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Characterization of the physico-chemical properties of the imidazopyridine derivative Alpidem. Comparison with Zolpidem

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Summary — Zolpidem and Alpidem are 2 new imidazopyridine derivatives that represent a novel chemical and therapeutic class in the treatment of sleep and anxiety disorders, respectively. The former is a hypnotic agent and the latter behaves as an anxiolytic drug. In contrast to the benzodiazepines, both compounds act selectively at the ω_1 (BZ_1) but not at the ω_2 (BZ_2) modulatory sites of the $GABA_A$ supramolecular complex. The lack of affinity for ω_2 (BZ_2) modulatory sites may account for the absence of myorelaxant effects of these drugs. In order to determine the parameters that are important for the mode of interaction of these compounds with ω_1 modulatory sites, their physico-chemical properties have been characterized and compared. Focus is directed on crystalline structures obtained from X-ray analysis, and electronic properties as electrostatic potential maps, atomic charges, delocalization effects, and electron densities obtained by *ab initio* molecular orbital calculations.

benzodiazepine / imidazopyridine / Alpidem / Zolpidem / mono-crystal X-ray diffraction / MO calculations / *omega* modulatory sites

Introduction

Since the discovery of receptors involved in the pharmacological action of benzodiazepines [1, 2], a tremendous amount of study has been made to discover compounds that differ structurally from benzodiazepines and which would act at the ω modulatory sites of the $GABA_A$–chloride ion channel supramolecular complex [3].

Among these new structures, imidazopyridines [4], *eg* Zolpidem and Alpidem (fig 1), represent a new chemical and therapeutic class which show high affinity and selectivity for ω subtypes [5–7]. Based on pharmacological criteria, a new nomenclature has been recently proposed to replace the names BZ_1 and BZ_2: ω_1 (BZ_1) and ω_2 (BZ_2) modulatory sites [8–10]. It has been found both in animal models and man that Zolpidem (*N*,*N*,6-trimethyl-2-(4-methylphenyl)-imidazo[1,2-*a*]pyridine-3-acetamide, hemitartrate) is a hypnotic agent [5, 11–13] and that Alpidem behaves as an anxiolytic drug [11, 14–17]. Receptor binding studies indicate that Zolpidem has a high affinity for ω_1 but neither for ω_2 nor peripheral site (peripheral benzodiazepine receptor subtype) [5], while Alpidem has a high affinity for ω_1 and for peripheral site but not for ω_2 modulatory site [6].

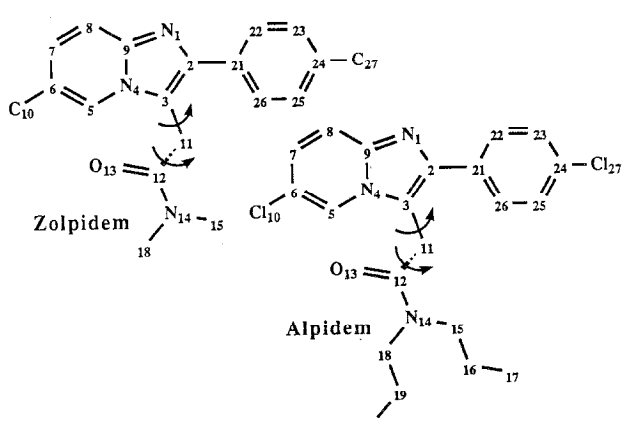

Fig 1. Planar structure formulae with atom numbering of Zolpidem and Alpidem.

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The aim of the present work was to characterize the physico-chemical properties of the imidazopyridine derivatives Alpidem and Zolpidem. We have first particularly discussed the crystalline structure of Alpidem (fig 1) in comparison with that of Zolpidem [4]. Taking these into consideration we have then scanned the conformational space by allowing torsions around the freely rotating single bonds in order to determine whether stable conformations other than that observed by X-ray diffraction might exist. Finally, we have focussed on the electronic properties, *ie,* electron densities, molecular electrostatic potential (MEP), atomic charges, delocalization effects, and charge transfer, by *ab initio* molecular orbital (MO) calculations. In these calculations, we have taken into account 2 different crystalline forms of Zolpidem that are present in the crystal unit of Zolpidem hemitartrate, the protonated and the non-protonated forms [4] in order to examine protonation effects on electronic properties. By comparison of the non-protonated crystalline forms of Zolpidem and Alpidem, the substitution effects in a series of analogues have been analyzed. The effects of the conformation on the electronic characteristics have been discussed considering the crystalline geometry of Alpidem and a plausible modified geometry [18] based on conformational results. Experimental and theoretical aspects have been ascertained by computer-aided molecular superimpositions of 3-D structures and molecular orbital topographies. These approaches have been considered in order to extract characteristic parameters of the pharmacophoric elements which should be considered in the study of the interaction modes with ω modulatory sites.

Results and discussion

Alpidem and Zolpidem have been synthesized by the Chemistry Department of Synthélabo Recherche.

X-ray structural analysis

In order to determine the structural and electronic parameters which might confer biological activity to the drugs under consideration, *ie* the imidazopyridine derivatives, it is logical to start with some experimental crystallographic bases. Thus, after determining the 3-D structure of Zolpidem [4], we determined the crystal structure of Alpidem by direct methods from single crystal X-ray diffraction, basing our analysis on 3 precise features for both compounds: i) their crystalline geometries; ii) their conformational behaviours; and iii) their electronic properties which could explain the observed crystal packing and suggest several modes of interaction with the given ω binding sites.

The atom numbering, bond lengths and valence angles of Alpidem are presented in figure 2; the atomic parameters and equivalent anisotropic thermal factors are given in table I. The vibrational ellipsoids are relatively small (fig 3), since the data were collected at a low temperature (–118°C). No co-crystallization is observed. The crystal packing (fig 4) presents no hydrogen bonding. However, one can observe an original stacking of the imidazo and pyridine rings. This parallel arrangement and the relatively short distance between these aromatic rings, 3.41 Å, is characteristic of a π–π interaction which could lead to a charge transfer between the considered moieties

