

A STRUCTURE-AFFINITY STUDY OF THE OPIOID BINDING OF SOME 3-SUBSTITUTED MORPHINANS

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SUMMARY

A ZINDO/1 quantum-chemical structure-affinity relationship study is presented for the interaction of a group of 3-substituted morphinans with mu, delta and kappa opioid receptors. From this and previous work it is concluded that:

1. Receptor selectivity is regulated by different electronic densities located sometimes at the same atomic center.
 2. The equations for the binding to the three opiate receptors are consistent with previous results obtained for other kinds of opiate molecules.
 3. Internal occupied molecular orbitals are very important in regulating receptor affinity (and selectivity).
 4. The role of molecular orbitals in the saturated rings needs further analysis through the study of more molecular systems interacting with opiate receptors.
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INTRODUCTION

Preparations of the opium poppy *Papaver somniferum* have been used for hundreds of years to relieve pain. In 1803, Sertürner isolated a crystalline sample of the main constituent alkaloid, morphine, which was later shown to be almost entirely responsible for the analgesic activity of crude opium. The rigid structural and stereochemical requirements essential for the analgesic actions of morphine and related opioids led to the theory that they produce their

effects by interacting with a specific receptor.

Today we know three types of opioid receptors: mu (μ), kappa (κ) and delta (δ) [1]. The μ -opioid receptor shows the highest affinity for morphine. Activation of these receptors inhibits GABA-containing interneurons, resulting in a net excitatory effect in the hippocampus. Localization of μ receptors is both pre- and post-synaptic and almost exclusively on GABAergic interneurons. Also, they are widely distributed in regions throughout the brain and spinal cord, including laminae I and II of the medullary and spinal dorsal horns, the striatum, the optic tract and the locus coeruleus. The κ -opioid receptor is primarily responsible for mediating the effects of the products of prodynorphin and possibly preproenkephalin. Localization of the κ receptor is primarily postsynaptic. δ -Opioid receptors are located postsynaptically on pallidostriatal feedback neurons. They also modulate nociception presynaptically in the periaqueductal gray matter. Additionally, receptor autoradiographic investigations have localized δ receptors to the external plexiform layer of the olfactory bulb, the nucleus accumbens, several layers of the cerebral cortex and several nuclei of the amygdala.

Recently a series of 10-ketomorphinan and 3-substituted-3-desoxymorphinan analogues have been synthesized [2]. The 3-substituted morphinans show different affinities for the three opioid receptor types. Following our research line on these kinds of receptors [3, 4], we present here a quantum-chemical study of the affinity and selectivity of a series of 3-substituted morphinans for the three opioid receptors.

METHODS, MODELS AND CALCULATIONS

As the method has been discussed thoroughly [3, 5, 6 and references therein], we shall present a very general sketch. Briefly, the equilibrium constant K can be expressed as:

$$\log K = a + b \log M_D + c \log \sigma_D + d \log(I_1 I_2 I_3) + e \Delta E \quad (1)$$

where a , b , c , d and e are constants, D refers to the drug molecule, σ is the symmetry number, M the drug's molecular mass, $I_1 I_2 I_3$ is the product of the three moments of inertia about the three principal axes of rotation, and ΔE is the morphinan-receptor interaction energy.

The interaction energy is evaluated using the Klopman-Peradejordi-Gómez approach [5] as:

$$\Delta E = W + \sum_i [E_i Q_i + F_i S_i^E + G_i S_i^N] + \sum_i \sum_m [H_i(m) D_i(m) + J_i(m) S_i^E(m)] + \quad (2)$$

where W , E , F , G , H , J , R and T are constants, Q_i , S_i^E and S_i^N are, respectively, the net charge, the total electrophilic superdelocalizability and the total nucleophilic superdelocalizability of atom i . The index m (m') refers to the contribution to the above properties of occupied (virtual) molecular orbital m (m'). $D_i(m)$ is the electronic density of atom i at MO m (or m'). We must mention that electrophilic superdelocalizabilities and electronic densities are always negative, nucleophilic superdelocalizabilities are always positive, and the net charges can be positive, negative or zero. The expression for ΔE in Eq. 2 comes directly from the statistical-mechanical definition of the equilibrium constant [5-7]. Electrophilic superdelocalizabilities (SDE) are related to electron-donor capacities, and nucleophilic superdelocalizabilities (SDN) are related to electron-acceptor capacities.

