*Pharmaceutical Research, Vol. 3, No. 4, 1986*

*Research Article*

# Conformation and Electrostatic Potential Surfaces of Opiates: Relationship to μ- and λ-Site Binding

**Terry P. Lybrand,[1,2] Peter A. Kollman,[1] Victor C. Yu,[1] and Wolfgang Sadée[1,3]**

*Received September 30, 1985; accepted February 15, 1986*

Molecular mechanics energy minimization and electrostatic potential surface calculations have been used to examine a series of opioid compounds that interact with the μ opoid receptor and a recently discovered high-affinity naloxone binding site (the λ site). The compounds studied include members of the morphinan, 4,5-epoxymorphinan, and benzomorphan families. All compounds bind with a high affinity at the μ opiate receptor site, but only the 4,5-epoxymorphinans bind tightly at the λ site. The results suggest that conformational differences in the various families do not satisfactorily explain the observed trends in binding affinity at the λ site. However, electrostatic potential surfaces for a representative subset of these opioids exhibit patterns that allow us to classify members as high-affinity or low-affinity λ-site ligands in good agreement with the experimental results. The procedures used in this work may be useful in defining characteristics that impart selectivity for opiate receptor subtypes such as the μ, δ, and κ receptors.

**KEY WORDS:** opiate receptor binding; opiate receptor, structure–affinity relationship; morphinans, conformation; morphinans, electrostatic potential surfaces; morphinans, molecular mechanics calculations.

## INTRODUCTION

Techniques of theoretical chemistry have been used to study the interaction of ligands with receptor sites of known structure for some time (1–6). Considerably less work has been done for ligand interactions with binding sites of unknown structure (7–11). Yet most ligand–receptor interactions of interest to medicinal chemists and pharmacologists fall in the latter category. The principal difficulty in applying theoretical chemical techniques to ligand binding problems where the receptor site is poorly characterized or completely unknown lies in the numerous possible conformations for most ligands. Without knowledge of the composition and three-dimensional structure of the receptor site, it is difficult to choose those ligand conformations that are suitable for ligand–receptor interactions. Some investigators have chosen to consider all conformations within 3–5 kcal of the global minimum energy structure as potential relevant binding conformations (12) and use all these structures in their search for physical properties (e.g., spatial orientation of potential hydrogen bond donors and acceptors, electrostatic properties, excluded volumes) that correlate with biological activity. However, if the ligands in question have more than a few degrees of conformational freedom, there are large numbers of potentially relevant binding conformations for the ligands. When many possible conformations for each ligand in question must be considered, it can become quite challenging to derive correlations between physical characteristics and biological properties.

If the ligands of interest are relatively rigid compounds with only limited conformational flexibility, the problem is more tractable. In these cases, it is feasible to obtain extensive energy minimization using empirical potential energy functions or even quantum mechanical techniques to analyze the conformational characteristics of the ligands. If one particular conformation or family of related conformations represents the biologically active entity, it may then be possible to correlate properties of various ligands in the pertinent conformation with their relative biological activity. The ideal case involves a series of extremely rigid ligands that differ from one another only slightly (e.g., methyl or chloro groups substituted for hydrogen in one position). Since all the ligands are rigid, conformational properties do not complicate the investigation. We have previously examined a series of neuroleptic compounds (clozapine and clozapine analogs) that closely approximate the ideal case (13). We were able to correlate rather widely varying pharmacological properties of these analogues with the varying patterns in their respective electrostatic potential surfaces. Encouraged by our results in the clozapine study, we decided to utilize similar techniques in an effort to rationalize the relative binding properties of a series of opioid compounds to high-affinity binding sites in the brain. Specifically, both 4,5-epoxymorphinans (e.g., oxymorphone, naltrexone) and morphinans (levorphanol) bind with a high affinity to the μ opiate receptor, while only 4,5-epoxymorphinans bind tightly to the newly discovered λ site in rat brain homoge-

[1] School of Pharmacy, University of California, San Francisco, California 94143.

[2] Department of Chemistry, University of Houston Central Campus, Houston, Texas 77004.

[3] To whom correspondence should be addressed.

0724-8741/86/0800-0218$05.00/0 

nates (14). The chemical structures of these and related opioid drugs are shown in Table I.

The λ sites have been shown to be stereospecific and are unique because of their instability *in vitro*. The μ site is believed to be the primary receptor responsible for mediating the analgesic effects of these drugs, while putative functions for the λ site remain unknown. However, our recent findings by autoradiography that the λ sites display a unique distribution in rat brain and, in particular, in hippocampal areas strengthen the notion that they have a physiological function (15). The objective in this study was to determine possible factors that explain why the μ receptor fails to differentiate the two classes of morphinans, while the λ site is exquisitely selective for 4,5-epoxymorphinans.

