A appears to have a moiety that is oriented into a new area not seen with many other κ-ligands (Figure 11). This area may suggest a new subpocket in the κ receptor. As an interesting example supporting this view, erinacine E, another natural κ-ligand,[34] appears to have the similar structural feature as well (see the right

Scheme 3. Alignment of a Group of κ-Agonists and Stereoview of the Assembly



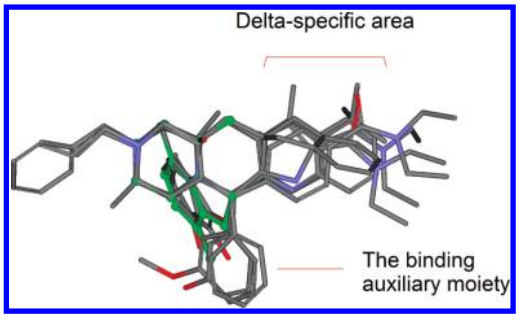


Figure 8. Assembly of all the aligned δ-agonists.

panel in Figure 11). In fact, a few of the other κ-ligands, such as GR-89690 and U50488H, may also hold moieties relevant to this subpocket. (See their structures in Scheme 3.)

Development of Backbone Conformational Models for Opioid Peptides. Upon the basic construction of agonist-assembly models for three types of small molecular opioids, in the following we will discuss how to correlate these results to the various opioid peptides. As we have mentioned at the beginning, opioids and opioid peptides bind at the same site of the opioid receptors, while the key segment of the morphine scaffold closely mimics the first peptide unit of Leu-Enk. Based on this consideration, presumably, opioids with certain extended moieties would mimic the second and beyond peptide units as well. And by tracing along the related key segments, we would possibly be able to map out the other related backbone segments with 3D conformations of opioid peptides.

On the other hand, we have seen the receptor-type specific areas in the above three agonist-assembly models, which are the common regions shared by many opioid ligands, and these regions appear to correlate closely with the receptor selectivity and affinity of those ligands. Presumably, these common regions would be the structural elements that are directly related to the backbones of the opioid peptides.

Thus, by combining the structural features of a number of large-sized small molecular opioids along with some conformationally restricted opioid peptides, three backbone conformational models of opioid peptide have been generated. With the κ-model as an example, the construction process is illustrated below.

Four large-sized nonpeptide κ-agonists were shown for the modeling (Figure 12). Among them, 6′-GNTI is a rather rigid molecule, while the other three are semirigid. The conformations of these ligands were set up with 6′-GNTI as the major reference compound. It can be clearly seen that the peptide backbone extends from unit 1−4 in the framework of all the agonists. The unit 1 forms the core structure (note that it assumes the 'μ-alignment' mode), while the units 2−4 extend through out the κ-specific area to account for the κ binding specificity.

The three peptide backbone conformational models, as shown in Figure 13a, indicate that all three types of backbones have the

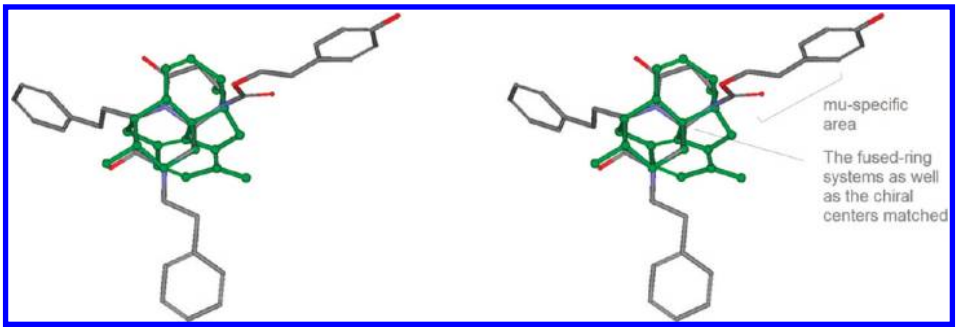


Figure 9. Stereoview of EM-mimic's alignment with morphine.

