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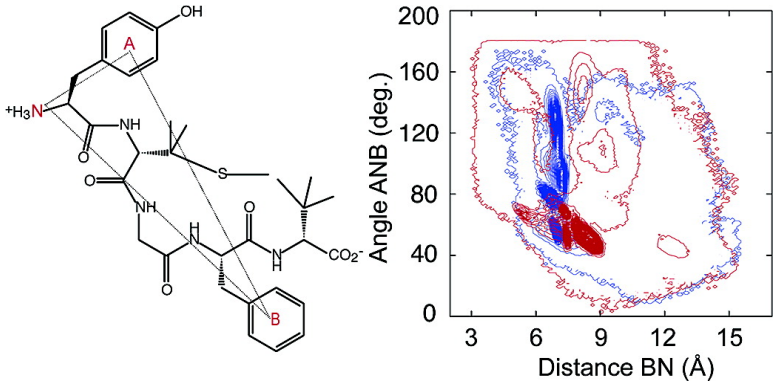

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Conformationally Sampled Pharmacophore for Peptidic δ Opioid Ligands

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Opioids represent the frontline treatment for acute pain, despite their side effects, motivating efforts toward developing novel opioid analgesics. To facilitate these efforts, a novel modeling approach, the conformationally sampled pharmacophore (CSP), has been developed that increases the probability of including the receptor bound form in the model. This method, originally used for developing a nonpeptidic δ opioid efficacy pharmacophore, is extended to peptidic ligands using replica exchange molecular dynamics simulation for conformational sampling. The developed 2D CSP indicates that the spatial relationship of the basic nitrogen and the hydrophobic moiety in the δ opioid ligands differentiates activity. In addition, results indicate that both peptidic and nonpeptidic ligands have the same binding mode with the receptor. Thus, the CSP approach distinguishes both peptidic and nonpeptidic δ opioid agonists and antagonists and is anticipated to be of general utility for the development of pharmacophores for species with multiple rotatable bonds.

Introduction

The δ opioid receptor system is involved in a number of biological processes such that agents acting on this system have significant therapeutic potential, including for the treatment of cocaine, amphetamine, and alcohol abuse and as immunosuppressants, among others.[1] δ Opioid ligands also have modulatory effects on the μ receptors and, hence, have important applications especially in the development of analgesics with reduced tolerance and dependence potential. Thus, significant efforts are ongoing to develop novel δ opioid ligands via rational drug discovery approaches.[2]

Computer-aided drug design (CADD) approaches allow for a rigorous understanding of the structural basis of the biological effects of molecules and can involve both ligand-based and target-based methods.[3−5] With the lack of an experimental 3D structure of the seven-transmembrane G-protein-coupled δ opioid receptor,[6,7] 3D models of the receptor have been developed for use in target-based CADD studies.[8−14] Alternatively, ligand-based approaches involving pharmacophore development[15−17] based on the structural features of existing ligands and QSAR analysis[18−22] have been undertaken. In particular, pharmacophore models that describe the spatial relationship of functionally important groups in δ opioid ligands are useful in the identification and development of new lead compounds; the development of such models has been undertaken by us[17] and others.[15,16,23−28] The methodology used for pharmacophore identification has traditionally involved the selection of low-energy conformers of different molecules in a data set, followed by the determination of geometric commonalities among them with respect to the atoms or groups thought to be essential for the interaction of the molecules with the receptor.[28] However, these approaches are limited in a number of ways by the inherent dynamic nature of molecules and the nature of their interaction with their receptors. Molecules at room temperature possess kinetic energy, thereby sampling a variety of conformations other than just the lowest energy conformation(s).[29] In addition, the lowest energy conformation obtained depends on the particular software and force field used in a given study.[30] But most importantly, the favorable interaction with the receptor may enable a molecule to overcome the conformational strain associated with assuming a higher energy conformation such that the bound conformation of a molecule need not be the lowest or among the low-energy conformers of the unbound molecule.[31]

To overcome these limitations, an approach based on the use of MD simulations for obtaining distributions of geometric information of molecules to be used in pharmacophore development, referred to as conformationally sampled pharmacophore or CSP, was developed. This approach was initially applied to a series of known nonpeptidic δ opioid agonists and antagonists,[17] from which a 2D CSP for prediction of δ agonists versus antagonists was developed. However, the natural δ opioid ligands are peptides, viz. the enkephalins,[32,33] and any opioid pharmacophore of general utility must