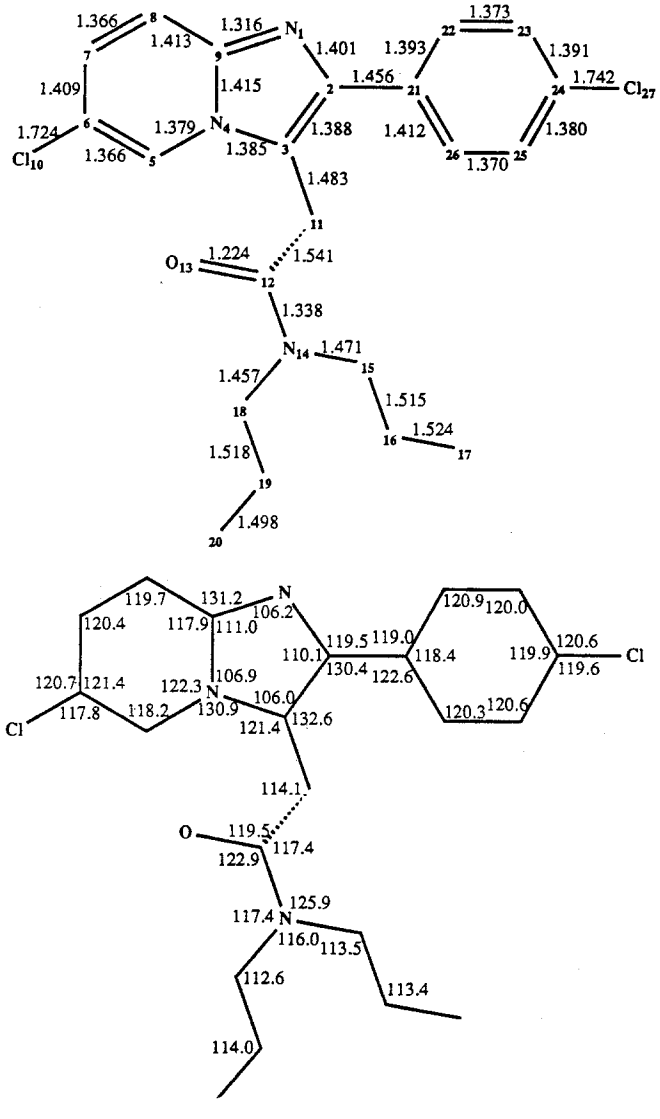

Fig 2. Atom numbering, bond lengths (Å), and valence angles (°) for Alpidem (maximum ESDs 0.009 Å and 0.9°).

Table I. Fractional atomic coordinates (x 10^4) and B_{eq} ($Å^2$) values with estimated standard deviations ESDs in parentheses for Alpidem.

	x/a	y/b	z/c	$B_{eq.}$	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
N(1)	3758(4)	3691(2)	2452(8)	1.91(7)	179(20)	353(20)	186(20)	-9(16)	34(16)	-40(16)
C(2)	4383(4)	3881(4)	1772(9)	1.88(8)	218(24)	335(24)	159(20)	-23(20)	45(20)	-38(20)
C(3)	4473(1)	4887(4)	1621(1)	1.78(8)	181(24)	322(24)	159(20)	-47(20)	21(19)	2(19)
N(4)	3905(4)	5325(2)	2232(8)	1.62(6)	157(19)	267(20)	192(19)	-17(16)	51(16)	-13(16)
C(5)	3709(4)	6299(4)	2325(9)	2.05(8)	196(24)	370(24)	181(21)	-53(20)	-8(20)	-4(20)
C(6)	3083(4)	6517(4)	2912(9)	2.30(9)	142(23)	445(28)	254(26)	-113(23)	-12(20)	24(21)
C(7)	2657(4)	5776(5)	3457(9)	2.76(10)	249(27)	500(32)	302(30)	-97(27)	75(24)	21(26)
C(8)	2846(4)	4810(5)	3353(9)	2.45(9)	185(26)	456(31)	317(27)	-8(24)	115(24)	-28(23)
C(9)	3472(4)	4553(4)	2695(9)	2.03(8)	185(24)	384(28)	199(24)	-19(20)	43(20)	-49(20)
Cl(10)	2843(2)	7738(1)	3018(5)	3.05(2)	313(6)	430(8)	410(8)	-93(5)	80(5)	100(5)
C(11)	5023(4)	5483(2)	967(9)	1.67(7)	150(21)	271(24)	183(23)	24(16)	-16(20)	42(16)
C(12)	5800(4)	5669(4)	2202(9)	1.67(7)	236(23)	216(19)	181(23)	-12(16)	51(20)	17(16)
O(13)	5967(4)	5202(2)	3475(6)	1.99(5)	238(17)	280(16)	216(17)	20(13)	20(15)	-38(13)
N(14)	6284(4)	6309(2)	1776(8)	1.72(6)	221(20)	216(16)	214(21)	4(15)	47(17)	-20(15)
C(15)	6144(4)	6847(2)	221(9)	1.67(7)	271(26)	188(20)	166(23)	16(16)	38(20)	-40(17)
C(16)	5978(4)	7929(4)	375(9)	2.29(9)	318(28)	199(21)	350(30)	-5(20)	75(24)	-30(20)
C(17)	5220(5)	8123(5)	835(9)	2.75(9)	278(28)	392(28)	357(31)	-15(24)	47(27)	-42(24)
C(18)	7063(4)	6373(4)	2820(9)	2.28(9)	188(24)	381(28)	245(26)	62(20)	-45(21)	-26(20)
C(19)	7591(5)	5577(5)	2448(9)	3.22(11)	298(31)	555(38)	353(35)	73(27)	51(27)	144(27)
C(20)	8382(5)	5573(8)	3582(12)	4.85(18)	260(35)	1024(64)	548(49)	348(45)	84(35)	49(35)
C(21)	4804(4)	3064(4)	1297(9)	2.04(8)	258(26)	351(26)	170(23)	6(20)	56(20)	-38(21)
C(22)	4484(4)	2125(4)	1194(9)	2.14(8)	210(26)	344(26)	227(24)	-2(20)	-1(21)	-95(20)
C(23)	4860(4)	1334(4)	741(9)	2.52(9)	245(28)	362(27)	293(28)	-16(21)	-37(24)	-24(20)
C(24)	5565(4)	1467(4)	355(9)	2.03(8)	249(26)	284(24)	227(24)	-1(20)	42(21)	12(20)
C(25)	5896(5)	2387(4)	478(9)	2.26(8)	274(28)	315(26)	258(27)	4(20)	47(24)	-20(21)
C(26)	5527(4)	3180(4)	936(8)	1.99(8)	240(26)	320(26)	194(23)	-35(20)	56(20)	-73(20)
Cl(27)	6036(2)	478(1)	-280(6)	3.12(2)	438(8)	284(5)	480(9)	-37(5)	144(6)	49(5)
H(5)	4180	6829	2082	3.40						
H(7)	2050	5911	3776	3.95						
H(8)	2588	4337	3537	3.71						
H(111)	5054	5220	8	3.08						
H(112)	4722	6013	644	3.08						
H(151)	6539	6953	-236	3.24						
H(152)	5898	6464	-434	3.24						
H(161)	6251	8350	1031	3.87						
H(162)	5963	8409	-731	3.87						
H(171)	5148	8968	988	4.18						
H(172)	5213	7645	1920	4.18						
H(173)	4771	7885	-71	4.18						
H(181)	7268	7168	2673	3.47						
H(182)	7010	6172	3848	3.47						
H(191)	7667	5763	1316	4.74						
H(192)	7549	4919	2580	4.74						
H(201)	8349	6144	3410	5.68						
H(202)	8842	5148	3238	5.68						
H(203)	8525	5414	4690	5.68						
H(22)	3952	2066	1456	3.63						
H(23)	4681	518	590	3.71						
H(25)	6499	2347	89	3.63						
H(26)	5859	3740	1173	3.32						

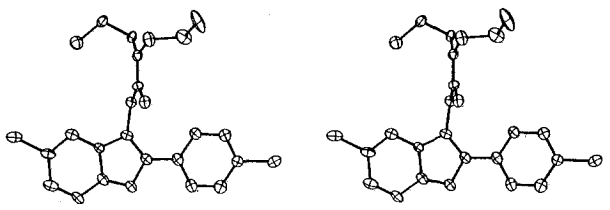

Fig 3. Stereoscopic view of the Alpidem molecular conformation with vibrational ellipsoids (probability 50%).

