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STRUCTURAL AND CONFORMATIONAL STUDIES OF 3,8-DIAZABICYCLO[3.2.1]OCTANE DERIVATIVES, SELECTIVE AGONISTS OF μ-OPIOID RECEPTORS

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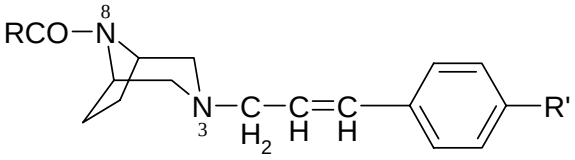
Abstract- Quantum mechanic calculations have been done on a set of 3,8-diazabicyclo[3.2.1]octane derivatives in order to elucidate their electronic structure in relation to the affinity towards the μ-opioid receptors. The conformations are compared with morphine, chosen for its μ-affinity and structural rigidity. The X-Ray crystal and molecular structures of 3-*p*-nitrocinnamyl-8-propionyl-3,8-diazabicyclo[3.2.1]octane (**1b**) and of two higher homologs 8-*n*-butyroyl- (**1c**) and pivaloyl- (**1d**) have been compared with the theoretical results.

The synthesis and the analgesic properties of 3,8-diazabicyclo[3.2.1]octanes were first reported by Cignarella *et al.*[1] Later on these compounds were shown to exert their activity through interaction with the opioid receptors, being fairly selective towards μ *vs* δ and κ subtypes.[2] The structural requirements for optimum activity seemed to reside in the presence of an aralkenyl group with a three carbon chain at N-3 and an acyl group at N-8. Amongst the most significant derivatives, the 3-*trans*-cinnamyl-8-propionyl derivative (**1a**) was 5 times more active *in vivo* (hot plate test) than morphine. Modifications of the aliphatic chain of the cinnamyl group generally retained the affinity of the model. Conversely, the introduction of a *p*-nitro group on the phenyl of **1a** gave compound (**1b**) with better μ/δ selectivity and analgesic potency than the model.[2] Moreover, **1b** seems to develop tolerance in a much slower way than morphine.[3] This paper describes the conformational studies and the X-Ray structures of **1b-d** (Scheme 1) related to their biological properties.

RESULTS AND DISCUSSION

Scheme 1



Compound	R	R'
1a	ethyl	H
1b	ethyl	*p*-NO_2
1c	*n*-propyl	*p*-NO_2
1d	*t*-butyl	*p*-NO_2

X-Ray structure determination

The molecular structure of **1b** is shown in Figure 1 and is characterized by the chair-conformation of the piperazine ring and the equatorial arrangement of the aralkenyl chain.

