adjusted to 3.5 with concentrated hydrochloric acid to give **20a**: 1.32 g (54%); mass spectrum (FD) m/e 432 ($M^+ + 1$). Anal. $C_{22}H_{41}NO_5S \cdot Na(-H)$.

4(*R*)-Hydroxy-5(*S*)-[(2-amino-2-carboxyethyl)thio]-6-(*Z*)-nonadecenoic Acid, Sodium Salt (20b). The reaction was carried out as described for **20a** from **9c** to give **20b**: 0.087 g (35%); mass spectrum (FD) m/e 432 ($M^+ + 1$). Anal. $C_{22}H_{41}NO_5S \cdot 1.5Na(-1.5\ H)$.

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Registry No. **1**, 3878-55-5; **2**, 1490-25-1; **3**, 13865-19-5; **4**, 98303-59-4; **5**, 98303-60-7; **6** (n = 11), 15510-55-1; **6** (n = 6), 13423-48-8; **6** (n = 16), 54907-67-4; **7a**, 98392-67-7; **7b**, 98303-61-8; **7c**, 98303-62-9; **8a**, 75290-62-9; **8b**, 1577-62-4; **9a**, 95405-68-8; **9b**, 95351-90-9; **9b** amide (detrifluoracetylated), 98303-63-0; **9c**, 98461-30-4; **9d**, 98461-31-5; **9e**, 98303-64-1; **9f**, 98330-11-1; **9h**, 98392-68-8; **10a**, 88903-81-5; **10b**, 88477-96-7; **11a**, 98303-65-2; **11b**, 98303-66-3; **12**·2Na, 98330-12-2; **13**·2Na, 98303-67-4; **14**, 95351-98-7; **15**·Na, 98303-68-5; **16**, 98303-69-6; **16**·xCF_3CO_2H, 98392-69-9; **17a**, 88903-82-6; **17b**, 88903-83-7; **18**, 98303-70-9; **19**, 98303-71-0; **20a**, 88847-39-6; **20a**·Na, 95462-55-8; **20b**, 88903-84-8; **20b**·3Na, 98392-70-2; LTD_4, 73836-78-9; $(Ph)_3P{=}CHCHO$, 2136-75-6; 1-bromotridecane, 765-09-3; triphenylphosphine, 603-35-0.

Factors Affecting Binding of *trans*-*N*-[2-(Methylamino)cyclohexyl]benzamides at the Primary Morphine Receptor

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In this paper, we describe the synthesis of a series of *trans*-*N*-[2-(methylamino)cyclohexyl]benzamides possessing morphine-like pharmacological properties. The affinity of the compounds for the agonist and antagonist states of the μ opioid receptor has been established by means of an in vitro binding assay. We have investigated the geometry and electronic structure of the molecules using molecular mechanics and an ab initio SCF-MO procedure with FSGO basis sets. Comparison to naloxone reveals properties of possible importance in receptor association. We have considered both the *S,S* and *R,R* isomers in the binding model. Statistical analyses imply that three factors play a significant role in binding: (1) membrane–water partitioning, (2) the capacity of the aromatic ring and amine N-substituent to act as electron acceptors, (3) the conformational energy required to attain the binding configuration.

Recent research in our laboratories has led to the discovery of a series of compounds, *trans*-*N*-[2-(methylamino)cyclohexyl]benzamides,[1] that exhibit morphine-like pharmacological properties, although there is little obvious structural resemblance to classical opiate analgesics. Owing to the novel arrangement of key structural features, the benzamide amines serve as useful probes to investigate factors affecting affinity for the primary morphine (μ) receptor. In this report, we discuss the synthesis of these compounds, the results obtained from tests of biological activity (including an in vitro receptor binding assay), and our efforts to determine the properties of these molecules that play an important role in drug–receptor association.

Among the diverse chemical agents with affinity for the morphine receptor, two common structural entities are found: a basic amino group and an aromatic ring.[2-4] Several investigators[3-7] have suggested that effective receptor interactions depend upon the drug assuming a conformation in which the key aromatic ring and basic nitrogen exhibit a spatial relationship similar to that of morphine. In certain classes of these agents, the presence of an allyl or cyclopropylmethyl substituent on the basic nitrogen causes the drug to act as a morphine antagonist or a mixed agonist–antagonist. The orientation of such an N-substituent with respect to other key features has been suggested as a factor in determining the degree of antagonist character.[6-8] These structure–activity relationships provide a basis for comparing the benzamide-amine geometry and electronic structure with that of a reference drug having high specificity for the μ receptor and strong affinity for both the agonist and antagonist states. We have selected naloxone as a suitable reference for structural comparisons and as the radiolabeled marker in the receptor binding assay.

In order to make detailed comparisons of the test and reference molecules, we have examined the geometry and electronic structure of the benzamide amines using the same computational methods employed in a previous study of naloxone.[9] An investigation of the "free-molecule" and "receptor-binding" conformational states was carried out by means of molecular mechanics,[10] a technique utilizing empirical potential functions to represent intramolecular interactions. Characterization of the ground-state elec-

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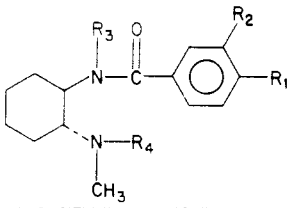
Table I. Physical Properties of Substituted Amides of *trans*-1,2-Diaminocyclohexanes



compd	R_1	R_2	R_3	R_4	formula[a]	mp, °C	recrystn solvent	anal	method of synthesis
1	Cl	Cl	CH_3	CH_3	$C_{16}H_{22}Cl_2N_2O$	97–98.5°	pet. ether (30–60 °C)–Et_2O	C, H, Cl, N	A
2	OH	H	CH_3	CH_3	$C_{16}H_{24}N_2O_2$	210–212°	MeOH–Et_2O	C, H, N	B
3	H	OCH_3	CH_3	CH_3	$C_{17}H_{26}N_2O_2 \cdot CH_3C_6H_4SO_3H$[b]	181–182°	MeOH–Et_2O	C, H, N, S	A
4	NO_2	H	CH_3	CH_3	$C_{16}H_{23}N_3O_3 \cdot CH_3C_6H_4SO_3H$[b]	210–211.5°	MeOH–Et_2O	C, H, N, S	A
5	CN	H	CH_3	CH_3	$C_{17}H_{23}N_3O$	130–131°	Et_2O	C, H, N	A
6	Cl	H	CH_3	CH_3	$C_{16}H_{23}ClN_2O \cdot C_4H_4O_4$[c]	172–173°	MeOH–Et_2O	C, H, Cl, N	A
7	OH	H	H	CH_3	$C_{15}H_{22}N_2O_2$	232.5–234°	MeOH–benzene	C, H, N	C
8	CF_3	H	CH_3	CH_2	$C_{20}H_{27}F_3N_2O \cdot CH_3SO_3H$	184–185.5°	MeOH–Et_2O	C, H, F, N, S	A
9	OH	H	CH_3	$CH_2CH=CH_2$	$C_{18}H_{26}N_2O_2$	oil		HR mass spec	A[d]
10	Cl	Cl	H	$CH_2CH=CH_2$	$C_{17}H_{22}Cl_2N_2O \cdot C_4H_4O_4$[e]	185–187°	MeOH–Et_2O	C, H, Cl, N	A
11	Cl	Cl	CH_3	$CH_2CH=CH_2$	$C_{18}H_{24}Cl_2N_2O \cdot CH_3SO_3H$	164–165°	MeOH–Et_2O	C, H, Cl, N, S	A
12	OCH_3	H	H	CH_3	$C_{16}H_{24}N_2O_2$	138–139°	Et_2O	C, H, N	A
13	OCH_3	H	CH_3	CH_3	$C_{17}H_{26}N_2O_2 \cdot CH_3C_6H_4SO_3H$[b]	196–197°	MeOH–Et_2O	C, H, N, S	A

[a] All the compounds were characterized by elemental analyses, IR, UV, NMR, and mass spectra. [b] *p*-Toluenesulfonate. [c] Maleate. [d] The acid chloride component was prepared from 4-hydroxybenzoic acid according to Schonenberger et al. [Schonenberger, H.; Holzheu-Eckardt, J.; Bamann, E. *Arzneim. Forsch.* **1964**, *14*, 3211] but without distillation. [e] Fumarate.

tronic structure was accomplished with ab initio quantum mechanics using the molecular fragment approach.[11] In all calculations, we have treated the cation formed by protonation of the amine nitrogen.

Adequate theoretical models cannot be constructed to describe solvation effects that undoubtedly influence drug–receptor association. However, an experimental model may be useful for simulating the process of removing the drug from solution and placing it at the receptor. In the present case, the procedures employed in the rat-brain homogenate binding assay do not free the morphine receptor from the synaptic membrane. If access to the receptor requires passage into a protein–membrane matrix of low polarity, the distribution of drug between the polar buffer medium and this matrix could be a major factor in determining the extent of equilibrium binding. As a test of this possibility, we measured the drug distribution coefficients in a buffer–octanol system and examined the correlation with receptor affinity constants. These findings will also be discussed in this paper.