(3.41 Å is the mean value of the elevation distances with regard to the above plane for the atoms involved in the π overlap). The possible charge transfer between the aromatic rings is schematized by dotted lines in figure 4.

Bond lengths within the imidazopyridine and phenyl rings are on the average slightly longer for Alpidem (fig 2 and table II) than for Zolpidem [4] considered in its non-protonated form as well as in its protonated form: + 0.073 and + 0.061 Å for the 10 imidazopyridine bonds of Alpidem *versus* that of non-protonated and protonated forms of Zolpidem, respectively, and + 0.024 and + 0.015 Å for the 6 phenyl bonds, respectively. These trends could be attributed to the substitution of both methyl groups of Zolpidem, in position 6 on the imidazopyridine and in *para* position on the phenyl, by 2 chlorine atoms in Alpidem. The chlorine atoms exert onto the entirely delocalized rings an attractive inductive effect in opposition to the donor inductive effect of the methyl groups. Concerning the amide function, differences between equivalent distances are relatively more marked: C(11)-C(12), + 0.041 and + 0.018; C(12)-O(13), – 0.020 and – 0.021; and C(12)-N(14), + 0.033 and + 0.023 Å, for Alpidem *versus* the non-protonated and protonated forms of Zolpidem. Alpidem presents a dipropyl amide function whereas Zolpidem presents a dimethyl amide function. No significant variations of the valence angles are observed between Alpidem and Zolpidem. In both cases, some sp^2 valence angles deviate from the standard value, especially around C(2) and C(3) to separate bulky steric groups: the phenyl group and amide side chain (fig 2).

Main torsion angles of the 3 considered analogues are presented in table III. The corresponding absolute values are very close to each other within a range of 10–15°, so that the global conformation is the same for these analogues. This conformation can be defined by a few dihedral angle values: between the imidazo and amide groups 76.5, 86.0, and 85.3° for Alpidem, non protonated Zolpidem, and protonated Zolpidem, respectively; between the imidazo and phenyl cycles, 14.7, 17.2, and 22.9°, respectively; and between the imidazo and pyridine fused rings, 1.6, 2.6, and 3.2°,

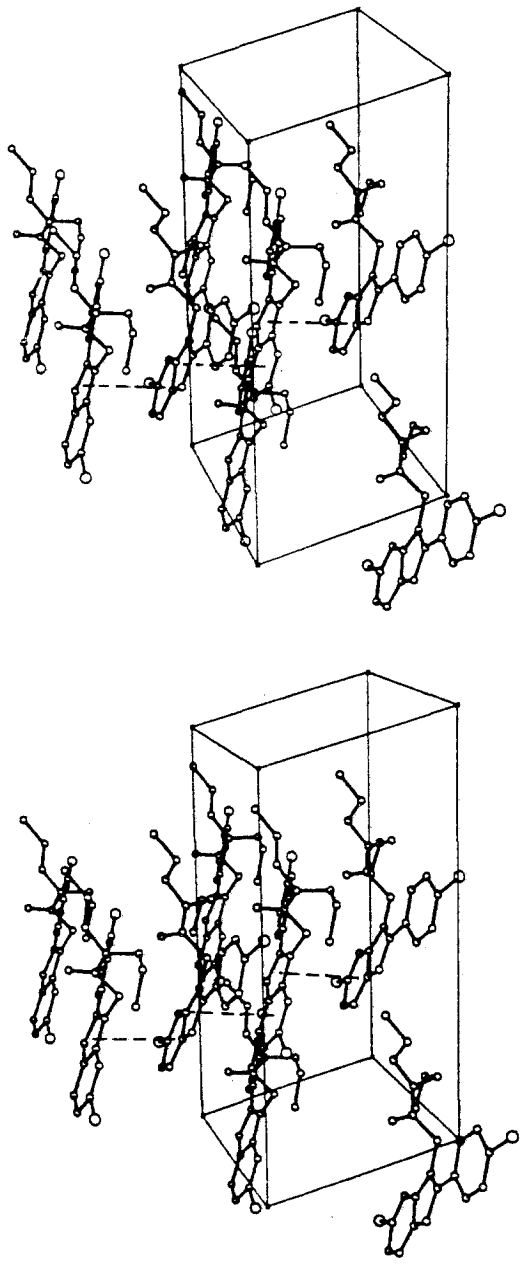

Fig 4. Stereoscopic view of the Alpidem molecular conformation and crystal packing. Dotted lines link the rings involved within a possible charge transfer.

respectively. The latter values just indicate that the bicyclic imidazopyridine is slightly twisted due to the presence of 2 voluminous substituents in the *ortho* position on the imidazo ring.

As noted for other systems involving a phenyl group linked to an aromatic heterocycle [18–19], the distance between C(2) and C(21) is relatively long:

Table II. Corresponding bond lengths (Å) for Alpidem, non protonated Zolpidem, and protonated Zolpidem.

	Alpidem	Zolpidem [4]	Zolpidem+H [4]
Imidazopyridine moiety			
N(1)-C(2)	1.401	1.391	1.380
C(2)-C(3)	1.388	1.381	1.368
C(3)-N(4)	1.385	1.384	1.391
N(4)-C(5)	1.379	1.388	1.372
C(5)-C(6)	1.366	1.345	1.373
C(6)-C(7)	1.409	1.407	1.432
C(7)-C(8)	1.366	1.352	1.363
C(8)-C(9)	1.413	1.422	1.378
C(9)-N(1)	1.316	1.303	1.345
N(4)-C(9)	1.415	1.393	1.375
Phenyl ring			
C(21)-C(22)	1.393	1.396	1.392
C(22)-C(23)	1.373	1.376	1.373
C(23)-C(24)	1.391	1.382	1.385
C(24)-C(25)	1.380	1.370	1.378
C(25)-C(26)	1.370	1.389	1.372
C(26)-C(21)	1.412	1.386	1.404
Amide function			
C(11)-C(12)	1.541	1.500	1.523
C(12)-O(13)	1.224	1.244	1.245
C(12)-N(14)	1.398	1.325	1.315

Table III. Main torsion angles (°) for Alpidem, non protonated Zolpidem, and protonated Zolpidem.

	Alpidem	Zolpidem [4]	Zolpidem+H [4]
C(2)-C(3)-C(11)-C(12)	-84.2(6)	99.7	-92.5
N(4)-C(3)-C(11)-C(12)	96.5(5)	-86.6	91.7
C(3)-C(11)-C(12)-O(13)	12.4(9)	-4.5	-12.9
C(3)-C(11)-C(12)-N(14)	-171.1(5)	178.9	167.7
C(11)-C(12)-N(14)-C(15)	-0.8(9)	-2.6	7.4
C(11)-C(12)-N(14)-C(18)	-170.6(6)	176.2	179.5
C(12)-N(14)-C(15)-C(16)	107.3(7)	-	-
N(14)-C(15)-C(16)-C(17)	-66.4(9)	-	-
C(12)-N(14)-C(18)-C(19)	82.4(9)	-	-
N(14)-C(18)-C(19)-C(20)	-175.7(7)	-	-
N(1)-C(2)-C(21)-C(22)	13.8(9)	-17.7	23.9
N(1)-C(2)-C(21)-C(26)	-166.1(6)	161.4	-154.8
C(3)-C(2)-C(21)-C(22)	-163.8(6)	162.3	-156.1
C(3)-C(2)-C(21)-C(26)	16.4(11)	-18.6	25.2

1.456(7), 1.462, and 1.467 Å for Alpidem, and the non-protonated and protonated Zolpidem, respectively. These bond lengths correspond to a single bond between sp^2 carbon atoms, indicating that there is no delocalization between both aromatic moieties and suggesting that the phenyl orientation is not imposed except under external constraints such as crystal packing or interaction with a receptor site.

