J|A|C|S
ARTICLES
Published on Web 02/19/2003

# 2D Conformationally Sampled Pharmacophore: A Ligand-Based Pharmacophore To Differentiate δ Opioid Agonists from Antagonists

Denzil Bernard, Andrew Coop, and Alexander D. MacKerell, Jr.*

*Contribution from the Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland 21201*

Received July 10, 2002; E-mail: amackere@rx.umaryland.edu

***Abstract:*** Pharmacophores are widely used for rational drug design and include those based on receptor binding sites or on known ligands. To date, ligand-based pharmacophores have typically used one or a small number of conformers of known receptor ligands. However, this method does not take into account the inherent dynamic nature of molecules, which sample a wide range of conformations, any of which could be the bound form. In the present study, molecular dynamics (MD) simulations were used as a means to sample the conformational space of ligands to include all accessible conformers at room temperature in the development of a pharmacophore. On the basis of these conformers, probability distributions of selected distances and angles in a series of δ specific opioid ligands were obtained and correlated with agonist versus antagonist activities. Individually, the distributions did not allow for unique agonist and antagonist pharmacophores to be identified. However, by extending the conformational analysis to two dimensions, a 2D conformationally sampled pharmacophore (CSP) for distinguishing δ receptor agonists and antagonists was developed. Application of this model to the compound DPI2505 suggests that it may have agonist activity. It is anticipated that the CSP method, which does not require alignment of compounds during pharmacophore development, will be a useful tool for obtaining structure−function relationships of ligands particularly in systems where the receptor 3D structure is not known.

## Introduction

The drugs of choice for the treatment of severe pain are the opioids,[1] the activities of which are mediated by their interaction with specific membrane bound G-protein coupled receptors (GPCR).[2,3] The existence of at least three major classes of opioid receptors, viz. μ, κ, and δ, has been well established.[4] These receptors have also been cloned and functionally characterized.[5−8] While agonists represent the majority of drugs for the treatment of chronic pain, the undesirable effects associated with them, such as respiratory depression,[9] development of tolerance and dependence,[10] nausea,[11] and constipation,[12] have directed considerable efforts toward the discovery of novel agents with fewer or no adverse side effects.

The development of selective peptide and non-peptide δ ligands over the past decade revealed some of the interesting physiological roles of the δ receptor including its modulatory effect on the μ opioid system.[13] Studies have shown that co-administration of a δ antagonist with a μ agonist reduces the development of tolerance and dependence to the μ agonist.[14−17] Slower development of tolerance has also been shown with the administration of a peptide with the dual profile of μ agonism and δ antagonism.[18] It has also been reported that δ receptor knockout mice develop no tolerance to morphine.[19] Thus, an agent with a dual profile of δ antagonism and μ agonism would be an excellent probe for the development of a potent medication for use in the treatment of chronic pain that would not require increasing doses, thereby limiting the undesired effects.

Our approach toward meeting this goal is the development of a pharmacophore for δ antagonism and using the model for

(1) Zieglgansberger, W.; Tolle, T. R.; Zimprich, A.; Hollt, V.; Spanagel, R. *Pain and the Brain* **1995**, *22*, 439−457.
(2) Knapp, R. J.; Malatynska, E.; Collins, N.; Fang, L.; Wang, J. Y.; Hruby, V. J.; Roeske, W. R.; Yamamura, H. I. *FASEB J.* **1995**, *9*, 516−525.
(3) Satoh, M.; Minami, M. *Pharmacol. Ther.* **1995**, *68*, 343−364.
(4) Pasternak, G. W. *Clin. Neuropharmacol.* **1993**, *16*, 1−18.
(5) Keiffer, B. L.; Befort, K.; Gavriaux-Ruff, C.; Hirth, C. G. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 12048−12052.
(6) Evans, C. J.; Keith, D. E.; Morrison, H.; Magendzo, K.; Edwards, R. H. *Science* **1992**, *258*, 1952−1955.
(7) Yasuda, K.; Raynor, K.; Kong, H.; Breder, C. D.; Takeda, J.; Reisine, T.; Bell, G. I. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6736−6740.
(8) Chen, Y.; Mestek, A.; Liu, J.; Hurley, J. A.; Yu, L. *Mol. Pharmacol.* **1993**, *44*, 8−12.
(9) White, J. M.; Irvine, R. J. *Addiction* **1999**, *94*, 961−972.
(10) Chakrabarti, S.; Wang, L.; Tang, W. J.; Gintzler, A. R. *Mol. Pharmacol.* **1998**, *54*, 949−953.
(11) Aparasu, R.; McCoy, R. A.; Weber, C.; Mair, D.; Parasuraman, T. V. *J. Pain Symp. Manage.* **1999**, *18*, 280−288.
(12) Mancini, I.; Bruera, E. *Sup. Care Cancer* **1998**, *6*, 356−364.
(13) Coop, A.; Rice, K. C. *Drug News Perspect.* **2000**, *13*, 481−487.
(14) Abdelhamid, E. E.; Sultana, M.; Portoghese, P. S.; Takemori, A. E. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 299−303.
(15) Hepburn, M. J.; Little, P. J.; Gingras, J.; Kuhn, C. M. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 1350−1356.
(16) Fundytus, M. E.; Schiller, P. W.; Shapiro, M.; Weltrowska, G.; Coderre, T. J. *Eur. J. Pharmacol.* **1995**, *286*, 105−108.
(17) Zhao, G.; Wu, D.; Soong, Y.; Shimoyama, M.; Berezowska, I.; Schiller, P. W.; Szeto, H. H. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 188−196.
(18) Schiller, P. W.; Fundytus, M. E.; Merovitz, L.; Weltrowska, G.; Nguyen, T. M. D.; Lemieux, C.; Chung, N. N.; Coderre, T. J. *J. Med. Chem.* **1999**, *42*, 3520−3526.
(19) Zhu, Y. X.; M. A., K.; Schuller, A. G. P.; Nitsche, J. F.; Reidl, M.; Elde, R. P.; Unterwald, E.; Pasternak, G. W.; Pintar, J. E. *Neuron* **1999**, *24*, 243−252.

rational drug design. Such a pharmacophore in combination with the extensive knowledge of the SAR of $\mu$ ligands can be used to predict $\delta$ antagonists with the desired dual profile. It will also provide a means for the de novo design of new ligands.

