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Computer-aided structure–affinity relationships in a set of piperazine and 3,8-diazabicyclo[3.2.1]octane derivatives binding to the μ-opioid receptor

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SUMMARY

Molecular modeling studies were carried out on a set of piperazine and 3,8-diazabicyclo[3.2.1]octane derivatives with the aim to highlight the main factors modulating their affinity for the μ-opioid receptor. Structure–affinity relationships were developed with the aid of molecular mechanics and semiempirical quantum-mechanics methods. According to our proposed pharmacodynamic model, the binding to the μ-receptor is promoted by the following physico-chemical features: the presence of hydrocarbon fragments on the nitrogen ring frame capable of interacting with one of two hypothesized hydrophobic receptor pockets; a 'correct' orientation of an *N*-propionyl side chain so as to avoid a sterically hindered region of the receptor; the possibility of accepting a hydrogen bond from a receptor site complementary to the morphine phenol oxygen.

INTRODUCTION

In previous papers the synthesis and the analgesic properties of 3,8-diazabicyclo[3.2.1]octanes [1] and related *cis*-2,6-dimethylpiperazines [2] were described. In recent years, the affinity towards μ-and δ-receptors of representative terms of these series was also evaluated [3, Fratta W., personal communication], finding that analgesic properties were correlated essentially to interactions with μ-receptors.

Among the variety of investigated compounds, the structures listed in Fig. 1 should be mentioned (their biological data are listed in Table 1). In these molecules as well as in other close analogs the μ-affinity is related to some structural requirements, also involving the N-substitu-

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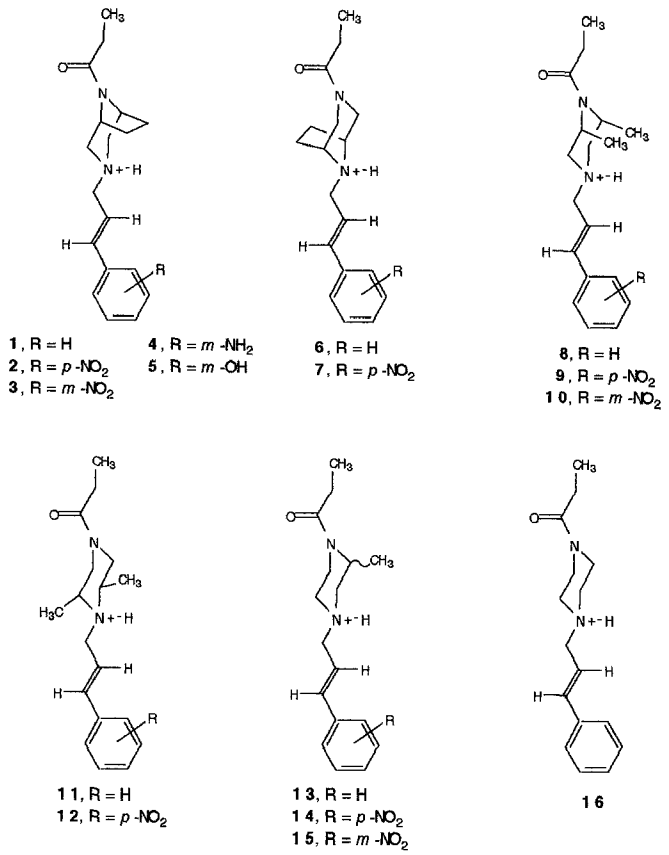


Fig. 1. Structures of the piperazine and diazabicyclooctane ligands.

ents. Particularly, a propionyl group and a three-carbon aralkyl or aralkenyl chain are needed for optimum activity.

From the comparison of the μ-affinity values of the benzene unsubstituted compounds (**1, 6, 8, 11, 13** and **16**) we notice that the potency depends on the particular type of nitrogen ring system.

The presence of a nitro group in *para* to the benzene ring is associated, except the pairs **8/9** and **13/14**, with a slight increment of affinity. When the -*p*-NO_2 group is replaced by the -*m*-NH_2 and -*m*-OH substituents, the binding to the receptor is weakened (compare **2** vs. **4** and **5**).

Compound **2** stands out in our set for its fairly high μ-potency combined with a remarkable analgesic activity (about eight times more effective than morphine). While **2** is nearly as active as its parent compound **1**, the removal of the -*p*-NO_2 group in the full agonist **7** unexpectedly leads to partial agonism in **6**.

In order to highlight the unclear aspects of the structure–affinity relationship in our data set, molecular modeling studies were conducted. Specifically, we attempted to address the following questions: do the compounds of our interest act at the μ-receptor similarly to classic opioids such as morphine?; can we assess their 'bioactive' conformations?; what are the main factors influencing their potency?

METHODS

Molecular models were constructed using standard bond distances and bond angles with the molecular modeling software package SYBYL [4] implemented on an Evans and Sutherland graphics system. The molecules were modeled in their nitrogen-protonated forms. Geometry optimizations were performed with the MAXIMIN2 command of SYBYL (using the TRIPOS [5] force field with neglect of electrostatics) or the MOPAC program [6] (selecting the AM1 [7] model).

Systematic conformational searches were carried out with the SYBYL/SEARCH option to detect low-energy conformations. These geometries were successively fully optimized with SYBYL/MAXIMIN2 to yield more accurate global minimum-energy values. The bioactive conformations of flexible molecules were determined through conformational searches constrained by interatomic distances measured in relatively rigid templates.