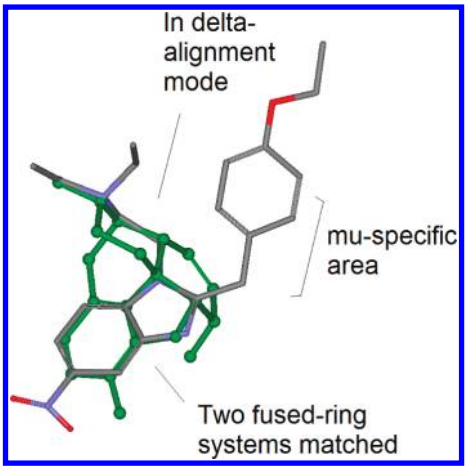


Figure 10. Etonitazene aligned with morphine.

same conformation at the first peptide unit, as aligned with morphine at the 'μ-alignment mode', while they are differentiated at the second unit and beyond. Both the δ- and κ-models have the second peptide units with nearly the same orientations but the opposite dihedral angles (the ψ^2) (see Figure 13b). The κ-model's second unit appears to align almost in the same orientation as the first unit (parallel to the first unit). And the μ-model has the second peptide unit in a quite different orientation, almost perpendicular to the first one (Figure 13a).

Assessment of the SARs of Special Opioid Peptides Based on the Three Backbone Conformational Models. Opioid peptides consist of a large family of peptides, often possessing 5−20 amino acid residues in their sequences.[2] As mentioned at the beginning, the receptor selectivity of most of the opioid peptides is decided by their address sequences. Therefore the current backbone conformational models cannot be directly applied to assess their structure−selectivity relationships, because the models cover only the message structure. However there are some special opioid peptides, which are shorter and conformationally restricted, such as cyclic opioid peptides and opioid peptides with rigid or modified residues.[23,35,36] Several of these opioid peptides present unique binding activities, and their SARs turn out to be well accessible through the backbone conformational models. As will be seen in the following discussions, the modeling results appear to be rather significant in understanding of some complicated or even confusing SARs of those peptides, which may hence serve well as examples for the validation of these new backbone conformational models.

Cyclic Peptides. Below are general guidelines with respect to the construction of 3D-structures of the cyclic peptides.

(1) Unless otherwise noted, the conformations of all the cyclic peptide ligands discussed here were examined with reference to the backbone conformational models of opioid peptides (Figure 13). For instance, for the conformation of the macrocycle of MABE the second peptide unit (D-A_2pr^2-Gly^3) was set according to the δ-model and the side chain moiety of D-A_2pr^2 according to the μ-model. Then the conformations of the rest of the macrocyclic ring as well as the related side chain groups were drawn arbitrarily followed by local energy minimization with the software ('clean geometry'). Since the backbone conformational models are deduced directly from the structures of morphine and many other highly active opioid ligands, they should be reliable in representing the active conformations of the opioid peptides.

(2) Whenever it is necessary we also used X-ray crystallography data as an additional reference for the modeling. The related cases will be detailed in the following discussions.

MABE (Tyr-c[($N^{\beta}CH_3$)-D-A_2pr-Gly-Phe-NH-CH_2-CH_2-])[37] is an Enk analog made by C-terminal-to-side chain cyclization via a *N*-methyl ethyldiamine bridge. MABE binds to both μ- and δ-receptors with high affinity (μ affinity, 1.6 nM; δ affinity, 2.1nM). Aligning the backbone of Tyr^1 of MABE (in the 'μ-alignment mode') with both the μ- and the δ-backbone conformational models, we can see that the two segments of the macrocyclic backbone of MABE that are directly connected to the $C^{\alpha}_{(D\text{-}A_2pr^2)}$ can be readily placed at the μ- and δ-specific areas and with a good match with the μ- and δ-selectivity related conformational models, respectively (see Figure 14), which would account as a structural basis for its dual receptor binding activities.

DPDPE (Tyr-c[D-Pen-Gly-Phe-D-Pen]) and DPLPE (Tyr-c[D-Pen-Gly-Phe-Pen]) are highly δ-selective agonists.[38] The two ligands are cyclic disulfides. Their cyclic backbones are 14-membered rings containing two D-Pen (or Pen) residues. The alignment is similar to MABE's, and the backbone of DPDPE's macrocyclic ring aligns well with the two models, (see Figure 15). However, since the gem-dimethyl groups of D-Pen^2 is involved in the μ-specific area, the μ-receptor binding would be disturbed presumably due to the significant steric repulsions caused by the gem-dimethyl groups for proper ligand−μ-receptor interactions. A similar explanation also can be applied to the case of DPLPE. Therefore, both DPDPE and DPLPE can only bind strongly to the δ-receptor.