A pharmacophore is a simple model describing the spatial relationship between atoms or functional groups in a ligand that are important for its interaction with a receptor. Many pharmacophores for $\delta$ receptor ligands have been developed; examples include Loew's pharmacophore[20] and the LMC pharmacophore,[21] which are primarily recognition pharmacophores. In addition, a qualitative pharmacophore based on energy minimized structures differentiating the pharmacological activity of $\delta$ opioid ligands has been presented.[22] The methodology used for pharmacophore identification typically involves the selection of low energy conformers of different molecules in a data set (sample 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. These approaches are, however, limited in a number of ways by the inherent dynamic nature of molecules and the nature of their interaction with biomolecules. Molecules at room temperature exhibit dynamic behavior due to kinetic energy,[23] thereby sampling a variety of conformations other than the lowest energy conformation(s). Importantly, the particular software and force field used in a given study can influence the calculated lowest energy conformation.[24] Finally, the bound conformation of a molecule need not be the lowest energy conformer of the unbound molecule, because the favorable interaction with the receptor or enzyme would enable it to overcome the conformational strain associated with assuming a higher energy conformation.

To overcome these limitations, we propose an approach based on the use of MD simulations of molecules for obtaining distributions of geometric information to be used in pharmacophore development. Probability distributions of selected geometric terms, rather than individual structures, are the basis of the pharmacophore. This approach is referred to as conformationally sampled pharmacophore or CSP, and it was applied in the present study to a series of known $\delta$-opioid nonpeptidic agonists and antagonists. The distributions of geometric information were analyzed in both one- and two-dimensions (2D), and from these efforts a 2D CSP allowing the prediction of $\delta$ agonists versus antagonists was developed. 2D CSP analysis was then applied to the compound DPI2505, indicating that the compound may have agonist activity.

## Computational Methods

A set of $\delta$ ligands, Figure 1, was model built using Sybyl 6.2 (Tripos, Inc.). All molecules were protonated at the basic nitrogen. In compounds **3**,**4** and **6**−**10**, the H atom was oriented over the plane of the hydrophobic group (B), shown in red in Figure 1, based on the known X-ray structure of naltrexone,[25,26] from which the antagonist naltrindole[27] and its analogues were derived. For **2**, two hydrogen configurations were modeled at the nitrogen bearing the allyl substituent, while for **1**, **5**, and **11**, four hydrogen configurations were obtained by the attachment of hydrogen in both configurations to the two basic nitrogens in these compounds. The structures were then energy minimized in Sybyl using the conjugate gradient method with Gasteiger−Hückel charges to a final gradient of 0.05 kcal/mol Å.

The molecules were ported into CHARMM[28,29] for treatment using the Merck-molecular force field (MMFF).[30,31] This treatment initially involved a 200-step adopted-basis Newton Raphson (ABNR) minimization followed by MD simulations. MD simulations were performed for 10 ns using Langevin dynamics[32] with a friction coefficient of 50 $ps^{-1}$ at a temperature of 300 K. SHAKE of all covalent bonds involving hydrogens[33] was performed allowing for an integration time step of 0.002 ps. Nonbond interactions were calculated using a constant dielectric with an infinite cutoff. A scaling factor of 0.75 was used to treat 1−4 interactions. Aqueous solvation, for energy minimization and dynamics, was treated via the generalized Born (GB) continuum solvent model[34] as implemented in CHARMM and specially optimized for MMFF.[35] GB models have been successfully used in a number of pharmacophore-based studies.[36−39] Coordinate sets were saved every 2 ps, from which the probability distributions were calculated. Probability distributions were determined on the basis of the number of conformations in a given bin divided by the total number of conformers in the entire sample (e.g., 5000 for each MD simulation of 10 ns). Bin widths were 0.1 Å for distances and 1° for angles. For compounds with multiple protonation states, data from the MD simulations on each of the protonation states were combined, from which a single probability distribution for those compounds was determined. MD simulations for all compounds were also performed at 600 K, and probability distributions were determined (see Supporting Information). In addition, gas-phase simulations were carried out for one protonation state of compound **2** and for compounds **6** and **9** using MMFF in a vacuum, and with CFF95[40,41] implemented in CHARMM, and results were compared with the results from the MMFF GB simulations (see Supporting Information).