Table 1. Peptidic δ Ligands Used in the Development of the CSP[a]

compound	sequence
	Agonists
1, DADLE	**Tyr**-D-Ala-Gly-**Phe**-D-Leu
2, deltorphin	**Tyr**-D-Met-**Phe**-His-Leu-Met-AspNH_2
3, deltorphin I	**Tyr**-D-Ala-**Phe**-Asp-Val-Val-GlyNH_2
4, deltorphin II	**Tyr**-D-Ala-**Phe**-Glu-Val-Val-GlyNH_2
5, DPDPE	**Tyr**-c[D-Pen-Gly-**Phe**-D-Pen]
6, Leu-enkephalin	**Tyr**-Gly-Gly-**Phe**-Leu
7, Met-enkephalin	**Tyr**-Gly-Gly-**Phe**-Met
	Antagonists
8, Dmt-Tic	**Dmt**-**Tic**-**OH**
9, ICI 174,864	$(H_2C{=}CCH_2)_2$-**Tyr**-Aib-Aib-**Phe**-Leu
10, TIPP	**Tyr**-**Tic**-Phe-Phe

[a] The pharmacophore points are the protonated nitrogens (N), the centroid of the phenolic group (A), and the centroid of the hydrophobic group (B) (see Figure 1). The A and B pharmacophoric moieties are shown in bold.

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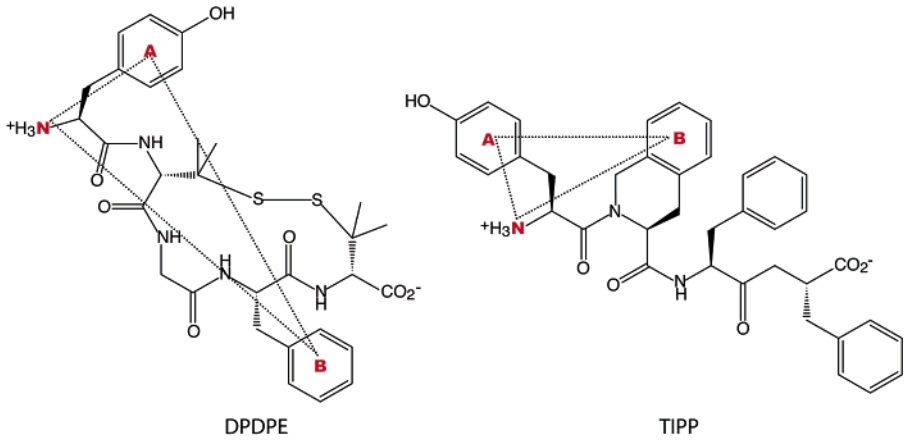

Figure 1. Peptidic δ opioid agonist DPDPE and antagonist TIPP used in CSP development. The pharmacophore points (in red) are the protonated nitrogen (N), the centroid of the phenolic group (A), and the centroid of the hydrophobic group (B).

Table 2. Percent of New Conformation Found with Respect to All Conformations Found after 10 ns for the Peptidic δ Opioid Ligands

	DADLE		deltorphin		deltorphin I		deltorphin II		DPDPE	
Parameter[a]	15 ns	20 ns	15 ns	20 ns	15 ns	20 ns	15 ns	20 ns	15 ns	20 ns
AB-ANB	8	12	3	5	0	3	22	28	11	16
AB−NAB	6	13	6	9	0	3	25	28	11	16
AB−NBA	9	11	2	3	0	6	22	24	5	8
BN-ANB	6	12	5	7	1	2	19	23	11	16
BN−NAB	6	12	6	9	0	1	14	16	9	14
BN−NBA	5	7	8	8	1	5	23	31	16	19
NA-ANB	1	3	0	3	2	6	1	3	0	1
NA-NAB	1	5	3	7	3	7	2	4	1	4
NA-NBA	2	4	8	9	2	12	13	16	0	4

	L-enkephalin		M-enkephalin		Dmt-Tic		ICI 174,864		TIPP	
Parameter	15 ns	20 ns	15 ns	20 ns	15 ns	20 ns	15 ns	20 ns	15 ns	20 ns
AB-ANB	0	1	0	3	2	3	9	22	3	5
AB−NAB	0	1	0	3	1	1	6	13	1	3
AB−NBA	0	1	1	3	2	4	5	11	2	3
BN-ANB	0	1	2	4	0	0	10	18	3	4
BN−NAB	1	2	2	4	0	0	7	14	2	3
BN−NBA	0	3	1	2	0	0	12	28	1	3
NA-ANB	2	3	2	3	3	7	6	12	3	4
NA-NAB	6	6	2	3	5	10	5	8	3	3
NA-NBA	0	10	2	2	3	9	4	10	3	3

[a] Distance angle combinations (e.g., AB−ANB) used as pharmacophore parameters are listed in the first column where AB, BN, and NA represent the distances and ANB, NAB, NBA represent the angles involving the pharmacophore points shown in Figure 1.

include these entities. Accordingly, in the present study we extend the CSP methodology to peptides that target the δ opioid receptor. These efforts include extensive conformational sampling of the peptides via replica exchange MD simulations,[34] from which a pharmacophore allowing for differentiation between δ opioid agonists and antagonists is developed and presented. While it has been suggested that the binding modes of peptidic and nonpeptidic δ opioid ligands may be different,[35−37] comparison of the developed CSP for peptidic ligands to the pharmacophore based on nonpeptidic δ opioids is found to be consistent with the same binding mode.