Moreover, as shown in the peptide backbone models (Figure 13), the orientation of the second peptide unit (the ψ^2)

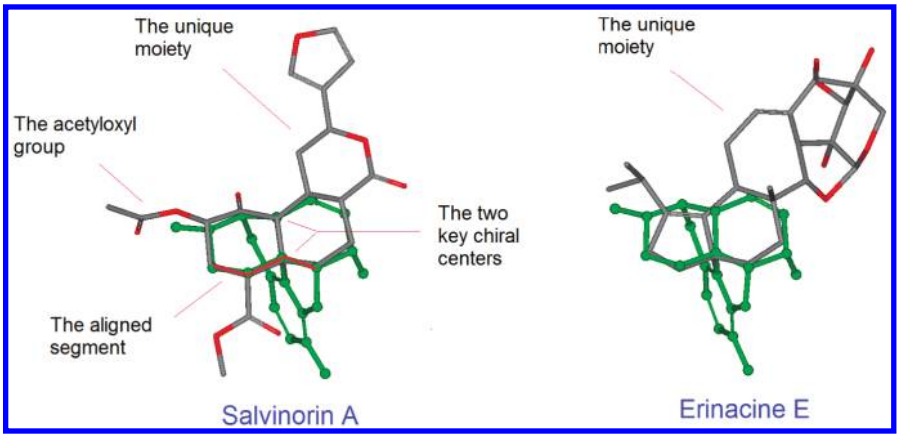


Figure 11. The alignment of salvinorin A and erinacine E with morphine.

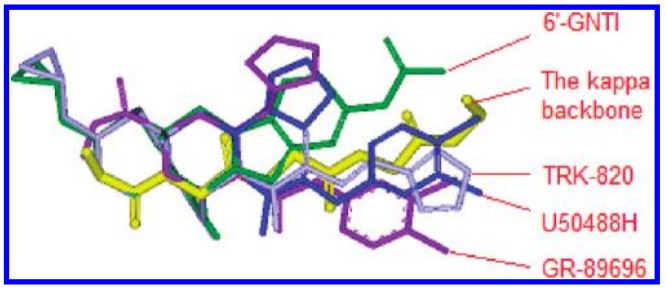


Figure 12. Construction of the backbone conformational model of κ-peptides.

is critical for the determination of receptor binding and selectivity of the three types of peptide ligands. Interestingly enough, this perspective from our modeling seems to have been well evidenced with previous SAR studies on DPDPE analogs.[39]

Although both [L-Ala^3]DPDPE and [D-Ala^3]DPDPE are closely related analogs, their δ-binding profiles are quite different from each other. While [L-Ala^3]DPDPE presented potent δ-affinity and -selectivity, the D-isomer was a poor and weak δ-agonist. The X-ray crystallographic data showed that these two ligands have quite different orientations of their second peptide units. That of [L-Ala^3]DPDPE was similar to the δ-model (see Figure 16), but [D-Ala^3]DPDPE's ψ^2 was about 180° rotated,[39] which conformational difference agrees well with their different δ-affinities.

According to the crystallographic data, however, the ψ^2 of DPDPE was different from that of [L-Ala^3]DPDPE but similar to [D-Ala^3]DPDPE's[39,40] (see Figure 16 for DPDPE's crystal structure. Note that the conformation of Tyr^1 of the crystal structure was adjusted so as to align properly with the backbone model, while the macrocyclic part remained unchanged). This result does not contradict but may further suggest the importance of ψ^2. DPDPE has a Gly^3 whose backbone is known to be conformationally flexible. Presumably, at the receptor's binding site the Gly^3-composed second peptide unit of DPDPE is able to assume a ψ^2 similar to the δ-model; hence, DPDPE still can achieve the high δ-binding affinity. In the case of [D-Ala^3]DPDPE, however, the rotatory freedom of ψ^2 is restricted due to the presence of the D-Ala^3 so that the backbone of this ligand cannot easily adapt to the δ-model-like conformation at the binding site, and thus it only shows weak δ-affinity.