Eq. (2) was derived assuming that the only important component of ΔE is the change in electronic energy. Only morphinan-related terms appear in Eq. (2) after some physically-based approximations were made [5]. In our previous studies it was shown that in the case of molecular orbital contributions, the first, second and third occupied (HOMO, HOMO-1 and HOMO-2, respectively) and virtual (LUMO, LUMO+1 and LUMO+2) molecular orbitals (MO) are involved. The insertion of Eq. 2 into Eq. 1 produces the final working equation. The moment of inertia term has been discussed in detail in another publication [7]. We shall say here that from the moment of inertia term a parameter called the "orientational effect" of the substituent is derived [7].

The selected molecules are shown in Fig. 1. The values of the equilibrium constants were taken from the literature [2].

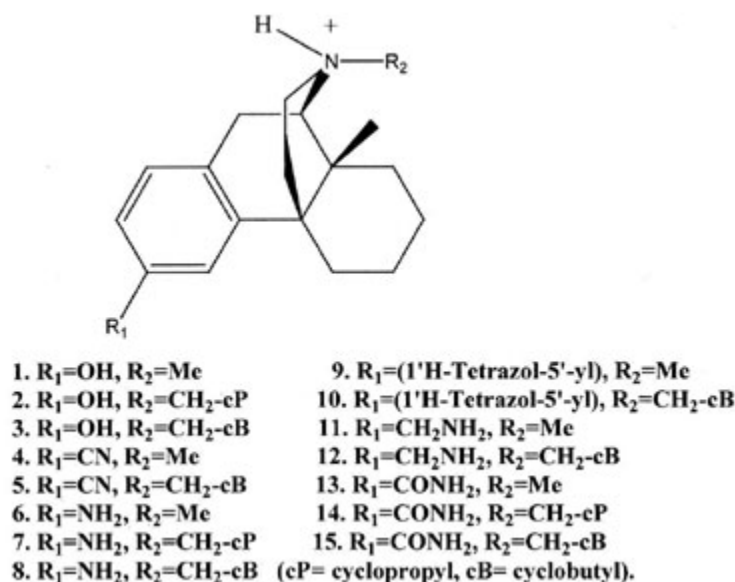


Fig. 1. Molecules employed in this study.

The molecules were studied in their protonated form. The geometry optimization and the calculation of the wave function were carried out using the Hyperchem package [8]. For full geometry optimization, AM1 semiempirical methodology was employed [9]. The method selected for calculating the wave function was Zerner's ZINDO/1 [10, 11]. This choice is justified because after AM1 geometry optimization ZINDO/1 is the only method producing positive nucleophilic superdelocalizabilities as required by the model [12].

We must stress here that the working equation must have a mathematical solution. The problem is that the number of molecules is generally less than the number of unknown quantities. For this reason statistical analysis is employed to find the best possible solution. The statistical fitting of Eq. 1 was performed by means of a stepwise regression technique with the above mentioned biological activities as dependent variables and the static reactivity indices of the atoms belonging to a common skeleton as independent variables. This common skeleton is depicted in Fig. 2. To these variables we added the substituent orientational effect whose values are shown in Table I. This is based on the hypothesis stating that the molecules interact with their receptors through a common skeleton, the substituents only modulating the electronic properties of the skeleton. On the other hand, and considering that in aromatic systems many independent variables are highly intercorrelated and stable estimates for the regression coefficients cannot be obtained via ordinary least squares methods, we employed the Ridge regression analysis [13-15]. The Statistical Software Package was employed [16].