Theoretical conformational analysis

The molecular geometry obtained by X-ray diffraction might not necessarily correspond to the only possible stable conformations. Theoretical calculations could show the existence of other energetic minima. The conformational space of both Alpidem and Zolpidem in their neutral form has therefore been investigated using the semi-empirical MO AM1 method. Figure 5 represents the energetic variations due to the modification of the amide function orientation by rotation around the 2 single bonds on both sides of methylene C(11). This rigid rotor approximation may be applied in these cases since the C(3)-C(11) and C(11)-C(12) bonds can be considered as real single ones (bond lengths between 1.48–1.54 Å) that link together 2 planar moieties (the imidazopyridine fused rings and the amide function). Starting with the crystalline conformations, the 2-D iso-energy maps display successive contours with an interval of 1 kcal·mol^{-1}. The white zones, *ie* energy > 30 kcal·mol^{-1}, correspond to forbidden conformations. The computed global minima are marked by * whereas the crystalline conformations (see torsion angle values in table III) are indicated by X. In the case of Alpidem, X_M corresponds to a modified conformation considered as the active one for the peripheral benzodiazepine binding sites [18]. This conformation will be used further to ascertain the influence of the conformation on the electronic properties.

Globally, the 2 maps show a similar pattern; 2 central regions including allowed conformations (dotted lines) in a space of forbidden conformations. From 10–30 kcal·mol^{-1} the iso-energy contours are very close to each other, indicating that the minimal energy zones are confined within 'abrupt cliffs'. In the case of Alpidem, the crystalline conformation (X) has a total energy close to that of the global minimum (*). For Zolpidem, the X conformation has a total energy < 1 kcal·mol^{-1} higher than the global minimum. Due to the presence of more voluminous groups on the tertiary amide function for Alpidem, 2 propyl chains instead of 2 methyl groups, the allowed conformational space is slightly restricted, as shown by the dotted contours (fig 5). However, the calculation does not include any geometrical optimization process; the propyl chains are considered as rigid entities and no bond length and bond angle rearrangement is allowed if particular steric or electronic interactions occur. In conclusion, one can assume that the conformational behaviours are closely related for both analogues.

Electronic properties analysis

An important notion in characterizing the ligand–receptor interactions at the molecular level is to analyze the complementarity of the molecular orbital topology, *ie* the nature (from the qualitative but if possible also from the quantitative points of view) of the interacting orbitals. With a properly oriented partner (see quantum theoretical MO calculations in the *Experimental protocols*), one easily depicts the nature of the highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO). For both

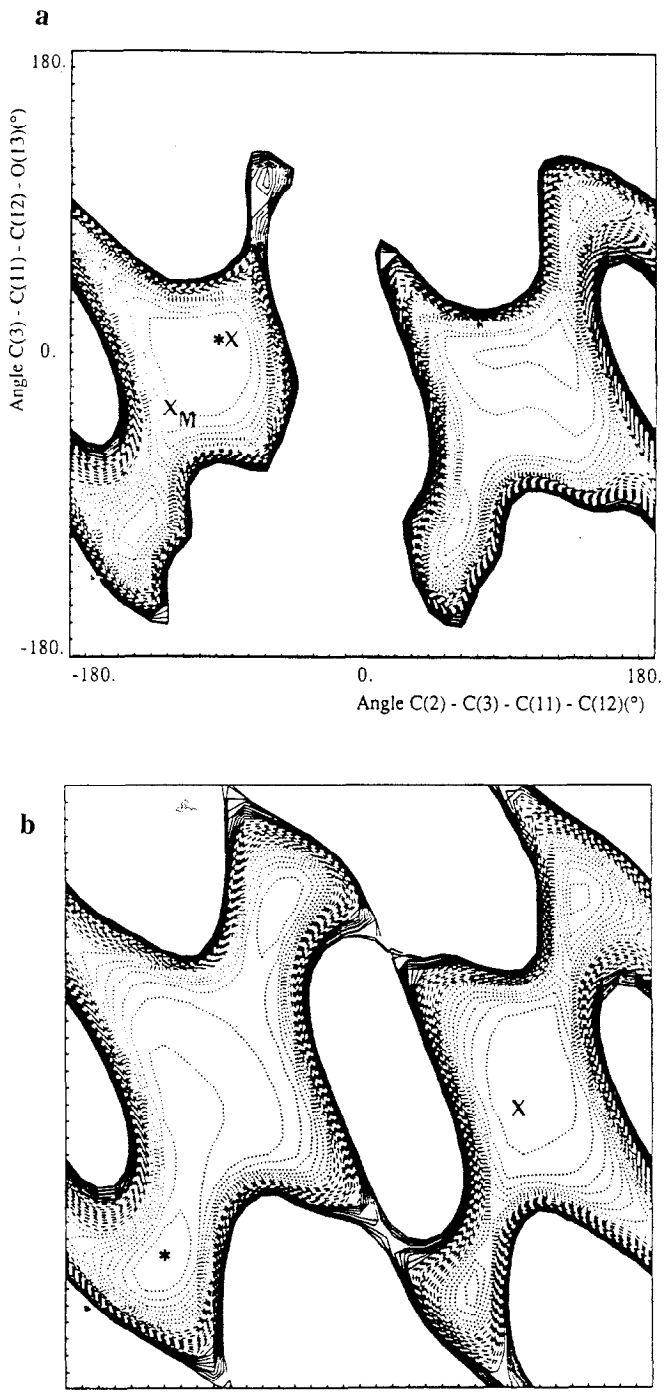

Fig 5. AM1 iso-energy contour map (kcal·mol^{-1}) for (a) Alpidem and (b) Zolpidem in its non protonated form. Point * corresponds to the absolute minimum energy; point X indicates the crystalline conformation. The contour-to-contour interval is 1 kcal·mol^{-1}; dotted lines indicate iso-energy contours up to 9 kcal·mol^{-1}, dashed lines, contours from 10 to 19 kcal·mol^{-1}, and solid lines, contours from 20–29 kcal·mol^{-1}.

analogues, Zolpidem and Alpidem, HOMO and LUMO are principally characterized by the $2p_{\pi}$ atomic orbitals (in our case $2p_y$ orbitals) of the imidazopyridine moiety with a weaker contribution of the phenyl group atoms. This is clearly visualized by computing the electronic density within various planes, for example situated 1.0 Å above or below the imidazopyridine cycle in order to obtain 2-D iso-density contour maps representing the HOMO and LUMO patterns.

