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Development of a common 3D pharmacophore for δ-opioid recognition from peptides and non-peptides using a novel computer program

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Summary

A unified three-dimensional (3D) pharmacophore for recognition of the δ-opioid receptor by families of structurally diverse δ-opioid ligands, including peptides and non-peptides, has been determined. An additional structural feature required for δ-selectivity was also characterized using a subset of these ligands that are highly selective for the δ-opioid receptor. To obtain these pharmacophores, we have used a recently developed computer program that performs systematic and automated comparisons of molecules to determine whether any common 3D relationships exist among candidate recognition moieties in high-affinity analogs. All the low-energy conformations of each ligand are included in these comparisons. The program developed should be applicable in general to molecular superimposition problems in rational drug design and to develop both 3D recognition and activation pharmacophores for any receptor for which high- and low-affinity analogs and agonists and antagonists have been identified.

Introduction

Opioids are potent analgesics that can also induce severe side effects. They exert their diverse physiological effects through specific membrane-bound receptors. In an attempt to enhance opioid potency at specific activation end points while eliminating undesirable effects, many studies continue to search for receptor-specific peptide and non-peptide ligands. It has been well established that there are at least three major opioid receptors [1], namely, μ, δ, and κ, that differ in their affinity for various opioid ligands and in their distribution in the nervous system. Recently, these three receptors have been cloned and functionally characterized [2–5]. However, there is not yet a three-dimensional (3D) structure for any of them. In the absence of an accurate description of the local environment at the binding site, the elucidation of structural/conformational requirements for ligand binding to each receptor still relies mainly on the characterization of structural commonalties among compounds which are able to bind at the same region of the receptor. From these common structural features, 3D pharmacophores, defined as the 3D arrangement of functional groups essential for receptor recognition or biological activity, can be developed.

Previous attempts to develop δ-opioid pharmacophores, both experimentally [6–18] and theoretically [16–26], have been reported. Although many peptide and non-peptide ligands with high δ-opioid receptor affinity have been identified and several pharmacophores for binding have been proposed, none of these previous studies have addressed the question of whether common binding determinants exist in structurally diverse families of δ-opioid ligands, particularly among peptides as well as flexible and relatively rigid non-peptides. In this paper, we describe computational studies used to explore this possibility of common recognition determinants in a collection of structurally diverse ligands with high δ-opioid binding affinity. As shown in Fig. 1, the families of δ-opioid ligands considered include fused-ring opioids, fentanyl analogs, SNC80, cyclic enkephalin-like peptides, and linear peptide antagonists containing a conformationally constrained phenylalanine-like residue (Tic) [13,14]. The binding affinities of these compounds are given in