Furthermore, the D-configuration of DPDPE's residue 2 appears to be rather important in this and in the other related cases because it determines the orientations of the two ring segments that are directly connected to C$^\alpha$(D-Pen^2) of the ring vs the core (Tyr^1). If L-Pen^2 were used, then the ring would have to flip over so that the two segments of the ring would switch their positions, which would cause the ligand's binding affinity and selectivity to change significantly (see the following CTP discussion).

CTP (D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Pen]-Thr-NH_2), CTAP (D-Phe-c[Cys-Tyr-D-Trp-Arg-Thr-Pen]-Thr-NH_2), and CTOP (D-Phe-c[Cys-Tyr-D-Trp-Orn-Thr-Pen]-Thr-NH_2) are three highly μ-selective antagonists.[41] Different from DPDPE and MABE, these ligands have a D-Phe^1 instead of an L-Tyr^1, which is quite different from many other cyclic peptide ligands. However, this feature may not be very critical in determining their binding selectivity, but the L-Cys^2 is. As pointed out above in the DPDPE discussion, the configuration at residue 2 is important for the orientations of the two C$^\alpha$-connected backbone segments of the macrocyclic ring. In this case, L-Cys^2 of CTP will cause a flipped-over conformation for its cyclic backbone ring as compared to that of DPDPE, so that the peptide backbone of L-Cys^2 will be oriented at the μ-specific area, while the disulfide bond moiety is placed at the δ-specific area, the opposite of DPDPE (see Figure 17). The disulfide bond with the gem-dimethyl groups of Pen^7 does not allow the ligand−receptor interactions at the δ specific site, and thus CTP as well as CTAP and CTOP have selectivity only for the μ-receptor, and these ligands do not bind to the δ opioid receptor. In addition, the larger sized (20-membered) rings appear to be another negative factor for δ-binding, because the large size increases the conformational flexibility of the backbone, and presumably the S−S bond site will be more affected. Thereafter, the larger sized rings of these ligands are improper for δ-alignment (see Figure 17, where the backbone of L-Cys^2 is well aligned with the μ-model but not the S−S bond segment with the δ-model).

CJ-15,208 (c[Phe-Pro-Phe-Trp])[42] is a cyclic tetrapeptide derived from nature that possesses dual κ- and μ- antagonistic activities (K_i: κ, μ, δ = 29, 130, 2000 nM, respectively). Interestingly, this cyclic peptide can be related to salvinorin A by properly aligning their backbone structures with each other (Figure 18). Not only the backbone but also the side chains of CJ-15,208 are in good accordance with those of salvinorin A. For example, the phenyl group of Phe^3 is matched with the pyrrolidine moiety of salvinorin A, while both moieties are found at the

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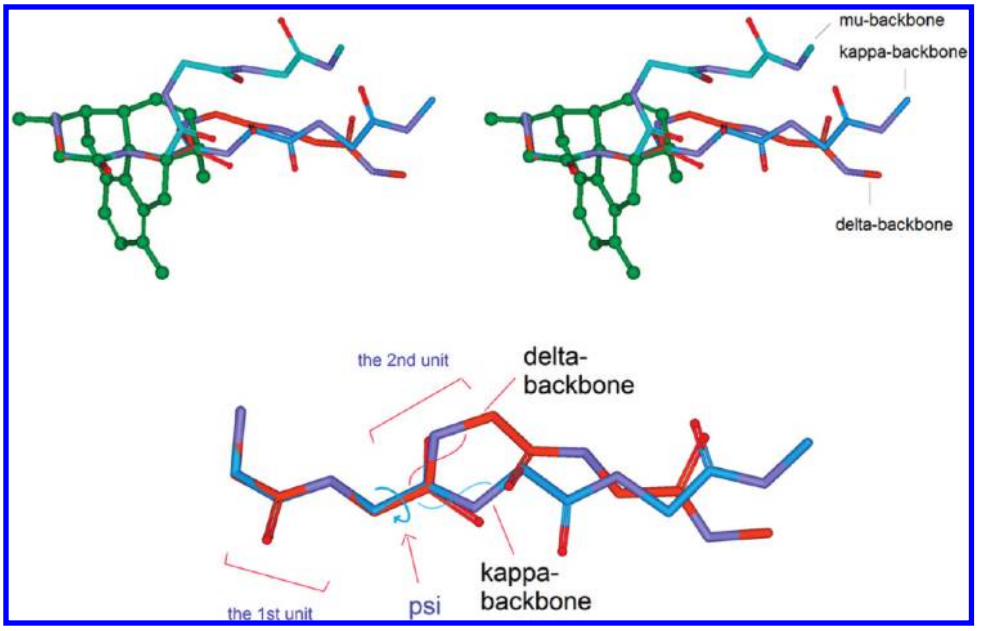