Computational Methods

Peptidic δ opioid ligands, seven agonists and three antagonists (Table 1), were model built using SYBYL, version 6.2,[38] and energy-minimized to a gradient of 0.05 kcal mol^{-1} Å^{-1}. The molecules were read into CHARMM[39,40] for treatment using the Merck molecular force field (MMFF),[41,42] and each molecule was then subjected to 200 steps of adopted basis Newton−Raphson minimization prior to MD simulations. Replica exchange MD simulations were carried out for 10 ns with four replicas between 300 and 400 K with exponential scaling. Exchanges were attempted every 100 steps. This exchange rate and the relatively small temperature difference between replicas allow faster equilibration yielding a 40% or greater acceptance rate for the exchanges that was considered satisfactory for the purpose of this study. Langevin dynamics[43] were performed with an integration time step of 0.002 ps, inclusion of all nonbond interactions (i.e., no atom truncation), and SHAKE of all covalent bonds involving hydrogens.[44] Aqueous solvation, for all energy minimization and dynamics calculations, was treated via the generalized Born continuum solvent model (GBSW) as implemented in CHARMM,[45,46] and the physiologically relevant protonated species of the ligands were used in the study.

GBSW was selected because of both efficiency and accuracy considerations. The GBMV[47,48] method implemented in CHARMM improves over traditional GB methods that use the Coulomb field approximation,

