Structure–Activity Relationships for Drugs Binding to the Agonist and Antagonist States of the Primary Morphine Receptor

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Thirty-three opiate drugs have been considered in an investigation of the geometric and electronic features required for association with the agonist and antagonist states of the principal morphine receptor. Conformational analysis was carried out by means of molecular mechanics, and electronic properties were calculated with an ab initio SCF-MO procedure using FSGO basis sets. Statistical analysis of receptor binding based on a free-energy model reveals several properties of the molecules under study that affect the stability of the drug–receptor complex. The results suggest that the same drug conformation is involved in binding at both the agonist and antagonist states of the receptor. A single set of drug features serves to rationalize association with both receptor states, but differences in binding-site topography are revealed by the relative importance of the various structural features in the regression equations for the two states.

Although the natural ligands of opioid receptors are peptides from the endorphin and enkephalin families, foreign ligands, such as morphine and related agents, have played an important role in the study of biochemical and physiological effects of this receptor system.[1,2] Since morphine possesses limited conformational freedom, investigators have found it to be a useful reference molecule for establishing the structural requirements of narcotic analgesics and antagonists.[3–9] The high-affinity binding site of morphine has been designated as the μ subtype of opioid receptor.[10,11] A variety of morphine agonists and antagonists associate with the μ receptor, including some that exhibit higher affinity for other opioid-receptor subtypes.[12–14] This paper describes efforts made to discover critical electronic and geometric factors affecting affinity for the μ receptor through detailed structural comparisons of 33 receptor ligands.

Snyder and co-workers[12,13] discovered that an increase of sodium ion concentration in the brain membrane receptor preparation enhances the binding of triated opiate antagonists while reducing the binding of triated opiate agonists. Other workers[15,16] have confirmed this finding. The sodium effect suggests that the primary morphine binding site exists in an equilibrium between two distinct conformations: the sodium-free agonist state and the sodium-loaded antagonist state. The pharmacological effects of a μ agonist presumably depend upon the fraction of the total receptor population that it stabilizes in the sodium-free state. Indeed, rank ordering of opioid drugs according to relative affinity for the two states of the μ receptor does correlate very well with observed agonist–antagonist activity profiles.[13,16] Although earlier investigators were only able to determine apparent μ-receptor affinities at high and low concentrations of sodium ion, recent developments in the analysis of binding have made it possible to determine actual affinity constants of a variety of drugs for the agonist and antagonist states of the μ receptor.[16] These affinity constants constitute a useful data base for the development of structure–activity relationships.

Drugs from several structural classes contain a benzene ring and tertiary amine nitrogen that may assume a spatial arrangement similar to that found in morphine. These substructures, which are found in antagonists as well as agonists, serve to define the essential pharmacophore for receptor recognition in the series under study. An extension of the pharmacophore to include N-substituent features that differentiate agonists from antagonists derives from the use of a potent μ ligand, naloxone, as the reference molecule for structural comparisons. Naloxone binds with high affinity to the agonist and antagonist states of the μ receptor, while morphine has low affinity for the antagonist state. Other molecules included in this investigation are representatives from the morphine (I), morphinan (II), 6,7-benzomorphan (III), and benzamide amine (IV) classes of μ-active drugs. Molecules from classes I–III may be compared to naloxone in a relatively straightforward manner owing to obvious similarities in structure. However, additional insight into factors affecting receptor binding may be afforded by considering μ-active opiates lacking a fused-ring structure. The inclusion of molecules in group IV provides an opportunity to investigate conformational constraints in receptor binding, as well as the effects of a variety of benzene ring substituents. The techniques employed may be applied to other μ opioid agents which have not been investigated in this study.

The μ receptor has an extended binding site that accommodates various ligands possessing a second aromatic ring. Among the opioid agents of this type with demon-

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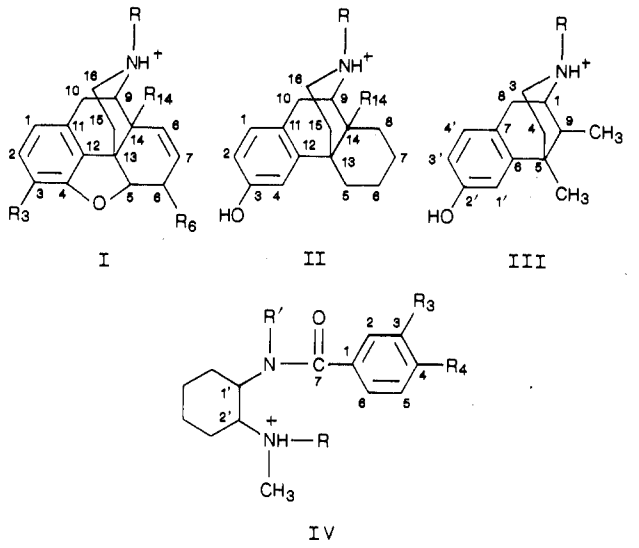

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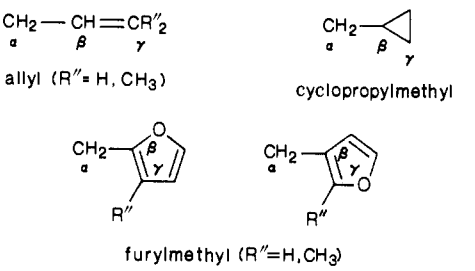
strated affinity for the receptor are the enkephalins, fentanyl, propoxyphene, phenazocine, etonitazene, and 7-(1-phenyl-3-hydroxybut-3-yl)-*endo*-ethenotetrahydrothebaine. Since the flexibility of these molecules precludes unambiguous specification of the relative positions of the two rings in binding, molecules with a second aromatic ring have been excluded from the series considered in this study. The existence of at least one secondary site for an aromatic ring makes it difficult to specify the preferred binding geometry of the 4-phenylpiperidines, which poorly match the morphine pharmacophore and weakly displace [^{3}H]naloxone from the μ receptor. Portoghese[17] has presented evidence that certain congeners of the 4-phenylpiperidines, the allylprodines, do not bind in the same manner as morphine and naloxone. Therefore, no representative molecules of this type are included in the drugs under investigation.

Methods

Receptor Association. Assays of receptor binding were performed in a buffered homogenate of rat-brain membranes with [^{3}H]naloxone as the displaceable marker. Analysis of the binding experiments was carried out in terms of a two-state model of the μ receptor. As described elsewhere,[16,18] the procedure yields values of the affinity constants, K_r for the agonist state and K_p for the antagonist state.

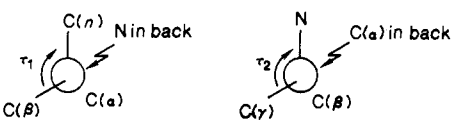
Geometry and Electronic Structure. X-ray crystallographic data were employed for the initial nuclear coordinates of morphine,[19] nalorphine,[19] naloxone,[20] cyclazocine,[21] and *trans*-*N*-[2-(dimethylamino)cyclohexyl]-*N*-methyl-3,4-dichlorobenzamide.[22] Congeners of these molecules were constructed from the skeleton of the parent molecule. No experimental coordinates were available for levorphanol or levallorphan, so three-dimensional molecular structures were developed by modifying the 1*R*,5*R*,9*R* isomers of metazocine and *N*-allyl-*N*-normetazocine.

Among the compounds under study, the N-substituent, R, may be either a methyl or one of the extended groups shown below:



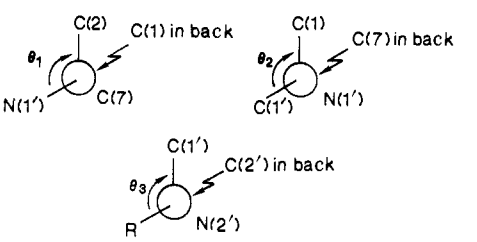
allyl (R″= H, CH_3) cyclopropylmethyl furylmethyl (R″=H,CH_3)

The orientation of an extended N-substituent may be described in terms of two torsional angles, τ_1 and τ_2, defined in the following Newman projections:



where C(n) is C(16) for molecules in classes I and II, C(3) in series III, and C(2′) in class IV.

Unlike the relatively rigid fused structures in classes I–III, the molecules in series IV exhibit several bonds about which rotation occurs. For conformations of interest in a benzamide amine, three torsional angles (θ_1, θ_2, and θ_3) serve to specify the relative orientations of major substructures within the molecule. The following diagrams illustrate these angles:



The geometry of every molecule was totally optimized by using the Duchamp molecular mechanics procedure.[23] Low-energy conformations of each molecule were found by using the same methods employed in previous studies of naloxone,[24] the benzomorphans,[25] and the benzamide amines.[18] All calculations were carried out on the N-protonated cation of each drug.