The lowest energy conformer for each molecule was obtained from the MD trajectories by selecting structures from the trajectory every 50 ps and subjecting each structure to 200 ABNR minimization steps followed by 50 steps of Newton−Raphson minimization. The resulting energy for each successive conformation was then compared, and the conformer with the lowest energy from the entire trajectory was selected. The conformation thus obtained was then minimized by the ABNR method separately in a vacuum and with the GB solvation

(20) Huang, P.; Kim, S.; Loew, G. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 21−28.
(21) Coop, A.; Jacobson, A. E. *Bioorg. Med. Chem. Lett* **1999**, *9*, 357−362.
(22) Brandt, W. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 615−621.
(23) McQuarrie, D. A. *Statistical Mechanics*; Harper Collinns Publishers: New York, 1976.
(24) Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 730−748.
(25) Amato, M. E.; Bandoli, G.; Grassi, A.; Nicolini, M.; Pappalardo, G. C. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1757−1762.
(26) Dain, A. C. L.; Madsen, B. W.; Skeleton, B. W.; White, A. H. *Aust. J. Chem.* **1992**, *45*, 635−640.
(27) Portoghese, P. S.; Sultana, M.; Takemori, A. E. *J. Med. Chem.* **1990**, *33*, 1714−1720.
(28) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187−217.
(29) MacKerell, A. D., Jr.; Brooks, B.; Brooks, C. L.; Nilsson, L.; Roux, B.; Won, Y.; Karplus, M. CHARMM: The Energy Function and Its Parametrization with an Overview of the Program. In *Encyclopedia of Computational Chemistry*; Schleyer, P. V. R., Allinger, N. L., Clark, T., Gasteiger, J., Kollman, P. A., Schaefer, H. F., III, Schreiner, P. R., Eds.; John Wiley & Sons: Chichester, 1988; Vol. 1, pp 271−277.
(30) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490−519.
(31) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 520−552.
(32) Allen, M. P.; Tildesley, D. J. *Computer Simulation of Liquids*; Oxford University Press: New York, 1989.
(33) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327−341.
(34) Qui, D.; Shenkin, P. S.; Hollinger, F. P.; Still, W. C. *J. Phys. Chem. A* **1997**, *101*, 3005−3014.
(35) Dominy, B. N.; Brooks, C. L. *J. Phys. Chem. B* **1999**, *103*, 3765−3773.
(36) Guarnieri, F.; Weinstein, H. *J. Am. Chem. Soc.* **1996**, *118*, 5580−5589.
(37) Barnett-Norris, J.; Guarnieri, F.; Hurst, D. P.; Reggio, P. H. *J. Med. Chem.* **1998**, *41*, 4861−4872.
(38) Bostrm, J.; Gundertofte, K.; Liljefors, T. *J. Comput.-Aided Mol. Des.* **2000**, *14*, 769−786.
(39) Barnett-Norris, J.; Hurst, D. P.; Lynch, D. L.; Guarnieri, F.; Makriyannis, A.; Reggio, P. H. *J. Med. Chem.* **2002**, *45*, 3649−3659.
(40) Maple, J. R.; Hwang, M.-J.; Stockfisch, T. P.; Dinur, U.; Waldman, M.; Ewing, C. S.; Hagler, A. T. *J. Comput. Chem.* **1994**, *15*, 162−182.
(41) Hwang, M. J.; Stockfisch, T. P.; Hagler, A. T. *J. Am. Chem. Soc.* **1994**, *116*, 2515−2525.

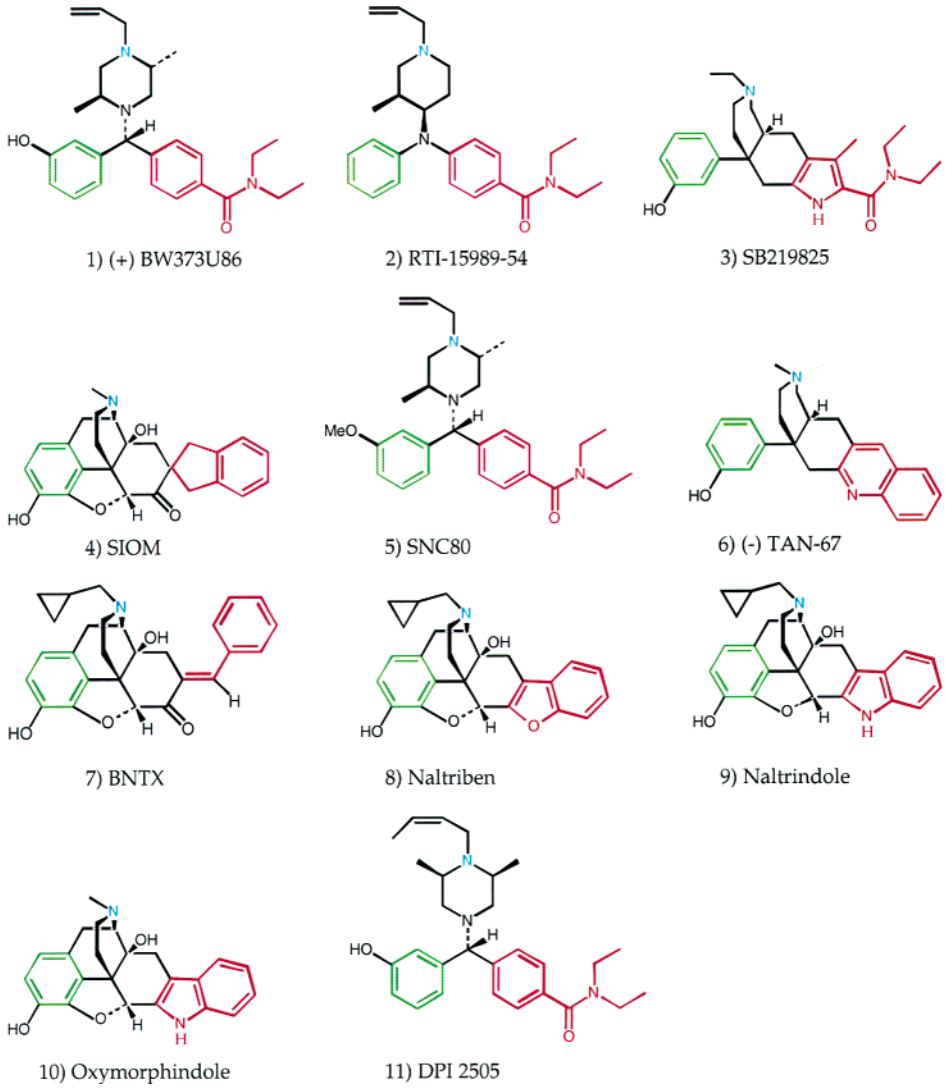


***Figure 1.*** Structures of the $\delta$ agonists (**1**−**6**) and antagonists (**7**−**10**) used for pharmacophore development and DPI2505 (**11**). The atoms used to define pharmacophore point A are colored in green, those used for point B are colored in red, and the N in blue denotes the basic nitrogen atom used as the third point.

model, to a gradient less than $10^{-5}$ kcal/mol Å, yielding a set of minimum energy conformers obtained in vacuo and in solvent.