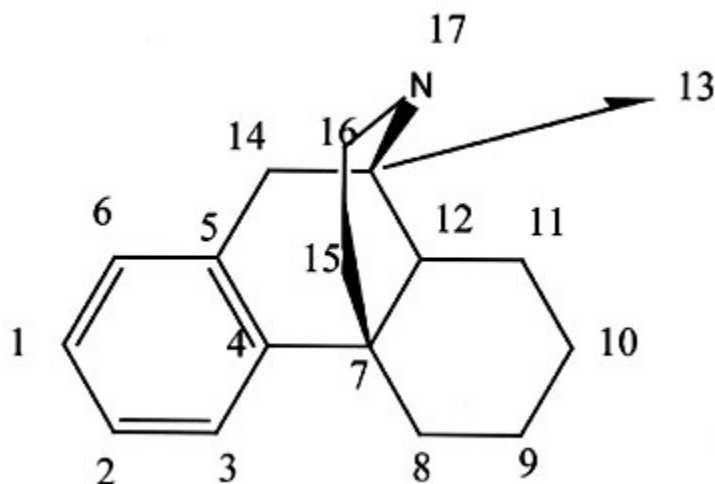


Fig. 2. Common skeleton numbering.

Table I. Values for the orientational parameters of the substituent ($uma \cdot \text{°A}^2$).

Substituent	O
OH	27.175
CN	86.955
NH_2	27.895
CH_2NH_2	133.366
$CONH_2$	187.174
1'H-Tetrazol-5'-yl	514.334
CH_3	32.782
Cyclopropylmethyl	532.570
Cyclobutylmethyl	398.966

RESULTS

The results of the statistical analysis for the interaction of the group of 3-substituted morphinans with mu, delta and kappa opioid receptors are presented below.

A. Mu receptor binding.

The best statistical equation found was:

$$\log K_i = -82.84 - 18.99S_6^E - 1.14S_2^N(LUMO) - 0.004O_N - 20.22D_{17}(LUMO+1) - \quad (3)$$

$$-8.76D_5(HOMO)+11.01S_{10}^E(HOMO-1)-27.07S_{15}^E(HOMO)$$

with $n = 15$, adjusted $R^2 = 0.98$, $SD = 0.21$, and $F(7,7) = 90.83$ ($p = 2 \cdot 10^{-6}$), where S_6^E is the total atomic electrophilic superdelocalizability of atom 6, S_2^N is the nucleophilic superdelocalizability of atom 2 at the LUMO, O_N is the orientational parameter of the N-substituent, $D_{17}(LUMO+1)$ is the virtual electronic density of atom 17 at the (LUMO+1), $D_5(HOMO)$ is the electronic density of atom 5 in the HOMO, $S_{10}^E(HOMO-1)$ is the electrophilic superdelocalizability of atom 10 in the (HOMO-1), and $S_{15}^E(HOMO)$ is the total atomic electrophilic superdelocalizability of atom 15 in the HOMO. Analogous meanings hold for equations 4 and 5.

Table II shows the experimental and calculated values. The result of Student's t test for the significance of the variables appearing in Eq. 3 is given in Table III. The squared correlation coefficient matrix for these variables is given in Table IV. The analysis of variance shows that this equation is statistically significant. From Table IV we must note that, as expected, the only highly correlated variables are those belonging to the same ring (S_6^E and $S_2^N(LUMO)$). Eq. 3 explains 98% of the variation of $\log K_j$.

Table II. Experimental and calculated $\log K$ values for m binding.

Substituent	O
OH	27.175
CN	86.955
NH ₂	27.895
CH ₂ NH ₂	133.366
CONH ₂	187.174
1'H-Tetrazol-5'-yl	514.334
CH ₃	32.782
Cyclopropylmethyl	532.570
Cyclobutylmethyl	398.966

Table III. Results of Students' t-test for the significance of the variables appearing in Eq. (3)

Variable	t	P
S_6^E	-6.93	$2 \cdot 10^{-4}$
$S_2^N(LUMO)$	-0.73	0.49
O_N	-9.88	$2 \cdot 10^{-5}$
$D_{17}(LUMO+1)$	-5.00	$1.5 \cdot 10^{-3}$
$D_5(HOMO)$	-7.20	$1 \cdot 10^{-4}$
$S_{10}^E(HOMO-1)$	6.27	$4 \cdot 10^{-4}$
$S_{15}^E(HOMO)$	-4.71	$2.1 \cdot 10^{-3}$