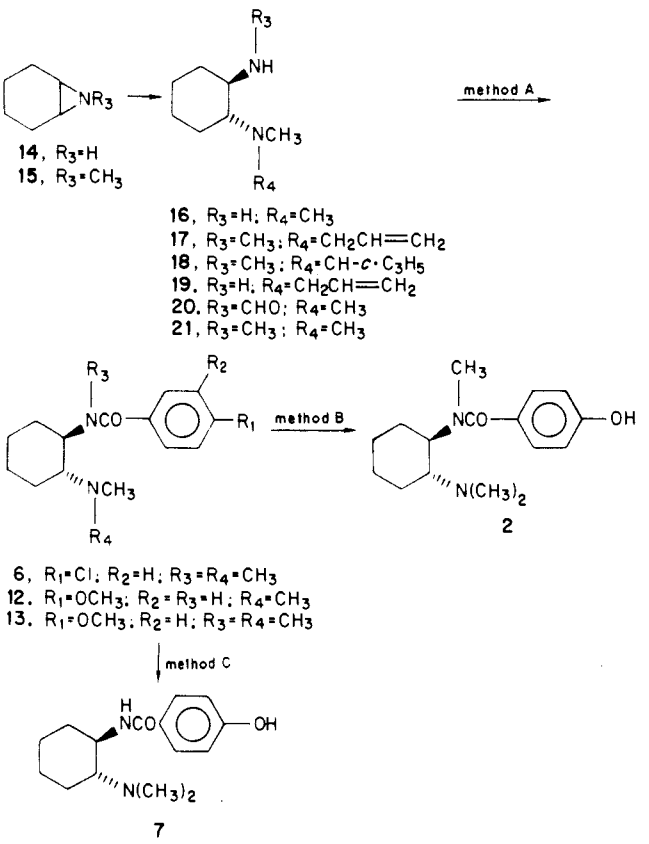
Methods

Synthesis. The required *trans*-1,2-diaminocyclohexane derivatives (Scheme I) were prepared by the reaction of the aziridine **14** with dimethylamine and *N*-allylmethylamine to give **16** and **19**, respectively.

The *N*-methylaziridine **15** was reacted with *N*-allylmethylamine and *N*-cyclopropylmethylamine to give **17** and **18**, respectively. Compound **21** was prepared by the reaction of **16** with ethyl formate, and the resulting formamide **20** was reduced with LAH to give **21**.

The amides were prepared by the condensation of the required *trans*-1,2-diaminocyclohexane with an acid chloride in the presence of triethylamine (method A) and are listed in Table I. In order to prepare compound **2**, **13** was subjected to demethylation with the sodium salt of ethane thiol (method B). Compound **7** was prepared by demethylation of **12** with boron tribromide (method C).

Scheme I



Pharmacology. Male CF-1 mice (18–22 g) were injected subcutaneously with a suspension of the test compound in 0.25% methylcellulose and tested for analgesia after a 15-min delay. As described elsewhere,[12] analgesic activity was assessed by timing the tail-flick response in-

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duced by a focused high-intensity light. After completion of this test, about 45-min postinjection, morphine sulfate (6.3 mg/kg of body weight) was given subcutaneously, and the mice were retested with the tail-flick procedure to determine if the compound might have narcotic antagonist properties. Blockade of morphine-induced elevation of tail-flick latency was scored as antagonism. Six mice were tested at each dose. When multiple doses were examined, the ED_{50} for each compound was calculated by the method of Spearman and Karber.[13] The upper and lower confidence intervals were never more than 2 nor less than 0.5 times the ED_{50}.

In addition to the assay for analgesia, test animals were observed for behavioral responses typically induced by morphine-like drugs, but not by compounds such as the benzeneacetamide amines[1] that elicit strong κ-receptor effects.

Receptor Binding Assay. Following decapitation, the brain of a rat, minus the cerebellum, was rapidly removed, rinsed in cold buffer, blotted dry, and weighed. The brain was then homogenized in three volumes (w/v) of 0.05 M tris(hydroxymethyl)aminomethane–HCl (THAM–HCl) buffer at pH 7.4 with use of a Brinkmann Polytron at setting 7 for 30 s. The homogenate was then centrifuged at 40000*g* for 10 min at 0 °C. The supernatant was discarded and the pellet was resuspended in 10 mL of buffer with the Polytron at setting 7 for 10 s. The final homogenate was diluted to 108 volumes (w/v) with buffer.

A portion of the homogenate (2 mL) was incubated with 0.1 mL of vehicle or drug, at various concentrations, and 0.1 mL of [^{3}H]naloxone, final concentration 1 nM (19.9 Ci/mmol; New England Nuclear Corp., Boston, MA). The concentration of sodium ion in the medium was adjusted to 50 mM in half of the samples by adding NaCl. In samples to which no NaCl was added, the concentration of Na^+ was found to be 0.28 (±0.07) mM by atomic absorption spectroscopy. Samples were incubated in triplicate at 25 °C for 40 min followed by a 15-min period on ice before filtering through Whatman GF/B glass-fiber filters. The filters were rinsed twice with 5 mL of 0.05 M THAM–HCl (pH 7.4). The filters were then placed in a liquid scintillation vial containing 15 mL of toluene–Triton X-100 (3:1) scintillation fluid and counted in a liquid scintillation counter to a counting error of 3%. The total concentration of bound [^{3}H]]naloxone was calculated as the mean of triplicate determinations using corrections for background radiation and counter efficiency.

Analysis of the binding experiments was carried out in terms of the two-state receptor model described by Cheney et al.[14] By means of an iterative nonlinear regression technique, the theoretical model was fit to the experimental binding data. The parameters determined in this procedure were the affinity constants, K_r for the agonist state and K_ρ for the antagonist state of the receptor.

Drug Distribution Coefficients. In the case of morphine-like analgesics partitioned between octanol and water, the distribution of total (protonated and unprotonated) drug between each phase is the appropriate equilibrium to consider in relationship to the binding study. The equilibrium constant is the drug distribution coefficient, P', defined as

$$P' = ([B] + [BH^+]_{oct}/([B] + [BH^+])_{aq} \quad (1)$$

where $([B] + [BH^+])_{oct}$ is the total concentration of both forms of the drug in octanol and $([B] + [BH^+])_{aq}$ is the total concentration of drug in water.

For partitioning, distilled water was buffered to a pH of 7.4 at 21 °C with THAM-HCl. The buffered aqueous phase and the 1-octanol (Aldrich) were mutually saturated by combining the two solvents and stirring constantly for 2 h at 0 °C. The phases were allowed to separate overnight followed by a centrifugation to remove any emulsion formed by the vigorous stirring.

Stock solutions of each compound, 10^{-4} to 10^{-5} M, were made to give an initial UV absorbance of ~2.0 for the B_{2u} band ca. 275 nm (ϵ 1500).[15] Absorbance data for four dilutions were used to construct a calibration curve for each compound by means of a least-squares fit of the following equation:

$$A = \epsilon bc \quad (2)$$

where A is the maximum absorbance of the B_{2u} band, ϵ is the molar extinction coefficient, b is the cell path length, and c is the molar concentration.

A volume ranging from 0.5 to 200.0 mL of 1-octanol was added to a quantity between 20.0 and 200.0 mL of stock solution contained in a 500-mL polyethylene centrifuge bottle. The volume ratio of the two phases was chosen so that the concentration of drug in the aqueous phase was about halved by the partitioning to yield an absorbance of ~1.0. The octanol–water system was shaken for 0.5 h in a 0 °C bath, followed by centrifugation and, finally, separation of the two layers via pipet.

The analysis of drug concentration in the aqueous phase after partitioning was made on a Cary 14 spectrophotometer using a cell path length of 10.0 cm; all measurements were zeroed against a blank. Due to the decreased solubility of 1-octanol in water with an increase in temperature from 0 to 20 °C,[16] it was necessary to use a water-jacketed UV cell cooled to 0 °C for absorbance determinations. Care was taken throughout the experiment to insure that all solutions remained at 0 °C.

The amount of compound that moves into the octanol layer is calculated from a simple difference, and the concentration in the octanol phase is given by

$$([B] + [BH^+])_{oct} = (m_{total} - m_{remain})/V_{oct} \quad (3)$$

where m_{total} is the amount of drug (mmol) added to the system, m_{remain} is the amount remaining in the aqueous phase after partitioning, and V_{oct} is the volume (mL) of octanol used in partitioning. Since the absorption measured in the UV is the sum of contributions from both protonated and unprotonated drug, we can directly use eq 1 to calculate P'.

Molecular Geometry. Nuclear coordinates for naloxone were taken from the crystal structure obtained by Karle,[17] and other low-energy conformations considered in this study were found by Cheney and Zichi.[9] Data from the X-ray crystallographic study[29] of *trans*-*N*-[2-(dimethylamino)cyclohexyl]-N-methyl-3,4-dichlorobenzamide (1) were employed to describe the skeleton of all benzamide amines under consideration. We have used the Duchamp[10] molecular mechanics procedure to determine the low-energy conformations of each drug.

In order to interact with the principal functional groups of the receptor in the same manner as naloxone, the benzamide-amine drugs should adopt a configuration in which

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Table II. Binding Affinity for Morphine Receptor and Analgesic Activity of Substituted Amides of *trans*-1,2-Diaminocyclohexanes

compd	agonist-state assoc constant, K_τ	antagonist-state assoc constant, K_ρ	K_τ/K_ρ	analgesic act.: ED_{50}, mg/kg	morphine antagonism: ED_{50}, mg/kg	morphine-like behavioral effects[a]
1	$1.1\ (0.4) \times 10^7$	$4.1\ (3.7) \times 10^5$	27	0.2	>100	+
2	$6.0\ (1.8) \times 10^4$	$3.3\ (0.3) \times 10^4$	2	>100	>100	–
3	$1.2\ (0.4) \times 10^5$	$1.1\ (0.5) \times 10^4$	11	28	>100	–
4	$5.8\ (1.5) \times 10^5$	$1.1\ (1.1) \times 10^4$	53	2	>100	+
5	$1.1\ (0.3) \times 10^5$	$8.1\ (2.3) \times 10^3$	14	3	>100	+
6	$2.6\ (0.7) \times 10^6$	$5.0\ (6.2) \times 10^4$	52	1	>100	+
7	$1.7\ (0.5) \times 10^4$	$6.8\ (0.8) \times 10^3$	2.5	>100	>100	–
8	$4.8\ (1.8) \times 10^5$	$4.7\ (0.6) \times 10^5$	1.0	0.4	>50	+
9	$5.3\ (2.2) \times 10^4$	$5.4\ (0.8) \times 10^4$	1.1	>50	>50	–
10	$3.1\ (1.1) \times 10^6$	$2.6\ (0.4) \times 10^6$	1.2	8.8	>100	+
11	$8.4\ (2.7) \times 10^6$	$1.3\ (0.3) \times 10^6$	6.5	20	>100	+
morphine	$1.5\ (0.4) \times 10^8$	$\sim 3 \times 10^5$	500	1.5	–	+
naloxone	$2.6\ (0.8) \times 10^8$	$9.8\ (1.3) \times 10^8$	0.3			–

[a] Straub tail, arched back, and increased locomotor activity.

key structural features exhibit the spatial relationships found in the reference molecule. The energy cost of forcing the aromatic ring and amine nitrogen in a test molecule to match the corresponding entities in naloxone was examined by means of the "extra potential" method described in ref 10. In this calculation, the test molecule was aligned with naloxone by minimizing its conformational energy under the constraint of three extra potentials linking carbons in the aromatic rings and one connecting the nitrogens of interest. In the case of benzamide amine with an allyl or cyclopropylmethyl group, three additional potentials were used to connect N-substituent carbons in the test molecule with the allyl carbons of naloxone. The force constants in the extra potentials were adjusted to allow close approach of designated atoms in the test molecule to their counterparts in naloxone without inducing severe distortion of bond lengths and bond angles in the benzamide-amine structure; as a result, the geometry changes that occurred in the matching process may be described primarily in terms of torsional angle variations.