## Results and Discussion

For the development of the pharmacophore, three groups were chosen, the protonated nitrogen (N), the centroid of an aromatic ring (A), and the centroid of the hydrophobic region (B), Figure 1. These are similar to those selected for determination of the Loew[20] and LMC[21] pharmacophores. While the endogenous ligands for the opioid receptors are peptides, nonpeptidic $\delta$ ligands were investigated due to the relatively lower degree of conformational freedom in these molecules. A set of prototypical $\delta$ agonists,[42,43] Figure 1 (**1**−**6**), and antagonists, Figure 1 (**7**−**10**), differing in the carbon−nitrogen skeleton, were selected for the study. DPI 2505,[44] **11**, which has been reported to be an antagonist, is an aryl piperazine compound structurally similar to the agonists such as **1**. This compound has been analyzed as a separate case in our studies.

Determination of a pharmacophore, based on the traditional method of analyzing a low energy conformation of the molecules in the data set, was done by obtaining the lowest energy conformation from the MD trajectories and then energy minimizing it both in vacuo and using an implicit solvent model. The distances and angles between the pharmacophore points in each molecule, including those with multiple protonation states, were calculated. Data for the individual compounds were tabulated (not shown), and the parameters for agonists, **1**−**6**, and antagonists, **7**−**10**, were combined into two separate sets. Table 1 gives the range of distances and angles obtained for the compounds in our set of known $\delta$ agonists and antagonists, and **11**, along with the Loew[20] and LMC[21] pharmacophores. From Table 1, it can be seen that the geometric parameters for the minimized structures are similar for the in vacuo and implicit solvent calculations. This is expected due to the relatively small number of flexible degrees of freedom in the studied compounds. The agonists in general appear to have greater conformational freedom as indicated by the wider range of values for

(42) Dondio, G.; Ronzoni, S.; Petrillo, P. *Exp. Opin. Ther. Pat.* **1997**, *7*, 1075−1098.
(43) Dondio, G.; Ronzoni, S.; Petrillo, P. *Exp. Opin. Ther. Pat.* **1999**, *9*, 353−374.
(44) Su, Y.-F.; McNutt, R. W.; Chang, K.-J. *J. Pharmacol. Exp. Ther.* **1998**, *287*, 815−823.

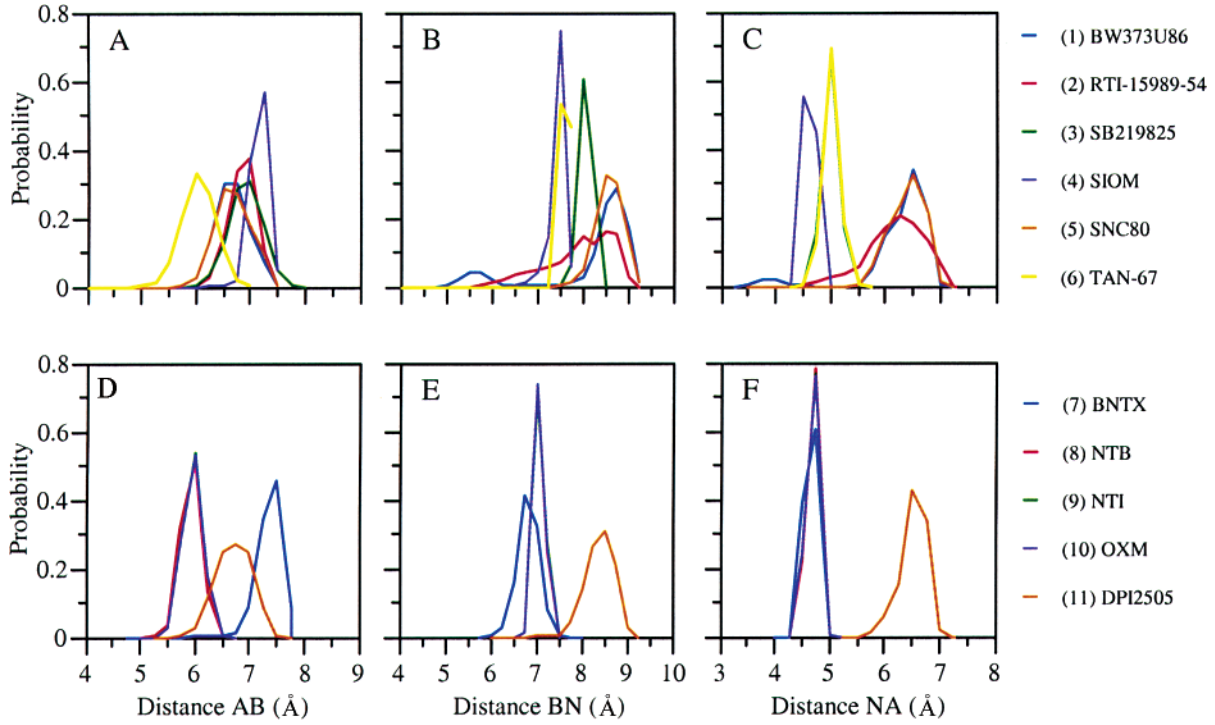


***Figure 2.*** Probability distributions of the distances between pharmacophore points A, B, and N from the MD trajectories. Distances for agonists (**1**−**6**) are shown in A, B, and C, and distances for antagonists (**7**−**10**) and DPI2505 (**11**) are shown in D, E, and F.