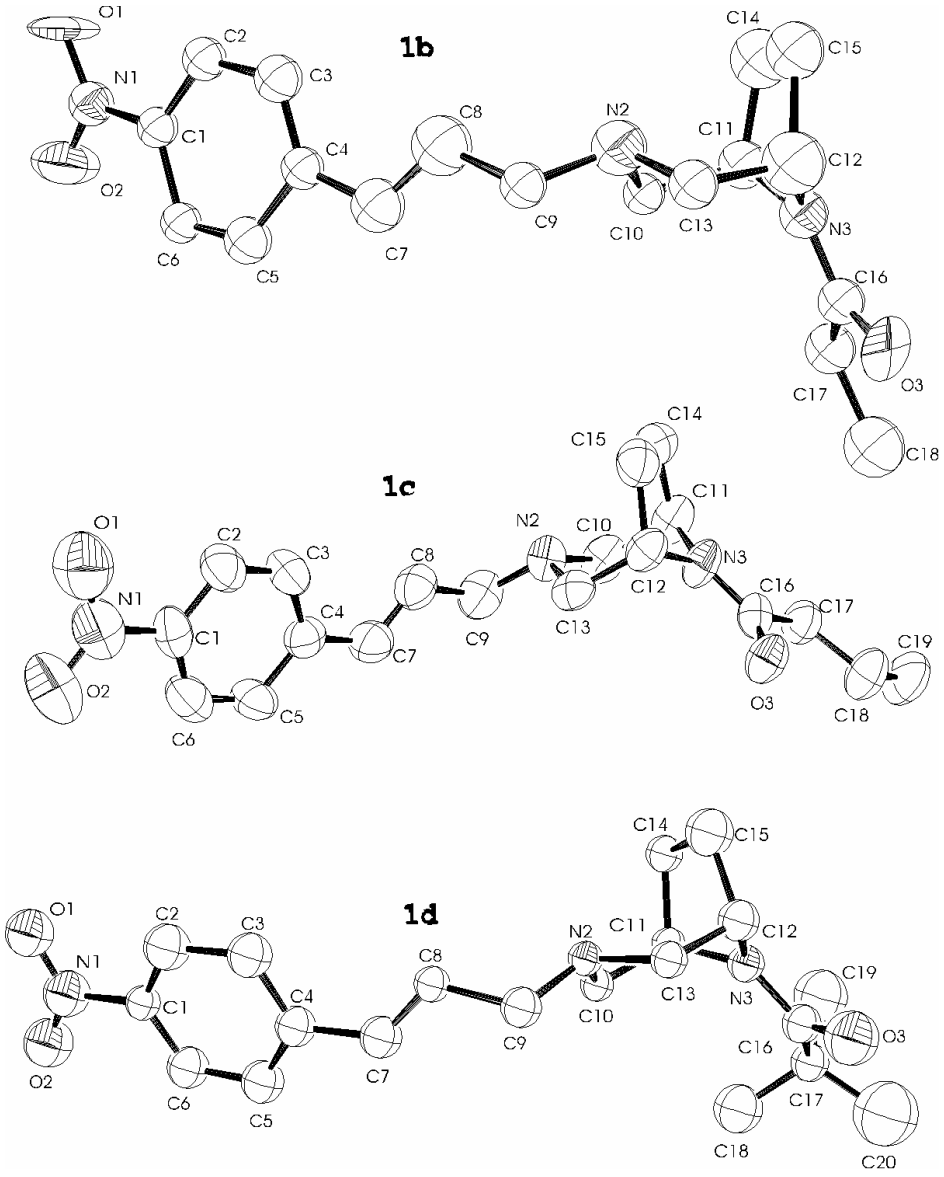


Figure 1. ORTEP view of **1b**, **1c** and **1d** showing the atom numbering.

Table 1. Selected torsion angles (°) for **1b**, **1c**, and **1d**.

		1b	**1c**	**1d**
τ_1	C(13)-N(2)-C(9)-C(8)	176(3)	173.1(5)	162(2)
τ_2	N(2)-C(9)-C(8)-C(7)	127(3)	-132.9(7)	125(2)
τ_3	C(9)-C(8)-C(7)-C(4)	171(3)	180.0(6)	174(2)
τ_4	C(8)-C(7)-C(4)-C(5)	178(3)	170.7(7)	162(2)

The mean plane calculated over the "planar" part of the chair shows perfect coplanarity of the atoms linked to the terminal nitrogens which are 0.704(9) Å [N(2)] and -0.766(9) Å [N(3)], apart from this plane. The ethylenic bridge C(14)-C(15) is nearly perpendicular to this plane. The aralkenyl chain presents a "trans-eclipsed" conformation (see Table 1).

Table 2. Significant bond distances (Å) and angles (°) for **1b**, **1c**, and **1d**.