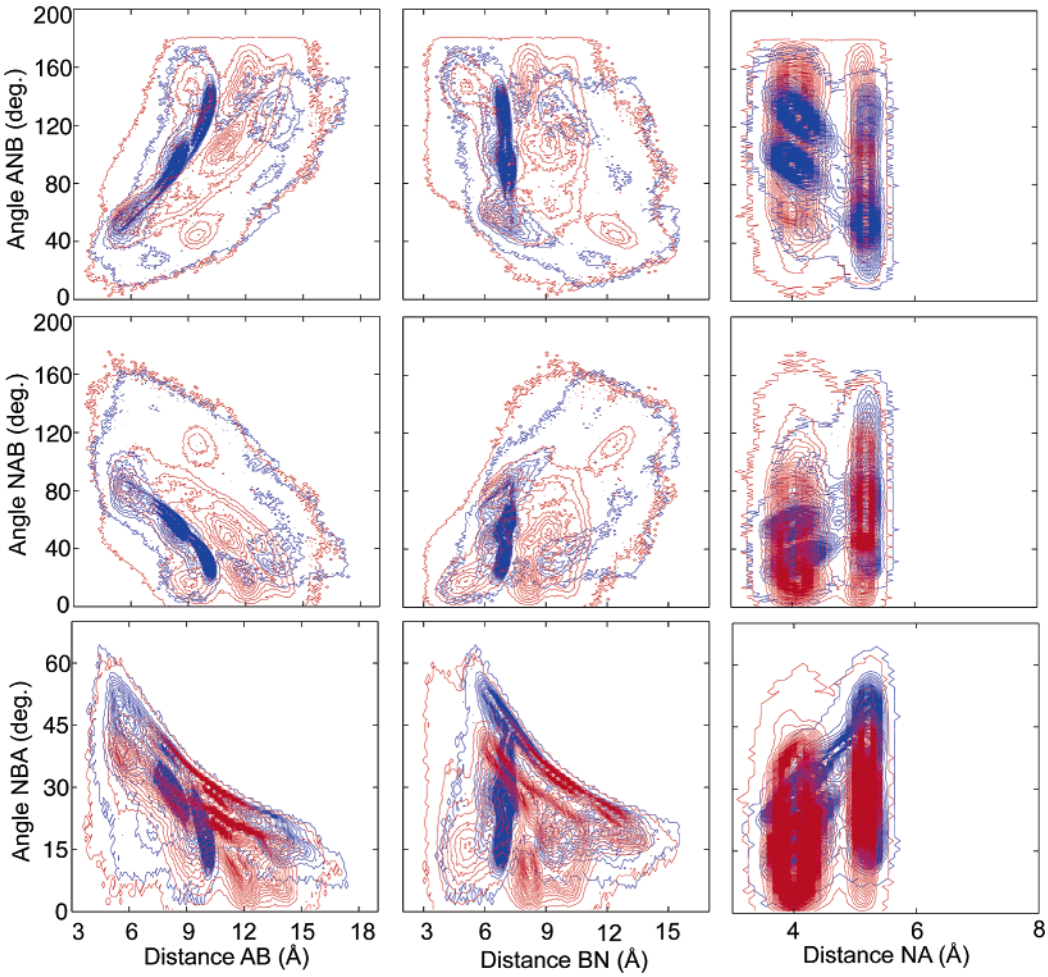

Figure 2. 2D probability distribution of distance angle combinations for peptidic δ opioid ligands. Agonist regions are in red, and antagonist regions are in blue. AB, BN, and NA represent the distances, and ANB, NAB, NBA represent the angles involving the pharmacophore points shown in Figure 1.

better reproducing results from Poisson−Boltzmann calculations; however, this method is time-consuming. The GBSW method used presently utilizes the GBMV formulation with a smoothing function to treat the dielectric boundary and is computationally less expensive than the GBMV method and was therefore used as the implicit solvent model for simulations. In addition, Langevin dynamics was employed because it includes the physical effects of solvent molecules not explicitly present in the simulations via the use of a frictional force to represent the drag due to solvent and a random force that approximates the effects of solute−solvent collisions due to thermal motion.

The angles and distances between the pharmacophoric points shown in Figure 1 and Table 1 were measured for all conformations obtained from the replica exchange simulations at all four different temperatures. The 2D analyses of the pharmacophoric parameters were performed using all combinations of angles with distances. Bin sizes for the 2D distributions were 0.1 Å and 1° for the distances and angles, respectively, with the probability contours generated in increments of 0.000 05 units and the lowest contour at 2×10^{-7}.

Results and Discussions

Conformational Sampling. The CSP approach to pharmacophore development is based on the inclusion of all accessible conformations of the molecules under study. In the case of peptides, owing to the large number of possible conformations, the application of MD simulations alone to access all possible conformations is challenging.[49] To overcome this limitation, replica exchange MD simulations were applied.[34] In the replica exchange approach, multiple MD simulations are run simultaneously, with the only difference being the temperatures of the simulations. Then, at selected time increments, the structures from the individual simulations are exchanged, with the acceptance of the exchanged conformations being based on a Metropolis criterion.[50] These exchanges lead to the increased sampling of conformational space, as required for the CSP approach. The successful application of this technique to study Met-enkephalin has been described previously.[51]

The CSP method, in principle, includes all possible conformations of a ligand in pharmacophore determination, and for the peptides, conformers from all four replicas were included for analyses to maximize the sampling of conformational space. The use of structures from the higher temperatures was deemed acceptable because we have previously demonstrated with nonpeptidic ligands that the use of high-temperature simulations at 600 K leads to greater sampling while still retaining discrimination between compounds of two different classes (i.e., δ opioid agonists and antago-