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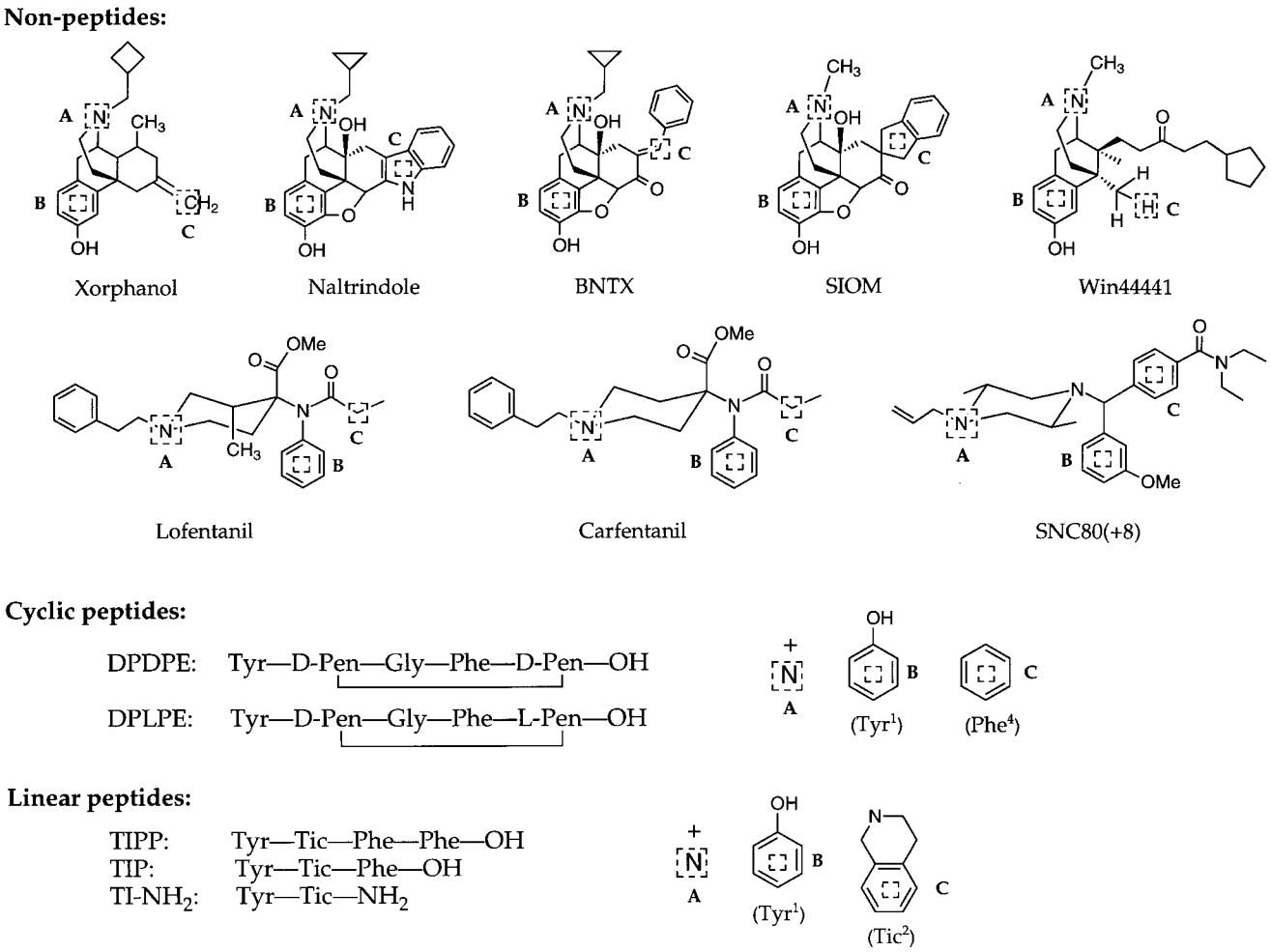

Fig. 1. Chemical structures of the δ-opioid ligands used for the study. The identified pharmacophoric atoms leading to the development of the δ-opioid recognition pharmacophore are labelled as A, B, and C in the figure.

Table 1. For the first time, linear and cyclic peptides as well as flexible and relatively rigid fused-ring non-peptide analogs were used together for comparison.

TABLE 1
BINDING AFFINITY AT THE δ-, μ-, AND κ-OPIOID RECEPTORS

Ligand	K_i (nm)		
	δ	μ	κ
Xorphanol	1.6	0.23	0.20
Naltrindole [10]	0.1	33.9	19.6
BNTX [30]	0.1	13.0	–
SIOM [6]	1.4	10.6	588
Win44441	1.1	0.05	0.09
Lofentanil [11]	0.2	0.02	0.6
Carfentanil [11]	3.3	0.02	43
SNC80(+8) [9]	1.0	2467	–
DPDPE [18]	13.1	2754	5000
DPLPE [18]	1.3	520	5000
TIPP [14]	1	1720	
TIP [14]	9	1280	
TI-NH_2 [15]	166	28 712	

The search for the form in which flexible molecules such as peptides bind to receptors is a challenging task because many low-energy conformations are accessible and they coexist in equilibrium. The complexity increases enormously when several diverse families of fairly flexible molecules are included, and the goal is to identify the common geometric arrangements of moieties that are determinants of receptor recognition or activation because all the low-energy conformations of each molecule should be included in the analysis. Previously, success in using the approach of systematic search to determine pharmacophores by a comparison of structures of a series of active compounds has been demonstrated [27,28]. In the current study, we have used a recently developed computer program to perform systematic and automated comparisons of the compounds shown in Fig. 1 by the use of their conformational profiles.