Table IV. Squared correlation coefficient matrix for the variables appearing in

Eq. 3

	S_6^N	$S_2^N(LUMO)$	O_N	$D_7(LUMO+1)$	$D_3(HOMO)$	$S_6^E(HOMO-1)$
$S_2^N(LUMO)$	0.86	1.0				
O_N	0.03	0.04	1.0			
$D_7(LUMO+1)$	0.003	0.00001	0.53	1.0		
$D_3(HOMO)$	0.02	0.05	0.10	0.14	1.0	
$S_6^E(HOMO-1)$	0.34	0.46	0.02	0.06	0.23	1.0
$S_{15}^E(HOMO)$	0.36	0.69	0.01	0.0001	0.11	0.42

B. Delta receptor binding.

The best statistical equation for this case is:

$$\log K_i = 33.93 - 3.08S_{12}^E(HOMO) - 22.78Q_2 + 16.13Q_5 - 0.002O_N - 2.77S_9^N \quad (4)$$

with $n = 12$, adjusted $R^2 = 0.96$, $SD = 0.25$ and $F(5,6) = 56.44$ ($p = 6 \cdot 10^{-5}$), where S_{12}^E (HOMO) is the electrophilic superdelocalizability of atom 12 in the HOMO, Q_2 is the net charge of atom 2, Q_5 is the net charge of atom 5, S_9^N and is the total atomic nucleophilic superdelocalizability of atom 9.

Table V shows the experimental and calculated $\log K_i$ values. The result of Student's t test for the significance of the variables appearing in Eq. 4 is given in Table VI. The squared correlation coefficient matrix for these variables is given in Table VII. The analysis of variance shows that this equation is statistically significant. Eq. 4 explains 96% of the variation of $\log K_i$.

Table V. Experimental and calculated $\log K$ values for d binding.

Molecule	$\log K(\delta)$ exp.	$\log K(\delta)$ calc.
1	0.62	0.79
2	0.28	0.12
3	0.77	0.75
5	3.28	3.25
6	3.18	3.06
7	2.18	2.18
8	2.26	2.42
11	3.40	3.16
12	2.61	2.72
13	1.11	1.10
14	0.00	0.07
15	0.60	0.55

Table VI. Results of Students' t-test for the significance of the variables appearing in Eq. (4)

Variable	t	p
$S_{12}^E(HOMO)$	-2.06	0.08
Q_2	-11.59	0.00002
Q_3	9.26	0.00009
O_N	-6.01	0.0009
S_9^N	-2.95	0.025

Table VII. Squared correlation coefficient matrix for the variables appearing in Eq. 4

	$S_{12}^E(HOMO)$	Q_2	Q_3	O_N
Q_2	0.29	1.0		
Q_3	0.53	0.85	1.0	
O_N	0.005	0.008	0.008	1.0
S_9^N	0.04	0.02	0.09	0.0009

Table X. Squared correlation coefficient matrix for the variables appearing in Eq. 5**C. Kappa receptor binding.**

The best equation obtained was:

$$\log K_i = -45.29 - 0.005O_N - 27.19D_8(HOMO-1) - 4.27D_7(HOMO-2) - 34.00D_{11}(HOMO-1) + 1.60S_1^N + 10.82S_3^E(HOMO-1) - 11.43Q_6 - 7.08S_{12}^E \quad (5)$$

with $n = 15$, adjusted $R^2 = 0.97$, $SD = 0.25$ and $F(8,6) = 58.74$ ($p = 4 \cdot 10^{-5}$),

where O_N is the N-substituent orientational parameter, $D_8(HOMO-1)$ is the electronic density of atom 8 in the (HOMO-1), $D_7(HOMO)$ is the electronic density of atom 7 in the (HOMO-2), $D_{11}(HOMO-1)$ is the electronic density of atom 11 in the (HOMO-1), S_1^N is the total atomic nucleophilic superdelocalizability of atom 1, is the electrophilic superdelocalizability of atom 3 in the (HOMO-1), Q_6 is the net charge of atom 6, S_{12}^E and is the total atomic electrophilic superdelocalizability of atom 12.