	1b	**1c**	**1d**
N(1)-O(1)	1.20(3)	1.221(9)	1.20(3)
N(1)-O(2)	1.23(4)	1.212(9)	1.20(3)
N(1)-C(1)	1.38(4)	1.461(8)	1.45(2)
C(4)-C(7)	1.42(5)	1.467(8)	1.46(2)
C(7)-C(8)	1.37(5)	1.309(9)	1.28(3)
C(8)-C(9)	1.57(5)	1.493(8)	1.51(3)
C(9)-N(2)	1.46(6)	1.455(7)	1.42(2)
N(2)-C(10)	1.49(6)	1.461(6)	1.47(3)
N(2)-C(13)	1.50(5)	1.465(6)	1.51(2)
C(12)-C(13)	1.50(7)	1.521(9)	1.48(2)
C(10)-C(11)	1.48(6)	1.517(7)	1.53(2)
N(3)-C(12)	1.43(6)	1.466(7)	1.48(2)
N(3)-C(11)	1.42(5)	1.470(6)	1.43(2)
C(11)-C(14)	1.50(4)	1.513(8)	1.53(3)
C(14)-C(15)	1.47(7)	1.534(8)	1.56(3)
C(15)-C(12)	1.59(6)	1.540(9)	1.51(2)
N(3)-C(16)	1.31(4)	1.334(6)	1.35(2)
C(16)-C(17)	1.56(6)	1.504(7)	1.54(3)
C(16)-O(3)	1.22(5)	1.234(6)	1.24(2)
O(1)-N(1)-O(2)	124(1)	122.3(6)	126(1)
C(4)-C(7)-C(8)	122(2)	126.5(6)	128(1)
C(7)-C(8)-C(9)	124(2)	125.6(6)	123(1)
C(8)-C(9)-N(2)	109(2)	111.2(5)	112(1)
C(10)-N(2)-C(13)	108(2)	111.4(4)	107(1)
N(2)-C(10)-C(11)	106(2)	109.5(4)	112(1)
C(10)-C(11)-N(3)	107(2)	107.4(4)	109(1)
C(9)-N(2)-C(13)	103(2)	111.8(4)	113(1)
N(2)-C(13)-C(12)	104(2)	109.7(4)	114(1)
C(13)-C(12)-N(3)	113(2)	106.8(4)	107(1)
C(11)-N(3)-C(12)	101(2)	103.4(4)	103(1)
N(3)-C(12)-C(15)	101(2)	101.8(4)	102(1)
C(12)-C(15)-C(14)	105(2)	103.7(5)	103(1)
C(15)-C(14)-C(11)	99(2)	103.7(5)	105(1)
C(14)-C(11)-N(3)	111(2)	101.7(4)	103(1)

The benzene ring is almost coplanar with the double-bond C(7)-C(8), as shown by the torsion angle τ_4, with a mesomeric effect on the adjacent bond C(4)-C(7) (see Table 2). The *p*-nitro group is slightly rotated with respect to the benzene plane with a dihedral angle of 13(3)°**.**

The 8-*N*-propionyl substituent is characterized by a C(11)-N(3)-C(16)-O(3) torsion angle of -169(3)°, where the carbonyl oxygen O(3) is "trans" to C(11), as well as for C(17) respect to C(12) with the torsion angle C(12)-N(3)-C(16)-C(17) of 180(3)°. The crystal packing is characterized by the C(7)-O(1)' contact ('= 1+x, y, z) of 3.46(4) Å allowing a distance C(7)-H(7)...O(1)' of 2.54(4) Å with an angle C(7)-H(7)...O(1)' of 159(3)°, involving the nitro-oxygen of the adjacent molecule. Similar interactions concern C(8)...O(3)'' (''= x-1,y,z) of 3.58(3) Å (propionyl carbonyl oxygen), with a distance C(8)-H(8)...O(3)' of 2.67(4) Å and an angle of 156(3)°.