This program, DistComp, was developed to determine 3D pharmacophores for the recognition and activation of receptors. It provides a procedure for identifying common spatial arrangements of selected moieties in a given set of

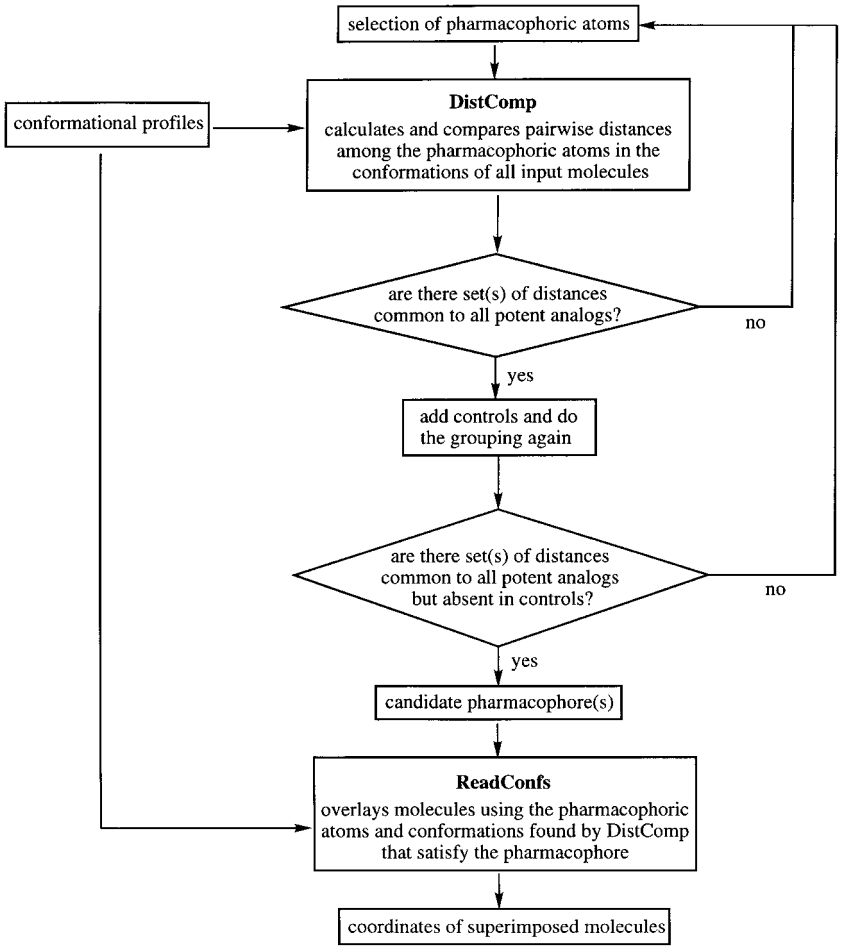

Fig. 2. Schematic description for DistComp and ReadConfs.

molecules using systematic analysis and automated comparisons of molecular conformations. No prior assumption of an active conformation is necessary. There is also no need for a rigid template. However, central to this procedure is the selection of sets of common functional moieties assumed to be important for recognition or activation. The validity of these candidate recognition or activation sites is then assessed by the program: for each hypothetical set of recognition or activation moieties selected, the program systematically determines whether any common 3D relationships among them exist in high affinity analogs that are absent in low affinity ligands for recognition, or exist in agonists but are absent in antagonists for activation. Each set of proposed chemical moieties that satisfies this requirement, together with the common spatial arrangements identified, comprise candidate 3D recognition or activation pharmacophores. Using this program we have identified a unique 3D recognition pharmacophore of the δ-opioid receptor common to both peptides and non-peptides. In addition, a subset of these ligands that are selective for the δ-opioid receptor was further examined for structural features required for δ-selectivity.