On the basis of the results obtained from a limited structure–activity study[18] involving comparisons of the benzamide amines to naloxone, the naloxone conformer illustrated in Figure 1 may be used to define the pharmacophore for both the agonist and antagonist states of the μ receptor. This structure is the 5*R*,9*R*,13*R*,14*S* isomer with $\tau_1 = 80°$ and $\tau_2 = 93°$. Other molecules were matched to this naloxone structure by using a set of three benzene ring carbons and the amine nitrogen as points of correspondence. In cases where the molecule possesses an extended N-substituent, three additional links were established to the allyl carbons of naloxone. The geometric comparison was accomplished by superimposing each rigid test molecule on naloxone in cartesian space via a proce-

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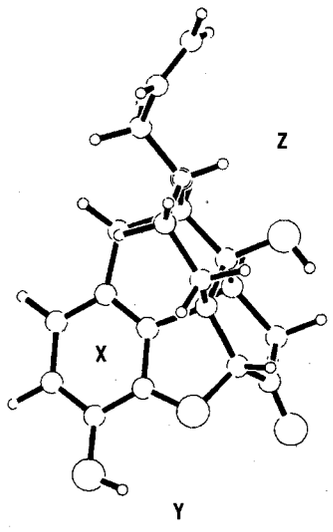

Figure 1. Pharmacophore conformation of naloxone eutomer showing putative receptor nucleophilic groups X, Y, and Z that interact, respectively, with the aromatic ring, 3-hydroxy, and cationic head of the drug.

dure designed to give a least-squares fit of the distance between matched points. In cases where no low-energy conformer provided a good match to naloxone (i.e., the mean distance between linked points exceeded 0.3 Å), the molecule was allowed to adapt its conformation in a molecular mechanics calculation with "extrapotential constraints" linking matched atoms. This method, described in detail elsewhere,[23] provides an estimate of the strain energy required to attain the naloxone-like binding configuration. Differences between the "free" and "constrained" configurations of the molecules under study could be described essentially by changes in torsional angles since the constraints were kept weak enough to prevent serious distortions of bond lengths and bond angles. If the drug was assayed as a racemic compound, both enantiomers were fit to the naloxone pharmacophore.

By means of the ab initio molecular fragment technique,[26] the ground-state electronic structure of each N-protonated cation was characterized in the conformation matching the naloxone pharmacophore. In this procedure, molecular orbitals (MO, ϕ_i) are written as linear combinations of floating spherical Gaussian orbitals (FSGO, G_s) by using the relationship

$$\phi_i = \sum_s c_{si} G_s \tag{1}$$

where the sum extends over all FSGO basis functions and c_{si} is a coefficient found by energy minimization of the wave function. The values of the MO energies, ϵ_i, are also found by this calculation. For large molecules, where it is impractical to employ extensive basis sets, the approximate results obtained with FSGOs serve to reveal trends in many properties of interest.

As a means of examining the capability of a drug to engage the receptor in Coulombic interactions, the molecular electrostatic interaction potential, $V(r)$, was calculated by using the following approximation:

$$V(r) = \sum_\alpha (Z_\alpha/|r - R_\alpha|) - \sum_s (q_s/|r - R_s|) \tag{2}$$

In eq 2, Z_α is the charge of nucleus α located at R_α and q_s is a partial electronic charge situated at R_s, which is the center of Gaussian G_s. The method of Shipman[27] was employed to partition the electronic charge among the FSGO basis functions.

The possibility that charge-transfer interactions contribute to drug–receptor-complex formation has also been considered. Since cations favor the approach of electron donors, special note has been made of the low-energy unoccupied molecular orbitals (LUMOs) that may serve as electron acceptors. The acceptor LUMOs of different drug molecules must have similar characteristics to permit interaction with a particular donor orbital in the binding pocket of the receptor. The regions of high density in an acceptor LUMO indicate where overlap with the donor orbital occurs, and the energy of the LUMO provides a measure of its readiness to receive electronic charge. In a simple Mulliken[28] charge-transfer complex, the donor orbital ϕ_d overlaps a single acceptor orbital ϕ_a'. However, there are circumstances where a manifold of LUMOs $\{\phi_u^*\}$ may engage in donor–acceptor interactions with ϕ_d since each member of the set shares some characteristics of the primary acceptor orbital, ϕ_a'. If ϕ_a' may be defined as one of a group of orthonormal localized orbitals $\{\phi_p'\}$ used in a transformation to represent $\{\phi_u^*\}$, the "effective" acceptor energy of the manifold may be given by

$$\epsilon_a^* = \sum_u (c_{au}')^2 \epsilon_u^* \tag{3}$$

where ϵ_u^* is the energy of ϕ_u^* determined in the SCF-MO calculations on the drug molecule and c_{au}' is the coefficient of ϕ_a' in the expansion of ϕ_u^*. The method employed to determine c_{au}' has been outlined elsewhere.[29]

Structure–Activity Relationships. Since many of the drugs under study exist as racemic mixtures, the system at equilibrium consists of the drug enantiomers, M and M′; the receptor R in either the agonist or antagonist state; and the drug–receptor complexes, C and C′, involving the eutomer and distomer, respectively. For the purpose of analysis, a hypothetical case is considered in which all receptors exist in the same state, so that the subscript on R and K may be omitted in the discussion. The resulting model will be applied individually to drug binding at the agonist and antagonist states of the receptor hereafter. The techniques of statistical mechanics (see supplementary material) may be employed to give the following expression for the equilibrium constant:

$$K = \frac{x q_C \exp(-\Delta\epsilon/kT) + (1 - x) q_{C'} \exp(-\Delta\epsilon'/kT)}{q_M q_R} \tag{4}$$

where x is the fraction of M in the racemic mixture of M and M′; q_i is related to the partition function for the ith molecule; $\Delta\epsilon$ and $\Delta\epsilon'$ are the dissociation energies of C and C′, respectively; k is Boltzmann's constant; and T is the absolute temperature. As a simplifying assumption, the value of x is taken to be $^1/_2$ for the racemic mixtures in this study. Therefore, eq 4 reduces to

$$K = [(q_C/q_M q_R) \exp(-\Delta\epsilon/kT) + (q_{C'}/q_M q_R) \exp(-\Delta\epsilon'/kT)]/2 \tag{5}$$

Although the molecules in the system are insufficiently characterized to use eq 5 in the calculation of the equilibrium constant, a model to account for the effects of various drug features on μ-receptor binding has been developed by restructuring the relationship in the form

$$\ln K = \ln \{[\exp(B) + \exp(B')]/2\} \tag{6}$$

where B and B' are free-energy-related terms associated

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with the eutomer and distomer, respectively. If Ω_r is a drug property or structural feature that affects the stabilization energy of the drug–receptor complex and ΔE is the energy required to attain the binding conformation, B may be expressed as

$$B = d_0 + \sum_r d_r\Omega_r - \Delta E/RT \quad (7)$$

where d_r is a parameter to be determined by regression. Under the conditions of the binding experiments, RT = 0.54 kcal/mol. A similar equation may be written for B' in which the rth regression variable and the conformational energy are denoted by Ω_r' and $\Delta E'$, respectively. If the bound population consists largely of the eutomer, eq 6 will be only weakly nonlinear and may be usefully rewritten as follows:

$$\ln K = B + \ln\{[1 + \exp(\Delta B)]/2\} \quad (8)$$

where

$$\Delta B = \sum_r d_r(\Omega_r' - \Omega_r) - (\Delta E' - \Delta E)/RT \quad (9)$$

Some of the molecules under study exhibit the essential pharmacophore in more than one low-energy conformational state. In such a case, the structure factor Ω_r and binding energy ΔE in eq 7 must be replaced by conformational averages. For example, if Ω_{rj} is the value of the rth structure factor for the jth conformation, the average, $\langle\Omega_r\rangle$, is given by

$$\langle\Omega_r\rangle = \sum_j \Omega_{rj} \exp(\sum_r d_r\Omega_{rj} - \Delta E_j/RT)/\sum_j \exp(\sum_r d_r\Omega_{rj} - \Delta E_j/RT) \quad (10)$$

Due to the nonlinearity introduced by eq 8 and 10, an iterative process must be employed to determine the magnitude of the coefficients d_r. The calculations have been performed by means of eq 8, beginning with a stepwise multiple linear regression computation in which the term $\exp(\Delta B)$ was ignored and the coefficients d_r in eq 10 were set to 0. In succeeding iterations the correction factor ΔB was determined from eq 9. When all coefficients d_r differed by less than 0.1% from one iteration to another, convergence was considered to have been achieved.

Results and Discussion

Geometric Comparisons. If an opiate drug assumes a naloxone-like binding configuration, those critical structural features that are common to both molecules will occupy the same sites on the receptor. The relative locations of other substituents at the binding site may be inferred from the structural comparisons to naloxone. This information may be helpful in discerning the effects of various substituents on receptor affinity.