Electronic Structure. We used the ab initio molecular fragment technique[11] to determine the ground-state wave function of the N-protonated benzamide-amine cations in the geometry matched to naloxone. In this procedure, molecular orbitals (MO, ϕ_i) are written as linear combinations of floating spherical Gaussian orbitals (FSGO, G_s) using the relationship

$$\phi_i = \sum_s c_{si} G_s \tag{4}$$

where the summation spans all FSGO basis functions, and c_{si} is a coefficient found by energy minimization of the wave function.

As an aid in analyzing the electronic structure, the molecular wave function was expressed in terms of symmetrically orthogonalized basis orbitals, χ_r, defined by

$$\chi_r = \sum_s S_{sr}^{-1/2} G_s \tag{5}$$

where $S_{sr}^{-1/2}$ is an element of the unitary transformation matrix that diagnolizes the FSGO overlap matrix.[18,19] Comparison of MO's between molecules was accomplished with the following index:[20]

$$\tau_{ij}^2 = |\sum_r C_{ri}^A C_{rj}^B|^2 \tag{6}$$

In this expression, C_{ri}^A represents the coefficient of χ_r in the ith MO of molecule A. Only corresponding structural moieties in molecules A and B give rise to significant terms in eq 6. If χ_r in molecule A has no counterpart in molecule B, the value of C_{rj}^B is taken to be zero. Depending upon the degree of similarity between ϕ_i^A and ϕ_j^B, the correlation index, τ_{ij}^2, will vary from 0 to 1 in magnitude.

An investigation has been made of the molecular electrostatic interaction potential $V(r)$, given approximately by

$$V(r) = \sum_\alpha [Z_\alpha / |r - R_\alpha|] - \sum_s [q_s / |r - R_s|] \tag{7}$$

where Z_α is the charge of nucleus α located at R_α and q_s is the Shipman[21] electronic point charge situated at R_s which is the center of Gaussian G_s. $V(r)$ indicates how the drug might engage the receptor in long-range electrostatic interactions.

Results and Discussion

Pharmacology and Receptor Binding. The biological test results for compounds 1–11 are summarized in Table II. At the doses tested, all of the congeners except those with R_1 = OH (**2**, **7**, and **9**) produced measurable analgesic activity. Compounds **1**, **6**, and **8** exhibit higher potency than morphine. In mice, the most active agents caused morphine-like behavioral effects such as Straub tail, arched back, and increased locomotor activity. None of the benzamide amines antagonized morphine at dosages as high as 50 mg/kg of body weight.

The values of K_τ and K_ρ in Table II reveal that the benzamide amines have significantly lower affinity for both states of the receptor than naloxone. In order to categorize analgesic agents according to the inherent degree of agonist character, we have defined an efficacy index in terms of the ratio K_τ/K_ρ. Uncertainty in the estimates of K_ρ does not permit compounds **1**, **4**, and **6** to be classified with confidence as pure agonists. However, the efficacy index for the other compounds under study generally places the series in a category with drugs such as cyclazocine (an antagonist–agonist, K_τ/K_ρ = 2.2) and pentazocine (an agonist–antagonist, K_τ/K_ρ = 37). Although the failure to observe morphine antagonism in the mouse assay seems to cast doubt upon this classification, the lack of antagonism may be explained by the competitive disadvantage of the test drugs resulting from low receptor affinity.

Since the endpoints of in vivo pharmacological assays are affected by processes of drug transport, metabolism, and excretion, as well as the involvement of other receptors, one cannot expect an absolute correspondence between analgesic potency and μ-receptor affinity. However,

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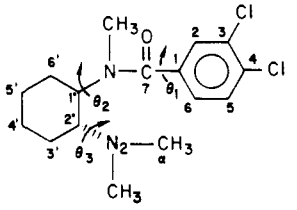
if the responses are mediated principally by the μ receptor, the benzamide amines with high values of *both* K_τ and K_τ/K_ρ should produce analgesia together with morphine-like behavioral effects. Conversely, an absence of these effects should be noted for those drugs with much lower values of K_τ and K_τ/K_ρ. The only benzamide amine that appears to be out of line with these expectations is compound 8. The exceptional analgesic activity of this compound may be due in part to association with other opioid receptors.

Drug Distribution Coefficients. We made experimental measurements of log P' for 11 drugs, including morphine and naloxone. Lack of sample made it necessary to estimate log P' for compounds 7 and 8 using empirically derived substituent contributions from the literature[22] and our own measurements on other opioid drugs. All of the benzamide amines except 7 exhibit greater lipophilicity than morphine under the assay conditions employed in this investigation. For a given iv dose, we expect the benzamide amines with high log P' to achieve a much greater concentration in the receptor compartment than morphine since passage of the blood–brain barrier is facilitated by lipid solubility. This seems to be the best explanation for the fact that some of the drugs under study exhibit analgesic potency similar to morphine in spite of much lower affinity for the agonist state of the μ receptor. Relationships between log P' and the activity indices for binding to the agonist and antagonist states of the receptor will be considered in a later section.

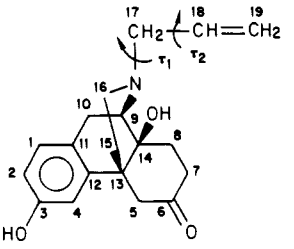
Molecular Geometry. In the crystal, 1 exists as a racemic compound. The cyclohexane ring has a chair form with the benzamide and amine substituents in equatorial positions. Three torsional angles,[30] θ_1 = ∠C(2)–C(1)–C(7)–N(1), θ_2 = ∠C(7)–N(1)–C(1′)–C(2′), and θ_3 = ∠C(1′)–C(2′)–N(2)–C(α), serve to describe the relative orientation of the major substructures in the molecule.



1(*R*,*R*)

The *R*,*R* form of 1 exhibits the following angles in the crystal: θ_1 = 58.3°, θ_2 = 118.2°, and θ_3 = 153.5°; corresponding angles in the *S*,*S* enantiomer are given by the formula $\theta_i(S,S) = 360° - \theta_i(R,R)$. Other geometries of each enantiomer considered in this study differ from the crystal conformation mainly in the values of θ_1, θ_2, and θ_3. When the conformational energy of the isolated molecule was minimized, these torsional angles were found to be θ_1 = 85.0°, θ_2 = 123.0°, and θ_3 = 151.8° in the *R*,*R* enantiomer. After rotating the benzene ring through 180° about the C(1)–C(7) bond, reminimization produced no significant change in the torsional angles or strain energy of the molecule—a result indicating that the meta chloro does not influence the conformational preferences of the molecule to any great extent.

Neither the *R*,*R* nor the *S*,*S* enantiomer in the crystal geometry provides a good simultaneous match-up of the benzene ring and N(2) with the corresponding entities in naloxone. However, by appropriate selection of the benzene ring orientation relative to the phenol ring of naloxone and application of the forced matching technique described in the Methods section, the *R*,*R* and *S*,*S* isomers were both superimposed on the reference drug at a cost in conformational energy of 2.8 and 2.4 kcal/mol, respectively. Both isomers yield good matches with naloxone in which corresponding atoms fall with 0.3 Å of one another. The overlay of aromatic rings in the case of the *R*,*R* form places C(1) of compound 1 near C(11) of naloxone, and the torsional angles describing the "binding" geometry are θ_1 = 261.0°, θ_2 = 113.8°, and θ_3 = 144.6°. For the *S*,*S* isomer, C(1) corresponds to C(12) of naloxone, and the angles take the following values: θ_1 = 270.9°, θ_2 = 245.3°, and θ_3 = 213.8°. The features of the matches involving the isomers of 2–7 are the same as those described for 1; however, the match of the *R*,*R* isomer of molecule 7 to naloxone was achieved with a slightly reduced strain energy of 2.5 kcal/mol.



naloxone

Since molecules 8–11 contain either a cyclopropylmethyl or allyl group, the matches to naloxone require the N-substituents to be overlaid in addition to the benzene rings and nitrogen atoms. Two torsional angles [τ_1 = ∠C(2)–N(2)–C(α)–C(β) and τ_2 = ∠N(2)–C(α)–C(β)–C(γ)] are necessary to specify the orientation of the N-substituent. Although the conformation of the cyclopropylmethyl substituent may be described using an arbitrarily selected ring carbon as C(γ), we will report the values of τ_2 for both possibilities in order to establish the relevant geometric and electronic comparisons of three carbon sequences in the N-substituents.