The rationale underlying the strategy is that the function of ligands depends mainly on the spatial positioning of several functional groups with respect to each other, i.e. the 3D arrangement of chemical functionalities such as charged atoms, hydrophobic groups, aromatic rings, hydrogen-bond donors and acceptors. The superimposition obtained is not based on the maximum steric overlap but on a few recognition moieties. The interaction of these moieties with complementary residues in the binding

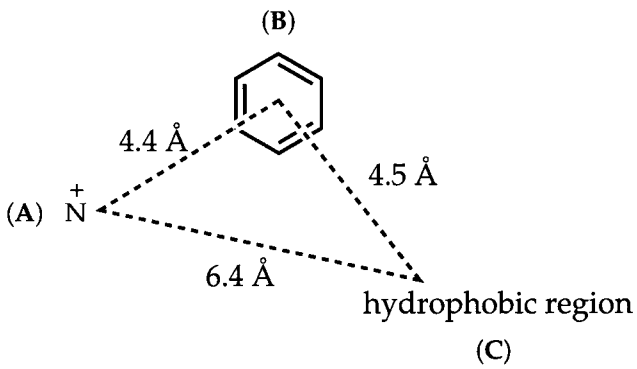

Fig. 3. Proposed 3D pharmacophore for δ-opioid recognition.