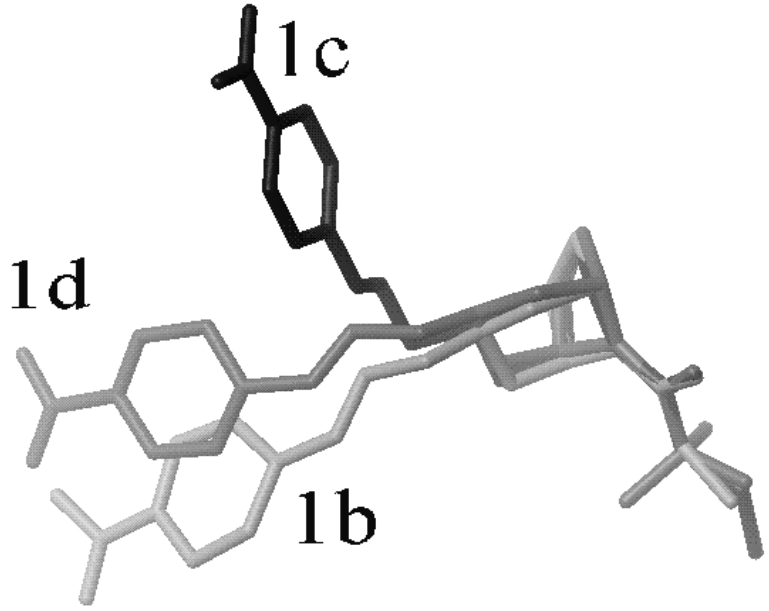


Figure 2. Superimposition of the structures (**1b**), (**1c**) and (**1d**).

Compound (**1c)**, differently from the previous one, has in 8 position a *n*-butyroyl group, which has an important steric impact, inducing conformational changes and a different molecular packing. The piperazine ring is in a chair-conformation, with the aralkenyl chain in equatorial orientation and in "trans eclipsed" conformation; consequently the orientation of the cinnamyl moiety is on the opposite side of **1b** as shown in Figure 2, where the structures of **1b**, **1c** and **1d** are superimposed.

The nitro group and the phenyl ring are almost coplanar. The presence in the structure of a crystallized water molecule, induces very important changes in the crystal packing. The H(4)-O(4)-H(41) water hydrogen atoms link together different molecules. H(4) interacts with O(3) (the butyroyl carbonyl oxygen) of the first molecule at a distance of 1.82(2) Å [O(4)-H(4)...O(3) 172(3)°], O(4)...O(3) is 2.827(6) Å and

H(41) links O(3)' ('= -x-1,-y,-z) of an adjacent molecule [O(4)...O(3)' 2.892(6) Å, O(4)-H(41)...O(3)' 1.99(2) Å, O(4)-H(41)...O(3)' 160(3)°]. Compound (**1d**) differs from the previous two having a pivaloyl group in 8 (see Scheme 1). The molecular geometry is characterized by the aralkenyl chain equatorially oriented in "trans eclipsed" conformation. The piperazine ring maintains the chair conformation, and the plane of the ethylenic bridge is inclined respect to the base of the chair of 68(2)°. In this molecule the phenyl ring and the aralkenyl chain are less coplanar, with minor electron delocalization. The molecular packing is determined by the intermolecular contacts H(12)...O(3)' of 2.35(3) Å ('= x, 1+y, z) with the tertiary carbon of the piperazine ring and the terminal chain carbonyl oxygen [C(12)...O(3)' 3.20(3) Å, C(12)-H(12)...O(3) 145(2)°] and the piperazine N(2) "lone pair" with the phenyl H(5) of an adjacent molecule [C(5)...N(2)" 3.48(2) Å, C(5)-H(5)...N(2)" 2.54(2) Å and C(5)-H(5)...N(2)" 161(1)° ("= 1/2-x, 3/2-y, z)]. This molecular packing does not imply significantly conformational changes respect to **1b**, although the steric hindrance of the lateral chain should have suggested important conformational changes.

Theoretical calculations

The conformational analysis on **1b-d** was carried out starting from the crystallographic results. The 3-nitrogen was considered to be protonated in order to simulate the structure possibly present at phisiological pH. The module annealing of the program TINKER[4] was used for calculations with a 3.5 Kcal window as energetic discriminant in order to obtain a minimum set of conformers. Among these the lower energy ones have been selected for further calculations of the energetic and geometric features of **1b-d** and morphine, as template for the μ-affinity and for its conformational rigidity, using semiempirical (MOPAC[5]) and *ab initio* (GAMESS[6]) methods.

Table 3. MOPAC[5] and GAMESS[6] calculations and experimental X-Ray values of the geometries of **1b-d**.