Our search for low energy conformers of compounds 9–11 produced six local minima in each case. Only the orientation of the *N*-allyl was found to vary significantly among the different conformers; the values of τ_1 occur in three groups (circa 65°, 175°, and 300°), and those of τ_2 fall into two clusters (80–112° and 220–271°). We also discovered six low-energy conformers of molecule 8, where a chain of three cyclopropylmethyl carbons could be related to the chain of allyl carbons in 9–11 as a result of similarities in τ_1 and τ_2. The torsional angles describing these conformers (denoted hereafter as E–J) and the associated energies found by total geometry optimization are given in Table III.

In naloxone, four conformations of the allyl group have been found that correspond to local minima in the potential energy surface generated by variations in τ_1 and τ_2.[9] Similar local minima exist for a series of benzomorphan drugs containing allyl, cyclopropylmethyl, and furylmethyl N-substituents.[23] Since geometry optimization of 8–11 yielded conformers that poorly matched the low-energy conformers of naloxone, we undertook a series of computations to force the benzene ring, N(2), and the allyl (or cyclopropylmethyl) onto the corresponding features of naloxone in Cartesian space using the seven-point model discussed in the Methods section. The findings of these calculations are summarized in Table III, where the matched structures are designated by the labels A–D.

(22) Goodford, P. J. *Adv. Pharmacol.* **1973**, *11*, 51.

(23) Cheney, B. V.; Zichi, D. A.; Miller, A. B. *Int. J. Quantum Chem. Quantum Biol. Symp.* **1983**, *10*, 43.

Table III. Conformational Energies (kcal/mol) and Torsional Angles (deg) of *N*-Allyl- and *N*-Cyclopropylmethyl Substituents in Naloxone-Matched and Isolated Benzamide Amines

	N-allyl[a]			*N*-cyclopropylmethyl[b]			
conformation[c]	τ_1	τ_2	ΔE	τ_1	τ_2	τ_2'	ΔE
A(*R*,*R*)[d,e]	51.2 (0.9)	229.5 (5.2)	7.2 (0.2)	61.8	210.9	280.4	8.0
A(*S*,*S*)[f]	298.3 (1.1)	120.1 (0.8)	5.6 (0.1)	293.6	95.8	165.2	5.6
B(*R*,*R*)[d,g]	322.6 (0.3)	102.5 (0.2)	6.8 (0.3)	313.4	113.5	182.5	4.2
B(*S*,*S*)[f]	306.4 (0.6)	272.1 (0.4)	6.0 (0.1)	309.3	258.6	191.1	5.0
C(*R*,*R*)[d,h]	287.5 (2.5)	107.4 (0.7)	6.7 (0.4)	297.6	113.6	179.5	5.6
C(*S*,*S*)[f]	167.5 (0.4)	101.8 (0.1)	3.6 (0.4)	173.7	101.5	169.0	2.8
D(*R*,*R*)[d,i]	289.7 (2.8)	116.8 (11.4)	4.4 (0.2)	303.6	119.0	185.4	4.6
D(*S*,*S*)[f]	166.1 (1.6)	220.7 (2.1)	4.4 (0.3)	178.4	216.4	284.6	3.1
E(*S*,*S*)[j]	298.4 (2.0)	105.2 (1.2)	2.2 (0.1)	299.2	100.1	169.9	2.7
F(*S*,*S*)[j]	303.0 (1.0)	264.5 (1.3)	2.6 (0.1)	298.1	274.7	205.7	3.9
G(*S*,*S*)[j]	175.1 (3.1)	112.2 (0.5)	0.9 (0.2)	72.7	94.7	163.2	0.4
H(*S*,*S*)[j]	175.1 (4.3)	271.0 (0.9)	1.2 (0.2)	176.6	268.0	208.7	0.7
I(*S*,*S*)[j]	67.7 (3.1)	80.8 (2.3)	0.5 (0.1)	74.3	83.8	152.8	0.0
J(*S*,*S*)[j]	64.3 (1.6)	245.7 (0.1)	0.0 (0.0)	65.1	248.9	179.0	0.7

[a] Averages obtained from molecules **9**–11. [b] In the cyclopropylmethyl group, τ_2' is the torsional angle with ring carbon C(γ') replacing C(γ) at the terminus of the N-substituent chain. [c] Geometries A–D were obtained through matches to the four low-energy conformers of (5*R*,9*R*,13*R*,14*S*)-naloxone: A (80°, 93°), B (77°, 248°), C (180°, 121°), and D (180°, 275°) where (τ_1, τ_2) specifies the torsional angles for the preferred allyl orientation (see ref 9) in the naloxone conformer. E–J were found through total geometry optimization of the isolated molecule in the six energy wells discovered by varying angles θ_1, θ_2, θ_3, τ_1, and τ_2. The θ angles were relatively unaffected by the nature of the N-substituent except for θ_3 in the matched (A–D) structures of the *R*,*R* enantiomer. Measures of the θ angles are given in subsequent footnotes. [d] θ_1 = 249.4 (3.9), θ_2 = 105.0 (2.0). [e] Allyl, θ_3 = 120.5 (2.6); cyclopropylmethyl, θ_3 = 133.7. [f] θ_1 = 273.5 (1.1), θ_2 = 249.8 (1.6), θ_3 = 206.4 (2.7). [g] Allyl, θ_3 = 151.3 (1.6); cyclopropylmethyl, θ_3 = 156.3. [h] Allyl, θ_3 = 125.6 (5.8); cyclopropylmethyl, θ_3 = 137.5. [i] Allyl, θ_3 = 135.3 (1.0); cyclopropylmethyl, θ_3 = 143.1. [j] For the *S*,*S* enantiomer, the following θ angles were obtained: θ_1 = 279.0 (1.0), θ_2 = 241.2 (0.3), θ_3 = 209.7 (0.1); values of the θ and τ angles in the *R*,*R* enantiomer are found by subtracting the *S*,*S* values from 360.

In treating the *S*,*S* isomer of molecules 8–11, conformations E(*S*,*S*)–H(*S*,*S*) provided the initial geometries for A(*S*,*S*)–D(*S*,*S*), respectively. Relaxation of the molecular geometry under the constraints of the extra potentials brought about a marked improvement in the fit to the pharmacophore of naloxone, although the changes in the torsional angles were not large as shown by the θ and τ values reported in Table III. For each matched conformation of the three allyl-containing *S*,*S*-benzamide amines, corresponding atoms in the test and reference molecules were generally separated by 0.1–0.5 Å; however, the C(α)–C(17) distance varied between 0.7 and 1.0 Å. The matches of the cyclopropylmethyl-containing molecule (8) to naloxone yielded separations between corresponding atoms of the order 0.2–1.2 Å, although the distances C(α)–C(17) and C(γ)–C(19) in matches involving conformers A(*S*,*S*), B(*S*,*S*), and D(*S*,*S*) were the only ones to exceed 0.8 Å. All of the matched *S*,*S* structures lie from 3 to 6 kcal/mol above the lowest energy conformer of the free molecule; C(*S*,*S*) has the least strain energy, while A(*S*,*S*) and B(*S*,*S*) possess the greatest.

In order to achieve a good match of all key features using the *R*,*R* isomer, the aromatic ring of the test molecule has to assume a different orientation on the phenolic ring of naloxone than the one taken by the *S*,*S* isomer. The actual ring arrangement for the molecules containing *N*-allyl or *N*-cyclopropylmethyl resembles that discussed previously for the *R*,*R* isomers of molecules 1–7. Although E(*R*,*R*) served as a reasonable initial guess to obtain A(*R*,*R*), the best starting point for all other matches was found to be J(*R*,*R*). With few exceptions, the *R*,*R* isomer fit the four conformations of naloxone with slightly less precision and with higher strain energy than the *S*,*S* isomer. The *R*,*R* matches involving the B form of 8 and the D form of **9**–11 were the only ones achieved at an energy cost equal to or lower than the corresponding *S*,*S* matches.

The foregoing analysis demonstrates that certain structural features in a benzamide amine can be accommodated to the key groups of naloxone at a relatively small conformational energy expense of 2–8 kcal/mol. By assuming a naloxone-like geometry, a benzamide amine will experience the same optimal interactions with critical receptor features as naloxone itself if the following two conditions hold: (1) the fit to the receptor does not involve other drug substructures than the ones considered in the matching of the ligands, (2) the electronic properties affecting receptor interactions of the matched structural features are the same in naloxone and the benzamide amine. A portion of the attractive interactions would necessarily pay the cost of maintaining the benzamide amine in the appropriate geometry, leaving the remainder to be expressed as net stabilization energy for the drug–receptor complex. This hypothesis is consistent with the observed low binding affinities of the benzamide amines relative to naloxone. Further evidence in support of the model will be provided in the following sections through comparisons of electronic structure and the establishment of specific structure activity relationships.

Key Features of Electronic Structure. If Mulliken charge-transfer interactions play a role in stabilizing the drug–receptor complex, the high-energy occupied and low-energy unoccupied MO's (HOMO's and LUMO's) are best suited to act, respectively, as electron donors and acceptors. In order to engage a common receptor, the reactive MO's of different ligands must have similar characteristics. Hence, we have attempted to discern whether any orbitals in each of the N-protonated benzamide amines resemble the three HOMO's (MO's 85–87) and three LUMO's (MO's 88–90) of N-protonated naloxone. Five naloxone reference orbitals are π or π^* orbitals with high density in the phenol moiety, and the sixth (the lowest unoccupied MO) is a π^* orbital localized on C(18) and C(19) of the *N*-allyl substituent. Comparison of a benzamide amine molecular orbital, ϕ_i^B, to a member, ϕ_j^N, of the reference set from naloxone was carried out by computing the τ_{ij}^2 correlation index. We considered the comparison to demonstrate a significant resemblance to ϕ_j^N of naloxone, if one or more ϕ_i^B could be found such that $\sum_i \tau_{ij}^2 > 0.6$. The calculations revealed that the naloxone MO's 89 and 90 correspond to a pair of π^* aromatic ring LUMO's in every benzamide amine. Although no other general correlations could be established involving either the reference HOMO's or LUMO's, we found a subset of four molecules (8–11) possessing one or more N-substituent

LUMO's similar to MO 88 of naloxone. For example, each allyl-containing benzamide amine has a π^* MO localized on C(β) and C(γ) that yielded a value of τ_{ij}^2 greater than 0.8 when compared with the lowest unoccupied orbital of naloxone. In addition, there is a significant resemblance between MO 88 in naloxone and three LUMO's in molecule 8 which exhibit major contributions from p-type basis functions in the cyclopropane ring. These comparisons indicate that the benzamide amines most resemble naloxone in their capacity to act as electron acceptors. Two distinct sites, the benzene ring and an allyl-like N-substituent, may serve as centers of attack for electron-donating receptor entities.