***Table 1.*** Measured Distances and Angles between the Pharmacophore Points for the Lowest Energy Conformers

| | distance (Å) | | | angle (deg) | | |
|---|---|---|---|---|---|---|
| | A–B | B–N | N–A | ANB | NAB | NBA |
| | | | gas phase | | | |
| agonists | 5.9−7.1 | 6.2−8.7 | 4.4−6.8 | 51−77 | 55−88 | 36−63 |
| antagonists | 5.8−7.3 | 6.5−7.0 | 4.4−4.6 | 56−81 | 62−83 | 37−41 |
| DPI 2505 | 6.7−6.8 | 5.8−8.3 | 6.3−6.8 | 52−65 | 51−79 | 48−64 |
| | | | solution[a] | | | |
| agonists | 5.9−7.1 | 6.8−8.8 | 4.4−6.8 | 47−75 | 62−90 | 37−61 |
| antagonists | 5.8−7.3 | 6.6−7.0 | 4.5−4.6 | 56−79 | 63−83 | 37−41 |
| DPI 2505 | 6.3−6.5 | 6.5−8.5 | 6.4−6.8 | 48−58 | 58−83 | 48−64 |
| Loew recognition[b] | 4.5 | 6.4 | 4.4 | | | |
| Loew selective[b] | 6.7 | 7.6 | 4.5 | | | |
| LMC recognition[c] | 5.9−6.7 | 6.7−8.3 | 4.5−4.6 | | | |

[a] Conformation obtained after using the generalized Born solvation model for energy minimization. [b] Reference 20. [c] Reference 21.

some of the distances and angles (e.g., distance NA and angle NBA), which is expected on the basis of the larger number of rotatable bonds in these molecules. However, there is significant overlap in the distance and angle ranges between the agonists and antagonists. For example, in the case of distance NA and angles NAB and NBA, the range of antagonist values are fully within the agonist values. Importantly, in no case is the range of values for the agonists unique from those for the antagonists. This shows that it is not possible to discriminate between the agonists and antagonists by the use of minimum energy conformations alone.

The distances obtained from our studies of the low energy conformers (Table 1) are comparable to those of the previous pharmacophores. The Loew[20] pharmacophores are generally in agreement with the ranges obtained in the present study. An exception is the AB distance for the Loew $\delta$-recognition pharmacophore, which is much smaller than the present data and the Loew $\delta$-selective pharmacophore. This is due to the use of an extended hydrophobic region to define the pharmacophoric point B in both our study and in the determination of the Loew $\delta$-selective pharmacophore. Similarly, the LMC[21] pharmacophore is in agreement with the data obtained from our study, with complete overlap of the distance ranges. These results further indicate the limitations in the currently accepted approach for pharmacophore development.

As discussed in the Introduction, the use of low energy conformers in the determination of a pharmacophore has significant drawbacks. To overcome these limitations, MD simulations were used as a means to identify accessible conformations of a molecule at room temperature for use in the determination of the pharmacophore. The distances and angles between the pharmacophore points were then obtained from each frame stored in the MD trajectories. This produced a range of values for each parameter representing the conformational space sampled by the different molecules. The data were converted to probability distributions for analysis, which are shown in Figures 2 and 3. Data for **11** were included with the antagonists for this analysis.

From the probability distributions for the individual distances and angles, Figures 2 and 3, respectively, it is evident that both the agonists and the antagonists sample overlapping regions of conformational space. For example, in the case of distance AB, both classes of compound sample the region of 5−8 Å. Such overlap is also seen for all of the angles in Figure 3. Comparison of the data in Figures 2 and 3 and those in Table 1 shows the range of distances and angles from the MD simulations to be significantly greater than that for the lowest energy conformers; this is the foundation of the CSP. Upon binding to a receptor, a ligand can assume a conformation that is higher in energy than the unbound global minimum energy conformation due to the favorable ligand−receptor binding energy. Although while in the absence of a 3D structure of the ligand−receptor complex,