The searches for steric energy global minima and pharmacophore-consistent conformations were performed by scanning properly specified torsional angles with 20° increments, starting from a local minimum conformer, applying a van der Waals scaling factor of 0.75 and an energy window of 10 kcal/mol. The distance constraints were associated to a ± 1.0 Å tolerance range.

Molecular superimpositions were performed by minimizing the root mean square distance

TABLE 1
BIOLOGICAL DATA

Cmpd	μ	δ	ED_{50}
1	7.43	6.15	1.1
2	7.89	5.60	0.6[a]
3	7.47	6.22	NT
4	7.04	6.15	NT
5	6.00	5.57	8.2
6	7.17	5.37	16.0
7	7.74	6.00	0.3[a]
8	7.17	5.89	1.2[a]
9	6.29	4.68	NT
10	6.60	5.15	NT
11	5.36[a]	NT	NA[a]
12	6.19[a]	NT	NT
13	6.47	5.30	1.9
14	5.77	3.54	NT
15	6.17	5.15	NT
16	5.40	4.19	NT
Morphine	7.82	6.95	5.0

[a] Data determined by Fratta, W. (personal communication); the remaining data were determined by Cignarella et al. [3]; μ is the −log of the inhibition constant (in nM) of the tested compound on [^{3}H]dihydromorphine [3] or [^{3}H-D-Ala^2-MePhe^4-Glyol^5-enkephalin (Fratta, W., personal communication) binding to Albino Swiss mice brain homogenates; δ is the −log of the inhibition constant (in nM) of the tested compound on [^{3}H]-D-Ala^2-D-Leu^5-enkephalin [3]; ED_{50} is expressed in mg/kg and is the analgesic activity determined in Albino Swiss mice at the peak effect time. NT = not tested; NA = not active at 20 mg/kg.

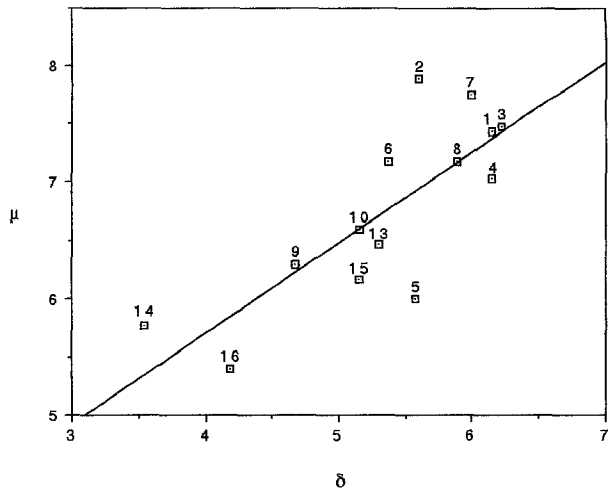


Fig. 2. The μ-affinity values of compounds **1–10** and **13–16** plotted versus the δ-affinity values.

(rmsd) between selected pharmacophoric points through the SYBYL/FIT option. Molecular volume manipulations were realized using the SYBYL/MVOLUME command.

RESULTS AND DISCUSSION

(1) Relationship between μ- and δ-receptor affinity

As it is clearly shown in Table 1, the compounds considered in this study are provided with a more or less pronounced μ/δ selectivity profile. All of them, except compounds **11** and **12**, which were not tested in vitro on the δ-receptor, exhibit highest affinity at the μ-receptor. It is interesting to note that the most potent term of the series, compound **2**, is also characterized by the highest μ-selectivity (specifically, it binds to the μ-receptor 192 times more tightly than to the δ-receptor).

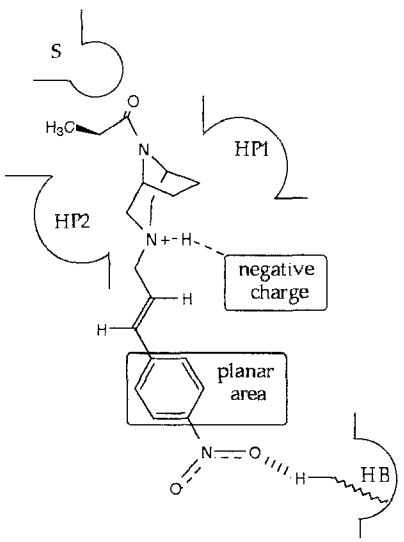
The following equation was derived by linearly correlating the μ- and δ-affinity values of compounds **1–10, 13–16**.

$$\mu = 0.775\ (\pm\ 0.377)\ \delta + 2.610\ (\pm\ 2.039)$$
$$n = 14,\ r = 0.791,\ s = 0.490,\ F_{1,12} = 20.023$$

In this equation, μ and δ correspond to the binding affinity data reported in Table 1, n is the number of data points, r is the correlation coefficient, s is the standard error, $F_{1,12}$ is the F-value used to evaluate the significance of the equation ($P < 0.001$), and the figures in parentheses are the 95% fiducial intervals.

In Fig. 2 the μ-affinity values are plotted versus the δ-affinity values. It can be easily noticed that compounds **2** and **5** significantly deviate from the interpolation curve and can therefore be considered as outliers.

The above relationship explains about 62% of the data variance and indicates that the physicochemical modulators of the potency at the μ- and δ-receptor subtypes are, overall, similar.



Scheme 1. Model of interaction at the μ-receptor: the HP1 and HP2 sites are hydrophobic pockets, the S site is a sterically hindered region and the HB site provides a hydrogen bond donor group.