The geometry of the cationic head in protonated naloxone does not correspond to that of the protonated benzamide amines with regard to the orientation of the N–H axis. Therefore, we have examined the molecular electrostatic potential, $V(r)$, of the molecules to discover what effect this might have on Coulombic interactions with charged groups on the receptor. Contour maps illustrating $V(r)$ in the region of the basic nitrogen atom of naloxone and molecule 11 are shown in Figure 1. A positive isopotential line in the contour map signifies that a +1 point charge located on its path would experience a repulsive interaction of the magnitude (in kcal/mol) indicated by the label. The maps in Figure 1 reveal no favorable avenues of approach for electrophiles in the vicinity of either molecule. On the other hand, a zone of high positive potential exists near the basic nitrogen of both molecules. In this zone, an electron-rich nucleophile (denoted by Z in Figure 1) would experience strong electrostatic attraction. Furthermore, the 60, 80, and 100 kcal/mol contour lines follow similar paths in the region of approach where Z would experience the least steric interference with drug moieties surrounding the cationic center. If Z were a neutral species with a large dipole moment, the gradient of the molecular electrostatic potential, not the magnitude, would determine the energy of interaction. Since the spacing between contour lines near Z is similar in the maps for naloxone and molecule 11, the gradients appear to be similar in the zone of interest. Therefore, we may conclude that the cationic centers of molecule 11 and naloxone would experience nearly the same attraction for Z, whether it were an anion or a dipole. This finding also holds for the other drugs under investigation since the molecular electrostatic potential maps of all benzamide amines are much alike in the immediate locale of Z.

Previous calculations of $V(r)$ in a study of naloxone demonstrated that the region near the 3-OH offers an extremely favorable environment for a nucleophilic receptor entity, Y, having the properties of a hydrogen-bonding H acceptor. among the benzamide amines under consideration, only the *R,R* isomer of molecules **2**, **7**, and **9** permits a phenol OH to lie near the indicated position of Y. Although none of the other molecules in the series offers the same opportunity for hydrogen bond formation, the computations of $V(r)$ indicate that Y should nevertheless experience attractive electrostatic interactions in every case; however, the nature of the ring substituent in the vicinity of Y does influence the degree of interaction.

Quantitative Structure–Activity Relationships. We have examined relationships between molecular properties and receptor affinity using the following model:

$$\ln K_i = d_{io} + \sum_r d_{ir}\Omega_r + \ln \{[\exp(-\Delta E_{Sj}/RT) + \exp(-\Delta E_{Rj}/RT)]/2\} \quad (8)$$

where K_i ($i = \tau$ or ρ) is the experimental association constant of the drug for the ith state of the receptor; Ω_r is a drug property that is independent of the isomeric form of the molecule; d_{ir} is a regression coefficient to be determined in the analysis; ΔE_{Sj} and ΔE_{Rj} designate the energy (in kcal/mol) required for the *S,S* and *R,R* isomers, respectively, to attain the binding geometry represented by the jth naloxone-matched state; and $RT = 0.54$ kcal/mol under the conditions of the binding assay.

Each of the four naloxone conformers must be considered a candidate for the preferred binding geometry at the agonist and antagonist states of the receptor. Therefore, we have carried out separate analyses using eq 8 for the benzamide amines in configurations A–D. The dependent variable considered in the analyses is a conformationally adjusted activity index, A_{ij}, given by

$$A_{ij} = \ln K_i - \ln \{[\exp(-\Delta E_{Sj}/RT) + \exp(-\Delta E_{Rj}/RT)]/2\} \quad (9)$$

A_{ij} provides a theoretical measure of the affinity for the ith state of the receptor under the condition that the entire population exists in the jth naloxone-matched conformation. If A_{ij} varies sufficiently with j, the analyses may give some insight regarding the preferred binding geometry of the molecules at the agonist and antagonist states of the μ receptor.

As a result of our drug distribution experiments and structural comparisons to naloxone, we have selected a set of four independent variables, Ω_r, to be considered in the statistical analyses. One of the variables is log P', and the other three are electronic reactivity indices to be discussed in the next paragraph. Values of the four variables are reported for the A geometry in Table IV, together with the receptor affinity indices employed in eq 9.

If an important contribution to drug–receptor stabilization energy arises from donor–acceptor interactions, the electron–acceptor capability of the benzamide-amine LUMO's may play a role in determining the observed variation in receptor affinity within the series. We discount the possibility of the benzamide amines participating as electron donors in the complex since no consistent correlation with any HOMO of naloxone was obtained. The energy, ϵ_u^*, of LUMO ϕ_u provides a convenient electronic index of acceptor capability; i.e., if a LUMO from one molecule has a density distribution comparable to a LUMO in another molecule, the overlap with a donor orbital will be similar, and the one with a lower value of ϵ_u^* will be the better acceptor. We have included three electronic variables ($\epsilon_{\phi 1}^*$, $\epsilon_{\phi 2}^*$ and ϵ_ν^*) corresponding to the energies of the LUMO's for which significant values of τ_{ij}^2 were found in the MO comparisons between the benzamide amines and naloxone. Since molecules 1–7 do not possess a π^* MO with the characteristics of MO 88 in naloxone, the acceptor capability of the amine N-substituent in this subseries has been estimated using the energy of the lowest lying σ^* MO with high density in the C(α)–H linkages.[23] Although molecule 8 possesses a set of three closely spaced LUMO's with density distribution on C(β) and C(γ) comparable to MO 88 of naloxone, we have calculated a single "effective" acceptor energy for the cyclopropylmethyl group using a procedure outlined elsewhere.[24]

The matrix of correlation coefficients corresponding to the set of benzamide amines in the A geometry is given in Table V. Both $A_{\tau A}$ and $A_{\rho A}$ correlate positively with log P' and negatively with $\epsilon_{\phi 1}^*$, $\epsilon_{\phi 2}^*$, and ϵ_ν^*. These findings are consistent with a system in which access to the receptor requires passage from a highly polar environment to one of low polarity, and in which interaction with the receptor may require the unoccupied drug orbitals to act as electron

(24) Cheney, B. V.; Tolley, T. *Int. J. Quantum Chem.* **1979**, *16*, 87.

Table IV. Calculated Results for Molecules in the A Geometry Compared To Observed Activities

	structural factors[a]				theor act.[b]		ln K_τ			ln K_ρ		
compd	log P'	$\epsilon_{\phi 1}$*	$\epsilon_{\phi 2}$*	ϵ_ν*	$A_{\tau A}$	$A_{\rho A}$	obsd	calcd[c]	obsd − calcd	obsd	calcd[d]	obsd − calcd
1	2.23	0.1417	0.1507	0.7561	21.0	17.7	16.2	17.5	−1.2	12.9	12.7	0.2
2	0.35	0.1974	0.2437	0.7561	15.7	15.2	11.0	11.1	−0.1	10.4	9.1	1.3
3	0.75	0.2317	0.2006	0.7561	16.4	14.1	11.7	12.5	−0.8	9.3	9.9	−0.6
4	0.83	0.1696	0.1601	0.7561	18.0	14.1	13.3	12.8	0.5	9.3	10.0	−0.7
5	0.45	0.1686	0.1493	0.7561	16.4	13.7	11.6	11.4	0.2	9.0	9.4	−0.4
6	1.53	0.1630	0.1708	0.7561	19.5	15.6	14.8	15.1	−0.3	10.8	11.4	−0.6
7	0.12[e]	0.1979	0.2422	0.7561	14.3	13.4	9.7	9.6	0.1	8.8	8.5	0.4
8	2.90[e]	0.1791	0.1863	0.3599	24.1	24.1	13.1	13.4	−0.3	13.1	11.7	1.4
9	1.77	0.1902	0.2341	0.1711	21.9	21.9	10.9	9.6	1.3	10.9	11.4	−0.5
10	3.18	0.1355	0.1422	0.1606	26.0	25.8	14.9	14.4	0.6	14.8	14.2	0.6
11	3.65	0.1351	0.1433	0.1626	27.0	25.1	15.9	15.9	0.0	14.1	15.1	−1.0
morphine[f]	0.11	0.2270	0.2295	0.7561	19.2[g]	13.0[g]	18.8	14.6	4.2	12.6	13.0	−0.4
naloxone[f]	1.06	0.2241	0.2184	0.1664	20.5[h]	21.8[h]	19.4	17.2	2.2	20.7	20.0	0.7

[a] The orbital energy, ϵ_u*, is reported as the mean of the values obtained from the enantiomers of the compounds. The standard error of the mean was calculated to be less than 10% in all cases. $\epsilon_{\phi 1}$* and $\epsilon_{\phi 2}$* are the energies of LUMO's localized in the aromatic ring which exhibit high correlations with MO's 90 and 89, respectively, of naloxone. ϵ_ν* is the effective acceptor energy of N-substituent LUMO(s) with sites of high density similar to MO 88 in naloxone. [b] The A_{ij} index, given by eq 9, provides a theoretical measure of the affinity for the ith receptor state when all molecules exist in the binding geometry specified by the jth configuration of the molecules. We employed A_{ij} as the dependent variable in the regression analyses. [c] Results obtained from eq 8 using the regression coefficients from Table VI (A). [d] Values calculated by eq 8 with the regression coefficients from Table VI (B). [e] Estimated using empirical substituent constants. [f] Not included in the regression analyses from which values of the coefficients in Table VI were obtained. [g] The calculated values of the conformational energy used in determining A_{ij} for morphine were $\Delta E_- = 0.0$ kcal/mol and $\Delta E_+ = 0.6$ kcal/mol. An axial orientation of the N-methyl group in (+)-morphine was required to match the N-methyl of (−)-morphine. [h] In the computation of the theoretical binding index of naloxone, the following calculated conformational energies were used for the A geometry: $\Delta E_- = 0.2$ kcal/mol for the 5R,9R,13R,14S isomer and $\Delta E_+ = 4.5$ kcal/mol for the 5S,9S,13S,14R isomer. In order to match the N-substituent chain of (−)-naloxone, the N-allyl of (+)-naloxone must assume a high-energy axial orientation on the piperidine ring.