Structural features of the 33 drugs under investigation are described in Table I, where the reference drug, naloxone, is listed as compound 1. In drugs from classes I and III, a low-energy conformer of the eutomer may be directly superimposed on the pharmacophore of naloxone with corresponding atoms separated by less than 0.3 Å. The drugs from class II, levallorphan (**13**) and levorphanol (**14**), also fit the naloxone pharmacophore without extrapotential constraints. An optimal match of the distomer in series I or III could only be obtained by reorienting the aromatic ring to bring the nitrogen atoms into proximity and placing the N-substituent in an axial position on the piperidine ring. The fit of the distomer with an axial N-substituent was obtained at an energy cost that ranged from 0.6 kcal/mol for R = CH_3 to as much as 4.3 kcal/mol for an extended R group. The matches shown in Figure 2 for the isomers of pentazocine (**16**) are typical of the pharmacophore fits achieved with drugs from classes I and III. Most eutomers in these classes, like (1*R*,5*R*,9*R*)-pentazocine, lie within the van der Waals envelope of (5*R*,9*R*,13*R*,14*S*)-naloxone except for the protrusions of differing N-substituents. On the other hand, the distomers resemble (1*S*,5*S*,9*S*)-pentazocine in that several groups extend beyond the van der Waals surface of the naloxone eutomer; the most notable are the phenolic hydroxy, the exposed backbone of the piperidine ring, the 5- and 9-methyls of a benzomorphan, and the C ring of a morphine-type drug.

The coordinates obtained through total geometry optimization of the isolated *trans*-*N*-[2-(dimethylamino)-cyclohexyl]-*N*-methyl-3,4-dichlorobenzamide (**26**) are given in the supplementary material. The lowest energy conformers of other benzamide amines were found to exhibit similar geometries with regard to the relative orientations of the benzene ring, amide moiety, and cyclohexyl ring. Since none of the benzamide amines included in this study has been resolved, both enantiomers have been considered in developing the binding model. Details of these calculations have been described elsewhere.[18]

No low-energy conformer of any drug in class IV could be made to fit the pharmacophore of naloxone. Nevertheless, by appropriate selection of the benzene ring orientation relative to the phenol ring of naloxone and application of the forced matching technique, the *R*,*R* and *S*,*S* isomers were both superimposed on (5*R*,9*R*,13*R*,14*S*)-naloxone at energy costs ranging from 2.4 to 8.0 kcal/mol depending on the nature of the N-substituent, R. Among the alternative ways of positioning the aromatic ring of the benzamide amine, one yielded a close approach of corresponding atoms in the pharmacophore (mean separation <0.6 Å) at a significantly lower energy than all others. In general, the *S*,*S* isomer overlaid the pharmacophore with somewhat greater precision and lower strain than the *R*,*R* isomer. Figure 3 portrays the matches achieved with the isomers of *N*-methyl-3,4-dichlorobenzamide allylamine **23** as typical examples of the fits obtained with molecules in class IV. The overlay of the *S*,*S* structure places C(1) and C(4) of the benzamide amine near C(12) and C(2), respectively, of (5*R*,9*R*,13*R*,14*S*)-naloxone. Depending upon the value of θ_1, a meta substituent on the benzene ring would lie in the vicinity of either O(3) or H(1) of naloxone. Among the groups that noticeably jut beyond the van der Waals surface of naloxone are a para substituent, a meta substituent in the H(1) position, the methyls on N(1′) and N(2′), and the C(3′)–C(6′) segment of the cyclohexyl ring. Superposition of the *R*,*R* isomer places C(1) and C(4) of the benzamide amine near C(11) and C(3), respectively, of the naloxone pharmacophore. In this case, a para substituent on the benzene ring occupies the site of O(3) in naloxone, and a meta substituent is in the neighborhood of either H(2) or O(4) depending upon the ring orientation. The moieties of the *R*,*R* isomer that extend beyond the envelope of naloxone are the entire cyclohexane ring, the amide oxygen, and a methyl attached to N(1′). Aside from *R*,*R* meta and *S*,*S* para substituents lying in the position taken by H(2) of naloxone, no exposed groups in the *S*,*S* and *R*,*R* isomers occupy the same region of space.

In the absence of information concerning the topography of the binding pocket, it is not possible to say a priori whether differences in the spatial requirements of the stereoisomers have a significant effect on the fit to the μ receptor. If the capacity of the pocket is sufficient to allow entry of both isomers of all compounds, selectivity for the eutomer would result from two factors: first, the higher strain energy required to hold the distomer in the optimal configuration for binding; second, the incorrect placement of key substituents on the distomer relative to reactive

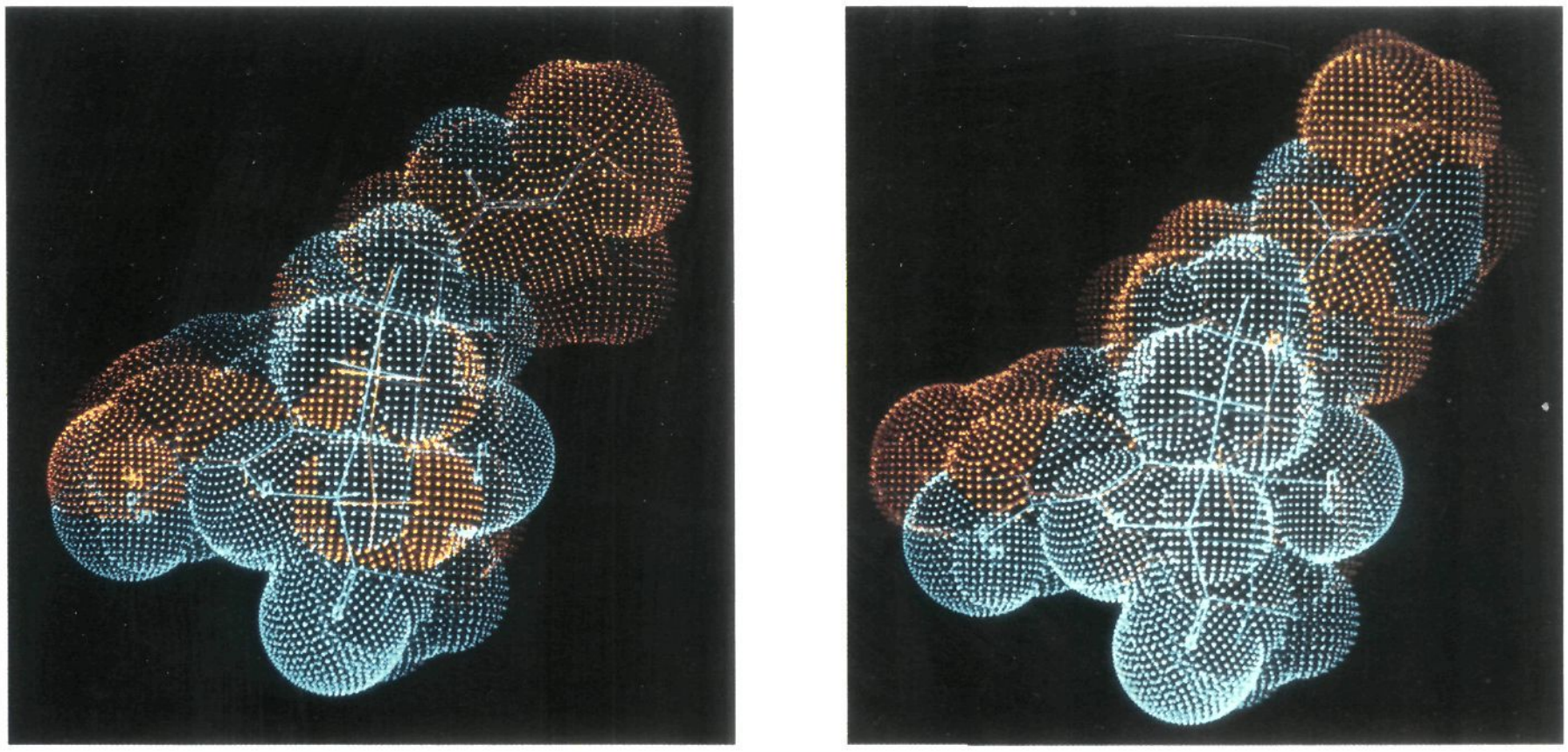


Figure 2. Calculated van der Waals envelope of pentazocine (**16**, orange) superimposed on naloxone (**1**, blue). The eutomer (A, left) and distomer (B, right) are the 1*R*,5*R*,9*R* and 1*S*,5*S*,9*S* isomers, respectively, of the benzomorphan molecule. The merged benzene rings are located at the lower left and the *N*-allyl groups at the upper right. The volume requirements of the molecules in the receptor binding pocket are suggested by the space-filling representation. In both cases, there is clear access to the benzene ring, the protonated nitrogen, and the *N*-allyl substituent. The phenolic hydroxy of the eutomer, but not the distomer, overlays the 3-OH of naloxone.