	τ_1	τ_2	τ_3	τ_4
1b X-Ray	176	127	171	178
chair[6]	168.6 (171.8)	115.7 (118.8)	179.8 (-179.5)	137.2 (179.8)
boat	69.1 (-70.2)	107.8 (110.6)	178.9 (179.4)	134 (179.7)
1c X-Ray	173.1	-133	180	171
chair	168.8 (168.7)	115.7 (99.4)	179.8 (-178.9)	137.3 (177.9)
boat	69.2 (-58.3)	108.2 (108)	178.9 (179.4)	134.1 (179.5)
1d X-Ray	162	125	174	162
chair	168.2 (-173.2)	115 (76.7)	179.6 (-178.3)	138.9 (179.2)
boat	73.9 (-74.2)	108.2 (101.1)	179.9 (-177.7)	135.2 (178.7)

(Results in parenthesis are from MOPAC[5])

The calculated structures in the hypothesis of a chair conformation of the piperazine ring are comparable with the X-Ray results, except for the τ_2 value which is totally different in **1c** (see Table 3)**.**

In general this conformation is preferred with respect to the boat except for **1d** where the difference of energy between chair and boat is reduced by the formation of H bond interactions between the carbonyl oxygen and the ammonium hydrogen (see Figure 3).

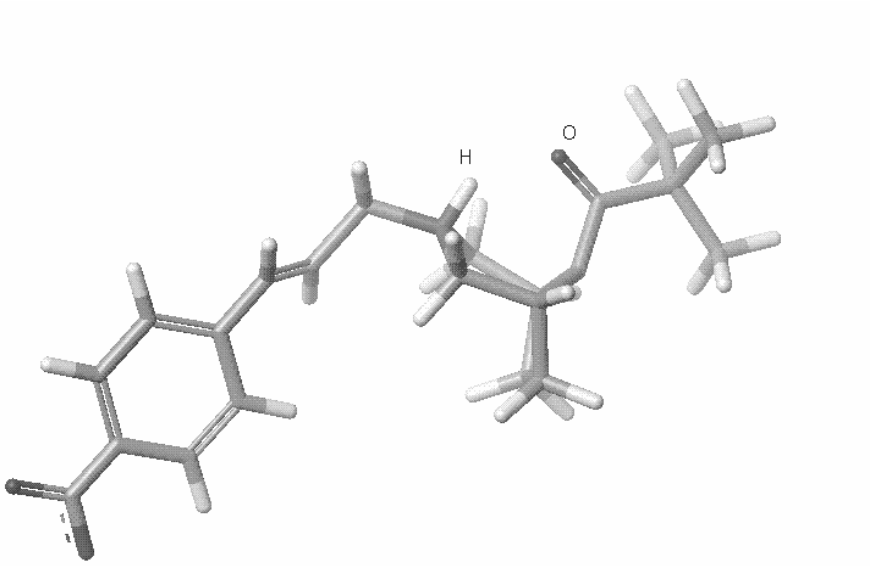


Figure 3. Theoretical boat conformation[6] of **1d**.

In Figure 4 are compared the geometric properties of the examined compounds in terms of van der Waals surfaces (A) and volumes (V). It is significant to observe that the polar surfaces are about the same, suggesting a similar polar contribution for the biological interactions.