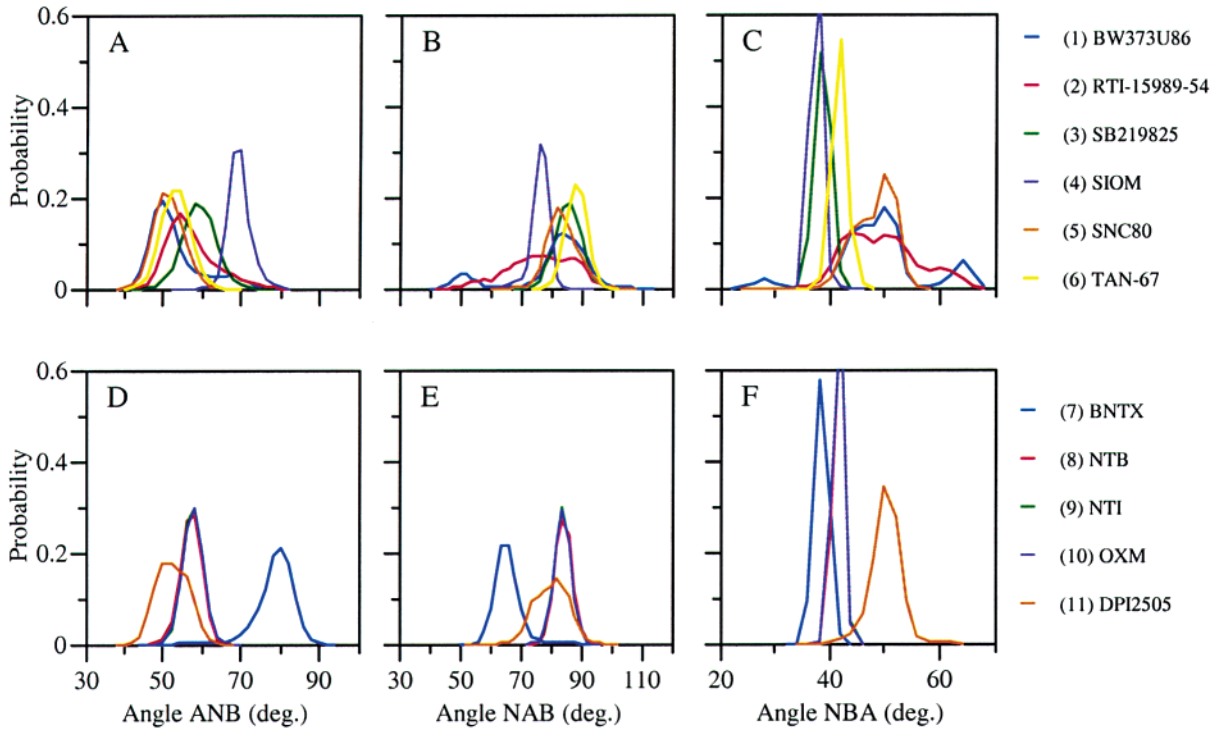


***Figure 3.*** Probability distributions of the angles between pharmacophore points A, B, and N from the MD trajectories. Angles for agonists (**1**−**6**) are shown in A, B, and C, and distances for antagonists (**7**−**10**) and DPI2505 (**11**) are shown in D, E, and F.

the ligand binding conformation cannot be determined, it may be assumed that the bound conformation will be sampled during a room-temperature MD simulation of the unbound ligand. Inclusion of all accessible conformations, therefore, may be considered a more robust approach for pharmacophore development. However, as is evident from Figures 2 and 3, in the present case, the individual distance and angle probability distributions do not allow for the differentiation of agonists and antagonists. This indicates that (1) the selected parameters defining the pharmacophore are not relevant to the biological activities or (2) the use of the individual terms alone is not adequate to obtain a rigorous structure−function relationship separating $\delta$ agonists from antagonists.

The individual parameters indicate the direct relation between pharmacophore points, but they do not describe the overall geometry of the molecules. So while individual distances or angles may overlap for two different molecules, it does not indicate that the overall geometry of the molecules is similar. For a definite representation of the conformational geometry, a combination of these parameters is required. Accordingly, 2D analyses of all possible combinations of angles with distances were performed.

Figure 4 shows the probability distribution for this 2D data for all possible permutations of the angles and distances. Results include the conformational requirements for both the agonists (red) and the antagonists (blue). The lowest contour corresponds to a region of less than $10^{-7}$ probability, while the denser contours indicate increasing probability in 0.005 increments. Inspection of all of the plots in Figure 4 shows overlap of the lowest contour; however, it is evident in many cases that the high probability regions are distinct for the agonists versus the antagonists. As can be seen in Figure 4A, distance AB versus angle ANB, the high probability regions are able to differentiate the conformational requirements for agonists and antagonists. Similar observations can be made from the other plots such as for distance BN versus angle NAB, Figure 4E. Plots of the distance BN versus the different angles offer the best discrimination (Figure 4B, E, and H), where it can be seen that the conformational space populated by the agonists and antagonists clearly differs. The poorest discrimination occurs in the plots where distance NA is along the *x*-axis (Figure 4C, F, and I), where in all cases the high probability regions have some direct overlap. In combination, these results imply that the structure of hydrophobic group B and its orientation relative to the other functional groups are critical for determining the biological activity of the $\delta$ ligands. This result is consistent with previous observations on the SAR of $\delta$ ligands.[22,45]

Some overlap exists in all of the plots for the low populated regions. This may be attributed to the fact that all molecules considered in the study exert their effects at the same receptor, that is, the $\delta$ receptor. Therefore, intuitively one may expect some degree of similarity in the conformational requirements for binding at the same receptor site. However, as noted above, there are significant differences in the conformational space sampled by agonists versus antagonists, allowing for the differentiation of the ligands based on their pharmacological activity.

The greater flexibility of the agonists as compared to that of the antagonists can be seen from the wider regions of conformational space sampled by these molecules. Also, for the agonists, multiple regions of high probability are seen, such as in Figure 4F and H. From 2D plots for the individual compounds (see Supporting Information), it was seen that among the agonists, the diaryl-piperazine compounds **1**, **2**, and **5** sampled almost identical regions, which in general were broad, and the other agonists **3**, **4**, and **6** populated more compact regions with

(45) Portoghese, P. S.; Sultana, M.; Moe, S. T.; Takemori, A. E. *J. Med. Chem.* **1994**, *37*, 579−585.