Figure 13. The peptide backbone conformational models: (a) Stereoview of the backbone conformational models; (b) κ- and δ-backbones showing the different ψ^2 angles.

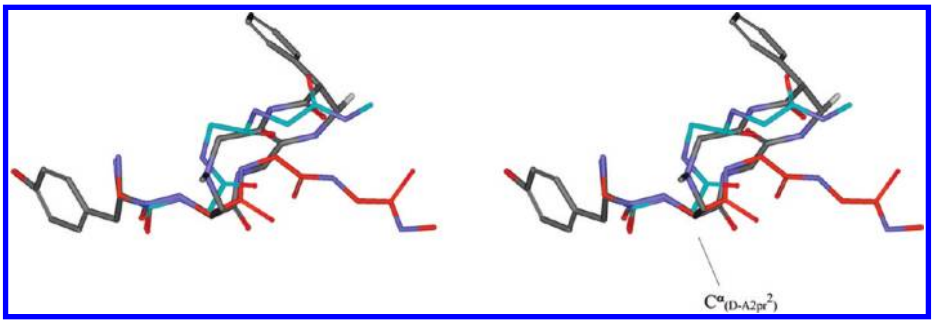

Figure 14. Stereoview of MABE alignment with μ- and δ-models (blue and orange, respectively).

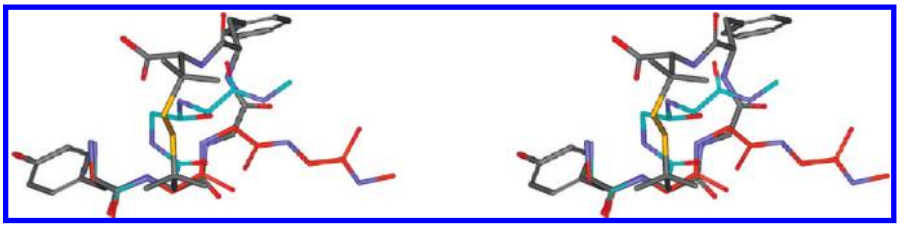

Figure 15. Stereoview of DPDPE alignment with μ- and δ-models (blue and orange, respectively).

unique κ-subpocket (see the previous discussion of salvinorin A). SAR studies showed that the binding affinity of CJ-15,208 is lost if the phenyl group of Phe^3 is removed (i.e., when Phe^3 was replaced with Ala^3; see compound **11** in the Table in Figure 18. Note that the compound numbering is the same as in the original publication).[42] It is particularly interesting to see that Trp^4 is necessary here; if Trp^4 is replaced with Ala^4, the binding affinity is lost (compound **14**). Meanwhile, a higher binding affinity is shown when Trp^4 is replaced with D-Trp^4 (compound **2**). These data can be well understood with the κ-alignment model. According to the model, the side chain of Trp^4 is located near the κ-specific area, so as to be the structural basis for the κ-selectivity. On the other hand, the D-Trp^4 epimer (compound **2**) has its side chain conformation closer to the κ-model so that it is able to have higher κ-affinity (see Figure 18).

In addition, CJ-15,208's μ-affinity can be partially understood as well with the μ-model. Both its Trp^4 side chain and its macrocyclic skeleton possess conformational flexibilities that will allow the indole ring to adopt a μ-model-like orientation at the binding site.