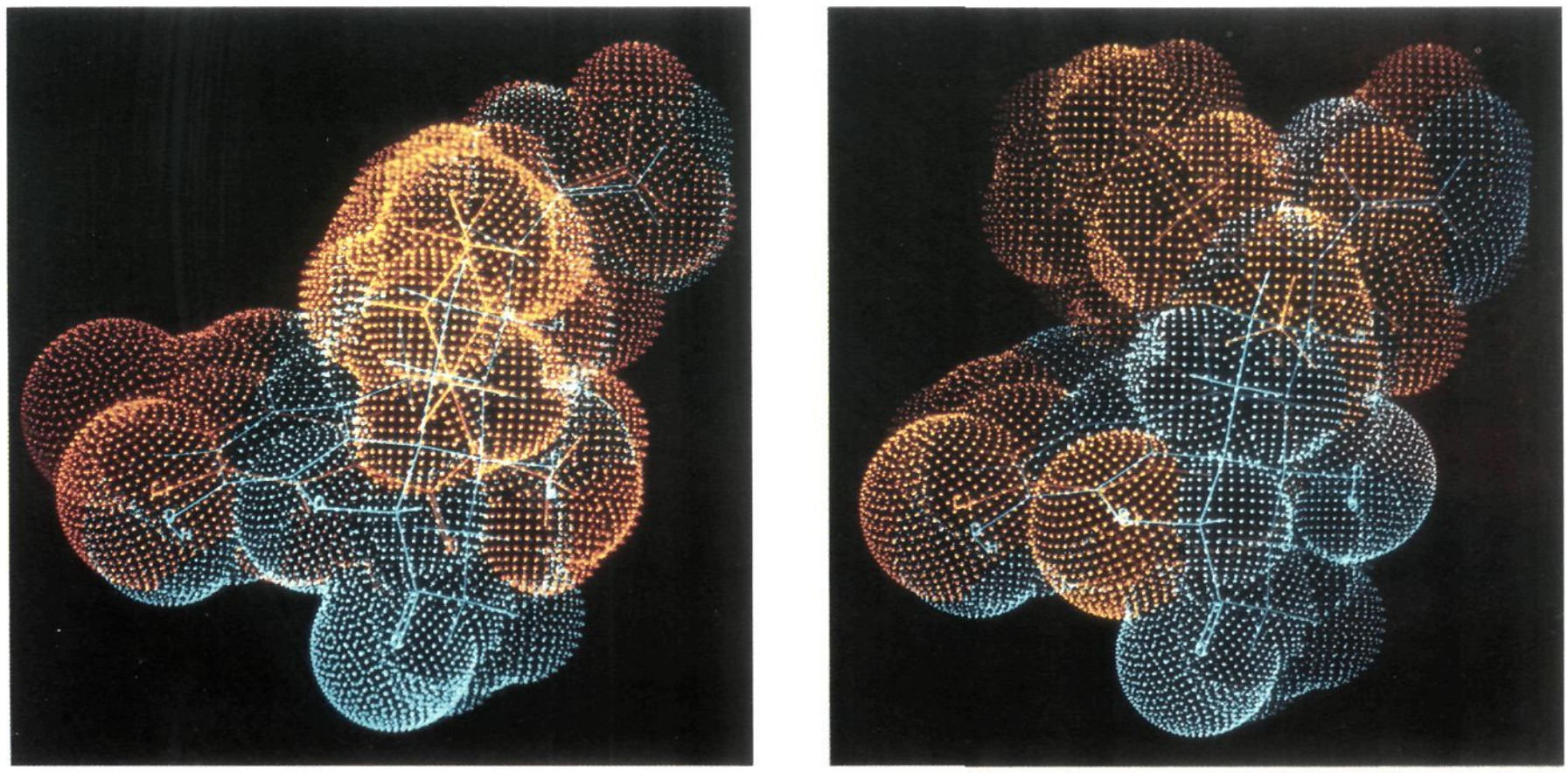


Figure 3. Match of *N*-methyl-3,4-dichlorobenzamide allylamine **23** (orange) to naloxone (**1**, blue) showing van der Waals surface of merged molecules. The 1′*S*,2′*S* isomer (A, left) is the eutomer, and the 1′*R*,2′*R* isomer (B, right) is the distomer. Naloxone has the same orientation as in Figure 2. Receptor entities in the binding pocket would have open approaches to the aromatic ring (lower left) and *N*-allyl group (upper right) of each molecule. However, the orientation of the *R*,*R* *N*-allyl is somewhat skewed relative to the corresponding feature of naloxone.

receptor groups within the binding pocket. The structure–activity model described above provides a basis for examining these factors through statistical analysis.

Pharmacophore Features. In order to develop quantitative structure–activity relationships, it is necessary to identify electronic properties that play a role in stabilization of the drug–receptor complex. The initial focus of this task must be the essential pharmacophore, which consists of the aromatic ring and protonated tertiary amine common to all drugs included in this study. After the key properties underlying receptor recognition and attachment have been determined, it may be possible to discern the effects of other moieties on the affinity for the two states of the μ receptor.

Several structural features required for high μ-receptor affinity have already been established in earlier studies.[18,24,25] The pertinent findings are summarized in this section, and a listing of the geometry-dependent structure factors for all molecules under consideration is given in Table I.

Electrostatic interactions undoubtedly make the greatest contribution to the stabilization of the drug–receptor