[Table VIII](#) shows the experimental and calculated values. The result of Student's t test for the significance of the variables appearing in Eq. 5 is given in [Table IX](#). The squared correlation coefficient matrix for these variables is given in [Table X](#). The analysis of variance shows that this equation is statistically significant. Eq. 5 explains 97% of the variation of $\log K_i$.

Table VIII. Experimental and calculated log K values for κ binding.

Molecule	Log K(κ) exp.	Log K(κ) calc.
1	0.36	0.42
2	-1.47	-1.84
3	-1.10	-1.11
4	2.30	1.97
5	0.78	0.65
6	2.04	2.11
7	-0.74	-0.96
8	0.26	-0.03
9	2.58	2.71
10	2.93	2.69
11	2.08	2.15
12	0.93	0.94
13	0.59	0.57
14	-0.74	-0.67
15	-1.06	-1.16

Table IX. Results of Students' t-test for the significance of the variables appearing in Eq. (5)

Variable	t	P
O_N	-8.98	0.0001
$D_8(HOMO-1)$	-3.24	0.02
$D_7(HOMO-2)$	-0.51	0.63
$D_{11}(HOMO-1)$	-4.32	0.005
S_1^N	5.63	0.001
$S_3^E(HOMO-1)$	3.96	0.007
Q_6	-3.41	0.01
S_{12}^E	-2.07	0.08

Table X. Squared correlation coefficient for the variables appearing in Eq. (5)

	O_N	$D_6(HOMO-1)$	$D_7(HOMO-2)$	$D_{11}(HOMO-1)$	S_1^N	$S_3^E(HOMO-1)$	Q_6
$D_6(HOMO-1)$	0.18	1.0					
$D_7(HOMO-2)$	0.07	0.85	1.0				
$D_{11}(HOMO-1)$	0.49	0.38	0.26	1.0			
S_1^N	0.01	0.36	0.46	0.01	1.0		
$S_3^E(HOMO-1)$	0.02	0.03	0.12	0.02	0.59	1.0	
Q_6	0.16	0.81	0.77	0.22	0.41	0.04	1.0
S_{12}^E	0.30	0.64	0.42	0.30	0.35	0.008	0.66

DISCUSSION

The conditions for a good binding to the receptor are based on the analysis of the signs of the different variables, the signs of the coefficients accompanying them, the sign of the constant, and the physical meaning of the variables appearing in the different equations.

0000a. Mu receptor binding.

Mu binding seems to be mainly orbital-controlled [17].

A variable-by-variable analysis of this equation shows that optimal affinity is related to (see Fig. 2):

1. A small value of the orientational parameter of the N-substituent, indicating that a methyl group is preferred.
2. A high electron-donor capacity of atom 6, a low electron-acceptor capacity of atom 2, and a low electronic density of the HOMO at atom 5, all these atoms belonging to the aromatic system.
3. At the level of the saturated rings a low electron-donor capacity of atom 10 at the (HOMO-1) level and a high electron-donor capacity of atom 15 are required.

0000b. Delta receptor binding.

This binding seems to be charge- and orbital-controlled.

The variable-by-variable analysis of this equation suggests that optimal affinity is related to:

1. A large value of the orientational parameter of the N-substituent, contrary to the mu binding case.
2. A positive net charge on atom 2 and a negative net charge on atom 5 at the level of the aromatic ring.
3. A low electron-donor capacity of atom 12 and a high electron-acceptor capacity of atom 9 in the saturated skeleton.

0000c. Kappa receptor binding.

Kappa binding appears to be charge- and orbital-controlled, with a predominance of the latter.

Optimal affinity is associated with:

1. A low value of the orientational parameter of the N-substituent, as in the case of mu binding.
2. A high electron-acceptor capacity of atom 1, a low electron-donor capacity of atom 3, and a negative net charge on atom 6 in the aromatic ring.
3. Low electron densities on atoms 7, 8 and 11 in different molecular orbitals and a high electron-donor capacity of atom 12 in the area of the saturated skeleton.