Notably, different from all the other cyclic peptides discussed here, CJ-15,208 has no exoring Tyr^1 as the core structure. Hence, its 3D structure drawing as well as its SAR modeling cannot be based on the above backbone conformational models. Instead,

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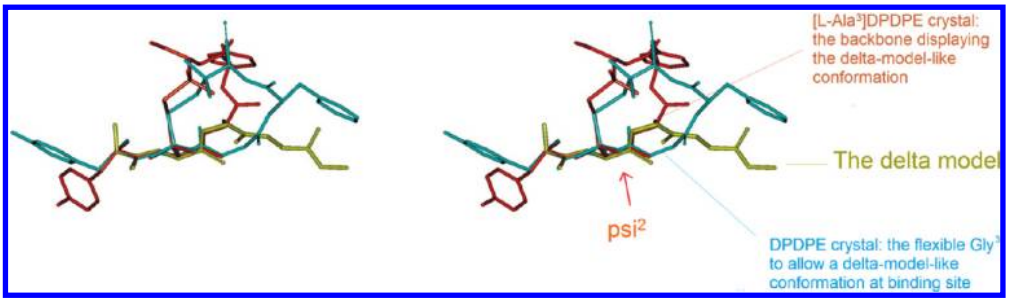


Figure 16. [L-Ala^3]DPDPE and DPDPE's crystal structures are compared with the δ-model.

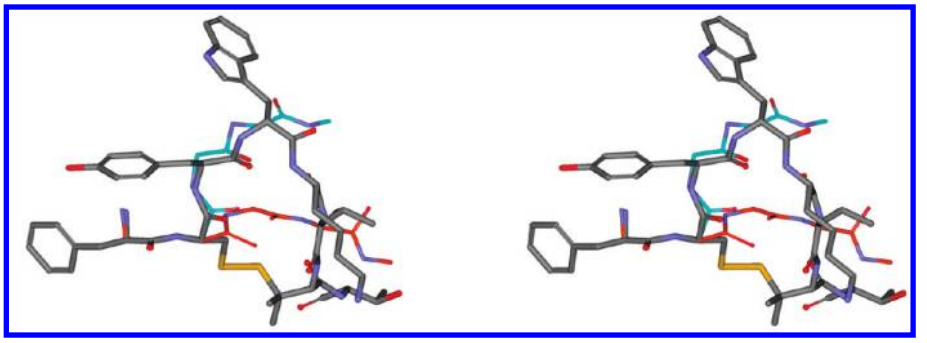
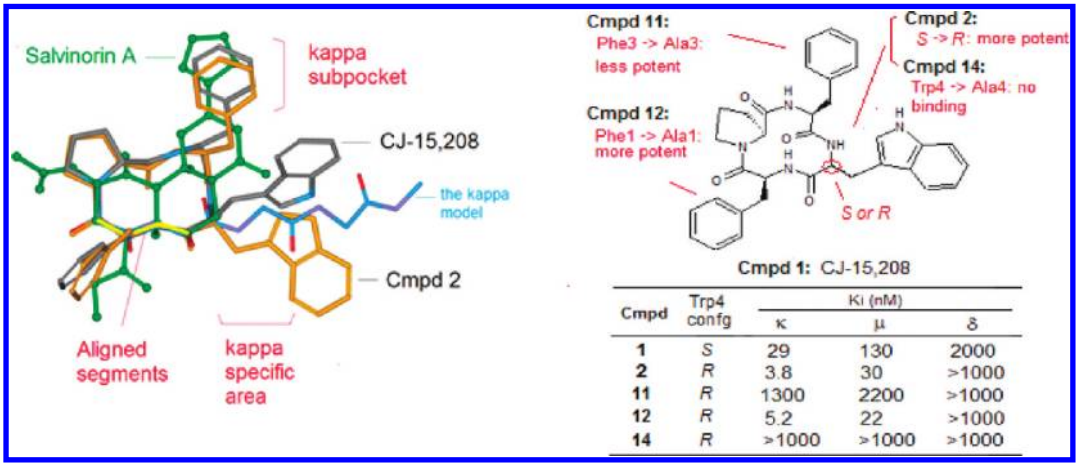


Figure 17. Stereoview of CTP alignment with μ- and δ-models (blue and orange, respectively).