Table I. Torsional Angles and Geometry-Dependent Structural Data

no.	class	isomer[a]	R[b]	τ_1	τ_2	ΔE	ϵ_ϕ*	ϵ_ν*	aromatic ring substituent factors[c] δ_H	δ_{R3}
1	I^{d-g}	5*R*,9*R*,13*R*,14*S*	A	80	93	0.2	0.2355	0.1512	0	0
		5*S*,9*S*,13*S*,14*R*		74	188	4.3	0.2104	0.1815	1	0
2	I^{d-g}	5*R*,9*R*,13*R*,14*S*	B	76	88	0.0	0.2376	0.3378	0	0
		5*S*,9*S*,13*S*,14*R*		70	181	3.8	0.2118	0.3565	1	0
3	I^{d-g}	5*R*,9*R*,13*R*,14*S*	C	–	–	0.0	0.2336	0.7561	0	0
		5*S*,9*S*,13*S*,14*R*		–	–	2.1	0.2117	0.7561	1	0
4	I^{e-g}	5*R*,9*R*,13*R*,14*S*	C	–	–	0.0	0.2256	0.7561	0	1
		5*S*,9*S*,13*S*,14*R*		–	–	2.1	0.2094	0.7561	1	0
5	$I^{e,f,i,j}$	5*R*,9*R*,13*S*,14*R*	C	–	–	0.0	0.1892	0.7561	1	0
		5*S*,9*S*,13*R*,14*S*		–	–	0.6	0.1939	0.7561	1	0
6	$I^{d,j,k}$	5*R*,6*S*,9*R*,13*S*,14*R*	A	79	93	0.3	0.2390	0.1563	0	0
		5*S*,6*R*,9*S*,13*R*,14*S*		69	191	2.4	0.2167	0.1774	1	0
7	$I^{d,j,k}$	5*R*,6*S*,9*R*,13*S*,14*R*	C	–	–	0.0	0.2372	0.7561	0	0
		5*S*,6*R*,9*S*,13*R*,14*S*		–	–	0.6	0.2168	0.7561	1	0
8	I^{i-k}	5*R*,6*S*,9*R*,13*S*,14*R*	C	–	–	0.0	0.1940	0.7561	1	0
		5*S*,6*R*,9*S*,13*R*,14*S*		–	–	0.6	0.2020	0.7561	1	0
9	$I^{h,j,k}$	5*R*,6*S*,9*R*,13*S*,14*R*	C	–	–	0.0	0.2307	0.7561	0	1
		5*S*,6*R*,9*S*,13*R*,14*S*		–	–	0.6	0.2155	0.7561	1	0
10	$I^{f,i-k}$	5*R*,6*S*,9*R*,13*S*,14*R*	A	78	94	0.3	0.1957	0.1544	1	0
		5*S*,6*R*,9*S*,13*R*,14*S*		72	184	2.4	0.2026	0.1766	1	0
11	$I^{d,f,j,k}$	5*R*,6*S*,9*R*,13*S*,14*R*	C	–	–	0.0	0.2349	0.7561	0	0
		5*S*,6*R*,9*S*,13*R*,14*S*		–	–	0.6	0.2170	0.7561	1	0
12	$I^{f,i,j,l}$	5*S*,9*R*,13*S*,14*R*	C	–	–	0.0	0.1907	0.7561	1	0
		5*R*,9*S*,13*R*,14*S*		–	–	0.6	0.1968	0.7561	1	0
13	II	9*R*,13*R*,14*R*	A	77	90	0.0	0.2322	0.1939	0	0
14	II	9*R*,13*R*,14*R*	C	–	–	0.0	0.2355	0.7561	0	0
15	III	1*R*,5*R*,9*R*	A	79	91	0.3	0.2326	0.1583	0	0
		1*S*,5*S*,9*S*		70	193	2.3	0.1913	0.1779	1	0
16	III	1*R*,5*R*,9*R*	A	80	93	0.0	0.2341	0.1927	0	0
		1*S*,5*S*,9*S*		70	188	2.6	0.1934	0.2195	1	0
17	III	1*R*,5*R*,9*R*	B	75	80	0.2	0.2341	0.3462	0	0
		1*S*,5*S*,9*S*		68	183	2.3	0.1946	0.3541	1	0
18	III	1*R*,5*R*,9*R*	C	–	–	0.0	0.2293	0.7561	0	0
		1*S*,5*S*,9*S*		–	–	0.6	0.1880	0.7561	1	0
19	III	1*R*,5*R*,9*R*	D	79	72	0.7	0.2321	0.2432	0	0
		1*S*,5*S*,9*S*		72	194	4.3	0.1913	0.2638	1	0
20	III	1*R*,5*R*,9*R*	D	78	71	0.5	0.2329	0.2579	0	0
		1*S*,5*S*,9*S*		72	194	4.3	0.1920	0.2812	1	0
21	III	1*R*,5*R*,9*R*	E	79	82	0.8	0.2344	0.2343	0	0
		1*S*,5*S*,9*S*		67	196	3.3	0.1951	0.2634	1	0
22	III	1*R*,5*R*,9*R*	E	77	74	0.7	0.2345	0.2513	0	0
		1*S*,5*S*,9*S*		68	197	3.0	0.1943	0.2736	1	0
23	IV^{m-p}	1′*S*,2′*S*	A	301	104	5.6	0.1334	0.1629	0	1
		1′*S*,2′*S*		301	104	5.6	0.1368	0.1629	1	0
		1′*R*,2′*R*		51	227	7.2	0.1436	0.1623	0	1
24	$IV^{i,m,n,q}$	1′*S*,2′*S*	A	297	106	5.6	0.1913	0.1723	1	0
		1′*R*,2′*R*		51	227	7.2	0.2337	0.1701	0	0
25	$IV^{i,m,n,r}$	1′*S*,2′*S*	B	294	96	5.6	0.1793	0.3693	1	0
		1′*R*,2′*R*		51	227	7.2	0.1845	0.3505	0	1
26	IV^{m-p}	1′*S*,2′*S*	C	–	–	2.4	0.1415	0.7561	0	1
		1′*S*,2′*S*		–	–	2.4	0.1450	0.7561	1	0
		1′*R*,2′*R*		–	–	2.8	0.1505	0.7561	0	1
27	$IV^{h,m,n,s}$	1′*S*,2′*S*	C	–	–	2.4	0.2300	0.7561	0	1
		1′*S*,2′*S*		–	–	2.4	0.2031	0.7561	1	0
		1′*R*,2′*R*		–	–	2.8	0.2068	0.7561	1	0
28	$IV^{i,m,n,q}$	1′*S*,2′*S*	C	–	–	2.4	0.1993	0.7561	1	0
		1′*R*,2′*R*		–	–	2.8	0.2435	0.7561	0	0
29	$IV^{i,m,n,t}$	1′*S*,2′*S*	C	–	–	2.4	0.1627	0.7561	1	0
		1′*R*,2′*R*		–	–	2.8	0.1601	0.7561	0	1
30	$IV^{i,m,n,u}$	1′*S*,2′*S*	C	–	–	2.4	0.1583	0.7561	1	0
		1′*R*,2′*R*		–	–	2.8	0.1494	0.7561	0	1
31	$IV^{i,m,n,p}$	1′*S*,2′*S*	C	–	–	2.4	0.1616	0.7561	1	0
		1′*R*,2′*R*		–	–	2.8	0.1706	0.7561	0	1
32	$IV^{m,o,p,v}$	1′*S*,2′*S*	A	297	106	5.6	0.1337	0.1620	0	1
		1′*S*,2′*S*		297	106	5.6	0.1371	0.1620	1	0
		1′*R*,2′*R*		52	236	7.2	0.1427	0.1592	0	1
33	$IV^{i,m,q,v}$	1′*S*,2′*S*	C	–	–	2.4	0.1939	0.7561	1	0
		1′*R*,2′*R*		–	–	2.5	0.2401	0.7561	0	0

[a] The eutomer is listed before the distomer. [b] N-Substituents are denoted as follows: A, allyl; B, cyclopropylmethyl; C, methyl; D, 2-furylmethyl; E, 3-furylmethyl. In classes I and III, the N-substituent of the distomer takes an axial position on the piperidine ring. [c] The variables δ_H and δ_{R3} indicate that the aromatic ring carbon corresponding to C(3) of naloxone in the matched structures carries a substituent other than hydroxy. If an H atom is attached to the carbon, $\delta_H = 1$; otherwise $\delta_H = 0$. If neither H nor OH is attached to the ring carbon

Footnotes to Table I (Continued)

of interest, $\delta_{R3} = 1$; otherwise $\delta_{R3} = 0$. [d] R_3 is OH. [e] R_6 is =O. [f] 7,8-Dihydro. [g] R_{14} is OH. [h] R_3 is OCH_3. [i] R_3 is H. [j] R_{14} is H. [k] R_6 is OH. [l] R_6 is H. [m] Torsional angles for the 1′*S*,2′*S* isomer are $\theta_1 = 273 \pm 1$ or 93 ± 1 depending on the placement of a meta substituent, $\theta_2 = 248 \pm 2$, and $\theta_3 = 209 \pm 3$. The angles for the 1′*R*,2′*R* isomer are $\theta_1 = 253 \pm 6$, $\theta_2 = 108 \pm 4$, $\theta_3 = 121 \pm 3$ if R is allyl, $\theta_3 = 134$ if R is cyclopropylmethyl, and $\theta_3 = 147 \pm 3$ if R is methyl. [n] R′ is CH_3. [o] R_3 is Cl. [p] R_4 is Cl. [q] R_4 is OH. [r] R_4 is CF_3. [s] R_4 is H. [t] R_4 is NO_2. [u] R_4 is CN. [v] R′ is H.

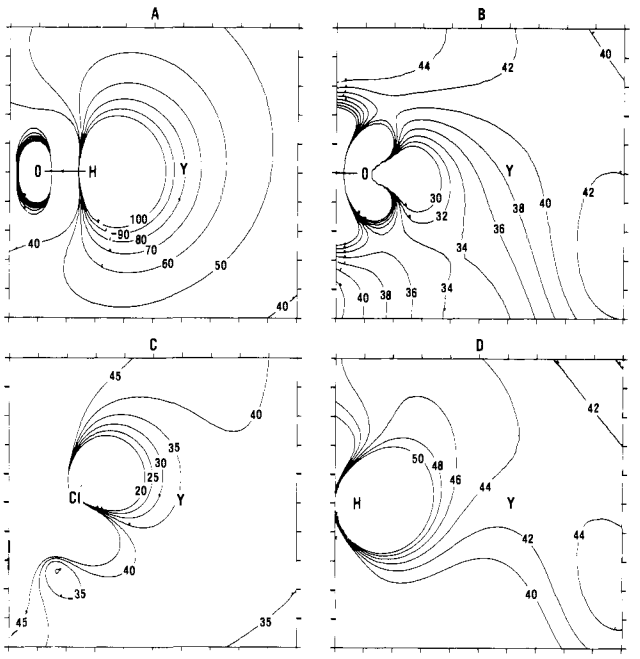


Figure 4. Contour maps of the molecular electrostatic potential near R_3 for the following molecules: (A) naloxone (**1**), (B) codeine (**9**), (C) 3,4-dichlorobenzamide amine (**26**), and (D) 3-deoxymorphine (**8**). The plane of each map is perpendicular to the aromatic ring. In map A, the origin is located at O(3) and the *x* axis lies along the O(3)–H bond. A polar, hydrogen-bonding receptor group would experience highly favorable interactions if its negative terminus were placed on the *x* axis at the position indicated by Y. The gradient of the potential is very steep near Y and favors an orientation of the dipole with the positive terminus directed away from O(3). In maps B–D, the spatial location of the plane relative to the aromatic ring is the same as in map A. When R is OCH_3 (B) or Cl (C), changes in $V(r)$ near Y are not large, and the slope of the potential requires reorientation of the receptor group so that the positive terminus of the dipole approaches the O or Cl atom. On the other hand, when the 3-substituent is H (D), the flatness of $V(r)$ near Y precludes effective interaction with the dipole.