The ligands examined here represented a more challenging problem than the clozapine analogues. There was greater substituent variation from one ligand to the next, and some of the ligands studied had a modest conformational flexibility. We were particularly interested in assessing the performance of our techniques in this analysis of ligand binding at two distinct opioid binding sites. We felt that if we could successfully rationalize the different behavior of these ligands at the μ receptor versus the λ site, we might be able to extend the approach to define characteristics that impart opiate receptor subtype selectivity to some ligands.

## METHODS

The approach used in this study involves an integrated application of molecular mechanics minimization, computation of electrostatic potentials, and interactive computer graphics analysis of three-dimensional structural features for the ligands. All energy minimization was performed using a Newton–Raphson algorithm in our molecular mechanics package AMBER (16). The potential function parameters used in these calculations were taken directly from the program MM2 developed by Allinger and Yun (17). Our potentials differed from the standard MM2 potentials only in that we employed atomic partial charges rather than bond dipoles to evaluate electrostatic interactions, and we did not include out-of-plane or stretch-bend components in the bond angle term. Thus, our expression for the potential energy is as follows:

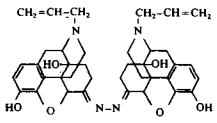
$$E = \sum_{\text{bonds}} K_b(R - R_o)^2 + \sum_{\text{angles}} K_a(\theta - \theta_o)^2 + \sum_{\text{dihedrals}} \frac{K_d}{2}[1 + \cos(n\Phi - \gamma)] + \sum_{\text{nonbonded}} \left[\frac{B_iB_j}{r_{ij}^{12}} - \frac{A_iA_j}{r_{ij}^{6}} + \frac{q_iq_j}{\epsilon_{ij}r_{ij}}\right]$$

where nonbonded interactions are evaluated for all atom pairs not involved in bond or bond-angle terms. The partial charges for the various molecules studied were determined from CNDO/2 calculations. We tested two dielectric constant models in these calculations: $\epsilon = 1.0$ and $\epsilon$ equal to the magnitude of the interatomic distance $r_{ij}$. The rationale for a distance dependent dielectic constant has been discussed previously (2,18). We discovered no notable structural differences with the two dielectric constant models.

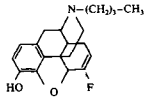
Table I. Chemical Structures

| | | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|
| Morphinans | | | | | |
| | Levorphanol | $CH_3$ | H | H,H | |
| | Levallorphan | $CH_2$–CH=$CH_2$ | H | H,H | |
| | S-20682 | $CH_2$–◁ | OH | O | |
| 4,5-Epoxymorphinans | Oxymorphone | $CH_3$ | OH | O | H |
| | Naloxone | $CH_2CH=CH_3$ | OH | O | H |
| | Naltrexone | $CH_2$–◁ | OH | O | H |
| | Dihydromorphine | $CH_3$ | H | H,OH | H |
| | INC 8339 | $(CH_2)_3$–$CH_3$ | H | F,F | H |
| | INJ 6471-3 | $CH_2$–◁ | H | F,F | H |
| | Nalmefene | $CH_2$–◁ | OH | $CH_2$ | H |
| | N-Noroxymorphone | H | OH | O | H |
| | Nalorphine | $CH_2$–CH=$CH_2$ | H | H,OH | H |
| | Morphine | $CH_3$ | H | H,OH | H |
| | 6-Acetylmorphine | $CH_3$ | H | H,$OCOCH_3$ | H |
| | N-Normorphine | H | H | H,OH | H |
| | Codeine | $CH_3$ | H | H,OH | $CH_3$ |