Cmpd	Trp4 config	Ki (nM) κ	μ	δ
1	S	29	130	2000
2	R	3.8	30	>1000
11	R	1300	2200	>1000
12	R	5.2	22	>1000
14	R	>1000	>1000	>1000

Figure 18. Alignment of CJ-15,208 with salvinorin A.

the backbone conformation of CJ-15,208 has been derived from the crystal structure of c[Ala-Pro-Phe-D-Trp],[42] a closely related analog (as CJ-15,208's crystal structure data are not currently available).

Peptides with Conformationally Restricted Residues. Endomorphin-1 (Tyr-Pro-Trp-Phe) and endomorphin-2 (Tyr-Pro-Phe-Phe) are endogenous ligands and have high μ-selective binding affinity.[43] The Pro^2 residue in their sequences is the key structural basis for their μ-selectivity, as it limits the conformation of the second peptide unit of the sequence.

As shown in Figure 19, at the backbone alignment with morphine (in the "μ-alignment mode"), the Pro^2 residue directly places the second peptide unit at the μ-specific area so as to account for the μ-selectivity. On the other hand, if the Pro^2 is replaced with a D-Pro^2, the orientation of the residues will be greatly changed so that the μ-affinity will be lost, which has been examined with a study on the related EM-1 diastereoisomers.[44]

Moreover, many other Pro^2-possessing peptides, such as morphiceptin (Tyr-Pro-Phe-Pro-NH_2), PL-032 (Tyr-Pro-Phe-D-Pro-NH_2), and PL-017 (Tyr-Pro-NMePhe-D-Pro-NH_2),[45] all have μ-selectivity, which may be understood as well with a similar backbone alignment.

TIPP-NH_2 (Tyr-Tic-Phe-Phe-NH_2)[46] and D-TIPP-NH_2 (Tyr-D-Tic-Phe-Phe-NH_2)[47] are a pair of diastereomers, and the only difference in their structures is the stereochemistry of residue 2. However their binding affinities are quite different. TIPP-NH_2 is a δ-ligand (K_i, δ; μ =3.0 nM; 78.8 nM),[46,47] while D-TIPP-NH_2 is a μ-ligand (K_i, μ; δ =7.3 nM; 520 nM).

Similar to etonitazene, the fused ring system of Tic^2 of both the ligands is the key moiety due to its rigidity and bulkiness and

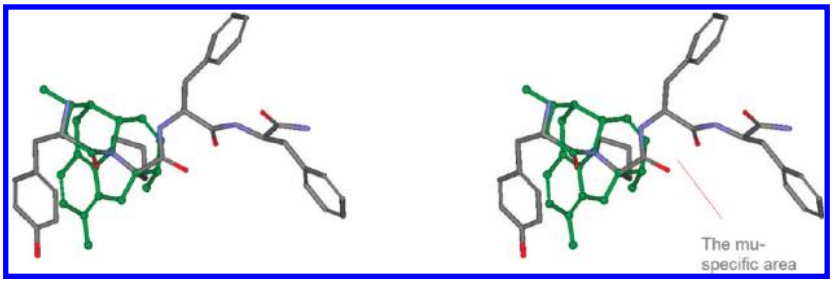


Figure 19. Modeling on endomorphins.

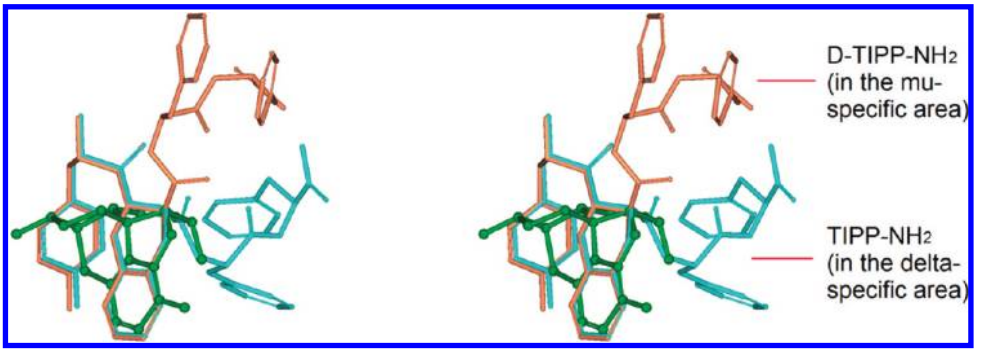


Figure 20. Modeling on TIPP-NH_2 vs D-TIPP-NH_2.