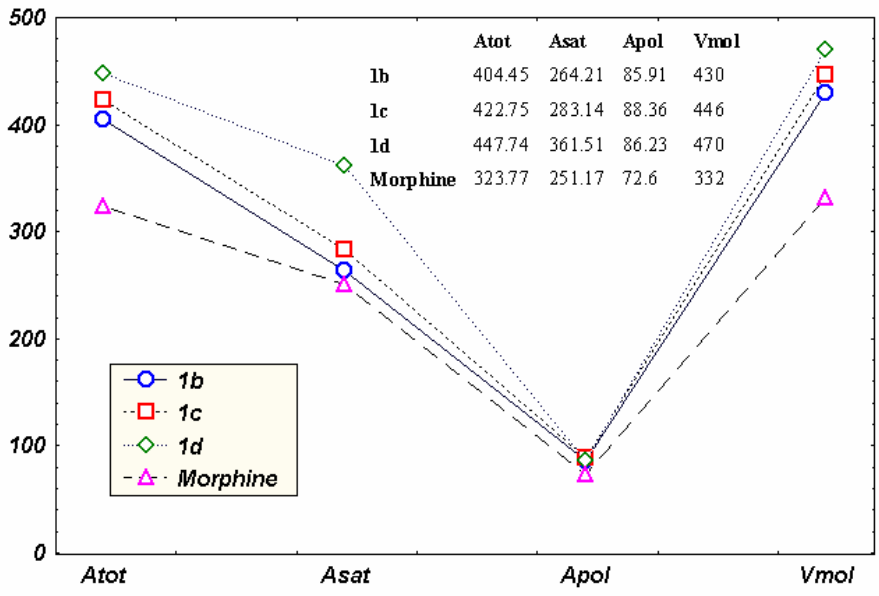
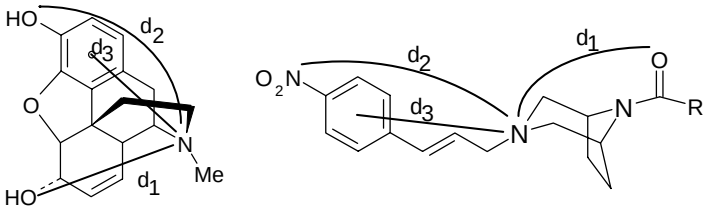


Figure 4. Geometric properties Surfaces A (Å^2) and volumes V (Å^3).
(tot = total, sat = sature, pol = polar, mol = molecular)

In Figure 5 are the distances between significant pharmacophoric groups important for the ligand-receptor interactions[7] obtained from X-Ray analysis and theoretical calculations. It is worth noticing that the distances between "important" pharmacophoric groups are different from morphine, mainly for d_1 and d_3 which seem interchanged.



	d_1	d_2	d_3
Morphine	6.44	7.12	4.37
1b X-Ray	4.37	8.86	6.22
chair[6]	4.58 (4.35)	8.79 (8.85)	6.06 (6.11)
1c X-Ray	4.56	8.79	6.09
chair	4.53 (4.29)	8.79 (8.84)	6.06 (6.1)
1d X-Ray	4.45	8.87	6.15
chair	4.36 (2.47)	8.65 (8.32)	5.99(6.1)

Figure 5. Scheme of morphine and **1b**, **1c**, **1d** with the significant distances of the pharmacophoric groups (in parentheses the values for boat conformation).