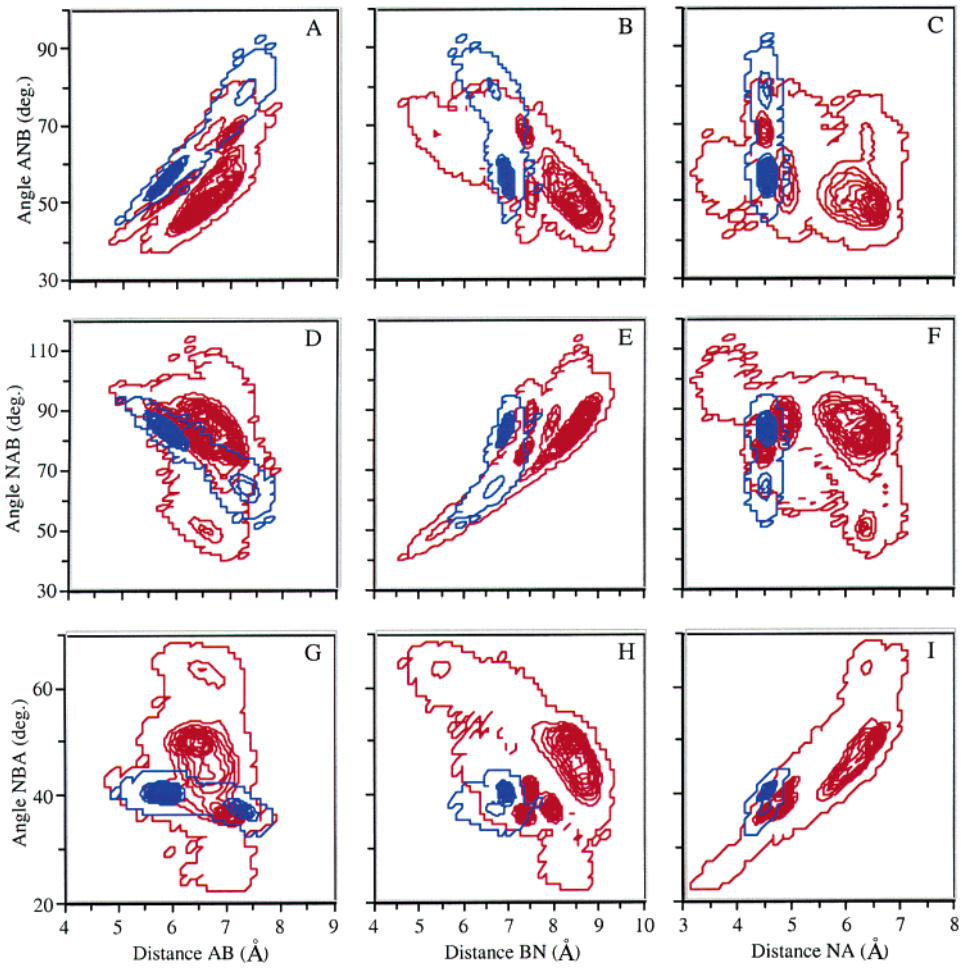


***Figure 4.*** 2D probability distributions of the distances and angles between the pharmacophore points A, B, and N. Contours in red are for the agonists (**1**−**6**), and those in blue are for the antagonists (**7**−**10**). Increments on the *z*-axis (probability) are 0.005 units. Each column corresponds to a single distance, and each row corresponds to a single angle.

some degree of overlap. In Figure 4, the contours for the agonists that are adjacent to those for the antagonists, in most cases, arise from compounds **3**, **4**, and **6** that are structurally more similar to the antagonists.

The aryl piperazine compound **11**, DPI2505,[44] was reported to be a $\delta$ antagonist in a study investigating the ability of $\delta$ ligands (peptidic and nonpeptidic) to reverse respiratory depression induced by alfentanil,[46] a $\mu$ agonist. Surprisingly, both $\delta$ agonists and antagonists were able to reverse the hypercapnia induced by alfentanil. In that study, it was found that administration of DPI2505 after the $\delta$ agonists, BW373U86, Deltorphin II, and DPDPE, or $\delta$ antagonists naltrindole and TIPP($\psi$), decreased the efficacy of these ligands in reversing the respiratory depression induced by alfentanil. The authors suggested that DPI2505 is perhaps a $\delta$ antagonist. While further details on the activity of this compound are still to be investigated, we found it to be an interesting candidate for the application of 2D CSP.

In Table 1, it can be seen that the individual distances and angles between the pharmacophore points for **11** lie in the same range as those for the agonists, for example, distance NA and angle NBA. This is also reflected in the one-dimensional probability distributions, Figures 2 and 3, where **11** shows profiles almost identical to those of agonist **1**. Figure 5 A, B, and C shows 2D plots for **11** for the three most discriminating combinations of parameters along with the probability distributions for the agonists (e.g., red in Figure 4). As is evident, there is almost complete overlap in the conformational space of DPI2505 with that of the agonists. Thus, the 2D analysis, which allows for discrimination of the agonists from the antagonists, indicates an agonistic activity. Therefore, we predict that, contrary to the suggested antagonist activity, this compound may possess agonist activity.

## Conclusions

In the present study, MD simulations are used to sample the conformational space accessible to selected $\delta$ opioid agonists and antagonists (Figure 1). This approach builds upon previous pharmacophore studies using MD- or Monte Carlo (MC)-based methods to sample the conformational space of ligands. For example, Guarnieri and Wilson developed a "smart" MC-based algorithm that was combined with simulated annealing to sample the conformational space accessible to a leukotriene,[47] and MC-based approaches have been used by other workers.[38] Similar to MC are torsion driving methods to sample conformational

(46) Aldrich, J. V. Analgesics. In *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed.; Wolff, M. E., Ed.; John Wiley & Sons: New York, 1996; Vol. 3, pp 321−441.

(47) Guarnieri, F. *J. Comput.-Aided Mol. Des.* **1995**, *16*, 648−653.