matches morphine's A- and E-rings. (In this match the Tyr[1] backbone is roughly aligned with morphine in the "δ-alignment mode"). Upon this alignment, the second and the third backbone units are placed directly at the μ-specific (D-TIPP-NH_2) or the δ-specific (TIPP-NH_2) area (Figure 20). These orientations nicely account for their different binding selectivity.

MATERIALS AND METHODS

All the opioid ligands and their related SARs data were found from the literature. The structure drawing and aligning were carried out with Accelrys DS Visualizer,[48] a molecular modeling software available from Accelrys Software Inc. However, the modeling study was essentially based on the visual examination of the 3D structures along with their SARs of various opioid ligands, a typical SAR analysis means in the conventional medicinal chemistry, rather than by any computational process.

SUMMARY AND CONCLUDING REMARKS

In this study we have applied a novel backbone alignment concept to assess the SARs of various opioid receptor ligands. This concept originated from a match of the backbone of the crystal structure of Leu-Enk to that of morphine, and the match suggested that morphine is a close mimic of the backbone of Leu-Enk. Based on this information three backbone alignment models have been developed corresponding to the three different types of opioid ligands, μ, δ, and κ, respectively.

For this kind of modeling the key step seems to be to identify a significant moiety (usually bulky and rigid) of the ligand that can be reasonably matched with part of morphine's scaffold. It appears that the major parts of morphine to be matched with are the C- and D-rings, and sometimes the A- and E-rings, but rarely the B-ring.

On the other hand, we did not apply any quantitative measure (such as root-mean-square deviation) to compare the fitting of alignment but only used visual inspection to see if the interested moieties were matched reasonably. As we believed in this study, it is more important to see how reasonable but not how precise the alignment is, so as to ensure the better correlation of the SARs of various ligands. This is because: (1) most of the 3D structures or conformations in this study were established not experimentally but randomly with only local energy minimization, and we would also partially adjust some of the conformations during the modeling process if necessary to facilitate the alignment. Thus it would make little sense to judge the quality of the fitting among the ligands by a quantitative standard; (2) sometimes the match took place between different cyclic systems with different sizes or ring-fusion patterns. Therefore significant deviations existed for those alignments, which would probably be overlooked when using a quantitative standard. Here qualitative visual inspection proved to be proper for this type of match. Although it was only a process of comparison and identification, the results were comprehensive and informative, where the most adequate alignment was decided by a well-balanced consideration among the various structural features, such as the ring type, the substitution pattern, the 3D conformation, and the stereochemistry of chiral centers, etc., but not by merely considering the best fitting among the individual bonds and fragments. Thus the best match would not mean the best fit, while sometimes the most reasonable match rendered only rough fitting of those apparent key segments (e.g., in the case of TIPP-NH_2-morphine alignment, see Figure 20).

In this study we have mostly focused on the backbone-related issues of opioid peptides but not on side chain effects. (We only discussed the side chain effects of a few unique examples, such as those of DPDPE, CJ-15,208, and EMs). Although the side chain effects are prominent to the SARs of opioid peptides, at this time

it is difficult for us to analyze them without the detailed information of the specific binding sites. To efficiently solve this issue, we may have to wait for the crystal structure data of opioid receptor−ligand complexes to be available.

Since using backbone alignment concept to assess the SARs of opioids appears to be novel, currently we are in the vigorous investigation of this approach. By disclosing these preliminary results here, we hope to draw the attentions of many other opioid researchers for a general validation and exploration of this method, which appears to be straight yet effective.

■ ASSOCIATED CONTENT

Supporting Information. In order to further validate the models, we have included a few latest examples of opioid ligands from the literature. Some of the structures appeared to be novel and interesting. The structures and the related modeling results are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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