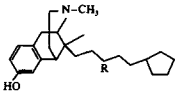
Naloxonazine



INJ7747-1



Benzomorphans



| | R |
|---|---|
| WIN 44441-3 | O |
| WIN 47885 | $H_2$ |

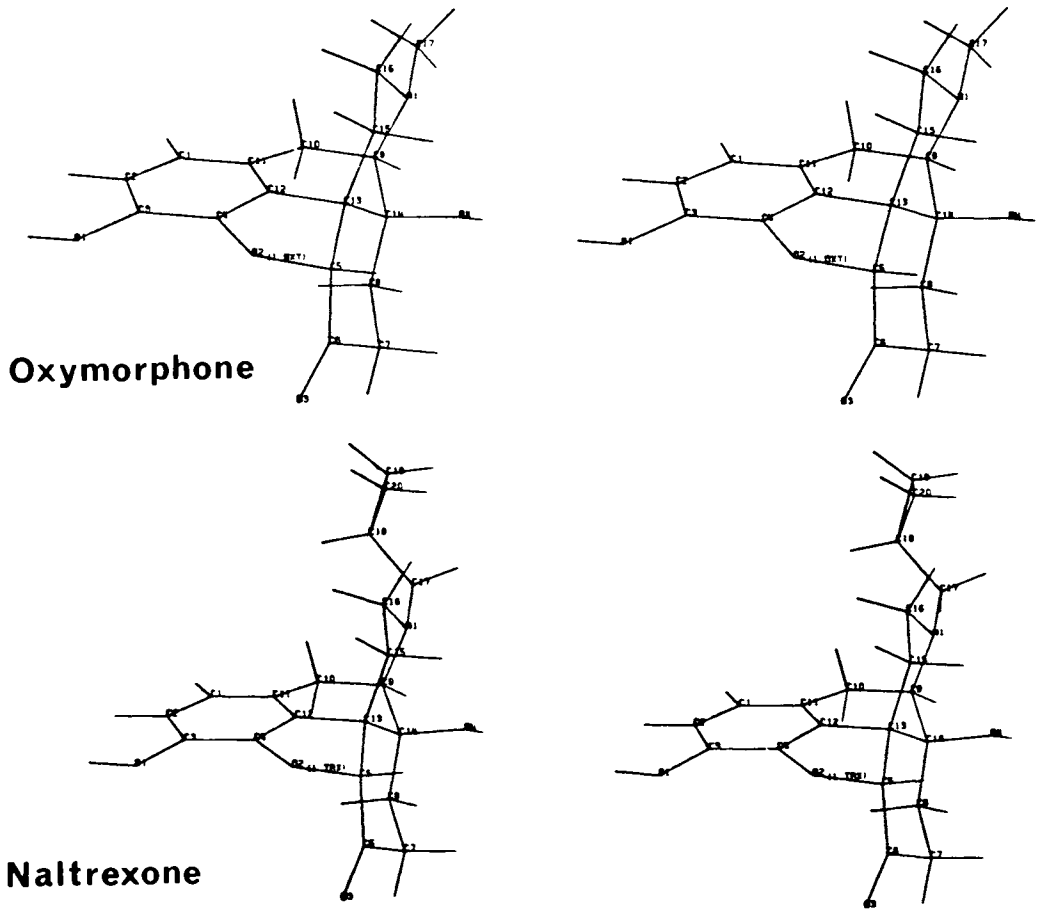



Fig. 1. Representative morphinans and 4,5-epoxymorphinans. Figures 1 through 4 represent stereopairs for viewing three-dimensional structures. Use stereoglasses or defocus your eyes.

All structural models for the opioid ligands were based on coordinates from X-ray crystallographic studies when available (19–23). Those compounds with no available X-ray coordinate data were model-built using crystal coordinates from closely related compounds for analogous sections of the molecules and standard bond lengths and angles for unique sections. All structures were then energy refined to remove any existing strain in the crystal structure or model-built compounds. Minimizations were considered converged when the root mean square derivative of the energy function with respect to the atomic coordinate change was $\sim 1.0 \times 10^{-4}$ kcal/mol Å or less.

Three-dimensional structural features for the various molecules were analyzed using MIDAS (24), an interactive computer graphics program designed for real-time manipulation of molecular structures using an Evans and Sutherland color PS2 picture system. Solvent accessible molecular surfaces were computed using an algorithm developed by Connolly (25) and displayed with MIDAS. Electrostatic potentials were computed using atom-centered partial charges taken from Mulliken populations calculated with the CNDO/2 formalism. Previous comparison with more sophisticated *ab initio* techniques for partial charge generation have shown that CNDO/2 Mulliken charges are adequate for examination of gross electrostatic potential features (13). The electrostatic potential was computed at a distance 1.4 Å above the solvent accessible molecular surface along a normal vector from each surface point. As discussed previously, the electrostatic potential beyond the molecular surface represents the potential experienced by a receptor site or another molecule as the ligand approaches.

Binding experiments were performed in rat brain membrane homogenates as described previously (14). Fresh homogenates were used at 0–4°C immediately after sacrifice for λ-site binding. $^3$H-Naloxone served as a tracer, in the presence of a diprenorphine blocker concentration ($3 \times 10^{-7}$ *M*) to eliminate μ, κ, and δ binding. Binding to μ sites was assessed with washed brain membrane homogenates at 20°C with $^3$H-naloxone. Under these conditions, λ-site binding of the tracer is largely suppressed, and $^3$H-naloxone labels primarily μ sites. Binding curves were fitted according to a procedure outlined previously (14).