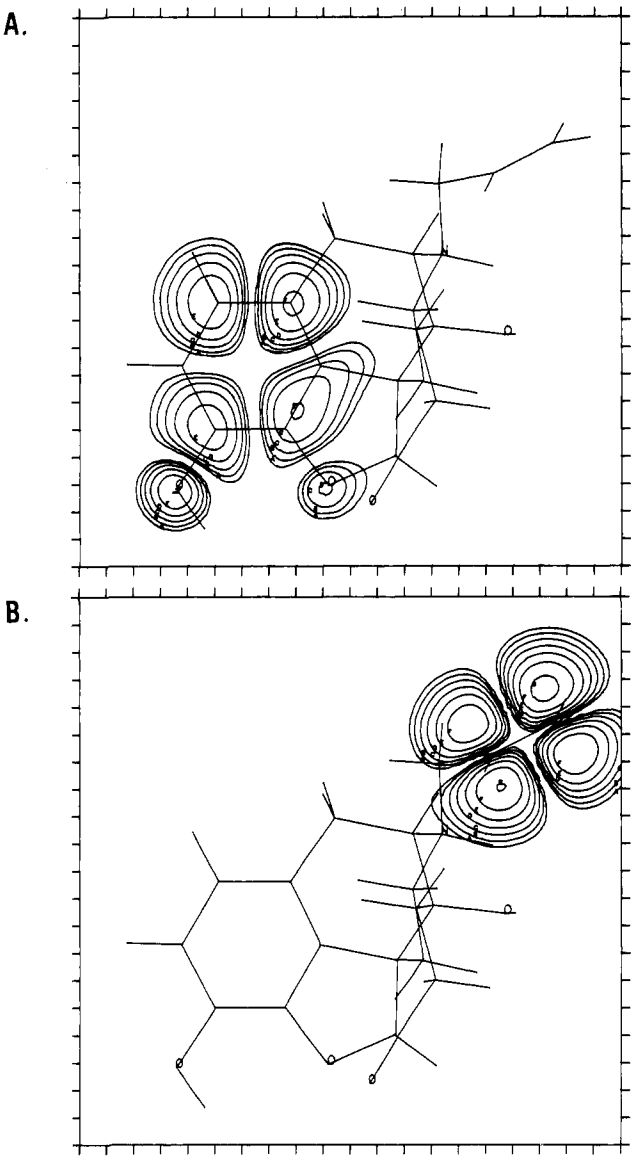


Figure 5. Contour maps of the principal electron-acceptor LUMOs in the aromatic ring (MO 90) and *N*-allyl (MO 88) of naloxone. (A) The plane of the map illustrating MO 90 is located 0.4 Å below the aromatic ring. (B) The plane for MO 88 is 2.8 Å above the ring.

complex. Computations of $V(r)$ reveal that the environment of the cations is favorable only to the close approach of electron-rich entities, i.e., anions or the negative termini of polar groups. The optimal site for a nucleophilic receptor group, Z, is near the protonated amine. Although the orientation of the amine N–H bond differs considerably among the molecules under study, the molecular electrostatic potential does not vary greatly in the region of approach for Z. Thus, Coulombic interactions involving the cationic head may be quite similar for all drugs in the series.

The contour map of $V(r)$ shown in Figure 4A exhibits an extremely favorable site for a hydrogen-bonding H acceptor, Y, near the 3-OH of the naloxone pharmacophore. However, contour maps in Figure 4B–D, obtained from molecules with other substituents in the corresponding site, reveal markedly different values of the electrostatic potential. If Y is a polar group, such as an OH, that is free to reorient in the electrostatic field of the drug molecule, it could associate weakly with Cl or OCH_3, but there would be essentially no attraction for H. Two structure factors have been defined to specify the effect of replacing the phenolic hydroxy with other entities: δ_H designates the presence of H in the site of the 3-OH, and δ_{R3} denotes either OCH_3 or Cl in that position.

Correlations between LUMO energies and receptor affinity obtained in previous work[18,25] suggest that charge-transfer interactions play an important role in stabilizing the drug–receptor complex. Two substructures, the aromatic ring and N-substituent, were implicated as separate participants in such interactions. The primary acceptor orbitals in the ring and an extended N-substituent are π^* MOs with sites of high density shown in Figure 5, where naloxone serves as the example. The capacity of the aromatic ring and N-substituent to act as electron acceptors has been measured in terms of the effective acceptor energies, ϵ_ϕ^* and ϵ_ν^*, calculated by means of eq 3 using the data provided in the supplementary material.

For the drugs with R = CH_3, the only N-substituent LUMOs with the capacity to act as electron acceptors are

Table II. Correlation Matrix of Variables[a]

	$\langle\Delta E\rangle$	$\langle\epsilon_\phi{}^*\rangle$	$\langle\epsilon_\nu{}^*\rangle$	$\langle\delta_H\rangle$	$\langle\delta_{R3}\rangle$	δ_{cyclop}	$\delta_{cis\text{-}Me}$	log P'	ln K_τ	ln K_ρ
$\langle\Delta E\rangle$	1.0	−0.74	−0.18	0.38 0.37	0.32	0.11	−0.16	0.54	−0.58	−0.33
$\langle\epsilon_\phi{}^*\rangle$		1.0	−0.14 −0.13	−0.57	−0.36 −0.35	0.09	0.25	−0.36	0.60	0.49
$\langle\epsilon_\nu{}^*\rangle$			1.0	0.32 0.31	0.11	−0.17	−0.30	−0.67	−0.37	−0.69
$\langle\delta_H\rangle$				1.0	−0.26 −0.27	−0.01	−0.24	−0.13	−0.76	−0.63
(δ_{R3})					1.0	−0.15	−0.15	0.17	−0.25	−0.19
δ_{cyclop}						1.0	−0.10	0.07	0.23	0.35
$\delta_{cis\text{-}Me}$							1.0	0.30	0.20	−0.01
log P'								1.0	0.11	0.27
ln K_τ									1.0	–
ln K_ρ										1.0

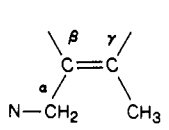
[a] If a matrix element for the agonist state differs from that of the antagonist state, r_{ij} (agonist state) is given first with r_{ij} (antagonist state) below it.

Table III. Results of Statistical Analysis Using Equation 6

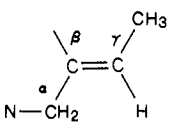
statistic	agonist state				antagonist state			
correlation coefficient (r)	0.97				0.98			
standard error of estimate (s)	0.92				0.95			
F value for analysis of variance	62.1				74.1			
prob > F	<0.01				<0.01			
regression parameter	parameter value	standard error of parameter	t value	prob > t	parameter value	standard error of parameter	t value	prob > t
d_0	28.62				28.56			
$d_{\Delta E}$	−1.08	0.16	−6.9	<0.01	−0.94	0.15	−6.1	<0.01
$d_{\epsilon\phi}$	−38.62	10.26	−3.8	<0.01	−27.18	9.68	−2.8	<0.01
$d_{\epsilon\nu}$	–	–	–	–	−9.58	0.78	−12.3	<0.01
d_H	−6.56	0.64	−10.2	<0.01	−4.36	0.62	−7.1	<0.01
d_{R3}	−4.69	0.68	−6.9	<0.01	−2.30	0.69	−3.3	<0.01
d_{cyclop}	2.47	0.60	4.1	<0.01	3.16	0.61	5.2	<0.01
$d_{cis\text{-}Me}$	−1.70	0.67	−2.5	<0.05	−4.79	0.63	−7.6	<0.01
$d_{\log P'}$	0.92	0.24	3.8	<0.01	–	–	–	–

high-lying C–H σ^* orbitals. The energy of the antibonding C–H orbital that is best suited to act as an acceptor has been calculated to be of the order 0.7561 hartree.[25] This value has been used as $\epsilon_\nu{}^*$ for all molecules containing *N*-methyl groups.

In the benzomorphan study,[25] it was found that molecules containing allyl-like N-substituents with a *cis*-methyl exhibit lower affinity for the antagonist state than the agonist state of the receptor. As shown in Figure 2, both



cis-methyl



trans-methyl

methyls attached to C(γ) in pentazocine extend beyond the van der Waals envelope of naloxone. Apparently, when the μ receptor assumes the antagonist configuration, the binding cavity cannot easily accommodate a bulky group in the region where the *cis*-methyl lies. A steric factor, $\delta_{cis\text{-}Me}$, has been included to account for this effect.

If the drugs under investigation were all members of a single structural class, the calculated strain energy required to maintain the molecule in the pharmacophore geometry could be treated reasonably as a variable with a known coefficient, $-1/RT$. Although the present investigation involves four classes, the effect of $\langle\Delta E\rangle$ was assessed in this manner during preliminary trials. However, the approximate procedures used to calculate ΔE may overestimate the values for the more strained molecules. As a check on this possibility, $\langle\Delta E\rangle$ was treated as a regression variable in the final run with the coefficient $d_{\Delta E}$ to be determined as a parameter.

Solvation Effects. The procedures employed in the rat brain homogenate assay do not free the μ receptor from the synaptic membrane. If access to the receptor requires passage from the polar buffer medium into a protein–lipid matrix of low polarity, the distribution of drug between these phases could be a major factor in determining the extent of equilibrium binding. As a test of this possibility, the drug distribution coefficient, log P', obtained from a buffer–octanol system was included as a variable for selection in the regression calculations.