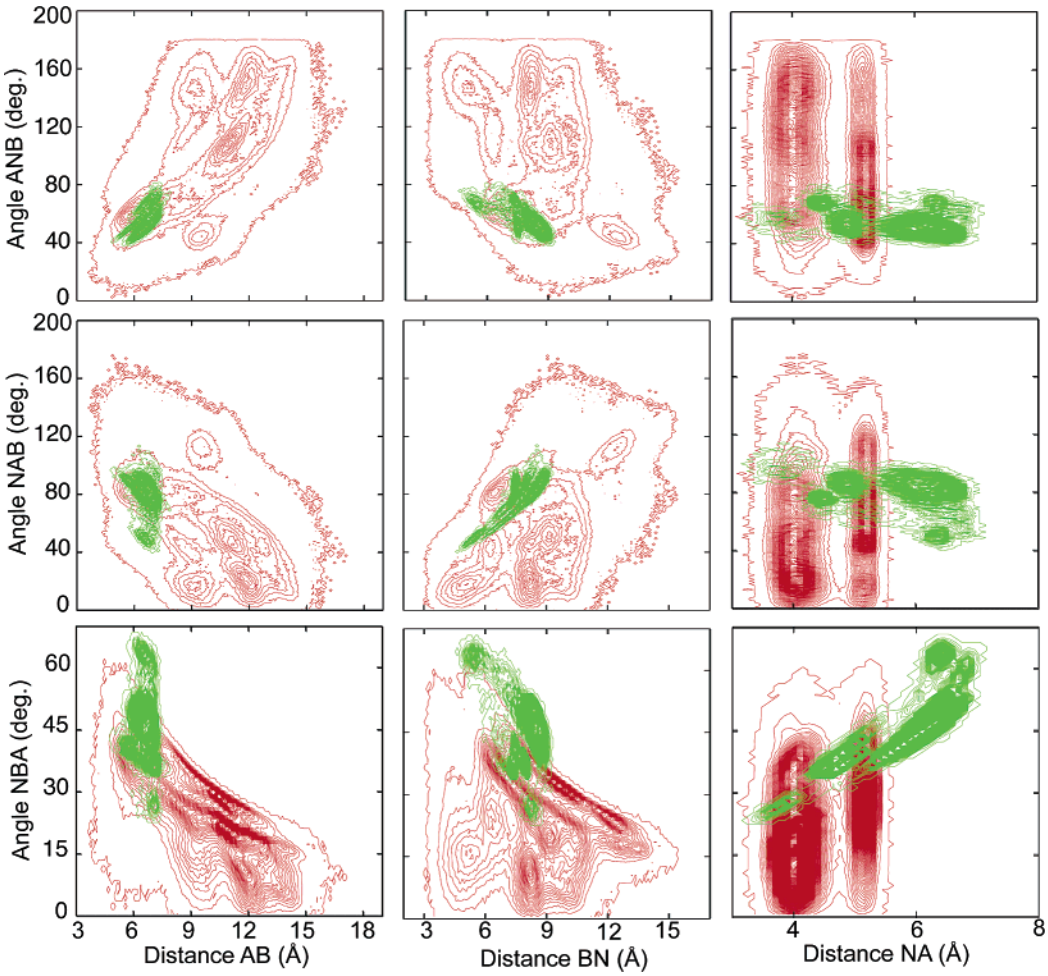

Figure 3. 2D probability distribution of distance angle combinations for peptidic and nonpeptidic δ opioid agonists. Peptidic agonist regions are in red, and nonpeptidic agonist regions are in green.

nists).[17] The distances and angles defining the pharmacophore features were obtained from each frame stored during the replica exchange MD simulations to yield a total of 200 000 conformers for each ligand, producing a range of values for each pharmacophore feature representing the conformational space sampled by the different ligands.

The extent of sampling of conformational space for the peptides was evaluated by extending all simulations to 20 ns. To test if sampling of new conformers was occurring as the simulations were extended, each conformer was defined as one value of a pharmacophore parameter, i.e., the combination of a distance and angle. Then, as the durations of simulations were increased, new conformations not previously sampled were identified. Table 2 shows the percentage of new conformers identified at 15 and 20 ns in comparison to the conformers identified in the first 10 ns of simulations for all the peptidic ligands. While ligands such as the enkephalins (**6** and **7**) and compound **10** show relatively low percentages of new conformers, others, such as the deltorphins (**2**−**4**) and compound **9**, indicate sampling of additional regions of conformational space. This indicates that longer simulations are required for complete sampling of conformational space and that the use of replica exchange simulations allows effective sampling of conformations, with new conformers being identified as the simulation length increases. While comprehensive sampling of conformational space is not achieved, analysis of the new regions of conformational space being sampled at >10 ns indicates them to not significantly extend beyond the periphery of the regions already sampled. Comparisons of the probability distributions from the full 20 ns simulations (Figure S1 of the Supporting Information) with the regions sampled in the 10 ns simulations (see below) support this conclusion. Importantly, the increased sampling does not alter the conclusions being made in the present work, such that, although full sampling of conformational space has not been achieved, the extent of sampling may be deemed adequate for the present study.

Pharmacophore Development. For the development of the pharmacophore three groups were chosen, the α amino nitrogen (N), the centroid of the phenolic aromatic ring (A), and the centroid of the hydrophobic region (B) (Figure 1 and Table 1). These are the same as those used in our previous study of the nonpeptidic ligands[17] and were originally selected on the basis of earlier work.[15,16] As may be seen, in the case of some of the peptidic ligands (e.g., compound **10**), multiple aromatic groups are present that may represent the hydrophobic moiety B. The choice of the aromatic group in this case was made on the basis of previous studies where the Tic moiety was found to satisfy required pharmacophore conditions for overlap of hydrophobic groups with other δ opioid ligands[24,52,53] in addition to