(2) Effect of the nitrogen ring system on the μ-affinity

The μ-affinity values of the benzene unsubstituted compounds vary within a two-orders-of-magnitude range. This indicates that the strength of the binding is heavily influenced by the particular type of the nitrogen ring frame. One of the possible reasons of the observed difference of affinity between **1** and **16** might be the possibility for the endoethylenic bridge of **1** to be accommodated into a small hydrophobic pocket of the receptor (HP1 site in Scheme 1).

Conformational searches for global minima showed that **8** exists in a largely preferred conformation with the 2,6-methyl groups having axial orientations. In such a conformation, the two methyl groups should be capable of interacting with the hydrophobic receptor site HP1 similarly to the endoethylenic bridge of **1**.

In the lowest-energy conformation of **11**, the 2,6-methyl groups are in equatorial positions.

The different conformational behavior of **8** with respect to **11** results from steric repulsion between the nearly coplanar amidic residue -C-CO-N1(-C2)-C6 and the 2,6-methyl groups of **8** when they assume an equatorial arrangement. Note that the steric energy difference between the 'axial' and the 'equatorial' conformers of **8** was estimated to be 4.9 kcal/mol by SYBYL/MAXIMIN2 and 7.1 kcal/mol by MOPAC/AM1. Regarding **11**, the 'equatorial' conformer is more stable than the 'axial' one by 2.0 kcal/mol or 4.8 kcal/mol using SYBYL/MAXIMIN2 or MOPAC/AM1, respectively. More precisely, these conformational energy differences refer to model structures in which the cinnamyl side chain was replaced by a methyl group (these models will be discussed later in detail).

The results of the conformational energy calculations are also in good agreement with those obtained by us in a previous work [8] where steric energies were estimated using the MM2(85) program [9] and supported by ^{1}H NMR experiments on compounds **8** and **11** in $CDCl_3$ solution.

The fact that compound **6** is nearly as potent as compound **1** indicates that the endoethylenic

bridge influences the binding affinity of the two isomers in a similar way. Provided that the endoethylenic bridge of **6** is not oriented at the receptor site similarly to that of **1** or to the 2,6-dimethyl groups of **8**, the equipotency of the isomers **1** and **6** may depend on the existence of a second hydrophobic pocket (HP2 site in Scheme 1) in the μ-receptor cavity able to accommodate the endoethylenic bridge of **6**.

The equatorial orientation of the two methyl groups in compound **11** would prevent this ligand from interacting at the HP2 site. Consequently, **11** exhibits a lower value of affinity compared with its isomer **8** and it is practically equipotent with the desmethyl analog **16**.

The 2-monomethylpiperazines **13–15** were tested as racemic mixtures. Nevertheless, their binding affinity data provide information which, in our opinion, supports the hypothesis that a hydrophobic pocket (HP1 site) exists in the μ-receptor cavity. The μ-affinity values referring to these 2-monomethyl derivatives are, on an average, about 0.5 units lower than the corresponding 2,6-dimethyl analogs **8–10** (see Table 1). Energy minimizations carried out on both 2-monomethyl and 2,6-dimethyl derivatives, either with SYBYL/MAXIMIN2, or with MOPAC/AM1, produced geometries with an extremely high degree of similarity as far as the *N*-propionylpiperazine system is concerned. These results allowed us to exclude the possibility that the lower potency of the 2-monomethyl derivatives, with respect to the 2,6-dimethyl derivatives, could reside in different steric or conformational properties. Therefore the observed 'extra-affinity' of these latter compounds is clearly due to the presence of an extra methyl group capable of interacting with the HP1 site of the receptor.

The fact that the μ-affinity values of the 2-monomethyl derivatives **13–15** are slightly lower than those of the 2,6-dimethyl analogs **8–10** supports the hypothesis that there is room for both methyl groups of **8–10** at the HP1 site. As a consequence, it appears that stereoselective interactions involving sterically hindered regions of the HP1 site can reasonably be excluded. No or slight differences of affinity between the *R* and *S* enantiomers of compounds **13–15** are therefore predictable since such differences would be the result of a more or less favorable position (*R* or *S*) at the piperazine ring of the 2-methyl group for interaction with the HP1 hydrophobic site.

(3) Receptor-bound conformation of the most potent compound

As a further step in our investigations, we tried to establish the μ-receptor-recognized conformation of **2**, the most potent compound in the data set, by following the *active analog approach* proposed by Marshall et al. [10]. The systematic conformational search on **2** was set up with distance constraints supplied by morphine (I in Fig. 3) which was preliminarily chosen as template for its high μ-affinity [11] and relative structural rigidity.

Several models of ligand–opioid receptor interaction have been developed [12–15] since the work of Beckett and Casy in 1954. All of them have invariably identified two fundamental pharmacophore substructures: (a) a basic nitrogen which is partially ionized at the physiological pH so as to associate with an anionic site of the receptor (probably through a salt bridge) and (b) a planar aromatic structure capable of van der Waals interactions with the receptor. In addition to these two necessary requirements, the presence of a hydrogen bonding substituent on the aromatic system increases the strength of the binding. According to this basic pharmacophoric model, the distances among the following atoms of morphine (**I**) and **2** were considered: the hydrogen atom (H) bound to the protonated nitrogens; two dummy atoms (DuA and DuB) placed at 1.0 Å along normals to the benzene rings passing through their centroids; the phenol