## RESULTS

Table II lists a number of opioid compounds along with their binding affinities to μ and λ sites. It appears that modification of the phenolic hydroxyl group to form an ether (morphine → codeine) destroys all affinity for the λ site and most of the μ affinity. This suggests that the phenolic OH group may bind in a region of extreme steric sensitivity (i.e., a region that is unable to accommodate groups bulkier than

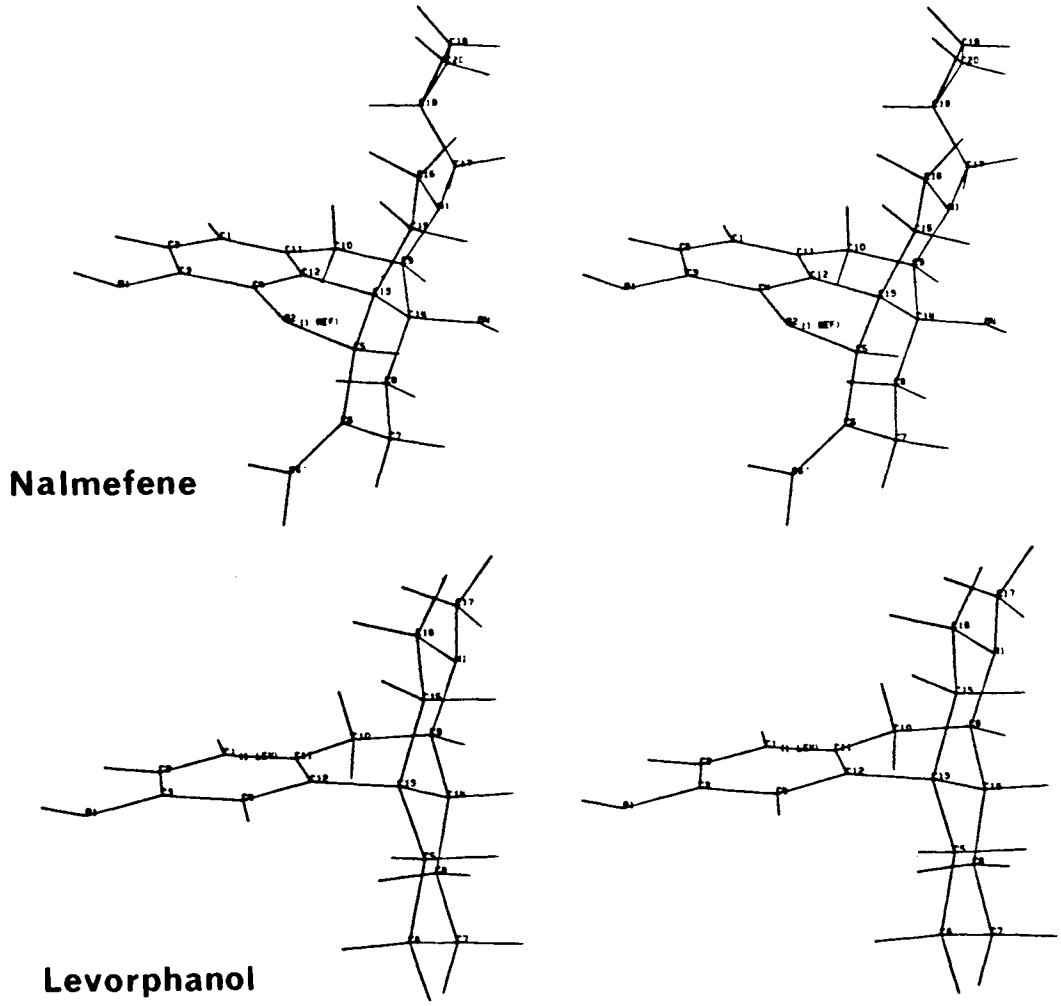



Fig. 1. Continued.

$-OH$). It is also possible that the phenolic $-OH$ group is a hydrogen bond donor at the binding site; the $-OCH_3$ derivative (codeine) is unable to function as a hydrogen bond donor. We also note that unsubstituted nitrogen (secondary amine) derivatives had substantially lower binding affinities to μ and, in particular, λ sites than their tertiary amine counterparts (e.g., oxymorphone vs *N*-noroxymorphone and morphine vs *N*-normorphine). This fact, together with the observation that large hydrophobic N substituents generally tend to enhance binding affinity, suggests a possible hydrophobic pocket at the binding site in the region where the nitrogen atom sits.