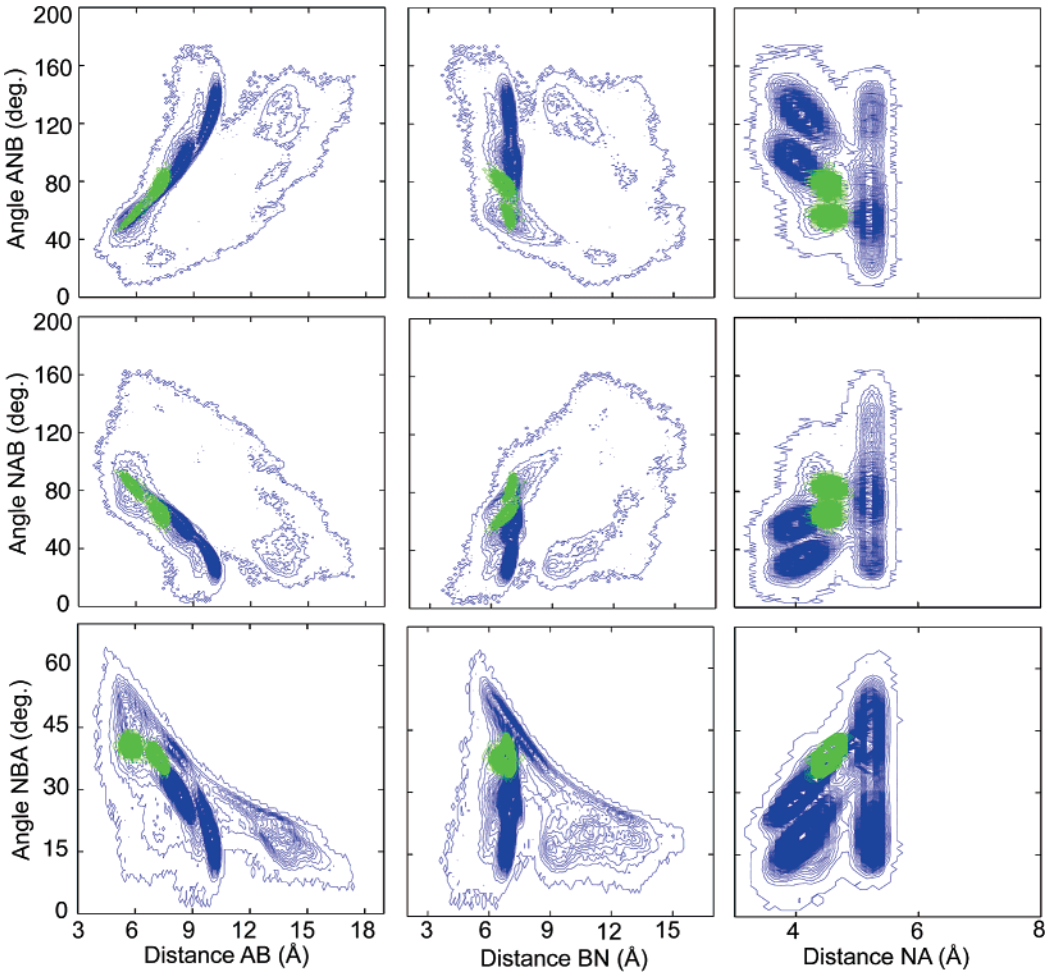

Figure 4. 2D probability distribution of distance angle combinations for peptidic and nonpeptidic δ opioid antagonists. Peptidic antagonist regions are in blue, and nonpeptidic antagonist regions are in green.

the observation that compounds such as Tyr-Tic-NH_2 and Tyr-Tic-Ala-OH also retain antagonist activity.[24]

In the previous application of the CSP method to the nonpeptidic ligands,[17] as well as in another study on peptides,[49] it was determined that single descriptors, in the form of one-dimensional probability distributions, were not adequate for distinguishing biological activity. Hence, the data were converted to 2D probability distributions, which were then shown to allow for discrimination of δ opioid agonists and antagonists. Accordingly, 2D probability distributions were used for pharmacophore development in the present study.

Figure 2 shows the 2D probability distribution for all possible permutations of the pharmacophoric angles and distances. The data represent the combined sampling of all agonists or all antagonists, allowing the overall conformational sampling of the two classes of δ opioid ligands to be analyzed. In general, the peptidic agonists (red contours) show somewhat greater flexibility than the antagonists (blue contours), as is evident from the larger distributions of the contours. The diffuse nature of the plots for peptidic agonists (red contours) reflect, in general, the larger conformational space sampled due to the greater number of residues in these ligands as well as the larger number of agonists. In many cases the antagonist sampling is focused in relatively small regions of conformational space compared to the agonists (e.g., AB vs NAB or ANB and BN vs NAB or ANB), although exceptions do exist (e.g., NA vs NBA or NAB). This trend is similar to the nonpeptidic ligands where the regions of conformational space sampled by antagonists were rather small (e.g., see Figures 3 and 4 below) in accordance with their rigid structures.[17] Also, as with the nonpeptidic ligands, significant overlap in conformational space of the agonists vs antagonists occurs, which is attributable to the requirements of binding to the same receptor. However, as with the nonpeptidic ligands, distinct high-probability regions are seen that are able to differentiate the conformational requirements for agonist versus antagonist. Plots of distances AB and BN versus the different angles offer better discrimination, while the poorest discrimination is seen with distance NA. The NA distances in the peptides are between the α amino nitrogen and the centroid A of the phenolic group (Figure 1, Table 1) of the same tyrosine residue. Because the phenolic moiety is, in general, known to be important for the activity of opioids,[54] it is expected that these distances would be rather similar and, hence, be the least discriminatory parameter. The pharmacophore descriptors involving the hydrophobic group B in the distance were also important for discrimination of non-peptide agonists and antagonists, implying that the orientation and structure of the B group is critical for determining the biological activity of the δ opioid ligands, while the relative orientations of the N and aromatic A groups are probably more important for binding. In addition, plots with angle NBA show greater overlap between agonists and antagonists