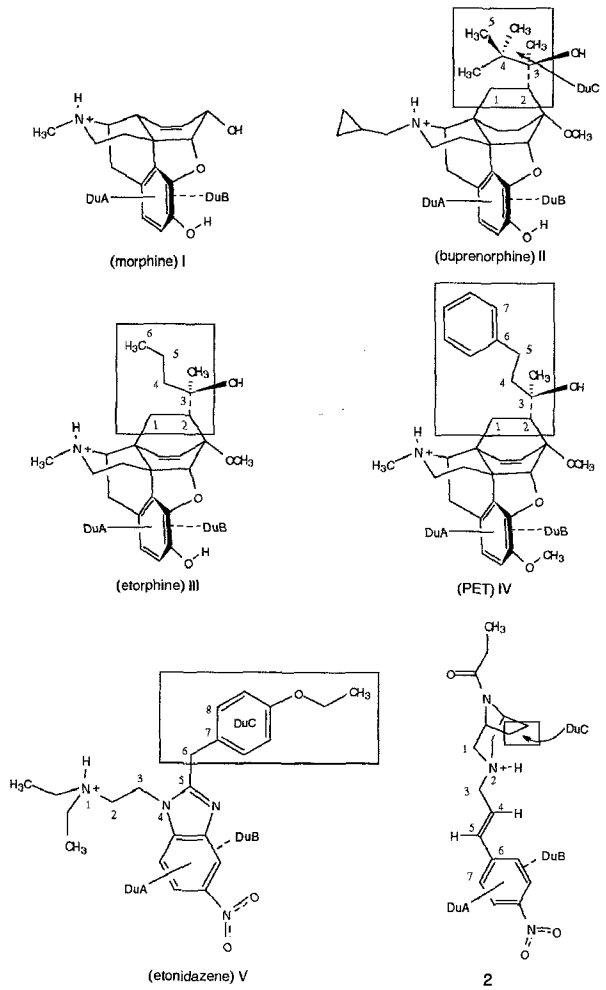


Fig. 3. Structures of classic opioid agents used for molecular comparisons with the piperazine and diazabicyclooctane derivatives. The structure of compound **2** is also reported. The dummy atoms DuA, DuB and DuC have been defined to perform, together with the H, O and N pharmacophore atoms, molecular superimpositions and conformational searches (the atoms defining the scanned torsional angles are indicated by conventional numbers). The hydrophobic substructures which are supposed to interact with the HP1 receptor site are highlighted by rectangles.

oxygen of morphine corresponding to one of the nitro oxygens of **2** (O). The hypothesis that one of the nitro oxygens of **2** can accept a hydrogen bond with a complementary site of the receptor (HB site) is reasonable since well-known opioid drugs, such as etonidazene (**V** in Fig. 3) possess a similar vital nitro group. Thus, the interatomic distances measured in morphine and successively used as constraints in the conformational search of **2** were d(H-DuA), d(H-DuB) and d(H-O) (the remaining three distances d(DuA-DuB), d(DuA-O) and d(DuB-O) being invariant in both structures). Three torsional angles in **2** (τ1, τ2 and τ4, defined in Table 2 on the basis of the conventional numbering scheme of Fig. 3) were scanned with 20° increments. The energetic and geometric filters rejected many of the theoretically possible conformations (2312). However, the

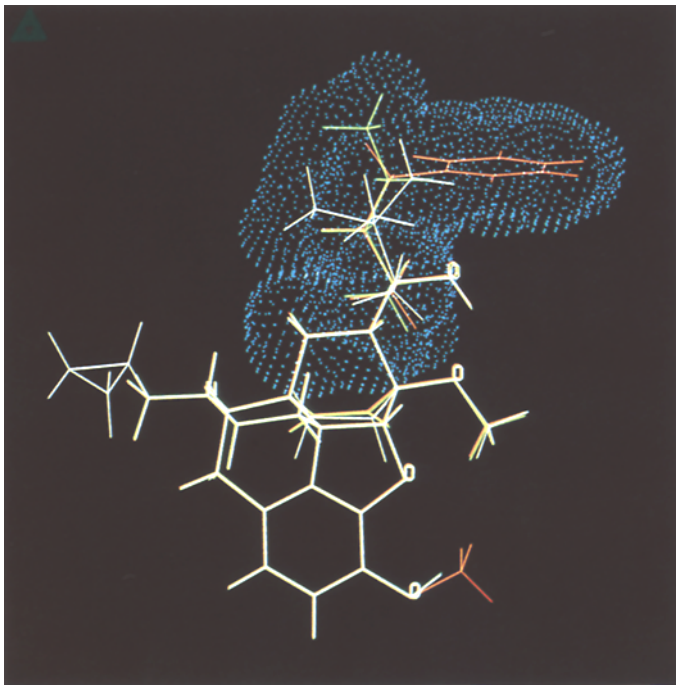


Fig. 4. Structures of buprenorphine (**II**, in white), etorphine (**III**, in green) and PET, (**IV**, in red) superimposed on their global minimum conformations. The blue dots display the union volume of their hydrophobic moieties which are supposed to fit into the HP1 receptor site.

number of output conformations was still too large to unambiguously select a unique receptor-bound conformation.

Since the importance of the hydrophobic endoethylenic bridge of **2** had already been established, we thought that such a substructure could be a useful discriminant to detect the bioactive conformation of this ligand. Several classic opioid agents are characterized by hydrophobic moieties which might in principle interact with the μ-receptor analogously to the endoethylenic bridge of **2**. Among these agents, the structures of buprenorphine [11], etorphine [11], the thebaine derivative PET [16,17] and etonidazene [18] appeared to us suitable to perform molecular comparisons with compound **2**. In fact, all of the four above-mentioned potent opioids (respectively, structures **II**–**V** in Fig. 3) have hydrophobic fragments (highlighted in Fig. 3 by rectangles) which

→

Fig. 5. The proposed receptor-bound conformation of compound **2** (in green) superimposed on buprenorphine (**II**, in white). It can be noticed that the endoethylenic bridge of **2** occupies the blue volume related to the hypothesized receptor hydrophobic pocket HP1.