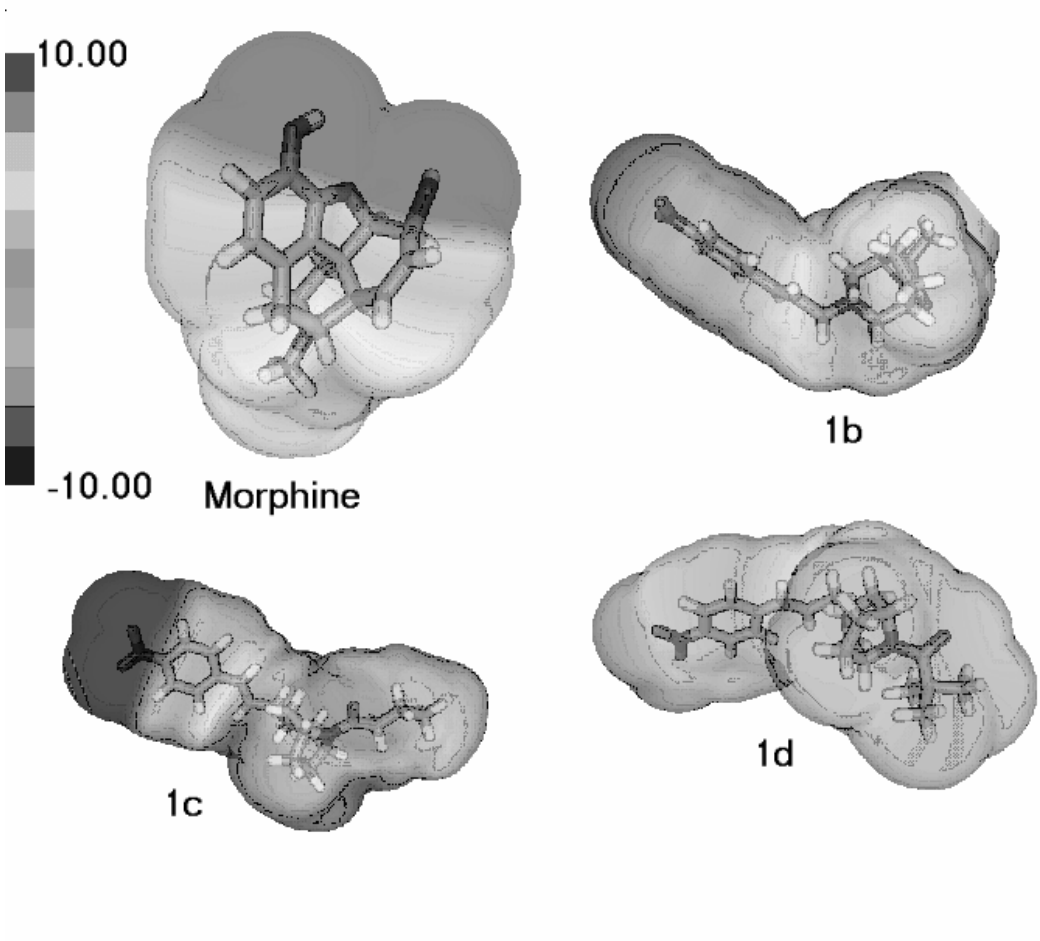


Figure 6. MEP Connolly surfaces[6] for morphine, **1b**, **1c** and **1d**.

The most significant information on the electronic properties have been obtained with Molecular Electrostatic Potential (MEP) analysis. The Connolly surfaces of the compounds compared to morphine are shown in Figure 6.

While the latter shows two distinct zones with opposite electrostatic potential related respectively to the aromatic and aminic portions of the molecule, the examined compounds have an additional zone with negative electrostatic potential around the carbamidic group as the aromatic zone. The considerations that can be made examining either the d_1, d_2, d_3 distances and the electrostatic potential maps evidence the presence of a more complex pattern with respect to morphine with the possibility of an interchange of the two zones having negative electrostatic potential.

Biological results

In the Table 4 are reported the inibition constants of morphine and **1a-d** towards μ-opioid receptors. Their affinity was found either comparable or sligthly lower than morphine despite the important electronic and geometrical differences discussed above. The binding studies have been made according to Barlocco *et al.*[8]

Table 4. Inhibition constants of morphine and compounds (**1a-d**) towards μ-opioid receptors.

Compound	K_i (nM)
Morphine	15
1a	37
1b	33
1c	21
1d	22

CONCLUSIONS

The compounds (**1b**), (**1c**), and (**1d**) have almost the same affinity towards the μ-receptors, but **1c** has a conformation different from **1b** and **1d**. Its molecular structure could be influenced in the crystal state by the presence of water (molecular ratio 1:1) responsible for strong hydrogen bond interactions that could affect the conformational changes. Compounds (**1b**) and (**1d**) have the same molecular conformation also in the presence of different steric hindrance at the substituent in 8 position but in absence of strong intramolecular interactions. However a comparison of the theoretical calculations (in vacum) shows equal values of the important torsion angles for the chair and for the boat conformation of the piperazine ring,

suggesting that possibly in solution the three compounds have identical conformation and then a comparable affinity towards the μ-receptor.