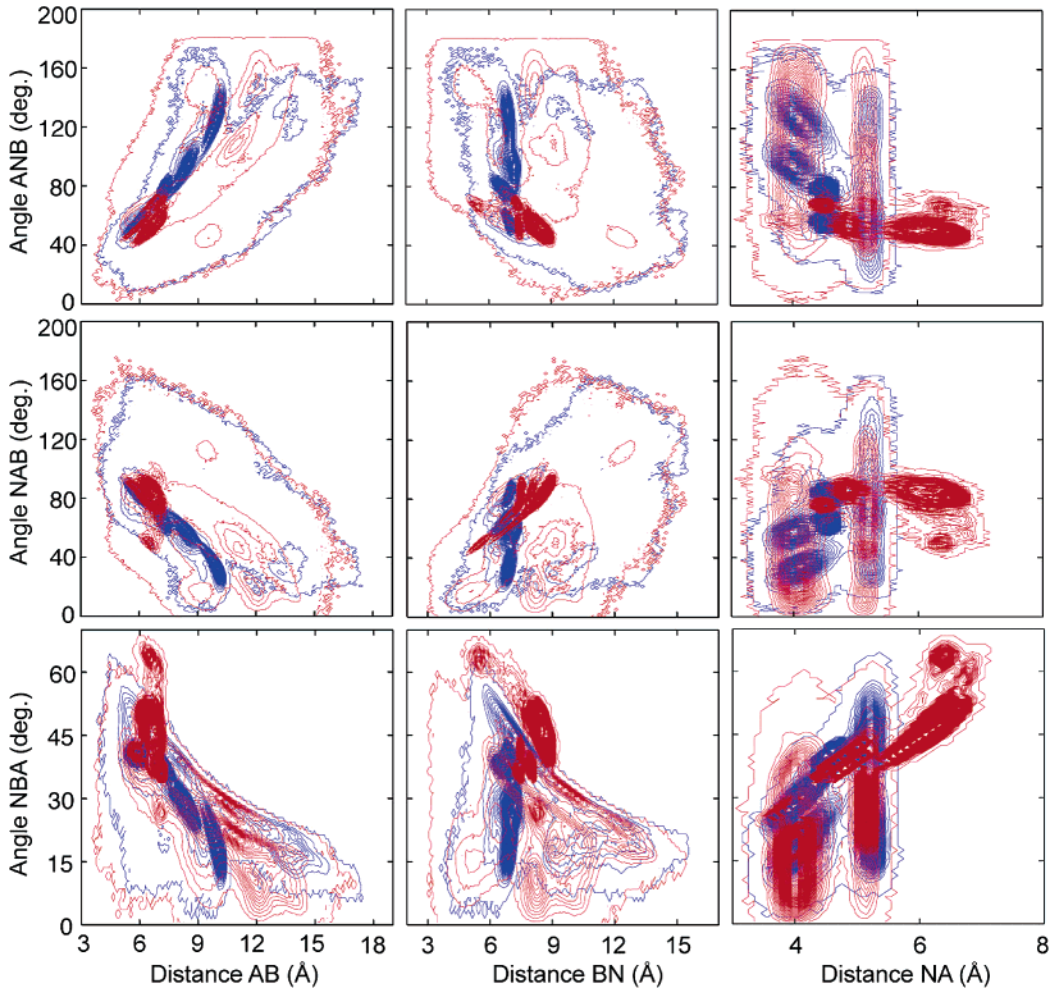

Figure 5. 2D probability distribution of distance angle combinations for both peptidic and nonpeptidic δ opioid ligands. Agonist regions are in red, and antagonist regions are in blue.

in the high-probability regions reflecting the restricted spatial orientation between the N and A groups required for binding to the δ opioid receptor.