Fig. 6. The proposed receptor-bound conformation of etonidazene (**V**, in red) superimposed to compound **2** (in green) and buprenorphine (**II**, in white). The blue dots are related to the hypothesized receptor hydrophobic pocket HP1 site.

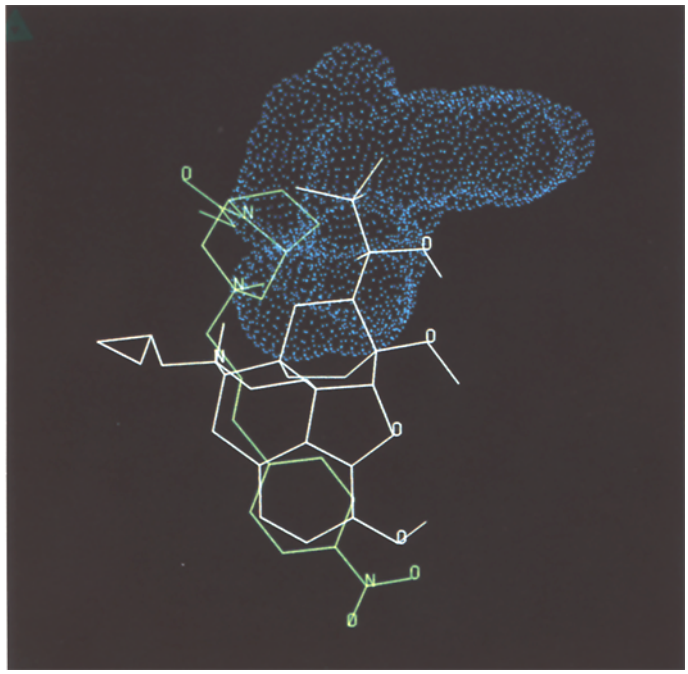


Fig. 5.

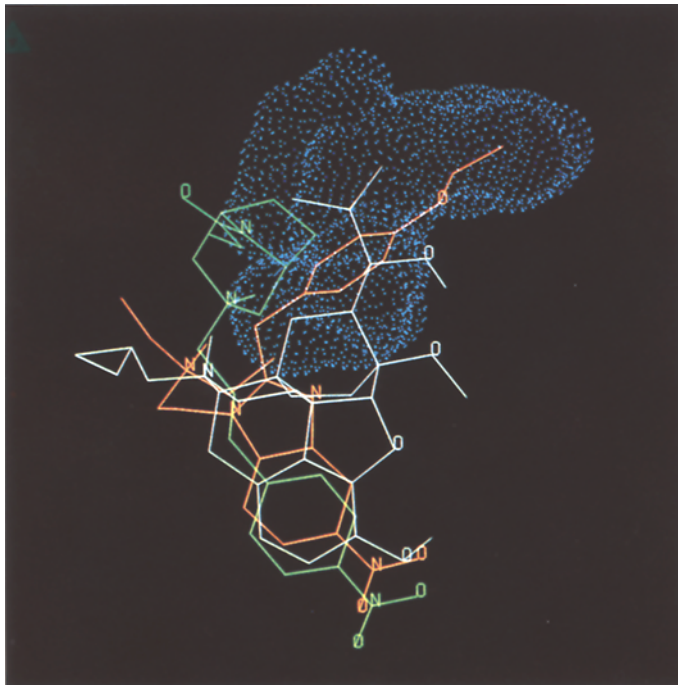


Fig. 6.

have been recognized to contribute to their high receptor affinities and which can potentially be accommodated in our proposed receptor HP1 site.

The global minimum conformers of structures **II–IV** (obtained by systematic conformational searches as previously described in the Methods section) were superimposed and the union volume of their biologically relevant hydrophobic residues was generated (see Fig. 4). Such a volume corresponds to a hydrophobic receptor site hypothesized by several authors [17,19,20] to exist in proximity to the C7-C8 atoms of endoetheno- and endoethanomorphinan derivatives such as buprenorphine, etorphine and PET.

In order to solve the ambiguities concerning the bioactive conformation of **2**, we introduced a further pharmacophore point in addition to H, DuA, DuB and O: the dummy atom DuC was used to describe approximately the position of hydrophobic residues complementary to the receptor HP1 site. In buprenorphine, DuC was generated as centroid of the carbon atoms highlighted in Fig. 3 by a rectangle. In compound **2**, the coordinates of DuC were calculated starting from those of the carbon atoms forming the endoethylenic bridge. The output conformations of **2**, derived from the previously described constrained conformational search, were superimposed on buprenorphine by minimizing the rmsd between the above listed set of five pharmacophoric points. Among these conformations, we picked up the one having the best fit on buprenorphine. The full geometry optimization of this conformation, performed with SYBYL/ MAXIMIN2, led to a global minimum conformer overlapping buprenorphine with an rmsd of 1.17 Å (0.53 Å by excluding the DuC fitting point). Such a conformation, within the level of approximation typical of this approach, represents the type of geometry that **2** might adopt at the μ-receptor. In Fig. 5 it is possible to see that the pharmacophoric elements of **2**, in its supposed bioactive conformation, overlap the corresponding ones of buprenorphine. Particularly, the endoethylenic bridge of compound **2** fits the hydrophobic region HP1 which other authors [17,19,20], as already mentioned, postulated to surround the C7-C8 bridge of endoetheno- and endoethanomorphinan analogs.