This variable-by-variable analysis is only a first approach because there is an interplay between all the variables in the regression equation. Therefore, it cannot be expected that one molecule possesses all the properties stated for an optimal binding affinity. For example, [figures 3](#) and [4](#) show different locations of the HOMO for molecules 13 and 1.

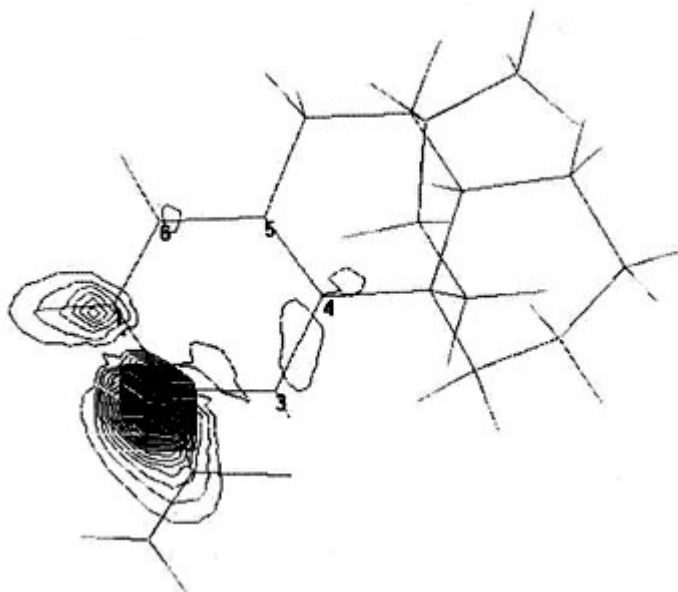


Fig. 3. Electronic density distribution map of the HOMO of molecule 13.

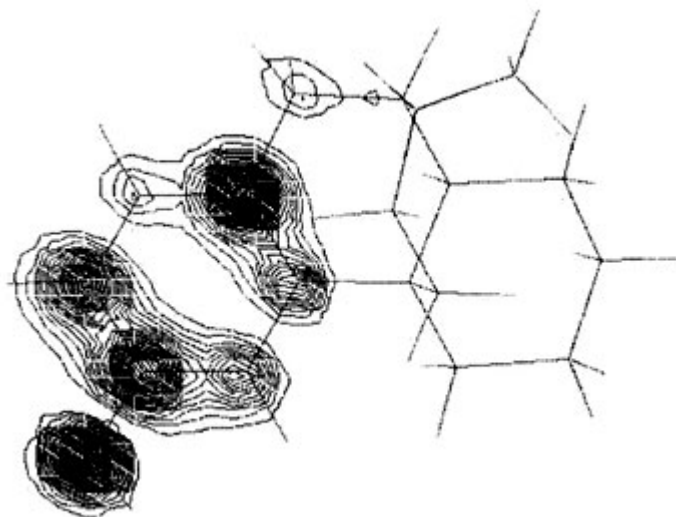


Fig. 4. Electronic density distribution map of the HOMO of molecule 1.

On the other hand, some words seem necessary regarding the indices appearing in the saturated rings. We do not believe that the indices represent charge transfer from or to $s - p_s$ molecular orbitals. We think that a better interpretation is that the presence of electron densities can interfere (through repulsive interactions with electron densities in the receptor) or facilitate (through penetration of the electron density into a region devoid of electrons) the drug-receptor interaction.

The examination of Eqs. 3 to 5 shows that the same molecules bind to the opioid receptors through different mechanisms. Nevertheless, some common features appear, such as the same requirement for the orientational parameter of the N-substituent for mu and kappa binding. This is probably due to the same phylogenetic origin of opioid receptors.

For the construction of the three interaction pharmacophores some restrictions apply arising from the results of Student's t-test (see Tables III, VI and IX) for the significance of the individual variables in the regression equation. For this case, we shall include only those variables for which the probability associated with the t-value is less than 0.01. We shall include the results coming from previous studies [3, 4].

The resulting pharmacophores are shown in Figs. 5 to 7. We incorporated in them all our previous results [3,4] to try to get a more complete view of the pharmacophores.