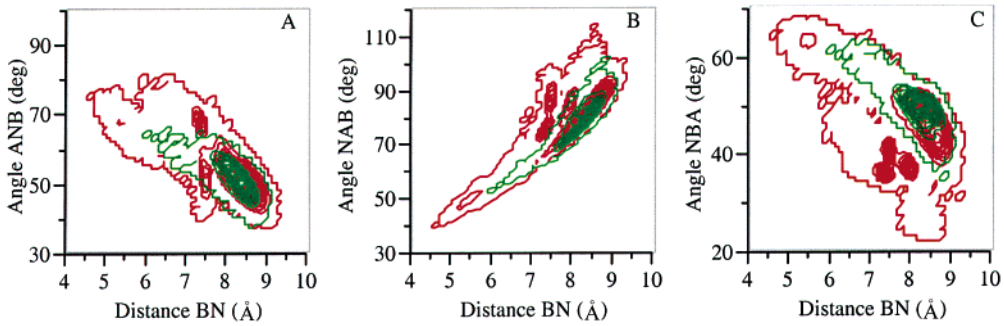


***Figure 5.*** 2D probability distributions of the angles between the pharmacophore points A, B, and N with distance BN for DPI2505 (**11**). The corresponding data for the antagonists from Figure 4B, E, and H are shown in red.

space,[48] often combined with genetic algorithms,[49,50] including studies on opioids.[51,52] MD has also previously been used to sample conformational space in the development of QSAR models.[53] However, in all of the previous studies, single or a small number of low energy conformations were ultimately selected for development of the pharmacophore. This selection typically involved identifying a "common" conformation among different molecules being studied, an approach in which alignment of the structures, or alternatively clustering based on RMS differences,[36,37,39] strongly influences the outcome. To avoid possible limitations associated with the selection of individual structures and structural alignments, the entire distributions are used presently to develop the pharmacophore. We term this approach the conformationally sampled pharmacophore (CSP). It should be noted that MD simulations at higher temperatures would sample an increased region of conformational space of the molecules and thereby increase the likeliness of including the bound conformation, although such an approach may make discrimination of the pharmacophores more difficult. CSP analysis of the present collection of compounds using MD simulations at 600 K yielded results similar to those in Figure 4 (see Supporting Information).

The CSP approach takes into account the dynamic nature of ligands, overcoming limitations in some traditional pharmacophore development methods. Most importantly, the inclusion of all accessible conformations in the pharmacophore increases the probability that the bound conformation of the ligand is included in the pharmacophore. A complicating factor of inclusion of all possible conformations in the pharmacophore is an inability to distinguish unique structural characteristics between, in the present case, $\delta$ agonists and antagonists (e.g., Figures 2 and 3). To overcome this, the pharmacophore analysis was extended to two dimensions (Figure 4). This extension allowed for discrimination of the $\delta$ agonists and antagonists. Application of this pharmacophore to DPI2505, previously suggested to be a $\delta$ antagonist, suggests that it may be an agonist. Novel $\delta$ ligands reported recently and molecules designed de-novo, using the pharmacophore described here, are being investigated to refine the existing model.

The developed CSP approach is analogous to the use of MD simulations in the development of a receptor-based dynamic pharmacophore, as recently described.[54] In that study, multiple protein conformations of HIV Integrase were used to develop the pharmacophore, and it was shown that known inhibitors fit the dynamic pharmacophore better than the static model. This again reflects the dynamic nature of the receptor−ligand interaction and the necessity of using multiple conformations of the receptor or ligand for pharmacophore development.

In the absence of high-resolution receptor structures, as in the case of the opioid receptors and other GPCR, computational models[55−57] can serve as a means for structure-based design of ligands. The CSP methodology described here should be similarly useful in the rational design of molecules based on receptor ligands. It should be particularly advantageous in the absence of receptor structures or models and also when the receptor bound ligand conformations are not known.

**Acknowledgment.** Support from NIDA (DA13583) and the Computer Aided Drug Design Center, School of Pharmacy, University of Maryland, Baltimore, is acknowledged.

**Supporting Information Available:** Figures of (1) the combined probability distributions for all agonists versus all antagonists from MD simulations at 600 K, (2) the probability distributions using different force field models for compounds **2**, **6**, and **8**, and (3) the individual probability distributions of all compounds in the present study (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA027644M

(48) Cinone, N.; Höltje, H.-D.; Carotti, A. *J. Comput.-Aided Mol. Des.* **2000**, *14*, 753−768.
(49) Filizola, M.; Harris, D. L.; Loew, G. H. *Bioorg. Med. Chem.* **2000**, *8*, 1799−1807.
(50) Harris, D. L.; Loew, G. H. *Bioorg. Med. Chem.* **2000**, *8*, 2527−2538.
(51) Filizola, M.; Villar, H. O.; Loew, G. H. *J. Comput.-Aided Mol. Des.* **2001**, *15*, 297−307.
(52) Filizola, M.; Villar, H. O.; Loew, G. H. *Bioorg. Med. Chem.* **2001**, *9*, 69−76.
(53) Hopfinger, A. J.; Wang, S.; Tokarski, J. S.; Jin, B.; Albuquerque, M.; Madhav, P. J.; Duraiswami, C. *J. Am. Chem. Soc.* **1997**, *119*, 10509−10524.
(54) Carlson, H. A.; Masukawa, K. M.; Rubins, K.; Bushman, F. D.; Jorgensen, W. L.; Lins, R. D.; Briggs, J. M.; McCammon, J. A. *J. Med. Chem.* **2000**, *43*, 2100−2114.
(55) Metzger, T. G.; Paterlini, M. G.; Portoghese, P. S.; Ferguson, D. M. *Neurochem. Res.* **1996**, *21*, 1287−1294.
(56) Metzger, T. M.; Paterlini, M. G.; Portoghese, P. S.; Ferguson, D. M. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 857−861.
(57) Pogozheva, I. D.; Lomize, A. L.; Mosberg, H. I. *Biophys. J.* **1998**, *75*, 612−634.