Statistical Analysis. All structure factors were included in the data set for analysis of binding at both states of the receptor. A stepwise regression process was employed to give an objective selection of the most important factors. After trial runs with the seven structure factors defined above, the three molecules (**2**, **17**, **25**) with R = cyclopropylmethyl remained as outliers. Therefore, an additional factor, δ_{cyclop}, was defined to account for the special effect of this N-substituent on binding. In the final runs, satisfactory agreement between observed and calculated activities was obtained by treating the *S,S* form of the benzamide amines as the eutomer and considering the distomer of all compounds as unsuited to fit the binding pocket of both receptor states. If this was not done, the outcome for both states of the receptor was poor since the *R,R*, 4-hydroxybenzamide amines (**24**, **28**, **33**) made excessive contributions to the calculated activity.

A summary of the results yielded by the statistical analysis is given in Tables II–IV. Cross-correlations among the independent variables (Table II) are significant in two cases, $\langle\Delta E\rangle$–$\langle\epsilon_\phi{}^*\rangle$ and $\langle\epsilon_\nu{}^*\rangle$–log P'. However, omission of any of these four variables from the data set led to fits with one or more outlying points. In addition to $\langle\Delta E\rangle$, five structure factors ($\langle\epsilon_\phi{}^*\rangle$, δ_{cyclop}, $\langle\delta_H\rangle$, $\langle\delta_{R3}\rangle$, and

Table IV. Observed and Calculated Measures of Receptor Binding

	ln K_τ			ln K_ρ			structure factors,[a] $\langle\Omega_r\rangle$							
no.	obsd	calcd	obsd − calcd	obsd	calcd	obsd − calcd	$\langle\Delta E\rangle$	$\langle\epsilon_\phi^*\rangle$	$\langle\epsilon_\nu^*\rangle$	$\langle\delta_H\rangle$	$\langle\delta_{R3}\rangle$	δ_{cyclop}	$\delta_{cis\text{-}Me}$	log P'
1	19.4	19.6	−0.2	20.7	19.8	0.9	0.2	0.2355	0.1512	0.0	0.0	0	0	1.06
2	21.9	21.6	0.3	21.2	21.3	−0.1	0.0	0.2376	0.3378	0.0	0.0	1	0	0.46
3	19.9	18.5	1.4	13.8	14.3	−0.5	0.0	0.2336	0.7561	0.0	0.0	0	0	−0.42
4	15.8	14.3	1.5	13.0	12.2	0.8	0.0	0.2256	0.7561	0.0	1.0	0	0	−0.23
5	16.0	14.4	1.6	12.4	11.1	1.3	0.0	0.1892	0.7561	1.0	0.0	0	0	0.40
6	19.7	19.5	0.2	19.9	19.6	0.3	0.3	0.2390	0.1563	0.0	0.0	0	0	1.22
7	18.8	18.9	−0.1	12.6	14.2	−1.6	0.0	0.2372	0.7561	0.0	0.0	0	0	0.11
8	14.1	14.1	0.0	10.3	11.0	−0.7	0.0	0.1940	0.7561	1.0	0.0	0	0	0.30
9	13.3	14.6	−1.3	11.4	12.0	−0.6	0.0	0.2307	0.7561	0.0	1.0	0	0	0.25
10	14.1	14.3	−0.2	14.6	16.4	−1.8	0.3	0.1957	0.1544	1.0	0.0	0	0	0.87
11	19.0	18.4	0.6	13.5	14.2	−0.7	0.0	0.2349	0.7561	0.0	0.0	0	0	−0.51
12	15.8	15.2	0.6	10.8	11.1	−0.3	0.0	0.1907	0.7561	1.0	0.0	0	0	1.35
13	21.6	21.4	0.2	20.8	20.5	0.3	0.0	0.2322	0.1939	0.0	0.0	0	0	2.14
14	20.8	20.5	0.3	15.6	14.9	0.7	0.0	0.2355	0.7561	0.0	0.0	0	0	1.03
15	19.6	20.1	−0.5	19.8	19.8	0.0	0.3	0.2326	0.1583	0.0	0.0	0	0	1.73
16	18.7	18.8	−0.1	15.1	14.9	0.2	0.0	0.2341	0.1927	0.0	0.0	0	1	1.58
17	22.2	22.0	0.2	21.3	21.2	0.1	0.2	0.2341	0.3462	0.0	0.0	1	0	0.97
18	18.1	19.5	−1.4	13.1	14.4	−1.3	0.0	0.2293	0.7561	0.0	0.0	0	0	0.47
19	19.9	20.5	0.6	19.2	18.6	0.6	0.7	0.2321	0.2432	0.0	0.0	0	0	2.49
20	19.1	19.1	0.0	14.1	13.8	0.3	0.5	0.2329	0.2579	0.0	0.0	0	1	2.68
21	19.4	19.8	−0.4	19.8	18.5	1.3	0.8	0.2344	0.2343	0.0	0.0	0	0	2.00
22	18.6	18.5	0.1	13.1	13.6	−0.5	0.7	0.2345	0.2513	0.0	0.0	0	1	2.25
23	15.9	15.1	0.8	14.1	14.9	−0.8	5.6	0.1338	0.1629	0.12	0.88	0	0	3.65
										0.10	0.90			
24	10.9	9.6	1.3	10.9	11.4	−0.5	5.6	0.1913	0.1723	1.0	0.0	0	0	1.77
25	13.1	13.5	−0.4	13.0	13.0	0.0	5.6	0.1793	0.3693	1.0	0.0	1	0	2.90
26	16.2	17.0	−0.8	12.9	12.0	0.9	2.4	0.1419	0.7561	0.12	0.88	0	0	2.23
										0.10	0.90	0	0	
27	11.7	12.2	−0.5	9.3	9.5	−0.2	2.4	0.2219	0.7561	0.30	0.70	0	0	0.75
								0.2244		0.21	0.79			
28	11.0	11.4	−0.4	10.4	8.6	1.8	2.4	0.1993	0.7561	1.0	0.0	0	0	0.35
29	13.3	13.3	0.0	9.2	9.6	−0.4	2.4	0.1627	0.7561	1.0	0.0	0	0	0.83
30	11.5	13.1	−1.6	9.1	9.7	−0.6	2.4	0.1583	0.7561	1.0	0.0	0	0	0.45
31	14.8	13.9	0.9	10.8	9.6	1.2	2.4	0.1616	0.7561	1.0	0.0	0	0	1.53
32	14.9	14.7	0.2	14.8	14.9	−0.1	5.6	0.1341	0.1620	0.12	0.88	0	0	3.18
										0.10	0.90			
33	9.6	11.2	−1.6	8.8	8.7	0.1	2.4	0.1939	0.7561	1.0	0.0	0	0	−0.12

[a] Variables that exhibit different conformational averages for the two states are listed with the agonist-state value first and the antagonist-state value below it.

$\delta_{cis\text{-}Me}$) were selected in the stepwise regression procedure for both states of the receptor. A seventh variable was also chosen in each case: namely, log P' for the agonist state and $\langle\epsilon_\nu^*\rangle$ for the antagonist state. All parameters d_r listed in Table III prove to be significant, and the analysis gives satisfactory measures for the quality of the fit. In particular, the values found for the standard error of the estimate, s, are of the same magnitude as the error in the measurements of ln K_τ and ln K_ρ. The conformational averages of the structure factors are given in Table IV, together with the observed and calculated measures of μ-receptor binding. The results are also plotted in Figure 6. Although there is scatter about the line of perfect correlation in each graph, no calculated value of ln K_τ or ln K_ρ exceeds the observed value by more than $\pm 2s$.

There are certain theoretical expectations that the results of the statistical analyses must satisfy if the proposed model properly represents the factors involved in the formation of the drug–receptor complex. For example, if charge–transfer contributes significantly to the stabilization energy, ln K_τ and ln K_ρ must be negatively correlated with the effective energy of the primary acceptor orbital(s).[29] Furthermore, if a molecule requires high strain energy to maintain the binding configuration, the net stabilization energy of the complex suffers as a consequence. Hence, the coefficient of $\langle\Delta E\rangle$ should be negative. Since the coefficients of $\langle\epsilon_\phi^*\rangle$, $\langle\epsilon_\nu^*\rangle$, and $\langle\Delta E\rangle$ carry a minus sign, the statistical analyses are in agreement with theory and give support to the proposed model. Although the sign of $d_{\Delta E}$ in the relationships for ln K_τ and ln K_ρ is correct, the magnitude of the coefficient is only half of the value of $1/RT$ at the assay temperature of 0 °C. This suggests that the procedures used to calculate $\langle\Delta E\rangle$ overestimate the strain experienced by the benzamide amines.