Concerning the neutral forms of both Alpidem and Zolpidem, the largest contributions (the largest coefficients of the atomic orbitals of the basic set within the considered molecular orbital) within the last 2 HOMOs and first 2 LUMOs are mainly π orbitals of imidazopyridine; just below these 2 HOMOs and above these 2 LUMOs, the molecular orbitals are mainly composed of π-type MOs of the phenyl group or amide function. More particularly for Alpidem, the principal contributions to the HOMO are the $2p_y$ orbitals of C(3), C(9), C(7), and C(2) so that the highest electronic iso-density contours appear mostly on the imidazo ring. The LUMO is principally composed of the pyridine ring atoms, particularly the C(5), C(8), and C(6) $2p_y$ orbitals. Therefore, within a charge transfer hypothesis, one can suggest that the imidazo 5-membered ring should be an electron donor (D) and that the pyridine part should be an electron acceptor (A). In fact, such an interaction mechanism is observed in the solid state (fig 4) showing an infinite (–D...A—D...A–) chain formed by particular aromatic plane stacking. Figure 6 represents a projection of the superimposed Alpidem molecules, as occurs in the crystalline state, on which we added the 2-D iso-density profiles (HOMO or LUMO) computed as mentioned above. The densest HOMO iso-contours of the imidazo ring (solid lines) globally recover the densest LUMO iso-contours of the pyridine cycle (dotted lines). However, neither the bonding and anti-bonding orbitals of the HOMO and LUMO nor the nodes correspond exactly. This observation is probably due to 2 main features: on the one hand, other forces (van der Waals, polarization, etc) participate in the molecular arrangement and in the crystal packing; and on the other hand, calculations are performed in the isolated state considering one partner only. Taking into account several partners forming the D–A chain in a unique calculation is practically impossible due to the huge size of such a system and consequently to the computing time required. Thus, the charge transfer is not unequivocally proven, but has a great probability of existing.

Concerning Zolpidem, we first compare the electronic pattern of its non-protonated form with that of Alpidem; both the HOMO and LUMO of these 2 analogues are practically identical. On the contrary, for the protonated form the contributions of the imida-

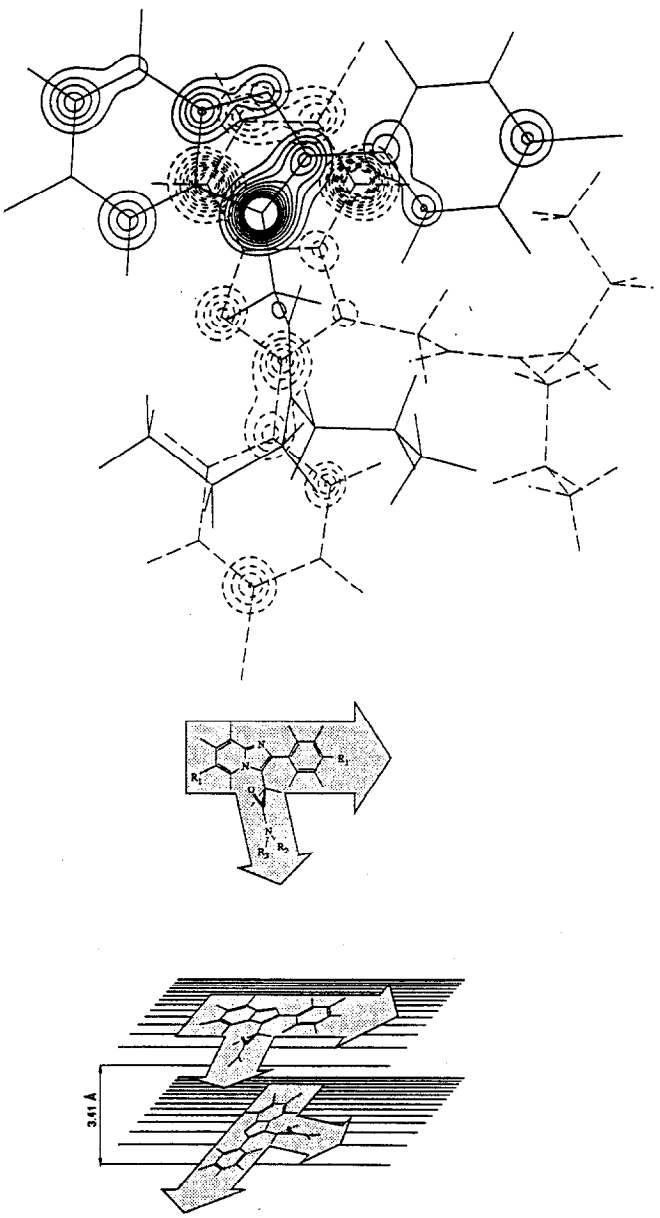

Fig 6. Superimposition of the molecular backbones as they appear in the crystalline state for Alpidem with projection of the HOMO (solid lines) and LUMO (dotted lines) 2-D iso-electron density contours. Ten contours are considered from 0.005 to 0.050 e. $Å^{-3}$ with a contour-to-contour interval of 0.005 e.$Å^{-3}$. This mode of perpendicular stacking presents an elevation distance of 3.41 Å between the planes.

zopyridine π-orbitals to the HOMO are lowered in favor of contributions of the phenyl and amide π orbitals, whereas they are higher in the LUMO. Moreover, let us recall that the crystal packing of Zolpidem is characterized by an alternant anti-parallel stacking of the 2 forms with 2 modes of superimposition showing elevation distances between the planes of 3.29 and 3.48 Å inversely related to the overlap amount for the imidazopyridine moieties [4]; these modes of aromatic plane interaction, *ie* anti-parallel, are different from that observed for Alpidem (figs 4, 6), *ie* perpendicular. The last 3 remarks led us to postulate that in the case of Zolpidem, we have to consider the entire bicyclic moiety of the protonated form as the electron acceptor and inevitably the same entity of the non-protonated form as the electron donor, thus leading to a ...D...A...D...A... pattern. Figure 7 summarizes the above considerations by presenting both superimposition modes of Zolpidem associated with the electronic densities of the HOMO (solid lines) and LUMO (dotted lines), *ie*, protonated and non-protonated forms. As noted for Alpidem, a strict comparison between nodes and bonding orbitals may not be considered; only global overlapping corroborates the charge transfer hypothesis.

In addition to the complementarity of the topography of HOMO and LUMO, their energy values and their gap can be examined (table IV). The lower the gap between the HOMO and the LUMO, the stronger the charge transfer. This charge transfer hypothesis is supported by comparison with the well-known hydroquinone–quinone complex. Hydroquinone and quinone have been considered separately under the same conditions as for Alpidem and Zolpidem (see *Experimental protocols*). The arrows in table IV indicate the considered charge transfer from the electron donor (D) to the electron acceptor (A).

Another simple electronic property to evaluate is the π-electron percentage on each bond as derived from the Mulliken population analysis, which permits a description of the composition, substitution, conformation and delocalization effects (fig 8). In all the cases considered, one observes an alternance of the π-overlap amount within the imidazopyridine ring avoiding a marked double bond character between N(4) and any of its 3 first neighbours. The formal double bonds arrangement as shown in figure 1 is respected. However, the π-overlap percentages around the N(4) atom are non-negligeable, *ie* between 11.5–17.9%, and show that its lone pair is fully delocalized in the entire bicyclic moiety, thereby explaining the sp^2 character of this nitrogen atom and the quasi-coplanarity of the imidazopyridine fused rings. Concerning the effect of protonation on N(1) for Zolpidem (fig 8a), it is observed that this process mainly implicates the N(1)-C(9) double bond ($\Delta \approx$ 10%) with a consecutive stronger delocalization of the pyridine ring. On the contrary, in the imidazo ring, the C(2)-C(3) double bond character is reinforced (27.8%), whereas for N(1)-C(2) and C(3)-N(4), the double bond character is lowered (13.0 and 12.7%).