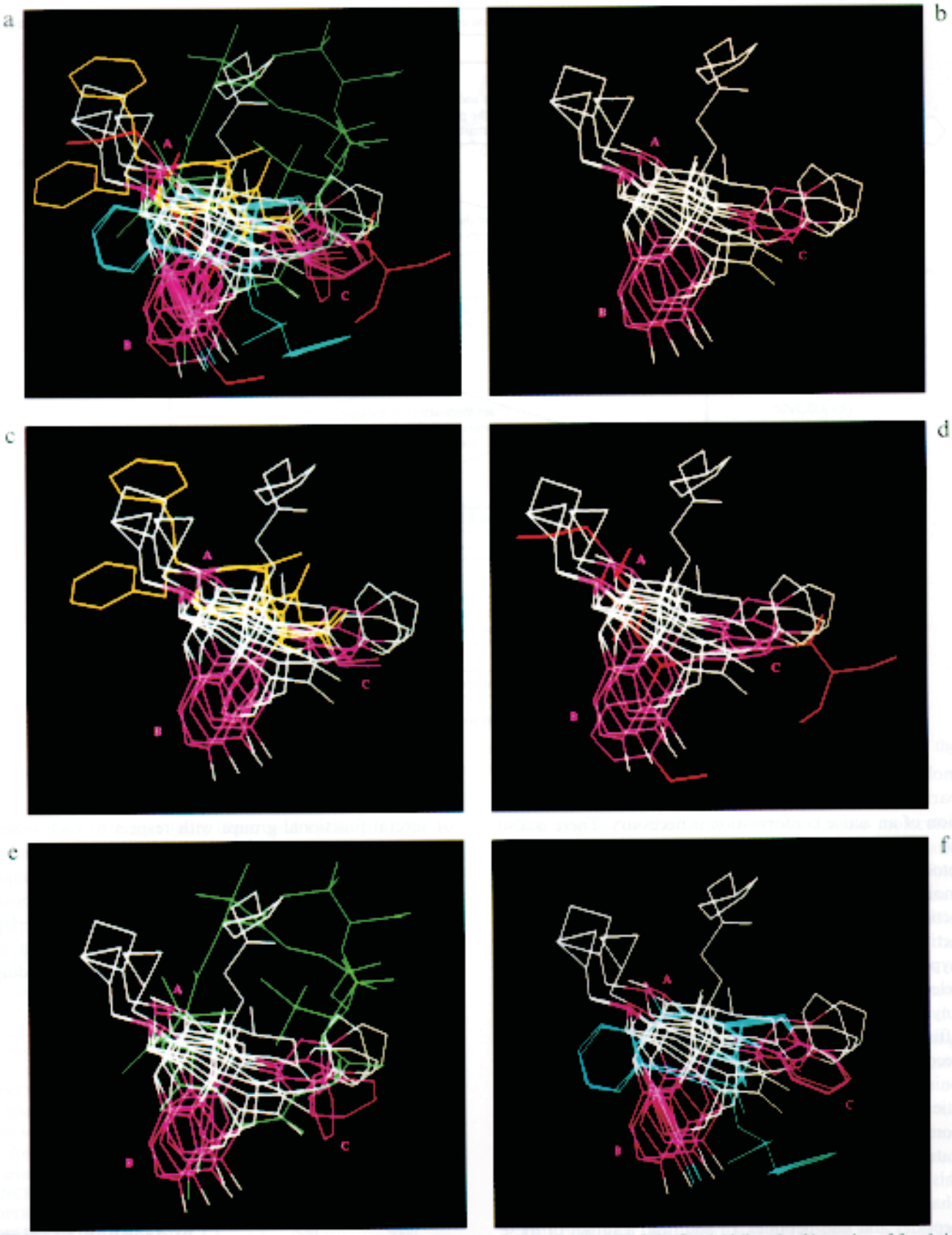

Fig. 4. Overlays using the proposed pharmacophoric atoms shown in Fig. 1. (a) Overlay of all the δ-opioid ligands; (b) overlay of fused-ring compounds only; (c) overlay of fused-ring compounds with fentanyl analogs; (d) overlay of fused-ring compounds with SNC80(+8); (e) overlay of fused-ring compounds with DPDPE and DPLPE; (f) overlay of fused-ring compounds with TIPP, TIP, and TI-NH_2. Magenta: recognition moieties; white: fused-ring compounds; yellow: fentanyl analogs; red: SNC80(+8); green: DPDPE and DPLPE; cyan: TIPP, TIP, and TI-NH_2.

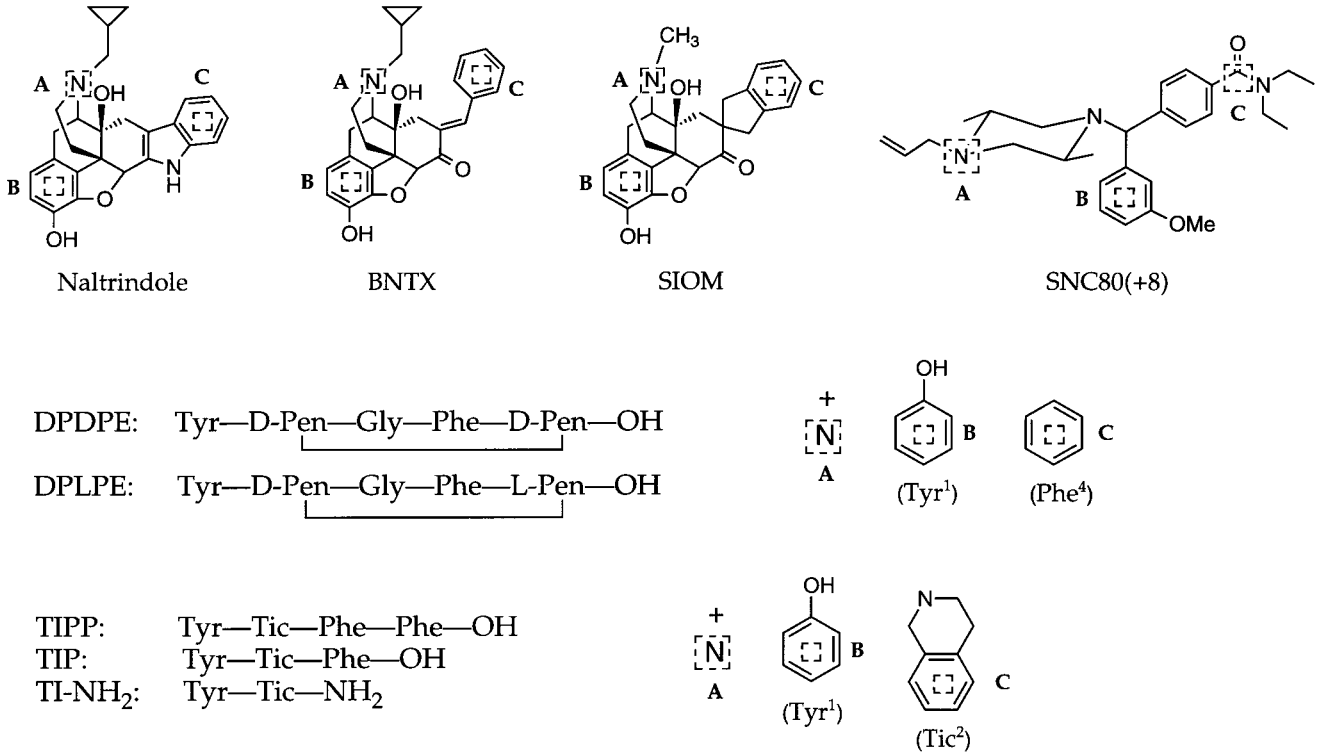

Fig. 5. Identified pharmacophoric atoms leading to the development of the δ-selective opioid recognition pharmacophore.

site of the receptor still allows a flexibility of binding of each ligand and does not require or assume that the binding sites of these diverse ligands are identical but only that they are in the same region of the receptor, i.e. that they are accommodated in a contiguous, partially overlapping, binding site region.