Several general qualitative assessments can be made simply by observing substitution patterns that radically alter λ-site binding affinity but have little or no effect on μ receptor affinity. 4,5-Epoxymorphinans tend to bind much more tightly than derivatives lacking the epoxy bridge at the λ site but not at the μ receptor. This difference in λ binding potency is particularly well documented by the much higher affinity of naltrexone ($IC_{50}$, 8 n*M*) than that of its close analogue S20684 without the oxygen bridge ($IC_{50}$, 3262 n*M*), while both compounds are potent μ antagonists ($IC_{50}$, 0.11 and 0.35 n*M*, respectively) (Table II). We have performed energy minimization calculations to examine the conformational effects produced by the presence or absence of the epoxy bridge (Fig. 1). Although we do find some differences in D-ring conformations between the two families, the overall deviations are not striking (Fig. 2). Computer graphics analysis of solvent accessible surfaces for the various molecules indicates that the gross steric consequences of the differing conformations are small. One notable difference that was initially intriguing concerned the orientation of potential hydrogen bond acceptors in the C6 position of the D ring. We observed different spatial orientations of the oxo- or hydroxy groups in the C6 position for high-affinity compounds as compared to ligands with a lower λ-site affinity. The spatial orientation of potential hydrogen bond acceptors appears to be unimportant, however. Compounds with mono- or difluorosubstitution at the C6 position of the D ring (INC 8339, INJ 6471-3, INJ 7747-1) display a high affinity for the λ site, and some affinity is retained in nalmefene, a compound with a methylene substituent in the C6 position. Neither the fluoro nor the methylene substituents allow for hydrogen bond formation with the binding site. Acetylation of morphine at the C6 position virtually eliminated λ-site affinity, while μ-site binding was unchanged. Similarly, the C6 hydrazone dimer naloxonazine displays a rather week λ-site affinity ($IC_{50\mu}$, 802 n*M*), while

Table II. $IC_{50}$ Values (n$M$) for Ligands at the λ and μ Binding Sites[a]

| | | |
|---|---|---|
| Oxymorphone | 2 | 1[b] |
| Naloxone | 3 | 0.6[c] |
| Naltrexone | 8 | 0.11[d] |
| Nalorphine | 11 | 0.9[c] |
| Morphine | 43 | 0.4–1[c,d] |
| Dihydromorphine | 104 | 0.75[d] |
| INC 8339 | 5 | 0.45 |
| INJ 6471-3 (NIH 9651) | 22 | 0.5[e] |
| INJ 7747-1 (NIH 9939) | 15 | 66[e] |
| Nalmefene[f] | 100 | 1[g] |
| Naloxonazine[h] | 802 | 5 |
| 6-Acetylmorphine | 1470 | 2 |
| S-20682[f] | 3262 | 0.35 |
| *N*-Noroxymorphone | 400 | 5 |
| *N*-Normorphine | 7000 | 4 |
| Levallorphan | >10,000 | 0.22[i] |
| Levorphanol | >10,000 | 0.3[c] |
| Codeine | >10,000 | 1000 |
| WIN 44,441-3 [(−)-isomer] | 63 | 2.2[j] |
| WIN 47,885 | 10,000 | 4 |

[a] $IC_{50\lambda}$ values were taken from Ref. 14 or were determined for the present study by the method described in Ref. 14 (using a 10-min incubation period). $IC_{50\mu}$ values were taken from the literature as indicated or were determined here using a standard rat brain membrane homogenate assay with $^3$H-naloxone that is described in Ref. 14. Reproducibility of the binding values obtained under identical conditions is usually within a range of ±20%. At the low tracer concentration used in the assays (14), $IC_{50}$ and $K_D$ values are similar in magnitude.
[b] From Ref. 31.
[c] From Ref. 28.
[d] From Ref. 29.
[e] From Ref. 27.
[f] Nalmefene (33) and S-20682 (34) are potent μ receptor antagonists.
[g] From M. E. Michel, Key Pharmaceuticals, Florida, personal communication ($IC_{50}$ value against $^3$H-DHM).
[h] Naloxonazine is an irreversible inhibitor of μ binding sites (35).
[i] From Ref. 30.
[j] From Ref. 32.

its $IC_{50\mu}$ is 5 n$M$. These results suggest that the λ site cannot accommodate bulky substituents at the C6 position, in contrast to the μ site.