Comparison of Binding Factors for the Two Receptor States. Since the drug features that affect association with the agonist and antagonist states are virtually identical, the change of receptor configuration must involve relatively minor shifts of the critical receptor groups within the binding pocket. However, as a consequence of this small local transformation, there is a significant allosteric effect on the Na^+ binding site, which is presumably the gate of the sodium ion channel.[30]

By comparing the coefficient d_r from the analysis of binding for the agonist state with that for the antagonist state, it is possible to determine which receptor configuration is favored by the presence of the drug feature Ω_r. To be considered significantly different, the values of d_r from the structure–activity relationships for the two states must differ by more than twice the standard error of the coefficient. According to this criterion, the factors that influence selectivity for the agonist and antagonist states are $\langle\epsilon_\nu^*\rangle$, $\delta_{cis\text{-}Me}$, $\langle\delta_H\rangle$, $\langle\delta_{R3}\rangle$, and log P'. On the other hand, the analysis suggests that $\langle\Delta E\rangle$, $\langle\epsilon_\phi^*\rangle$, and δ_{cyclop} play a major role in the formation of a stable drug–receptor

(30) Pert, C. B.; Garland, B. L. In *Receptors and Hormone Action*; Birnbaumer, L., O'Malley, B. W., Eds.; Academic: New York, 1978; Vol. III, pp 535–551.

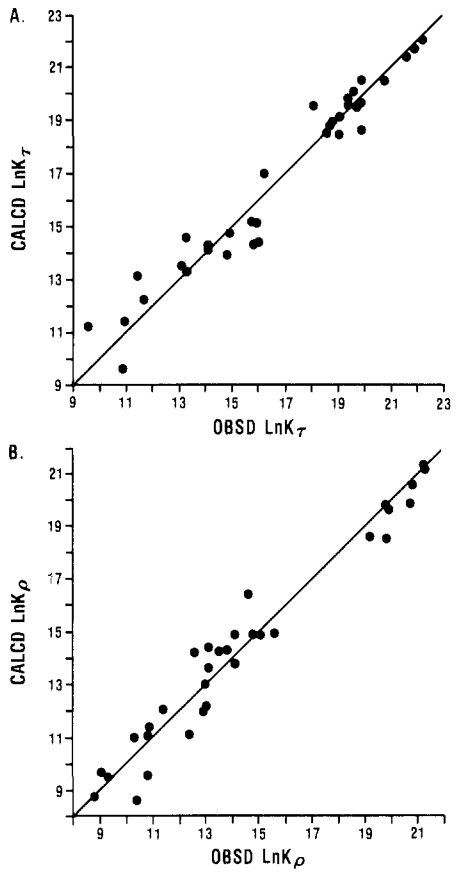


Figure 6. Comparison of experimental and calculated indices for binding at the agonist (A) and antagonist (B) states of the opioid μ receptor. The points are shown in relation to the theoretical line of unit slope.

complex without favoring either state to any great extent.

Replacement of the phenolic hydroxy in the pharmacophore by hydrogen, methoxy, or chlorine has a detrimental effect on binding at both states of the receptor. However, the values of d_H and d_{R3} indicate that the substitution leads to a greater loss of affinity in the case of agonist-state binding. Apparently, the Y–HO hydrogen bond is weakened by the transformation from the agonist to the antagonist configuration, while the less effective interactions involving H, OCH_3, and Cl remain relatively unchanged. Thus, the phenolic hydroxy seems to enhance both the potency and intrinsic activity of μ agonists when it falls in the receptor site where the 3-OH of naloxone binds. The weak binding of the 4-hydroxybenzamide amines (**24**, **28**, **33**) may be attributed to the fact that the *p*-OH of the *S,S* isomer does not lie near enough to Y for effective hydrogen bonding.

All allyl-like N-substituent with good electron-acceptor properties, as measured by $\langle \epsilon_v^* \rangle$, markedly increases the affinity of the drug for the antagonist state of the μ receptor. However, there appears to be a steric restriction in the binding pocket near the terminus of the allyl-like group since a *cis*-methyl has an adverse effect on the association with both receptor configurations. The values of the coefficient $d_{cis\text{-Me}}$ from the relationships for ln K_τ and ln K_ρ suggest that the site occupied by this methyl is more constricted in the antagonist than the agonist form of the receptor.

Since log P' was selected as a regression variable only in the case of the agonist state, the binding-site environment of this receptor configuration appears to favor the more lipophilic molecules. However, due to the cross-correlation between log P' and $\langle \epsilon_v^* \rangle$ in the set of drugs under study, the effects of the two factors cannot be entirely dissociated. Therefore, the analysis cannot be construed to mean that lipophilicity is unimportant for antagonist-state binding, nor can charge-transfer interactions involving the N-substituent be ruled out as a factor in the stabilization of the agonist-state complex.

Conclusion

Most drugs that act at the opioid μ receptor contain a benzene ring and basic tertiary amine with the spatial arrangement found in morphine. Furthermore, the N-substituent, R, plays a major role in determining whether such a drug will act as a morphine agonist (e.g., R = methyl) or antagonist (e.g., R = allyl). In this study, efforts have been made to discern the electronic properties that confer agonist or antagonist character upon the drug molecule in action at the μ receptor.

The intrinsic activity of a μ-receptor drug may be attributed to the fraction, f_τ, of the receptor population that it stabilizes in the agonist (sodium-free) state. Since f_τ depends upon the relative magnitude of the affinity constants, K_τ and K_ρ, for the agonist and antagonist states of the receptor, the results of binding experiments serve to rationalize the agonist/antagonist ratio observed in pharmacological assays for analgesia and morphine antagonism. The link between pharmacological effects and structural features of the drug molecule has also been established through analysis of the binding data for the agonist and antagonist states of the receptor.

Characterization of the geometry and electronic structure of the 33 drugs included in this study was accomplished by means of molecular mechanics and an ab initio quantum mechanical procedure using FSGO basis sets. The critical features involved in receptor association were suggested by structural comparisons of the molecules to naloxone, a drug with high affinity for both states of the receptor. These features were then investigated further as regression variables in the development of binding models with the values of ln K_τ and ln K_ρ as the dependent variables. The statistical analyses reveal that the features required for binding to the agonist form of the receptor are also needed for attachment to the antagonist form. However, the importance of certain features differs for the agonist and antagonist states as a result of variations in the configuration of the receptor binding pocket.

Electrostatic effects involving the cationic head of the drug molecule and charge-transfer interactions involving the aromatic ring as an electron acceptor have been implicated as factors underlying association with both states of the μ receptor. If the molecule contains an extended N-substituent, the spatial arrangement of the aromatic ring and the N–C(α)–C(β)–C(γ) chain must resemble that found in the (5*R*,9*R*,13*R*,14*S*)-naloxone conformer with τ_1 = 80° and τ_2 = 93°. The terms involving the strain energy, $\langle \Delta E \rangle$, in the structure–activity equations indicate that it is necessary to maintain the same binding conformation at both states of the receptor. Furthermore, the similar effects of the *N*-cyclopropylmethyl on ln K_τ and ln K_ρ may be best explained if neither the bound molecule nor the binding pocket changes drastically when the receptor alters configuration. On the other hand, the transformation from the agonist to the antagonist state does affect the strength of certain critical interactions between the drug and receptor. Charge-transfer contributions involving an allyl-like N-substituent as the electron acceptor are far more important for binding at the antagonist than the agonist state. A *cis*-methyl attached to C(γ) in the N-substituent seems to experience a more crowded environment in the antagonist form of the receptor. Although replacement

of the phenolic hydroxy corresponding to the 3-OH of naloxone by H, OCH_3, or Cl has an adverse effect on the affinity for both states, the substitution destabilizes the agonist-state complex to a greater extent.

The information concerning key structural factors obtained in this study serves to rationalize the agonist–antagonist behavior of opiates in the morphine, morphinan, benzomorphan, and benzamide amine classes. Furthermore, the model may be useful for examining μ-receptor ligands that were not included in this investigation.

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Registry No. 1, 465-65-6; **2**, 16590-41-3; **3**, 76-41-5; **4**, 81165-08-4; **5**, 32295-31-1; **6**, 62-67-9; **7**, 57-27-2; **8**, 51269-51-3; **9**, 76-57-3; **10**, 69663-74-7; **11**, 509-60-4; **12**, 55592-68-2; **13**, 152-02-3; **14**, 77-07-6; **16**, 89575-86-0; **17**, 63903-61-7; **18**, 25144-79-0; **19**, 38047-74-4; **21**, 56498-83-0; **23**, 67579-46-8; **24**, 98587-43-0; **25**, 98587-46-3; **26**, 82657-23-6; **27**, 67579-34-4; **28**, 98587-47-4; **29**, 98587-45-2; **30**, 111060-35-6; **31**, 67579-11-7; **32**, 67579-41-3; **33**, 67579-15-1.

Supplementary Material Available: Electronic data used in calculation of effective acceptor energies for the aromatic ring and N-substituent, coordinates of the calculated minimum energy conformer of *trans*-*N*-[2-(dimethylamino)cyclohexyl]-*N*-methyl-3,4-dichlorobenzamide, and statistical mechanical description of equilibrium model (12 pages). Ordering information is given on any current masthead page.