Methods

Conformational profiles

The conformational profiles of the compounds included in the present study were previously calculated in our laboratory [24–26], using a strategy described in detail elsewhere [29]. Briefly, this procedure consists of repeated cycles of high (900 K) and low (300 K) temperature molecular dynamics (MD) simulations combined with the energy minimization of structures obtained during the MD trajectories. These cycles are continued until no new low-energy conformers are generated. All these calcula-

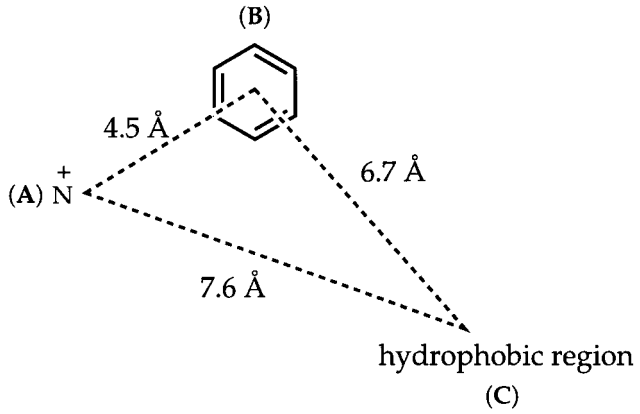

Fig. 6. Proposed 3D pharmacophore for δ-selective opioid recognition.

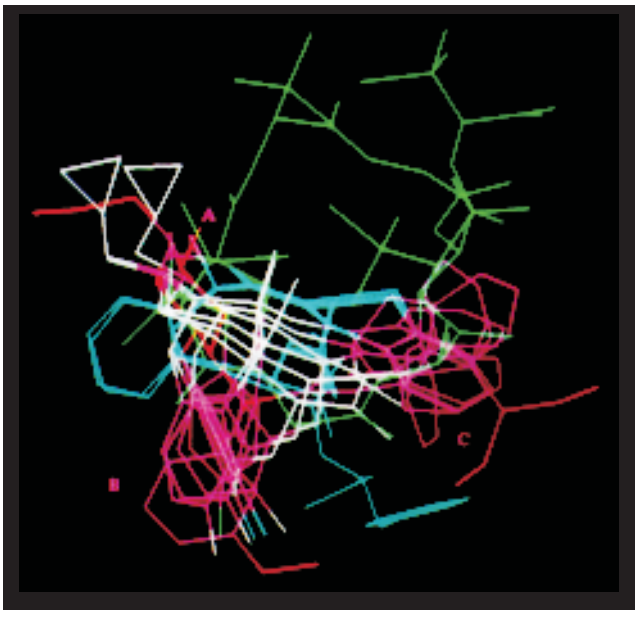

Fig. 7. Overlay of all the δ-selective opioid ligands using the proposed pharmacophoric atoms shown in Fig. 5. Magenta: δ-selective recognition moieties; white: naltrindole, BNTX, and SIOM; red: SNC80(+8); green: DPDPE and DPLPE; cyan: TIPP, TIP, and TI-NH_2.

tions were performed using Quanta/CHARMm. Using this procedure, conformational libraries were generated for all the compounds included in this study (Fig. 1) [24–26]. With the exception of the more rigid fused-ring analogs, each compound had many low-energy conformers within 6 kcal/mol from the lowest energy found, all of which were used for the analysis.

Method for the determination of 3D pharmacophores

The program DistComp was developed in order to identify 3D pharmacophores in terms of common geometric arrangements of recognition or activation moieties in structurally diverse ligands using libraries of their accessible conformations. The program requires as input the conformations of each ligand stored in a csr format of QUANTA/CHARMm as well as the selection of sets of candidate chemical moieties common to each ligand as possibly important sites for recognition or activation.