A systematic conformational search on etonidazene (**V** in Fig. 3), conducted similarly to that already performed on compound **2**, led us to define for **V** a receptor-bound conformation in which the hydrophobic *p*-ethoxyphenyl substituent occupies the HP1 site (see Fig. 6). This conformation has 3.3 kcal/mol of steric energy above the global minimum conformer (SYBYL/MAXIMIN2) and overlaps buprenorphine at the pharmacophore atoms with an rmsd of 0.50 Å (0.42 Å by excluding the DuC fitting point).

Table 2 lists the most significative torsional angles defining the bioactive conformations of

TABLE 2
MAIN TORSION ANGLES[a] OF THE RECEPTOR-BOUND COMFORMATIONS OF COMPOUNDS II–V AND **2**

Cmpd	τ1	τ2	τ3	τ4	τ5
II	54.0°	47.9°			
III	53.5°	174.9°	−169.3°		
IV	49.3°	164.5°	−61.5°	110.0°	
V	52.6°	−109.5°	−0.9°	160.7°	87.8°
2	−178.4°	−120.5°	176.2°	149.4°	

[a] The torsion angles are defined as follows on the basis of the atom numbering scheme reported in Fig. 3: τ1 = τ(1,2,3,4); τ2 = τ(2,3,4,5); τ3 = τ(3,4,5,6); τ4 = τ(4,5,6,7): τ5 = τ(5,6,7,8).

compounds **II–V** and **2**; Table 3 lists the distances among the pharmacophoric atoms of compounds **II, V** and **2**.

Recently, Cometta-Morini et al. [21] have proposed a model of μ-pharmacophore for the fentanyl class of compounds. Because of some similarities between these fentanyl derivatives and the compounds we have investigated, we thought that it was worth comparing the structures of compound **2** and fentanyl in their hypothesized bioactive conformations. The receptor-bound conformation of fentanyl that we have used was obtained by building a molecular model with torsional angles taken by reports of the above authors [21,22] and energy-minimizing the input geometry with the SYBYL/MAXIMIN2 option. The obtained molecular model of fentanyl was then aligned on the template buprenorphine by minimizing the rmsd between the pharmacophore points H, DuA and DuB. Figure 7 shows fentanyl and compound **2** superimposed on their proposed receptor-recognized conformations. To be emphasized is the fact that the benzene ring of the fentanyl *N*-phenethyl moiety and the *N*-propionyl-diazabicyclooctane assembly of compound **2** occupy distinct regions of space. Clearly our postulated HP1 site does not correspond to the receptor hydrophobic domain that Cometta-Morini et al. [21] postulated as complementary to the *N*-phenethyl chain of fentanyl.

(4) Effect of the benzene ring substituent on μ-affinity

The receptor-bound conformation of **2** was considered as a valuable model to analyze how the μ-affinity could be influenced by the type of substitution on the benzene ring. The molecular models of compounds **1, 3, 4** and **5** were built by simply replacing the -*p*-NO_2 group in the structure of **2** with the -H, -*m*-NO_2, -*m*-NH_2, and -*m*-OH substituents, respectively, and fully optimizing the so obtained geometries with SYBYL/MAXIMIN2.

First, it should be noted that one of the nitro oxygens of the -*m*-NO_2 derivative **3** is very close to the pharmacophoric oxygens of **2** and buprenorphine (on the left in Fig. 8). This is perfectly consistent with the observed similar μ-affinity values of **3** and **2**. The equivalence of the *para* and *meta* positions of the nitro group should be also the reason for the close affinity values of **9** and **10**.

The substituents -*m*-NH_2 and -*m*-OH, potentially capable of accepting hydrogen bonds, are instead oriented away from the positions of the above-mentioned oxygen atoms (see, for instance, the structure of **5** superimposed on buprenorphine on the right in Fig. 8). This somewhat less optimal alignment may explain the low affinity values displayed by **4** and **5**. The fact that compound **1**, which does not bear any substituent on the benzene ring, is more potent than **4** and

TABLE 3
DISTANCES (in Å) AMONG THE PHARMACOPHORIC POINTS H, O, DuA AND DuB IN THE RECEPTOR-BOUND CONFORMATIONS OF COMPOUNDS **II, V** AND **2**

Cmpd	d(H-O)	d(H-DuA)	d(H-DuB)	d(O-DuA)	d(O-DuB)	rmsd[a]
II	7.76	5.20	5.61	3.00	2.96	0.00
V	7.90	4.44	5.35	3.65	3.66	0.53
2	8.93	6.15	6.29	3.68	3.64	0.42

[a] rmsd is the root mean square distance resulting from the fitting of the molecule on structure **II** at the pharmacophoric points H, O, DuA and DuB.

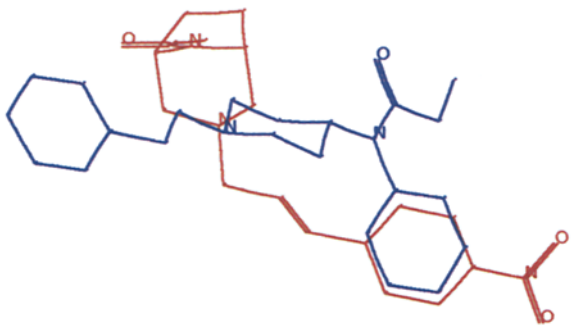


Fig. 7. The receptor-bound conformations of fentanyl (in blue) and compound **2** (in red) mutually aligned through fitting on buprenorphine (not shown).