While increased steric sensitivity of the λ site in certain regions (i.e., C6 binding region) can account for differential binding of a few ligands at the μ receptor and λ sites, we were unable to discover any obvious conformational properties that rationalized the binding data for most of these ligands. We next examined electrostatic potential surfaces for a representative subset of compounds. The electrostatic potentials were displayed on the solvent accessible surface using a scaled coloring algorithm (13). Briefly, the surface points associated with the regions of most negative potential were colored green, while those surface points associated with regions of most positive potential were colored red. Then, all points of intermediate potential were colored automatically by a scaling algorithm within MIDAS. Thus, points of relatively neutral potential were blue, points of moderately positive potential were purple to violet, etc. This coloring scheme has been used by us extensively and has proven to allow for the greatest discrimination between regions of varying electrostatic potential.

Utilizing the color-coded electrostatic potential surfaces, we analyzed a subset of high- and low-affinity λ-site ligands using the graphics software MIDAS. Distinctive differences in electrostatic potential characteristics of high- and low-affinity compounds were clearly evident. High-affinity ligands, such as oxymorphone, displayed a ridge of strongly negative potential from the phenolic oxygen through the epoxy ether bridge to the C6 substituent (carbonyl oxygen in oxymorphone; Fig. 3). Ligands with a low to moderate λ-site binding affinity (e.g., nalmefene) displayed a discontinuous or weakly negative potential across the same region, and compounds with no detectable binding affinity (e.g., levorphanol; Fig. 4) exhibited a relatively neutral or weakly positive potential in this region. Most of the low-affinity (λ) compounds lack electronegative substituents at the C6 position and/or the 4,5-epoxy bridge, so it is not surprising that they do not exhibit continuous strong negative potential across this region. Other regions of these molecules displayed no perceivable consistent differences in electrostatic potential characteristics between high- and low-affinity λ ligands.

One ligand that displayed a respectable affinity for the λ site did not seem to fit the patterns outlined above. Win 44,441-3 is a benzomorphane derivative that lacks an epoxy bridge since no D ring is present in the molecule. Molecular mechanical energy minimization and computer graphics analysis of this molecule suggest that it may be able to adopt conformations that place the side-chain carbonyl oxygen in the same relative spatial orientation to the phenolic −OH group as is observed for the C6 substituent in the other high-affinity λ-site ligands. The resultant electrostatic potential surface is not unlike those for other ligands of comparable λ-site affinity. The lack of any λ binding affinity of a Win 44,441-3 analogue without the side-chain carbonyl (Win 47,885) further supports the need for an electronegative substituent in that location. In contrast to the dependence of λ binding on the presence of the side-chain carbonyl, there was no such requirement for strong biding to the μ site ($IC_{50\mu}$ values for WIN 44,441-3 and WIN 47,885 (racemic) are 2.2 and 4 n$M$, respectively) (Table II).

## DISCUSSION

We have found that molecular electrostatic potential surface characteristics allow us to classify compounds as high- or low-affinity λ-site ligands in good agreement with experimental binding data. The strongly negative electrostatic potential across the front edge of the surface is characteristic of all high-affinity ligands and is either disrupted or absent in the low-affinity compounds. The differences in electrostatic potential surface characteristics appear to be completely unrelated to μ receptor binding affinity.

We discovered no conformational characteristics that would allow discrimination between high- and low-affinity λ-site ligands. Molecular (i.e., steric) considerations do appear to be important in certain regions of the ligands (e.g., C6 substituents), but a larger collection of compounds with more substituent variation in these regions would be necessary to address fully the importance of steric factors in distinguishing strong μ- and λ-site binders. The fact that no significant conformational differences exist between these ligands can be used to rationalize the uniformly strong

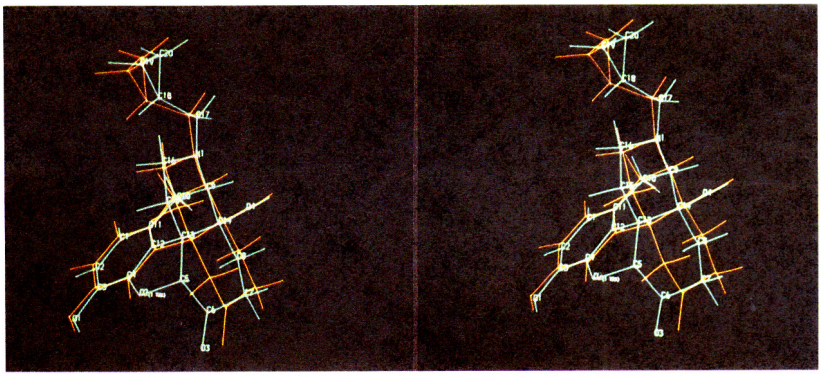


Fig. 2. Comparison of conformational differences between a 4,5-epoxymorphinan (naltrexone; white molecule) and a morphinan (S-20682; red molecule). These two molecules differ structurally only in that naltrexone has a 4,5-epoxy bridge.