The selection of candidate recognition or activation moieties, represented as pharmacophoric atoms to be used in DistComp, can be based on the structure–activity relationships (SAR) studies, if any, reported in the literature or on the high affinity or activity ligands with the fewest possible candidate recognition or activation moieties. A pharmacophoric atom could be an actual atom, a centroid of a group of atoms, or an extension point, defined as a point at which a complementary receptor atom could be located. An example of an extension point is the position of a proton donor in the receptor, expressed as an extension of a carbonyl group acting as the proton acceptor in the ligand for hydrogen-bond formation.

Each set of pharmacophoric atoms selected represents a hypothesized mode of the ligands interacting with the receptor for recognition. Its validity is tested through the analysis using DistComp, as shown in Fig. 2. Specifically, within DistComp, for each set of proposed recognition or activation moieties, the distances among them in all low-energy conformers of all molecules are calculated. Using these distances, the conformers of each molecule are clustered into families based upon a distance tolerance, usually ±1.0 to ±1.5 Å. Pairwise distance comparisons using the same tolerance are then performed to determine whether similar 3D relationships of the candidate moieties exist in all high affinity analogs that are absent in the low affinity analogs for recognition, or exist in agonists but are absent in antagonists for activation. Each set of candidate moieties selected that passed this test, together with the common geometric arrangements found for them, then constitute candidate 3D recognition or activation pharmacophores. This process is continued for different proposed sets of recognition or activation moieties until as many candidate pharmacophores as possible are identified. The greater the structural diversity of ligands included in this analysis, the fewer the candidate pharmacophores obtained and the more reliable and useful the results.

For the purpose of graphics display, a supplemental program ReadConfs has also been developed to superimpose the identified recognition or activation moieties using one conformer for each compound that has the lowest energy among the conformations satisfying the requirements of the 3D pharmacophore. ReadConfs requires as input the csr files containing the conformations of each ligand as well as the output of DistComp. The results of ReadConfs are the coordinate files for molecules superimposed using the pharmacophoric atoms and the calculated rms deviations. The flow chart shown in Fig. 2 provides a schematic description for DistComp and ReadConfs.

Results and Discussion

For the present work, the selection of pharmacophoric atoms was made using xorphanol as a guide because it has high affinity at the δ-receptor, is conformationally rigid, and has the fewest candidate recognition moieties, namely two polar groups, an aromatic ring, and two hydrophobic regions. Using DistComp, all combinations of these five possible recognition moieties were investigated and only one led to a common set of distances present in all high affinity ligands, including the peptides and non-peptides used in this study. This set of pharmacophoric components consists of a protonable amine (A), an aromatic ring (B), and a hydrophobic region (C), which are shown in Fig. 1 for all the compounds. The identification of these moieties as important for the recognition of the δ-opioid receptor is consistent with the known experimental SAR for both cyclic enkephalins [12] and linear peptide antagonists [13,14], which suggests the importance of an N-tyramine region (N-terminal and tyrosine ring) and the phenylalanine residue (hydrophobic region) for δ-opioid receptor recognition.

As a result, using the set of pharmacophoric atoms shown in Fig. 1, a unique 3D pharmacophore (Fig. 3) for δ-opioid recognition has been found by the use of our novel procedure of systematic analysis and automated comparison of molecular conformations. This pharmacophore is in general agreement, but is not identical to those obtained previously in our laboratory by a separate consideration of non-peptides [26], cyclic peptides [24], and linear peptides [25].

The overlays of the ligands by superimposing the pharmacophoric atoms identified (Fig. 1) are shown in Figs. 4a–f. The fused-ring compounds (white) are shown in every figure to provide cross reference. It is seen from these figures that a good spatial overlap of the proposed recognition moieties (magenta) has been obtained for all the molecules. In fact, all calculated rms deviations are less than 1.1 Å. It is also seen that the superimposition of these recognition moieties does not correspond to maximum steric overlap of the ligands. The fused-ring com-

pounds (white), fentanyl analogs (yellow), and SNC80 (red) occupy only the central region of the binding site. The cyclic DPDPE and DPLPE (green) and the linear peptides (cyan) occupy additional regions on the top and bottom of this central region. Therefore, the binding pocket in the δ-opioid receptor should be able to accommodate larger non-peptide as well as peptide analogs. This is indeed supported by the newly discovered [7] *N*-benzylnaltrindoles as δ-opioid receptor-selective antagonists.