EXPERIMENTAL

Chemistry

Melting point were determined on a Büchi 510 capillary melting points apparatus and are uncorrected. Analysis indicated by the symbols were within ±0.4 of the theoretical values. ^{1}H-NMR spectra were recorded on a Bruker AC200 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane. TLC on silica gel plates was used to check product purity. Silica gel 60 (Merck; 70-230 mesh) was used for column chromatography.

Compounds (**1a**,**b**) were previously reported.[2] Compounds (**1c**,**d**) were prepared according to the same procedure. For **1c**, Anal. Calcd for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.23. Found: C, 66.58; H, 7.15; N, 12.35. Oil: δ (ppm) ^{1}H-NMR ($CDCl_3$): 1.1 (t, 3H, J = 6.8 Hz); 1.9 (m, 6H); 2.3 (d, 2H, J = 7.0 Hz); 2.8 (d, 2H, J = 7.0 Hz); 3.2-3.4 (m, 4H); 4.6 (app s, 2H); 6.4 (m, 1H); 6.6 (d, 1H, J = 10.5 Hz); 7.5 (d, 2H, J = 8.8 Hz); 8.1 (d, 2H, J = 8.8 Hz).

For **1d**, Anal. Calcd for $C_{20}H_{27}N_3O_3$: C, 67.20; H, 7.61; N, 11.75. Found: C, 67.35; H, 7.55; N, 11.63. mp = 128-129°C: δ (ppm) ^{1}H-NMR ($CDCl_3$): 1.3 (s, 9H); 1.8 (m, 4H); 2.3 (d, 2H, J = 7.0 Hz); 2.8 (d, 2H,J = 7.0 Hz); 3.2 (d, 2H, J = 6.8 Hz); 4.6 (app s, 2H); 6.4 (m, 1H); 6.6 (d, 1H, J = 10.5 Hz); 7.5 (d, 2H, J = 8.8 Hz); 8.1 (d, 2H, J = 8.8 Hz).

X-Ray Crystallography

A summary of the data collection and refinement process for the three compounds are in Table 5. The orientation matrix and cell dimensions were determined by least squares refinement of the angular positions of 20 reflections. The H atoms were introduced in calculated positions with unique fixed isotropic thermal value. In **1c** the water protons were introduced at the observed positions. Structures were refined by full-matrix least squares using anisotropic temperature factors for non H atoms in **1c**, while in **1b** and **1d** the poor quality of the crystals allowed only a rigid body isotropic refinement for the benzene ring and isotropic for the remaining non H atoms. The programs used for structure solution and

refinement were respectively MULTAN82,[9] PARST,[10] and SHELX76.[11] ORTEP[12] was used for the drawings.

Table 5: Crystallographic data.

Compound	**1b**	**1c**	**1d**
Empirical Formula	$C_{18}H_{23}N_3O_3$	$C_{19}H_{27}N_3O_4$	$C_{20}H_{27}N_3O_3$
Molecular weight	329.40	361.44	357.45
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1$	$P2_1/a$	$P2_1/a$
Cell parameters (Å)			
a	8.365(3)	9.861(1)	11.887(3)
b	6.9935(7)	16.637(3)	6.210(2)
c	14.896(2)	11.852(4)	26.251(6)
β(°)	102.00(2)	94.24(2)	95.32(2)
Volume (Å^3)	852.4(3)	1939(7)	1929(1)
D_{calc} (Mg m^{-3})	1.28	1.24	1.23
Z	2	4	4
Temperature (°C)	23	23	23
Radiation (λ MoKα, Å)	0.71069	0.71069	0.71069
Diffractometer/scan	Enraf-Nonius CAD-4(ω/2θ)	Enraf-Nonius CAD-4(ω/2θ)	Enraf-Nonius CAD-4(ω/2θ)
Scan width	1.2 + 0.35 tanθ	1.2 + 0.35 tanθ	1.2 + 0.35 tanθ
θ range (°)	2-25	2-25	2-25
Reflections collected	1754	3790	4774
Reflections observed I>2σ(I)	754	1199	734
R(Rw)	0.12(0.13)	0.047(0.054)	0.09(0.095)

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