Table V. Correlation Matrix for the Benzamide Amines in the A Geometry

	log P'	$\epsilon_{\phi 1}$*	$\epsilon_{\phi 2}$*	ϵ_ν*	$A_{\tau A}$	$A_{\rho A}$
log P'	1.0	−0.69	−0.57	−0.79	0.99	0.93
$\epsilon_{\phi 1}$*		1.0	0.77	0.40	−0.69	−0.54
$\epsilon_{\phi 2}$*			1.0	0.17	−0.53	−0.31
ϵ_ν*				1.0	−0.86	−0.94
$A_{\tau A}$					1.0	
$A_{\rho A}$						1.0

acceptors. On the basis of the magnitude of the correlation coefficients, variation in $A_{\tau A}$ and $A_{\rho A}$ is highly correlated with log P' and ϵ_ν, while the correlation with $\epsilon_{\phi 1}$* is weaker but still noteworthy. Since log P' also tends to vary negatively with $\epsilon_{\phi 1}$* and ϵ_ν* in the series under study, it is not possible to dissociate the effects of drug distribution from electron-acceptor capacity in developing a quantitative structure-activity relationship. The correlation matrices for geometries B–D (not shown) exhibit the general features noted in the matrix for geometry A.

Using eq 8 as the model, we employed stepwise multiple regression analysis for each candidate geometry of the drug molecules to examine binding at the agonist and antagonist states of the μ receptor. In all four cases, a single regression variable, log P', sufficed to give the best fit for the agonist state, while two variables, log P' and ϵ_ν*, were required for the antagonist state. A summary of the statistical analyses corresponding to the A geometry is shown in Table VI, and the comparison of calculated to observed values of ln K_i ($i = \tau$ or ρ) is given in Table IV. The calculated values of ln K_τ and ln K_ρ for all benzamide amines in the A geometry are in reasonable agreement with the experimental findings. Since the electronic indices, ϵ_u*, show relatively minor variation from one conformation to another, differentiation among the possible binding conformations arises primarily from the strain energy contributions to A_{ij} in molecules 8–11. As shown in Table VII, the strain-energy effects caused extremely high estimates of ln K_τ for molecule 8 in geometries B–D. Although strain-energy contributions did not lead to outliers in any calculation of ln K_ρ, the effects were revealed more subtly in the value of the coefficient of ϵ_ν* obtained in the regression equations for the different binding geometries. We found that the equations for geometries A and B exhibit a dependence upon ϵ_ν* which is consistent with the result obtained in a benzomorphan study,[23] but the equations for geometries C and D do not show the same

Table VI. Statistical Analyses Using Eq 8 and the Structural Data for the Benzamide Amines in the A Configuration: (A) Agonist-State Binding, (B) Antagonist-State Binding

A

structural factor, Ω_τ	regression parameters, $d_{\tau r}$	standard error	F	prob > F
log P'	3.4	0.2	363.4	0.0001
intercept	14.6			
multiple correlation coefficient (r^2)			0.98	
standard error of estimate (s)			0.7	
F value for analysis of variance (F)			363.4	
prob > F			0.0001	

B

structural factor, Ω_τ	regression parameters, $d_{\rho r}$	standard error	F	prob > F
ϵ_ν*	−9.9	1.7	34.2	0.0004
log P'	1.9	0.4	26.1	0.0009
intercept	20.7			
multiple correlation coefficient (r^2)			0.97	
standard error of estimate (s)			0.9	
F value for analysis of variance (F)			143.6	
prob > F			0.0001	

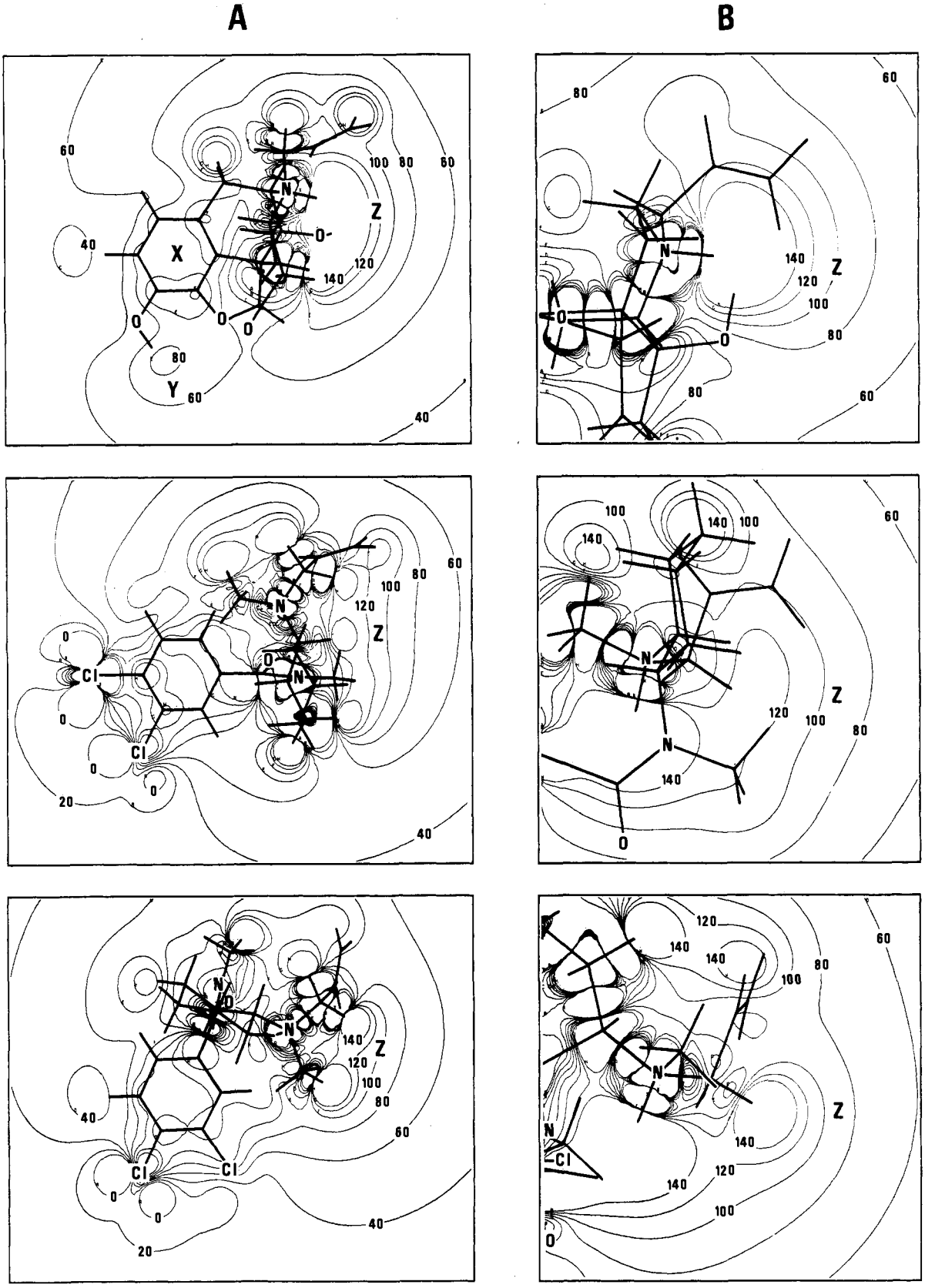


Figure 1. Molecular electrostatic potential contour maps of naloxone (top), the *S,S*, enantiomer of molecule 11 (center), and the *R,R* enantiomer of molecule 11 (bottom). The three A-geometry structures are shown in the matched orientation. For naloxone, the origin is at C(3), the benzene ring lies in the *xy* plane, and N is located at point (4.43, 3.35, 1.26), where coordinates are expressed in A. (A) *xy* plane at $z = 1.26$ Å. (B) *xz* plane at $y = 3.35$ Å. X, Y, and Z are hypothetical electron-rich receptor entities which interact with the aromatic ring, the 3-OH of naloxone, and the protonated amino group, respectively.

dependence. This evidence suggests that the A conformation may be the preferred binding geometry for both states of the μ receptor, although the B geometry may not be ruled out in the case of the antagonist state. On the other hand, configurations C and D are less likely to be relevant structures for μ activity.