5 and less potent than **2** may be the result of a balance between the favorable hydrogen bonding ability and the unfavorable hydrophilic nature of the -*m*-NH_2 and -*m*-OH substituents. In other words, the tendency of the extremely hydrophilic substituents of compounds **4** and **5** to be surrounded by an aqueous environment would not be compensated by the possibility of hydrogen bonding at the receptor. Thus, although **1** cannot form a hydrogen bond with the receptor, the H atom is definitely more hydrophobic than the NH_2 and OH groups.

An enhancement of affinity due to the -*p*-NO_2 substituent can be observed in the following pairs of ligands: **1/2, 6/7** and **11/12**. In contrast, the pairs **8/9** and **13/14** show a surprising inverted order of affinity which probably results from differences in the binding mode at the receptor site.

In order to verify whether the geometric properties of our ligands could play a role in determin-

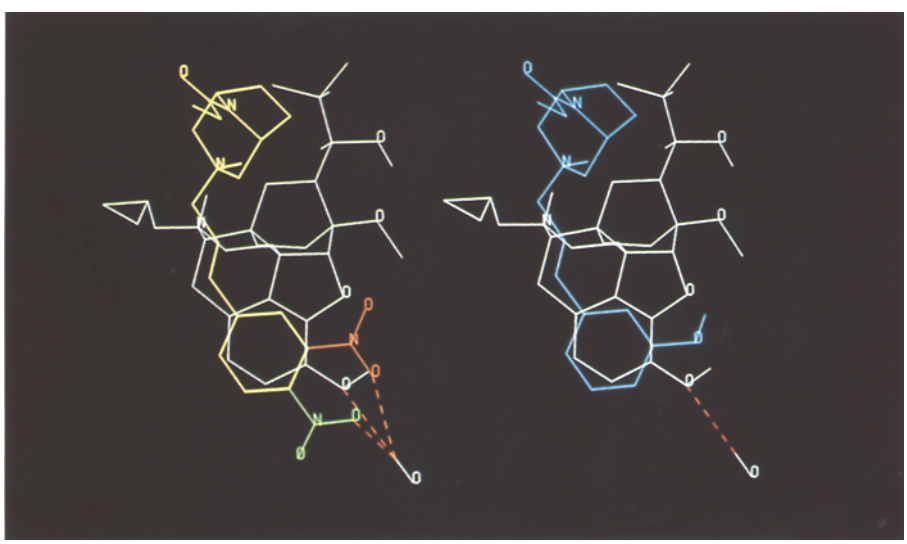


Fig. 8. On the left, compounds **2** (in green) and **3** (in red) are superimposed on buprenorphine (**II**, in white). On the right, compound **5** (in blue) is superimposed on buprenorphine (**II**, in white). The red dashed lines indicate hydrogen bonds between the benzene ring substituents of the ligands and the hypothesized hydrogen bond donor group of the receptor (HB site). Note that compound **5** does not form any hydrogen bond with the HB site.

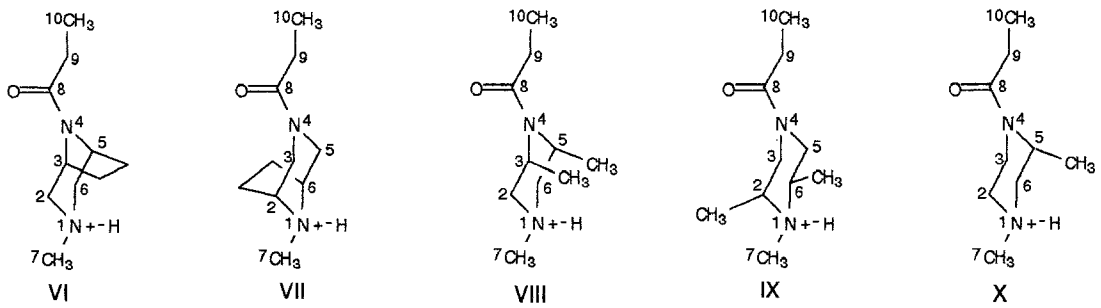


Fig. 9. Structures used to model the *N*-propionyl piperazine and diazabicyclooctane ring systems.

ing such an unexpected structure–affinity relationship pattern, the geometries of the 'simplified' structures **VI–X**, reported in Fig. 9, were analyzed. In using such model structures we assumed that the intramolecular interactions involving the cinnamyl moieties could be neglected. Structure **X** was constructed arbitrarily in the *S* configuration to model compounds **13–15** which were actually tested in vitro as racemic mixtures. The methyl groups are oriented axially in structures **VIII** and **X** while equatorially in structure **IX**.

First, structures **VI–X** were energy-minimized by means of the TRIPOS force field. The resulting geometries were successively used as input coordinates for MOPAC/AM1 full optimizations (the calculations were set up including the 'MMOK' keyword for the amidic bond molecular-mechanics correction). The two methods gave very similar results: the mean value of the rmsd between the heavy atoms of differently optimized geometries was 0.08 Å. Then, the five structures, obtained as output from SYBYL/MAXIMIN2, were superimposed with their common residue H-N^+-C(H_3).