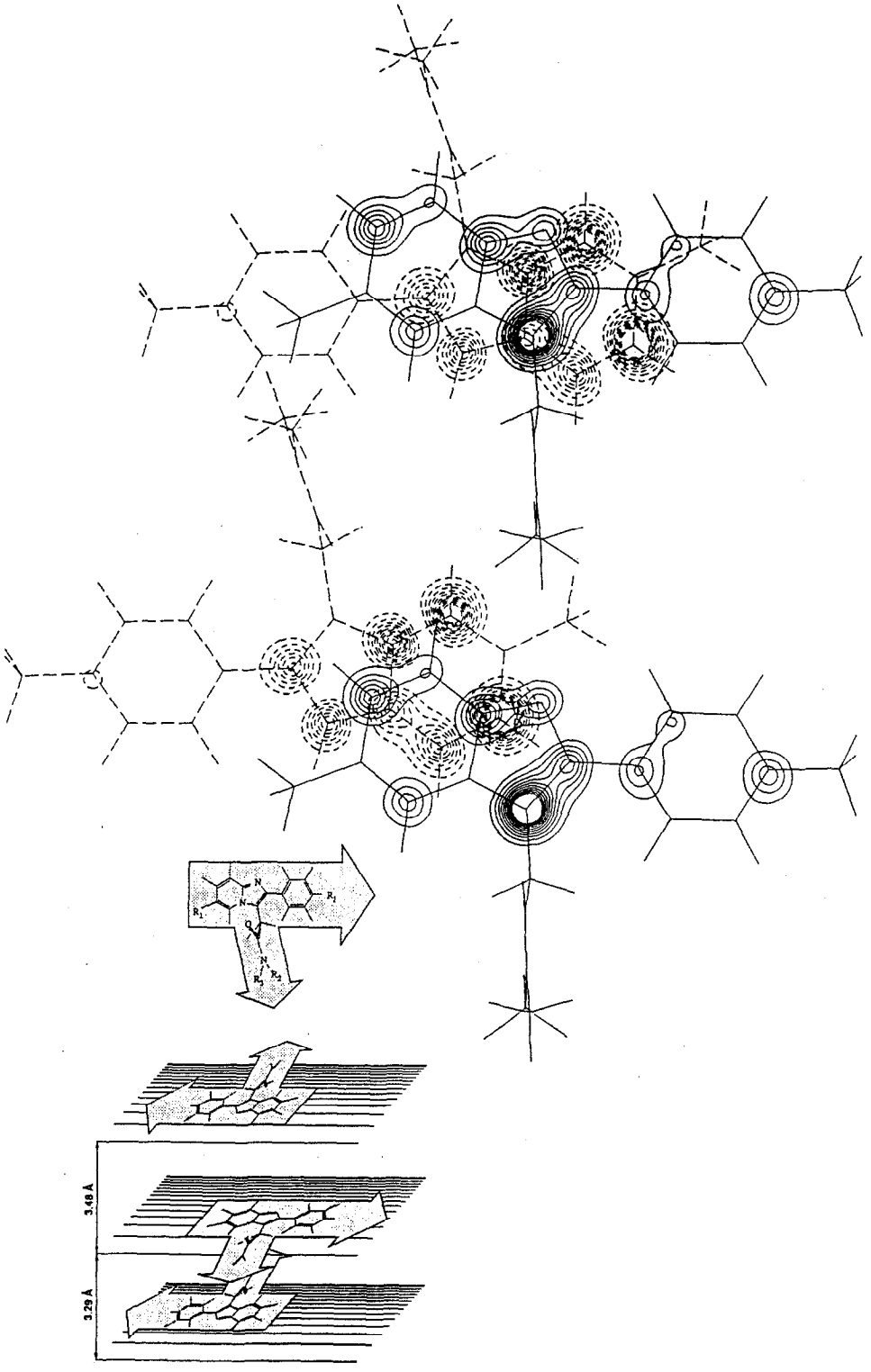

Fig 7. Superimpositions of the molecular backbones as they appear in the crystalline state for Zolpidem with projection of the HOMO of the non protonated form (solid lines) and of the LUMO of the protonated form (dotted lines) 2-D iso-electron density contours. Same contour definitions as in figure 6. Both modes of anti-parallel stacking are visualized with (a) 3.48 Å and (b) 3.29 Å between the planes.

Table IV. Total energy and HOMO and LUMO energies (atomic units) for Alpidem, non-protonated Zolpidem, and protonated Zolpidem, hydroquinone, and quinone. Arrows indicate the possible charge transfer from the electron donor (D) towards the electron acceptor (A).

	Alpidem	Zolpidem	Zolpidem+H	Hydroquinone	Quinone
E_{tot}	-1943.141	-957.952 *	-958.445 *	-375.544	-374.328
E_{LUMO+3}	0.2672	0.2924	0.1619	0.5204	0.4779
E_{LUMO+2}	0.2578	0.2869	0.1101	0.5026	0.3282
E_{LUMO+1}	0.2207	0.2490	0.0773	0.2877	0.2780
E_{LUMO}	**0.1810**	**0.2124**	**0.0223**	**0.2519**	**0.1379**
D → A	↑				
E_{HOMO}	**-0.2225**	**-0.2102**	**-0.3637**	**-0.2237**	**0.2993**
E_{HOMO-1}	**-0.2333**	**-0.2219**	**-0.3674**	-0.2908	0.3298
E_{HOMO-2}	-0.2753	-0.2643	-0.4018	-0.3725	0.3475
E_{HOMO-3}	-0.2986	-0.2705	-0.4136	-0.4022	0.3617

*The difference between these values gives the protonation energy: 309.5 kcal/mol.

No significant variation is observed between both neutral forms of Zolpidem and Alpidem, indicating that the major effect of the replacement of the methyl group by a chlorine atom is inductive rather than mesomeric. Similarly, 2 propyl in place of 2 methyl substituents for the tertiary amide function do not induce significant changes in the π-overlap populations. The modification in the Alpidem conformation (as mentioned above in the *Theoretical conformational analysis* section) does not lead to any significant change (fig 8b). As observed for other similar systems [18, 19], let us finally note that there is a low π-overlap between the phenyl group and the imidazopyridine fused rings of 5.7–6.3%, corroborating the fact that there is no significant delocalization between these 2 moieties.