As a check on the model, we have employed the regression equations obtained in this study to calculate ln

Table VII. Parameters, d_{ir}, and Deviations of Outliers Obtained in the Analysis of μ Receptor Binding Using Eq 8 with the Benzamide Amines in Geometries B–D

geometry	affinity constant, K_i	regression parameter, d_{ir}			obsd ln K_i – calcd ln K_i		
		intercept	log P'	ϵ_ν*	molecule 8	morphine[a]	naloxone[a]
B	K_τ	14.7	3.3 (0.3)		−2.9[b]	4.2[b]	1.9
	K_ρ	21.7	1.6 (0.3)	−11.0 (1.5)	−1.1	−0.5	−0.3
C	K_τ	15.2	2.1 (0.3)		−2.3[b]	3.7[b]	2.6[b]
	K_ρ	16.3	1.7 (0.3)	−3.9 (1.4)	−0.8	−0.5	4.0[b]
D	K_τ	15.1	2.3 (0.3)		−2.3[b]	3.8[b]	3.2[b]
	K_ρ	17.3	1.6 (0.3)	−5.3 (1.5)	−0.8	−0.5	3.9[b]

[a] Not included in data set used to determine regression equation. [b] The magnitude of the deviation exceeds 2s, where s is the standard error of the estimate.

K_i for naloxone and morphine. The values reported for geometry A in Table IV show that ln K_τ is seriously underestimated for both drugs, while ln K_ρ is in reasonable agreement with observation. Our examination of other naloxone geometries (see Table VII) revealed that conformer B gave results similar to A, but conformations C and D yielded poor estimates for both ln K_τ and ln K_ρ. The poor calculated values of ln K_τ indicate that the simple model for agonist-state binding of the benzamide amines does not extend to morphine and naloxone. However, since none of the active benzamide amines possesses a hydroxy group corresponding to the 3-OH of morphine and naloxone, a modification of the model to include a 3-OH substituent factor might serve to reconcile the calculated and observed values of ln K_τ for all compounds considered in this study. The existence of a 3-OH effect in receptor binding has been demonstrated experimentally by comparison of compounds such as 3-deoxymorphine and morphine.[14,25] The satisfactory calculations of ln K_ρ using the equations for geometries A and B makes it unnecessary to postulate additional substituent effects to rationalize the activity of naloxone and morphine at the antagonist state of the receptor. In order to use statistical methods for examination of additional substituent effects (such as the 3-OH), studies on an expanded series of μ-active drugs will be required.

Conclusion

We have employed molecular mechanics and an ab initio SCF-MO procedure using FSGO basis sets to investigate the geometry and electronic structure of various benzamide amines. Since these agents exhibit affinity for the opioid μ receptor and produce morphine-like pharmacological effects in animals, we have compared the molecules to naloxone, a drug possessing high affinity for both the agonist and antagonist states of the μ receptor. Spatial matching of the benzene ring and amine nitrogen atom to the corresponding features of naloxone required torsional angle adjustments costing 2.4–2.8 kcal/mol in energy. We found that the N-protonated cations of the benzamide amines and naloxone resemble each other electronically in two key aspects: (1) the features of a pair of π* MO's centered in the benzene ring and (2) the value of the molecular electrostatic potential near the cationic head. Those benzamide amines with an N-allyl or N-cyclopropylmethyl were matched to each of the four low-energy naloxone conformations by adjusting the N-substituent, as well as the aromatic ring and amine nitrogen. The total energy expended in making the required geometry changes was calculated to fall in the range from 2.8 to 8.0 kcal/mol depending upon the naloxone conformer used as reference. We discovered low-energy unoccupied π* MO's resembling the LUMO of N-protonated naloxone in the allyl- and cyclopropylmethyl-substituted benzamide amines. As a result of the similarities in the spatial location and electronic structure of key features, the cations of the benzamide amines and naloxone could interact strongly with electron-rich receptor entities situated near the benzene ring, the protonated nitrogen, and an allyl-like N-substituent.

The experimental measure of receptor affinity, ln K_i (where $i = \tau$ for the agonist state and $i = \rho$ for the antagonist state), exhibits significant correlations with certain benzamide-amine properties—in particular, the drug distribution coefficient, log P', and the energies, $\epsilon_{\phi 1}$* and ϵ_ν*, of LUMO's localized in the benzene ring and N-substituent. The correlation with log P' indicates that access to the binding site requires passage from the highly polar buffer medium into, or through, a region of lower polarity; while the correlations involving the LUMO energies suggest that formation of the drug–receptor complex involves charge-transfer interactions, where the benzene ring and N-substituent act as electron acceptors. For the series of benzamide amines included in this study, the relative importance of drug partitioning and donor–acceptor interactions cannot be clearly assessed due to the rather strong covariance of log P' and the two ϵ_u* indices.

By means of stepwise regression techniques, we examined a model that takes into account the strain energy required to attain the hypothetical binding conformation represented by one of the four naloxone-matched geometries. In the case of agonist-state receptor binding, a single regression variable, log P', sufficed to give a highly significant fit. The most satisfactory results were obtained with the molecules in the A configuration. In the case of antagonist-state binding, two regression variables, ϵ_ν* and log P', were required in the fitting process. The best overall fits were achieved with configurations A and B. Of interest is the possibility that both states of the receptor interact with the drugs in the A geometry. The relationship between ln K_ρ and ϵ_ν* is consistent with the results obtained in a study of benzomorphan congeners.[23]

In summary, this study allows us to make several observations regarding the potency and efficacy of the benzamide amines as μ-active opioid drugs. First, the relatively low affinity of the benzamide amines for the μ receptor can be attributed to the low population of molecules in the high-energy naloxone-like binding conformation. This is particularly true for molecules 8–11, where difficulty in attaining the proper orientation of the amino N-substituent adversely affects the affinity for both states of the receptor. Second, although the benzamide amines with $K_\tau/K_\rho \approx 1$ did not antagonize morphine at the highest doses tested, these compounds are predicted to exhibit antagonist character against a μ agonist with lower affinity for the receptor. Third, since the R,R and S,S stereoisomers of 1–7 match naloxone with small differences in strain energy, both isomers are predicted to associate with the receptor if the fit to the binding site involves only the

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pharmacaphore used in establishing the matches. This is in contrast to naloxone, where the activity resides mainly in the (-) optical antipode. Fourth, antagonist character has a marked dependence on the electron-acceptor properties of the substituents on the amine nitrogen. Thus, molecules 1–7 are primarily agonists due to the weak acceptor capacity of the *N*-methyl group. Fifth, some differentiation in the agonist–antagonist character of the benzamide amines may be attributed to the effect of log P', since ln K_τ varies more strongly with the drug distribution coefficient than ln K_ρ.

We have applied the techniques described in this paper to a larger series of opioid μ agonists and antagonists, including compounds from a variety of chemical classes. The results of the extended study will be published at a later date.

Experimental Section

Melting points were taken in a capillary tube and are corrected. IR spectra were determined in Nujol by using a Perkin-Elmer Model 421 recording spectrophotometer. UV spectra were determined in 95% EtOH by using a Cary Model 14 spectrophotometer. Mass spectra were recorded by using a CH-4 Atlas mass spectrometer. The silica gel used for chromatography was obtained from E. Merck A. G., Darmstadt, Germany. NMR spectra were recorded on a Varian XL-100 and A-60A spectrometer equipped with a Varian variable-temperature probe; chemical shifts were recorded in parts per million downfield from Me_4Si.

Synthesis of Intermediates. The following compounds were prepared according to the literature: 7-azabicyclo[4.1.0]heptane,[25] *trans*-*N*,*N*-dimethyl-1,2-cyclohexanediamine,[26] and N-methyl-7-azabicyclo[3.1.0]heptane.[28]

***trans*-*N*,*N*'-Dimethyl-*N*-allyl-1,2-cyclohexanediamine.** A mixture of *N*-methylazabicyclo[4.1.0]heptane (8.64 g, 0.078 mol), *N*-allylmethylamine (11.05 g, 0.156 mol), 16.6 mL of H_2O, and 0.2 g of NH_4Cl was stirred and heated in an oil bath maintained at 115–117 °C for 16 h. The mixture was cooled, saturated with solid NaOH, and extracted well with ether. The ether extract was dried ($MgSO_4$) and evaporated with use of a Vigreux column, and the residue was distilled at bp 104–105 °C mm 7.27 g (51% yield).[30]

***trans*-*N*,*N*'-Dimethyl-*N*-cyclopropylmethyl-1,2-cyclohexanediamine** was prepared according to the above procedure with use of *N*-methylazabicyclo[4.1.0]heptane and *N*-cyclopropylmethylamine, bp 127–129 °C (13 mm), 37% yield.[31]

***trans*-*N*-Methyl-*N*-allyl-1,2-cyclohexanediamine** was prepared according to the above procedure with use of 7-azabicyclo[4.1.0]heptane and *N*-allylmethylamine, bp 100–102 °C (14 mm), 34% yield.[31]

***N*,*N*,*N*'-Trimethyl-1,2-cyclohexanediamine.** A solution of *trans*-*N*,*N*-dimethyl-1,2-cyclohexanediamine (5.12 g, 0.036 mol) and 100 mL of ethyl formate was refluxed 17 h and evaporated. The product, *trans*-*N*-[2-(dimethylamino)cyclohexyl]formamide was distilled at bp 104 °C (0.1 mm) 5.2 g (85% yield).[31] A solution of the above *N*-formyl compound (4 g, 0.0235 mol) in 50 mL of ether was added during 5 min to a solution of LAH (4 g) in 250 mL of ether and the mixture was refluxed 17 h. It was cooled in ice, decomposed in succession with 4 mL of H_2O, 4 mL of 15% NaOH, and 12 mL of H_2O, stirred 1 h at room temperature, and filtered. The cake was washed with ether, and the solvent was distilled through a Vigreux column. The residue was distilled at bp 86–87 °C (14 mm), 3 g (82% yield).[31]

Method A. *trans*-*p*-Chloro-*N*-[2-(dimethylamino)cyclohexyl]-*N*-methylbenzamide Maleate (1:1) (6). A solution of *p*-chlorobenzoyl chloride (0.875 g, 5 mmol) in 10 mL of ether was added dropwise during 10 min to a solution of *N*,*N*,*N*'-trimethylcyclohexane-1,2-diamine (0.78 g, 5 mmol) in 50 mL of ether containing triethylamine (0.505 g, 5 mmol) while the temperature was maintained at 20–26 °C. The resulting suspension was stirred at room temperature for 17 h. Saturated $NaHCO_3$ solution (25 mL) was added, the ether layer was separated, and the aqueous was extracted once with ether. The combined ether extract was washed with H_2O and saturated salt solution, dried ($MgSO_4$), and evaporated. A solution of the crude solid in 10 mL of 2% MeOH–$CHCl_3$ was chromatographed on 130 g of silica gel with use of the same solvent as eluent. The first fraction (500 mL) and fractions 2–18 (25 mL each) gave no material. Fractions 19–46 (25 mL each) gave 1.1 g, which was crystallized from ether to give 1 g: mp 121–122 °C; UV λ_{max} 220 nm (ϵ 12 550), sh 271, sh 277 (542); IR 2780 (*N*-alkyl), 1625 (C=O), 1600 (C=C), 1570, 1515, 1490; aromatic 850; NMR ($CDCl_3$) δ 1.0–2.0 (m, 8, CH_2's), 2.06, 2.29 (2 s, 6 NMe_2), 2.5 (center of multiplet, 1, CHN), 2.77, 2.94 (2 s, 3, $CONCH_3$), 7.38 (s, 4, aromatic). Anal. ($C_{16}H_{23}ClN_2O$) C, H, Cl, N.