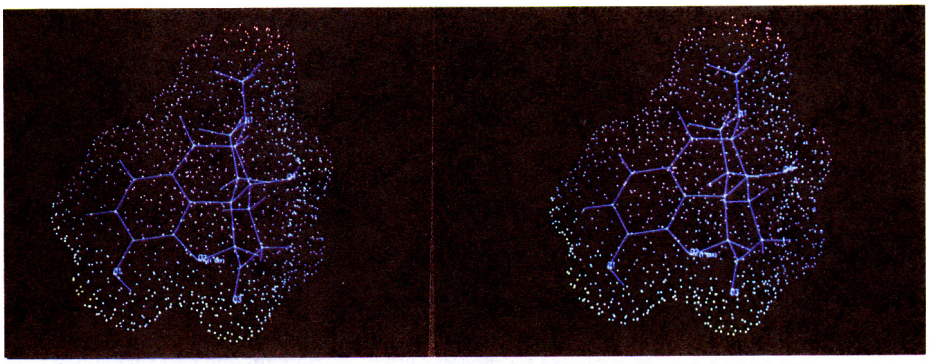


Fig. 3. Electrostatic potential surface for oxymorphone. Light green regions of surface represent strongest negative potential, while red regions of surface indicate strongest positive potential.

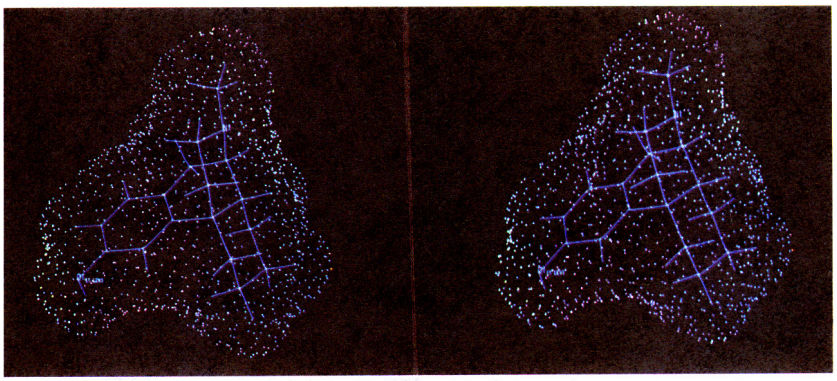


Fig. 4. Electrostatic potential surface for levorphanol. Color code as described in the legend to Fig. 3.

binding of these ligands at the μ site. For example, our results for high-affinity μ-site ligands are consistent with one pharmacophore model for the μ receptor site (9,26).

Our models suggest that the λ site may differ from the μ receptor site in that the λ site is particularly sensitive to the gross electrostatic potential surface characteristics of a ligand relative to the μ receptor. Additionally, the λ site appears to impose greater steric constraints on potential ligands. We feel that the results reported here, along with previous work by our group and others, illustrate the potential utility of theoretical chemical techniques in the study of drug–receptor interactions at binding sites of unknown structure and composition. The key feature in our work has been the integration of conformational analysis using molecular mechanics with electrostatic potential surface calculations and interactive color computer graphics studies. The absence of any one component may greatly diminish the chances for success in a study of this nature. Such studies are, as discussed above, more likely to yield useful information when the compounds in question are rigid or semirigid molecules. The procedures we have outlined will probably have far less chance for success if the ligands of interest are

conformationally "floppy" molecules, with many degrees of internal freedom and numerous local minimal energy conformations.

Since most opiate receptor ligands are relatively rigid molecules and because binding data for various receptor subtypes are available for a wide number of compounds, we are optimistic that an approach such as that outlined in this paper may allow for the elucidation of characteristics that impart opiate receptor subtype (e.g., μ, δ, κ, σ) selectivity.

## ACKNOWLEDGMENTS

This work was supported in part by Public Health Research Grant DA 01095 from the National Institute on Drug Abuse and Grant GM-29072 from the Institute of General Medical Sciences. Terry P. Lybrand is Robert Wood Johnson Memorial Fellow 1983–1984 of the American Foundation for Pharmaceutical Education.