The Mulliken analysis also provides access to the gross charge of atoms within the molecule. Atomic charges have been commonly used for relative molecular bonding studies as they show trends which appear chemically realistic and compatible with concepts such as the electronegativity of the atoms within the molecule. In this sense, they therefore represent an electronic property which can be useful for the description of interactions such as those in ligand–receptor complex formation. Considering the neutral forms of both Alpidem and Zolpidem (bold in fig 9) one observes a great similarity in the charge pattern except, logically, for the heavy atoms carrying the different susbstituents C(6), C(15), C(18), and C(24). Moreover, modifying the conformation of Alpidem with respect to the energy minimum does not relevantly alter the charge distribution (fig 9b). The protonation on N(1) for Zolpidem mainly affects the

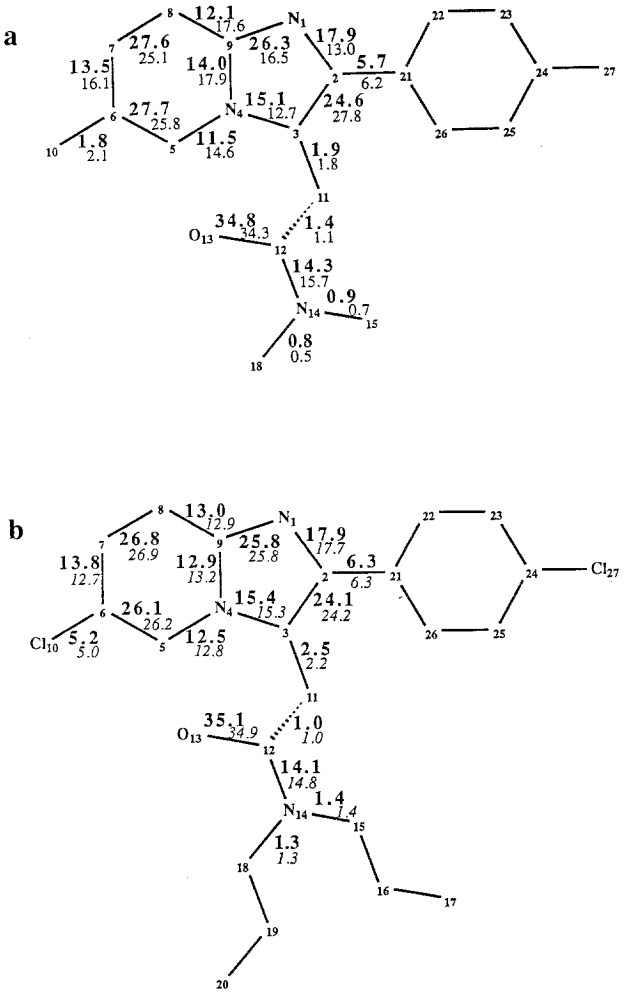

Fig 8. *Ab initio* STO-3G, or STO-3G*, π-overlap population *versus* the total one (%) derived from the Mulliken analysis for (a) Zolpidem in its non-protonated (bold) and protonated (normal) form, and for (b) Alpidem in its crystalline (bold) and modified (italic) conformation.

nearest heavy neighbour atoms but not the nitrogen itself (fig 9a). All hydrogen atoms contribute weakly to absorbing the positive unit charge; the hydrogen atoms' overall charge equals 2.13 e including 0.28 e for the added hydrogen atom, H(1), *versus* 1.50 e for the non-protonated Zolpidem.

Variations are more marked for the atomic charges derived from the surrounding molecular electrostatic potential (fig 10). We may recall that atomic charges computed in such a manner which is more dependent on the overall conformation than the Mulliken approach, are certainly more adapted to reflect the intermolecular–electrostatic interactions. A first comparison with the Mulliken type-charges may be made by examining the replacement of the methyl

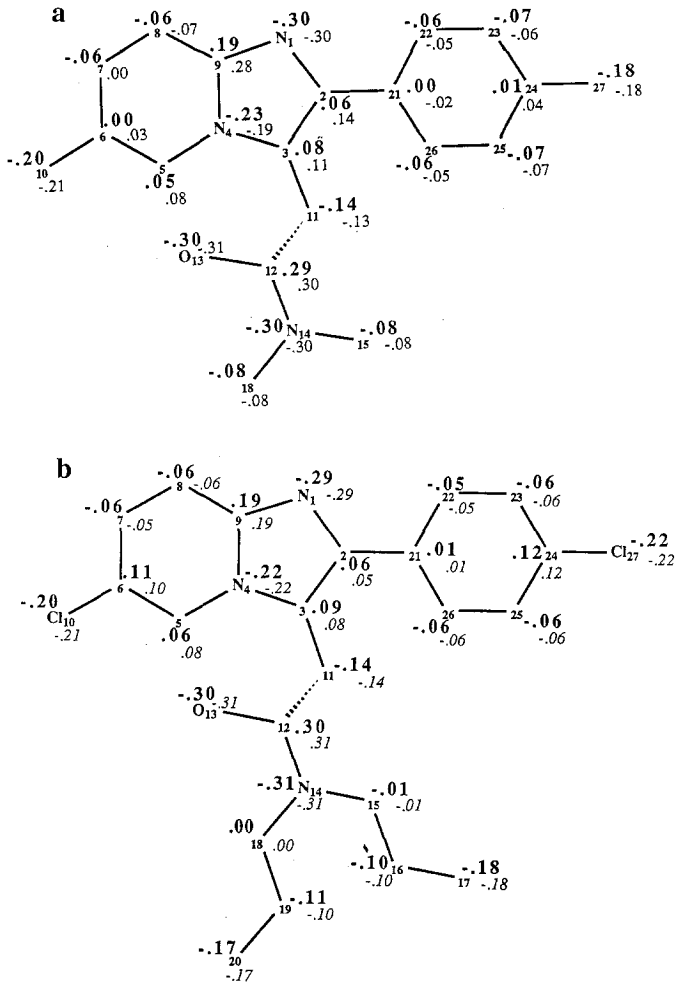

Fig 9. *Ab initio* STO-3G, or STO-3G*, atomic charges (electron) issued from the Mulliken population analysis for (a) Zolpidem in its non-protonated (bold) and protonated (normal) form, and for (b) Alpidem in its crystalline (bold) and modified (italic) conformation.