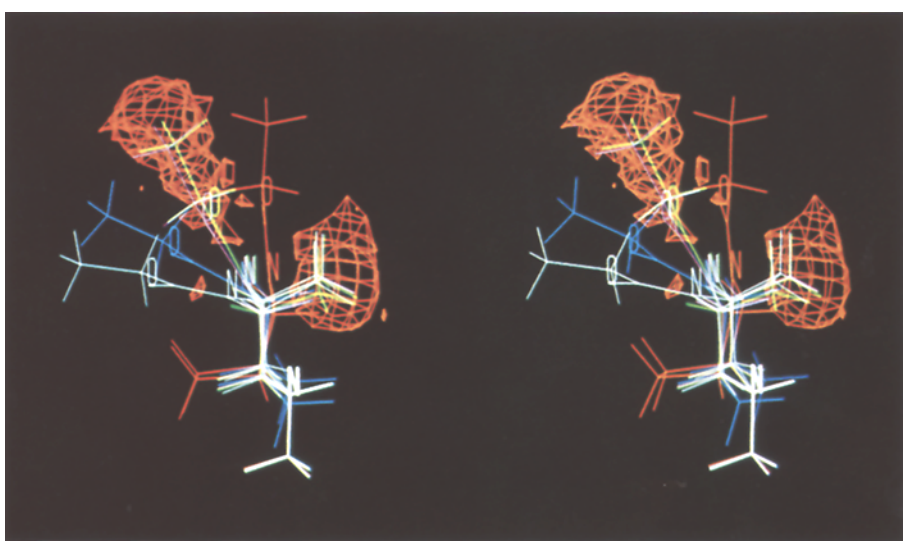


Fig. 10. Stereo-pair picture of structures **VI** (in white), **VII** (in red), **VIII** (in green), **IX** (in blue) and **X** (in magenta) superimposed with their common residue H-N^+-C(H_3). The 'extra-volume' of **VIII** and **X** is depicted in red.

TABLE 4
TORSION ANGLES[a] OF STRUCTURES **VI–X** OPTIMIZED THROUGH THE TRIPOS FORCE FIELD

Cmpd	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5
VI	55.2°	−72.2°	−177.0°	176.2°	−172.9°
VII	51.8°	−27.4°	176.0°	−172.0°	167.0°
VIII	45.3°	−35.4°	−177.5°	−176.5°	178.8°
IX	55.1°	−63.1°	−175.1°	176.5°	176.7°
X	51.2°	−44.5°	178.3°	−173.8°	177.2°

[a] The torsion angles are defined as follows on the basis of the atom numbering scheme reported in Fig. 9: $\phi_1 = \tau(1,2,3,4)$; $\phi_2 = \tau(2,3,4,5)$; $\phi_3 = \tau(3,4,8,9)$; $\phi_4 = \tau(4,8,9,10)$; $\phi_5 = \tau(7,1,2,3)$.

Table 4 lists the dihedral angles defining the conformations of structures **VI–X** optimized through the TRIPOS force field. The torsional angle characterized by the highest variance within the set of considered structures is ϕ_2. Such a torsional angle heavily determines the type of 3D arrangement assumed by the *N*-propionyl side chains.

According to the procedure proposed by Sufrin et al. [23], the 'extra-volume' of **VIII** and **X**, corresponding, respectively, to compounds **8** and **13**, was generated. More specifically, the following logical operation on the molecular volumes (Vol) was performed: (Vol-VIII ∩ Vol-X)–(Vol-VI + Vol-VII + Vol-IX).

As shown in Fig. 10, the *N*-propionyl substituents of **VIII** and **X** are nearly coincident in a unique region of space corresponding largely to the generated extra volume. The remaining part of this extra volume is associated to the overlapping 2-methyl groups which slightly increase the size of **VIII** and **X** along an ideal line passing through the C2 and C6 atoms of the piperazine ring.

The above findings are compatible with the hypothesis that compounds **1, 6** and **11** bind to the receptor by fitting their propionyl groups into sites of relative steric tolerance. In contrast, the ligands **8** and **13** cannot probably occupy the receptor cavity in the same way without giving rise to repulsive interactions between their *N*-acyl substituents and a sterically hindered region of the receptor (S site in Scheme 1). It has already been discussed in the second paragraph of this section that the HP1 receptor cavity is large enough (see also Fig. 5) to accommodate the axial 2-methyl groups of structures **8** and **13**. The presence of a sterically 'forbidden' region of the receptor that we have termed S site may lead compounds **8** and **13** and the corresponding nitro derivatives **9/10** and **14/15** to bind to the receptor with a slightly different mode (which our pharmacophore model cannot predict). Therefore, the different binding mode of these relatively low potent nitro derivatives may be characterized by lack of interaction between the nitro oxygen of the ligand and the postulated HB site. According to this hypothesis, the effects of the *N*-propionyl and the aralkenyl side chains on the binding affinity would be 'cooperative' rather than 'additive'.

We want to point out that the molecular alignment shown in Fig. 10 should be considered only as a 3D comparison among ligands in the formulation of structure–affinity relationships and not as a description of the actual relative orientations of the compounds in the receptor cavity.

CONCLUSIONS

Our studies have revealed that our set of ligands act at the μ-receptor site like classic opioid

agents such as morphine and etonidazene. In addition to well-known pharmacophoric structural elements (that is, an aromatic system placed at an appropriate distance from a tertiary ionizable aminic function), other requisites must be fulfilled for a high μ-affinity. Specifically, an endoethylenic bridge or two structurally analogous methyl groups, positioned so as to fit one of two postulated hydrophobic receptor pockets, enhance the binding; a *para-* or a *meta*-nitro group on the benzene ring further increases the binding affinity by accepting a hydrogen bond from a site of the receptor complementary to the phenol group of morphine. The geometry of the nitrogen ring frame may also modulate the affinity by determining the orientation of the *N*-propionyl moiety. The derived structure–affinity relationships suggest that the effects of the *N*-propionyl and the aralkenyl side chains on the binding affinity are 'cooperative' rather than 'additive'.

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