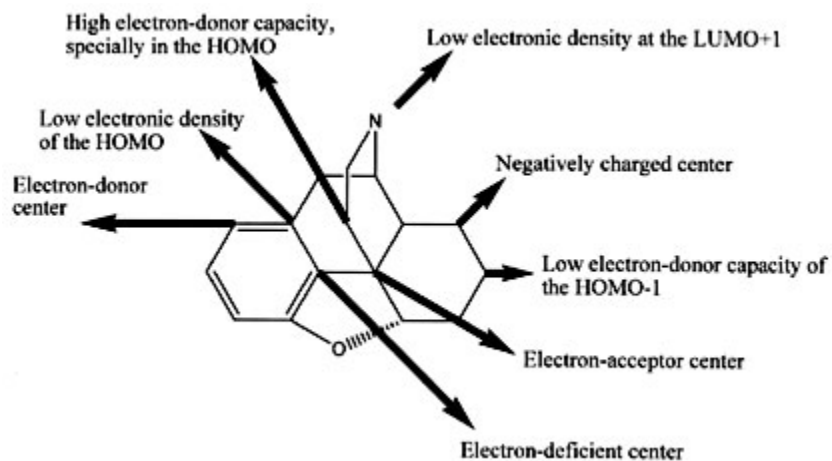


Fig. 5. Mu interaction pharmacophore.

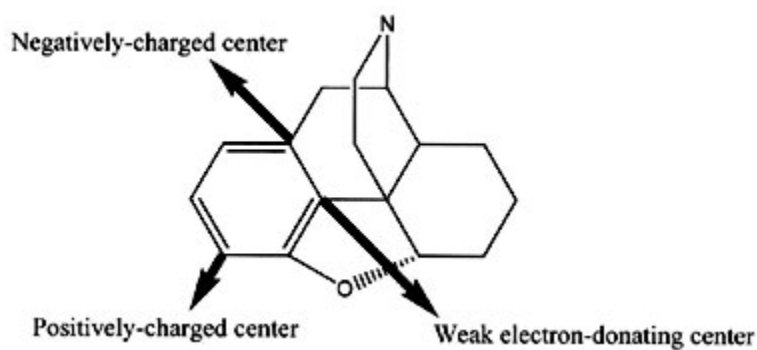


Fig. 6. Delta interaction pharmacophore.

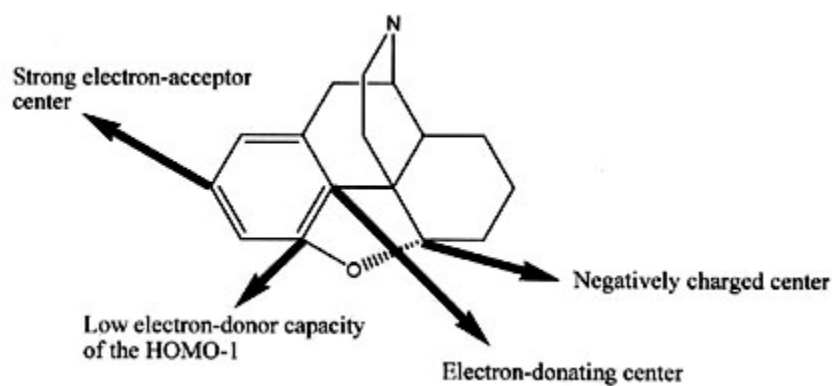


Fig. 7. Kappa interaction pharmacophore.

In conclusion, we have shown, starting from a model-based method [18] that:

1. Receptor selectivity is regulated by different electronic densities located sometimes at the same atomic center.

2. The equations for the binding to the three opiate receptors are consistent with previous results obtained for other kinds of opiate molecules [3,4].
3. Internal occupied molecular orbitals are very important in regulating receptor affinity and selectivity.
4. The role of molecular orbitals in the saturated rings needs more analysis through the study of more molecular systems interacting with opiate receptors.

Finally, a comment about the evolution of the opioid receptors. It has been shown that the order of receptor-type evolution is kappa, then delta, and most recently the mu opioid receptor. Also, analyses suggest that conserved receptor domains determine the type selectivity of vertebrate opioid receptors [19]. This information could be of interest for future docking studies.

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