The peptidic ligands have greater conformational freedom than the nonpeptidic ligands and, as expected, sample larger regions of conformational space (Figures 3 and 4). In general, the peptide antagonists encompass the conformational space of the non-peptide antagonists (Figure 4), whereas in the case of the agonists there exist regions that are sampled only by the nonpeptidic agonists (Figure 3). This is seen especially in combinations of the distance NA, where the peptidic ligands are restricted to shorter NA distances, as opposed to the nonpeptidic agonists. It can also be seen that in all combinations with angle NBA there are distinct regions in conformational space accessible to the non-peptide ligands that are not accessible to the peptidic ligands. These regions inaccessible to the peptidic ligands may involve conformations that lead to the differential behavior of peptidic and nonpeptidic δ opioid ligands. However, combinations of the AB and BN distances with angles NAB and ANB give distinct regions of agonist space that are part of both the peptidic and nonpeptidic ligands, suggesting that these regions may encompass the bioactive conformers for the peptides and nonpeptides. These overlapping regions also suggest that the peptidic and nonpeptidic ligands have similar binding modes. Importantly, these results show that both the peptidic and nonpeptidic ligands sample similar regions of conformational space such that a CSP that includes both classes of ligands should be possible.

The combined CSP for peptidic and nonpeptidic δ opioid ligands is shown in Figure 5. It is clearly seen that distance BN gives the best discrimination between the agonists and antagonists with reasonable discrimination occurring with all three angles. This discrimination is based on the regions of high probability of the agonists versus the antagonists; if the low-probability regions are considered, distinct sampling of conformational space is not observed (note that the first contours correspond to a probability of 2×10^{-7}). This result suggests that higher probability, lower energy conformations of the ligands are involved in ligand−receptor interactions. Notably, the discriminatory ability of the BN distance also occurs with the nonpeptidic ligands alone.[17] Thus, it is evident that the spatial relationship of the hydrophobic B regions of the ligands with respect to the basic nitrogen, N, is of primary importance for the discrimination between δ opioid agonists and antagonists, consistent with previous observations.[27,28,53,55]

Conclusions

As opposed to traditional methods that use the low-energy conformers of molecules, the CSP approach, in principle, includes all accessible conformations in determining the pharmacophore, taking into account the

dynamic nature of ligands and their interaction with receptors. This method was originally used in the development of a δ opioid activity pharmacophore using nonpeptidic ligands, subsequently used in a study of the peptide compstatin and related compounds,[49] and the present study extends the application of the approach to peptidic δ opioid ligands. An important part of the extension of the approach to peptidic ligands is obtaining adequate sampling for the accessible conformations of the peptides due to their large number of rotatable bonds. This was achieved via the application of MD simulations combined with the replica exchange method. Analysis of the extent of convergence indicates that although full sampling of all possible conformations is not achieved, adequate sampling is performed to allow the application of the 2D CSP to this important class of peptidic ligands.

The 2D CSP determined for the peptidic ligands is consistent with that obtained previously for nonpeptidic δ opioid ligands and is able to distinguish the δ opioid agonists from the antagonists. Comparisons between peptidic and nonpeptidic ligands indicate that they share significant regions of conformational space, thereby leading to overlaps in the measured 2D pharmacophoric probability distributions. Such overlap is expected because all the ligands bind to the same receptor and support a model where both classes of ligands interact with the δ receptor via the same binding mode. However, there exist high-probability regions that are primarily sampled by agonists versus antagonists and vice versa for both the peptidic and nonpeptidic ligands. The spatial relationship of the hydrophobic region B, primarily with respect to the basic nitrogen N, is once again seen to harbor the structural requirements for discrimination of δ opioid activity. Finally, the observation that discrimination occurs in high-probability regions of the 2D distributions indicates that lower energy conformations, although not necessarily local or global minima in solution, are involved in receptor binding.

The CSP method does not require information for the receptor bound conformation of ligands and is thus advantageous in the development of ligand-based pharmacophores in general. Inclusion of all accessible conformations at room temperature increases the probability that the bound conformation of the ligand is included in the pharmacophore, and in the case of peptides, which have multiple low-energy conformers, this is particularly important. The present study further validates the method as being applicable to peptidic ligands, and importantly, a model that encompasses both nonpeptidic and peptidic ligands has been developed. The availability of the present pharmacophore is expected to facilitate the design of novel δ opioid antagonists that may be used as novel analgesics that minimize the side effects of current therapeutics.

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Supporting Information Available: Figure of the conformational probability distribution following 20 ns of simulation. This material is available free of charge via the Internet at http://pubs.acs.org.

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