The free base was converted to the salt with equimolar amount of maleic acid in ether. Crystallization from methanol–ether gave colorless prisms, mp 172–173 °C. Anal. ($C_{16}H_{23}ClN_2O \cdot C_4H_4O_4$) C, H, Cl, N.

Method B. *trans*-*N*-[2-(Dimethylamino)cyclohexyl]-*p*-hydroxy-*N*-methylbenzamide (2). A solution of ethanethiol (0.54 g, 8.6 mmol) in 10 mL of DMF was added during 10 min to a suspension of sodium hydride (0.361 g, 8.6 mmol of 50% dispersion in mineral oil, washed with petroleum–ether, 30–60 °C) and stirred for 1 h. A solution of *trans*-*N*-[2-(dimethylamino)cyclohexyl]-*p*-methoxy-*N*-methylbenzamide (13, free base) in 30 mL of DMF was added and the mixture heated at 90 °C for 3 h. The solvent was evaporated, and the residue was treated with 5 mL of H_2O and 8.6 mmol of HOAc, followed by $NaHCO_3$ to bring the pH to 7. The product was extracted well with $CHCl_3$; the extract was dried ($MgSO_4$) and evaporated. Crystallization from ether gave 0.247 g of colorless prisms. Recrystallization from MeOH ether gave 0.187 g, mp 210–212 °C.

Method C. *trans*-*N*-[2-(Dimethylamino)cyclohexyl]-*p*-hydroxybenzamide (7). A solution of boron tribromide (4.31 g, 0.017 mol) in 25 mL of CH_2Cl_2 (dried over molecular sieves, 4 Å) was added dropwise during 15 min to a solution of compound 12 (0.95 g, 0.0034 mol) in 25 mL of CH_2Cl_2 while the temperature was maintained at −50 to −70 °C. The resulting oily mixture was allowed to warm to room temperature during ca. 40 min and stirred for 20 h. It was concentrated at room temperature in vacuo to a small volume. CH_2Cl_2 (50 mL) and water was added, followed by solid $NaHCO_3$ to pH 7. The mixture was saturated with NaCl and extracted with ethyl acetate (10 × 50 mL). The extract was dried ($MgSO_4$) and evaporated to give 0.636 g. Crystallization from MeOH–benzene gave colorless rods, 0.469 g, mp 232.5–234 °C. Anal. ($C_{15}H_{22}N_2O_2$) C, H, N.

Acknowledgment. D. J. Duchamp provided the X-ray crystallographic structural data for molecule 1. The pharmacological assays were carried out under the auspices of P. F. VonVoigtlander. Expert technical assistance in the synthesis of the compounds, receptor binding assays, and theoretical calculations was provided, respectively, by L. G. Laurian, C. Barsuhn, and A. B. Miller. We appreciate the contributions of these individuals to our study.

Registry No. 1, 82657-23-6; (*R*,*R*)-1, 98717-00-1; (*S*,*S*)-1, 98717-01-2; 2, 98587-47-4; 3, 67579-34-4; 3·4-$MeC_6H_4SO_3H$, 67579-35-5; 4, 98587-45-2; 4·4-$MeC_6H_4SO_3H$, 98587-48-5; 5, 67579-77-5; 6, 67579-11-7; 6·maleate, 67579-12-8; 7, 98587-44-1; (*R*,*R*)-7, 98717-02-3; 8, 98587-46-3; (*R*,*R*)-8, 98717-03-4; (*S*,*S*)-8, 98717-04-5; 8·$MeSO_3H$, 98587-49-6; 9, 98587-43-0; (*R*,*R*)-9, 98717-05-6; (*S*,*S*)-9, 98717-06-7; 10, 67579-41-3; (*R*,*R*)-10, 98717-07-8; (*S*,*S*)-10, 98717-08-9; 10·fumarate, 67579-42-4; 11, 67579-46-8; (*R*,*R*)-11, 98717-09-0; (*S*,*S*)-11, 98717-10-3; 11·$MeSO_3H$, 67579-47-9; 12, 67579-25-3; 13, 67579-70-8; 13·4-$MeC_6H_4SO_3H$, 67579-71-9;

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(31) This compound was characterized by UV, IR, NMR, MS, and GC.

14, 286-18-0; 15, 51066-08-1; 16, 67198-21-4; 17, 67198-25-8; 18, 67198-32-7; 19, 67579-93-5; 20, 67198-27-0; 21, 67198-26-9; *N*-allymethylamine, 627-37-2; 4-methoxybenzoyl chloride, 100-07-2; 3,4-dichlorobenzoyl chloride, 3024-72-4; 4-hydroxybenzoyl chloride, 28141-24-4; 3-methoxybenzoyl chloride, 1711-05-3; 4-nitrobenzoyl chloride, 122-04-3; 4-cyanobenzoyl chloride, 6068-72-0; 4-chlorobenzoyl chloride, 122-01-0; 4-(trifluoromethyl)benzoyl chloride, 329-15-7; *N*-(cyclopropylmethyl)methylamine, 18977-45-2.

Pyrimidinones. 1. 2-Amino-5-halo-6-aryl-4(3*H*)-pyrimidinones. Interferon-Inducing Antiviral Agents

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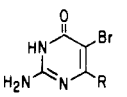
Interferon induction and antiviral activity was discovered with 2-amino-5-bromo-6-phenyl-4(3*H*)-pyrimidinone. An analogue study incorporating a series of 2-amino-5-substituted-6-arylpyrimidinones revealed that the most potent interferon inducers were mono- and difluorophenyl analogues. These same analogues were also potent antiviral agents against Semliki Forest virus and herpes simplex type 1. In addition the monomethoxyphenyl analogues were potent antiviral agents but weak interferon inducers. Relatively modest structural changes led to dramatic changes in bioactivity. There was a relatively poor correlation between levels of circulating interferons induced and systemic antiviral activity.

The utility of interferons in mediating an antiviral state in the virus-infected host is well established.[1,2] One approach to antiviral therapy with interferon (IFN) in the infected host is through the use of interferon inducers.[2,3] Although much of the focus in this area has been on high-molecular-weight, ionic polymers such as polynucleotides (poly(IC)) and pyrans, research and clinical evaluation has also included several low-molecular-weight interferon inducers, such as fluorenones and aliphatic diamines.

In 1976, 2-amino-5-bromo-6-methyl-4(3*H*)-pyrimidinone (ABMP) was reported to induce circulating serum levels of interferons when administered orally (po) or intraperitoneally (ip) to rodents and cats.[4] Although it compared favorably with poly (IC) and tilorone in terms of circulating IFN levels and hyporesponsiveness,[5] it exhibited a toxicity-limiting crystal deposition in the renal papillae of rats upon chronic administration.[7] Subsequent studies demonstrated that the analogous 6-phenylpyrimidinones did not possess this toxic liability[6] and, furthermore, exhibited enhanced IFN-inducing and antiviral potency and activity.[6,8]

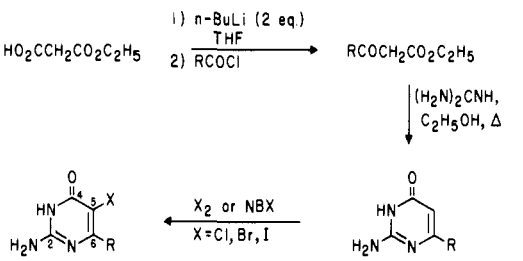
Further biological evaluation of an initial lead candidate in this second-generation pyrimidinone series, 2-amino-5-bromo-6-phenyl-4(3*H*)-pyrimidinone (ABPP), served to unravel an intriguing spectrum of immunomodulatory activity[9-11,16] that may be related to its antiviral[12,13] and antitumor activity.[14,15] In efforts to elucidate the structure–activity relationship (SAR) profile of these bioactivities in this pyrimidinone series, we have systematically varied synthetically accessible points in the generic molecule. We report herein the effects of molecular modifications at the 6-position of the 2-amino-5-halo-4-pyrimidinone structure upon antiviral activity (SFV and HSV-1) and IFN induction in mice.



R = CH_3 – ABMP
R = C_6H_5 – ABPP

Chemistry. 2-Amino-6-substituted-4-pyrimidinones are readily prepared by condensation of an appropriate β-keto ester with guanidine (Scheme I).[6,17] The β-keto esters are prepared in high yield by acylation of dilithio ethylmalonate[18,19] followed by protonation. The introduction

Scheme I



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