We have also investigated the structural features required for δ-selectivity using only those ligands highly selective for the δ-opioid receptor. The selectivity of the compounds can be determined from comparisons of their binding affinities at different receptors. It is seen from Table 1 that compounds that bind selectively to the δ-receptor include naltrindole, BNTX, SIOM, SNC80(+8), and all the cyclic and linear peptide analogs. The difference between high affinity selective and nonselective δ ligands is that, while both satisfy the requirements for δ-receptor recognition, the selective analogs have additional features that are unfavorable for μ and κ receptor recognition. As shown in Fig. 1, all three δ-selective fused-ring compounds (naltrindole, BNTX, and SIOM) have an extended hydrophobic region beyond moiety C, which is absent in the nonselective ligands. This has guided us to use this extended hydrophobic region as an alternative third moiety C for δ-selective recognition, in searching for common geometric arrangements of the three components already determined as recognition requirements. The set of pharmacophoric atoms shown in Fig. 5 was then identified using DistComp. The resultant 3D pharmacophore is displayed in Fig. 6 for δ-selective binding. A comparison of Figs. 6 and 3 clearly shows that the extended hydrophobic region C is the origin of δ-selectivity, namely the longer distances between it and the other two recognition moieties that can be accommodated at δ but are apparently unfavorable for μ and κ recognition. The superimposition of the extended hydrophobic regions (C, magenta) in all the δ-selective ligands can be clearly seen in Fig. 7. The calculated rms deviations using the pharmacophoric atoms shown in Fig. 5 are all less than 0.9 Å. Thus, the pharmacophore shown in Fig. 3 represents the requirements for *nonselective* δ-opioid binding, while that in Fig. 6 represents the requirements for δ-*selective* binding. The presence of an extended hydrophobic region C shown in Fig. 6 is proposed as the determinant for δ-selectivity.

Among the previous pharmacophore models proposed for δ-opioid peptide agonists and antagonists [22,23], common identified key structural elements include a positively charged N-terminal NH_3^+ group, the aromatic ring and phenolic hydroxyl of tyrosine, and the aromatic ring of the phenylalanine or Tic residue. The distances between the two aromatic ring centers in these models are 5.7 Å [22] and 6.5 Å [23] individually. Our models proposed in this study differ from these previous ones in that (i) 3D pharmacophores for δ-receptor recognition with and without δ-selectivity are defined separately; (ii) the tyrosine residue is replaced by an aromatic ring; (iii) the phenylalanine or Tic residue is replaced by a hydrophobic region which does not need to be an aromatic ring; and (iv) since only peptides which are δ-selective were used in the previous studies, the δ-selective pharmacophore developed here is more similar to the previous ones [22,23] in that the distance between the aromatic ring and the hydrophobic region is 6.7 Å. By using a more diverse set of ligands including high affinity nonselective δ ligands, we have determined that this distance can be as short as 4.5 Å. In addition, the hydrophobic region is somehow forgiving, tolerable to less hydrophobic components such as that in Win44441 (Fig. 1) and SNC80(+8) (Fig. 5).

Conclusions

We have developed a computer program for the systematic analysis and automated comparison of molecular conformations for the development of a 3D pharmacophore for receptor recognition or activation. Using this program we have characterized the molecular determinants for δ-opioid recognition, represented as a 3D pharmacophore, from families of structurally diverse δ-opioid ligands. The additional structural requirement for δ-selectivity was also determined using the same method but from only the highly δ-selective ligands. The 3D recognition pharmacophores developed here, shown in Figs. 3 and 6, provide a reliable basis for the search of 3D databases for the discovery of novel compounds that could be high affinity and selective or nonselective δ-opioid ligands.

The program developed can be used to characterize both recognition and activation 3D pharmacophores for any receptor for which high and low affinity analogs and agonists and antagonists have been identified. It is also applicable to any general molecular superimposition problem, especially when the molecules are fairly flexible, in rational drug design.

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