## REFERENCES

1. G. Wipff, J. Blaney, P. Weiner, A. Dearing, and P. A. Kollman. *J. Am. Chem. Soc.* **105**:997–1005 (1983).
2. J. M. Blaney *et al. J. Am. Chem. Soc.* **104**:6424–6434 (1982).
3. T. P. Lybrand, S. C. Brown, R. H. Schafer, and P. A. Kollman. Submitted for publication (1985).
4. K. J. Miller, R. Brudzinsky, and S. Hall. *Biopolymers* **19**:2091–2121 (1980).
5. G. R. Pack and G. H. Loew. *Biochim. Biophys. Acta* **519**:163–172 (1978).
6. C. Hansch, R. Li, J. M. Blaney, and R. Langridge. *J. Med. Chem.* **25**:777–784 (1982).
7. M. Froimowitz, P. Salva, G. J. Hite, G. Gianutsos, P. Suzdak, and R. Heyman. *J. Comput. Chem.* **5**:291–301 (1984).
8. W. E. Klunk, B. L. Kalman, J. A. Ferrendelli, and D. F. Covey. *Mol. Pharmacol.* **23**:511–518 (1983).
9. C. Humblet and G. R. Marshall. *Drug Dev. Res.* **1**:409–434 (1981).
10. S. K. Burt, G. H. Loew, and G. M. Hashimoto. *Ann. N.Y. Acad. Sci.* **367**:219–239 (1981).
11. H. Weinstein, R. Osman, J. P. Green, and S. Topiol. In P. Politzer and D. G. Truhlar (eds.), *Chem. Appl. At. Mol. Electrostat. Potentials, Proc. Symp. Role Electrostat. Potentials Chem.*, 1981, pp. 309–334.
12. M. Froimowitz. *J. Med. Chem.* **25**:1127–1133 (1982).
13. P. Weiner, J. Blaney, R. Langridge, R. Schaefer, and P. Kollman. *Proc. Natl. Acad. Sci. USA* 79:3754–3758 (1982).
14. J. Grevel and W. Sadée. *Science* **221**:1198–1201 (1983); V. C. Yu, A. E. Jacobson, K. C. Rice, and W. Sadée. *Eur. J. Pharmacol.* **101**:161–162 (1984); J. Grevel, V. C. Yu, and W. Sadée. *J. Neurochem.* 44:1647–1656 (1985).
15. D. C. Perry and W. Sadée. Abstract, Int. Narcotics Res. Conf., North Falmouth, Mass. (1985).
16. P. Weiner and P. A. Kollman. *J. Comput. Chem.* **2**:287–303 (1981).
17. N. L. Allinger and Y. H. Yun. *User's Manual for MM2 and MM2P* (1980).
18. A. Hopfinger. *Conformational Properties of Macromolecules,* Academic Press, New York, 1973, pp 59–63.
19. I. L. Karle. *Acta Cryst.* **B30**:1682–1686 (1974).
20. R. J. Sime, M. Dobler, and R. L. Sime. *Acta Cryst.* **B32**:2937–2940 (1976).
21. D. Canfield, J. Barrick, and B. C. Giessen. *Acta Cryst.* **B35**:2806–2809 (1979).
22. T. G. Cochran and J. E. Abola. *Acta Cryst.* B31:919–921 (1975).
23. I. L. Karle, R. D. Gilardi, A. V. Fratini, and J. Karle. *Acta Cryst.* **B25**:1469–1479 (1969).
24. L. Gallo, C. Huang, and T. Ferrin. *UCSF, MIDAS Molecular Interactive Display and Simulation,* University of California, San Francisco.
25. M. Connolly. *QCPE Bull.* **1**:75–76 (1981).
26. D. S. Fries and P. S. Portoghese. *J. Med. Chem.* **19**:1155–1158 (1976).
27. A. E. Jacobson. In L. S. Harris (ed.), *Problems of Drug Dependence,* NIDA Research Monograph 43, 1982, pp. 389–398.
28. K.-J. Chang, E. Hazum, and P. Cuatrecasas. *Proc. Natl. Acad. Sci. USA* 77:4469–4473 (1980).
29. E. A. Barnard and C. Demoliou-Mason. *Br. Med. Bull.* **39**:37–45 (1983).
30. P. L. Wood, S. E. Charleson, D. Lane, and R. L. Hudgin. *Neuropharmacology* **20**:1215–1220 (1981).
31. S. H. Snyder, C. B. Pert, and G. W. Pasternak. *Ann. Intern. Med.* **81**:534–540 (1974).
32. P. L. Wood, C. Pilapil, M. Thakur, and J. W. Richard. *Pharm. Res.* **1**:46–48 (1984).
33. C. B. Nash and R. W. Caldwell. FACEB Abstract 3987 (1984).
34. E. Freye, E. Hartung, and G. K. Schenk. *Pharmacology* **26**:110–116 (1983).
35. E. F. Hahn, M. Carroll-Buatti, and G. W. Pasternak. *J. Neurosci.* **2**:572–576 (1982).