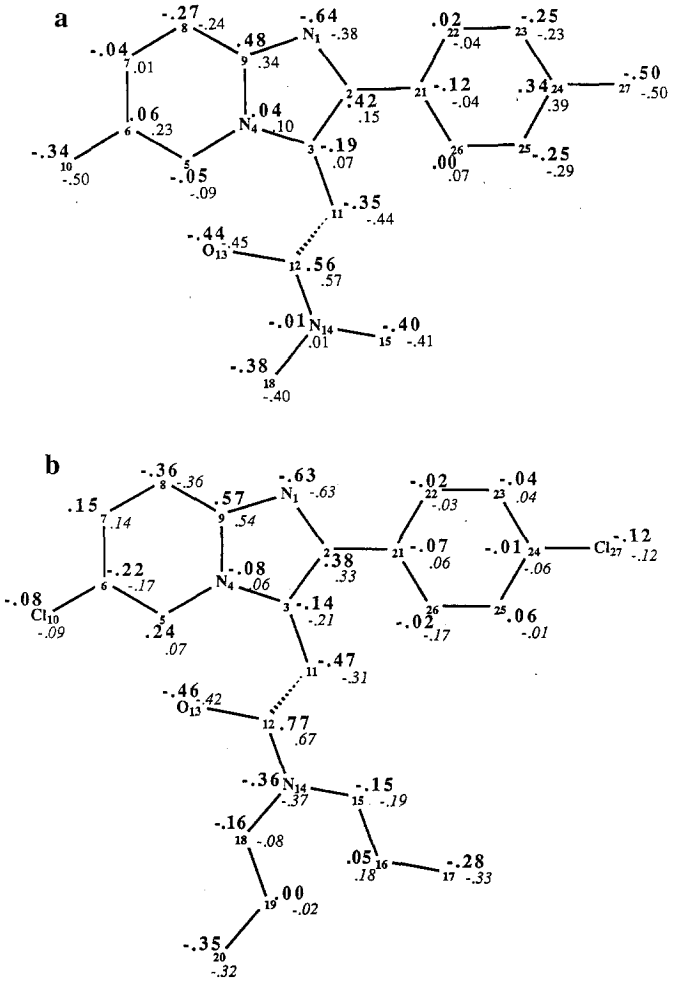

Fig 10. *Ab initio* STO-3G, or STO-3G*, atomic charges (electron) derived from the surrounding molecular electrostatic potential for (a) Zolpidem in its non-protonated (bold) and protonated (normal) form, and for (b) Alpidem in its crystalline (bold) and modified (italic) conformation.

groups by chlorine atoms. The potential derived model (PDM) confers relatively weak negative charges to the chlorine atoms (fig 10b), between –0.08 and –0.12 e, compared to the Mulliken results, between –0.20 and –0.22 e (fig 9b). Concerning the total charge on the methyl groups, PDM provides values between –0.05 and –0.11 e *versus* 0.00–0.08 e for the Mulliken approach. The PDM charges for a methyl group and for a chlorine atom are very similar and close to 0.0 e so that they can be regarded as non-polar substituents, whereas the Mulliken partitioning attributes a relatively more marked negative character to the chlorine substituent. The MEP values in the vicinity of a chlorine atom substituting an aromatic ring are generally very slightly negative [18] and values are close to zero for a methyl group. Thus, PDM seems to be more efficient in accounting for the similar hydrophobic character of methyl and chlorine substituents. Another difference with the Mulliken data is that, when considering the PDM approach, one also observes that a substitution influences not only the carrier atom but also may affect the charge pattern till the 4th neighbour. Moreover, the variation depends on the nature of the substituent; replacing the methyl group in position 6 of the imidazopyridine bicyclic part by a chlorine atom leads to increased positive and negative charges on the pyridine ring while the same replacement in the *para* position of the phenyl group gives rise to atomic charges close to 0.0 e on the entire 6-membered ring.

The protonation effect in Zolpidem (fig 10a) is logically strong on N(1); $\Delta \approx 0.26$ e, and extends to longer distances, in particular to the C(6) and C(10) atoms; $\Delta \approx 0.17$ and 0.16 e, respectively, as opposed to the Mulliken results which do not show such an effect. As all the computed electrostatic potential values are positive close to the skeleton of the protonated form, the total hydrogen atom charge is higher, *ie* 3.06 e including 0.34 e for the added H(1) *versus* 2.32 e for the non-protonated form.

Considering the allowed conformational change for Alpidem, one observes that such a modification induces no significant differences (fig 10b), except for the atoms closely involved in this variation of conformation, *ie*, charges on C(5), C(11), and C(26); Δ = 0.14, 0.16, and 0.15 e, respectively. In fact, there are just local charge rearrangements and, very often, changes in heavy atoms are counterbalanced by the hydrogens carried.

When hydrophobic propyl chains replace less hydrophobic methyl groups, the effect is perceived mainly by the N(14) and C(12) atoms; their charge is markedly altered; $\Delta \approx 0.35$ e and $\Delta \approx 0.20$ e, respectively, and slightly by C(11); $\Delta \approx 0.12$ e. Regarding the interaction ability of the entire molecules, it should be noted that C(12) is embedded in the molecular volume and that N(14) is entirely delocalized towards the oxygen atom. In fact, O(13) is the unique atom of the amide function which is capable of interacting in a direct manner, *ie*, hydrogen bonding, with an amino acid residue. Let us also note that its charge remains unchanged, between –0.42 and –0.46 e for both analogues in any form or conformation. In other words, if the receptor site can accommodate a more voluminous groups, for example dipropylamide, the ligand affinity would be as strong. Thus, atomic charges at strategic points seem to be constant in the imidazopyridines as also showed by the N(1) charge, –0.64 and –0.63 e for the non-protonated forms. Moreover, according to the pK_a values, 6.2 and 5.6 for Zolpidem [4] and Alpidem (George *et al*, manuscript in preparation), respectively, one can finally suggest that these analogues are on the base form at physiological pH (7.4) when they interact with a common site.

Conclusions

Thanks to precise experimental observations of the 3-D structure of 2 analogues, Alpidem and Zolpidem, we were able to establish a precise identity card describing the structural, conformational, and electronic properties of these imidazopyridine derivatives. X-ray diffraction has revealed a very similar spatial arrangement of the groups within the considered analogues. Variations for dihedral angles between planar moieties do not exceed 9.5°. Crystal packings always include stacking of aromatic regions emphasizing the π–π interaction importance in the molecular recognition process by the ω receptor sites. The application of *ab initio* MO methods to the study of the electronic structure of the imidazopyridine moiety and their substituents provides an explanation of the observed crystal packings and a hypothesis regarding the existence of charge transfer between the imidazopyridine fused rings, considering the HOMO and LUMO topography overlap. Furthermore, conformational freedom is relatively limited to a range of $\pm$ 40° for each rotation around each single bond considered. The conformational analyses confirm the correct choice made in a previous study [18] of an Alpidem-modified conformation for the interaction with peripheral (ω_3) benzodiazepine binding sites. A following paper (Georges *et al*, manuscript in preparation) will show the importance of the amide function orientation for the interaction of the considered molecules with the central ω_1 receptor sites.

Owing to 2 conformations for Alpidem and 2 forms for Zolpidem, conformation, protonation, and substitution effects have been discussed in terms of bond lengths, π overlap percentages, and Mulliken and PDM atomic charges. These observations have allowed the similarities and differences between the considered analogues to be pointed out. Particularly, PDM charges that take into account the overall conformation of the molecules are more differentiated than Mulliken charges and should be considered in intermolecular–electrostatic interactions. So the hydrophobic character of chlorine atoms, methyl and propyl substituents and phenyl groups has been well demonstrated. The PDM charges have been used within 3-D steric and electronic semi-quantitative superimpositions to construct a peripheral interaction model [18]. On the other hand, atoms able to form hydrogen bonds like N(1) and O(13) show constant atomic charge values in any bio-active state of the considered ligands, –0.63 and –0.44 e respectively. N(1) could lead to a direct interaction by hydrogen bonding with the central-type receptor whereas that direct link is accomplished by O(13) with the peripheral binding sites, as noted by several authors [20–22] who have already proposed different recognition models.

Experimental protocols

X-ray measurements and crystallographic data

Alpidem: $C_{21}H_{23}N_3OCl_2$; M = 404.3; crystallization by slow evaporation of toluene at 278 K; crystal system, monoclinic; space group, Cc; $Z = 4$; a = 17.897(3), b = 13.610(3), c =

8.460(1) Å, β = 104.32(1)°; V = 1996.6 Å^3; F(0,0,0) = 848.0; D_c = 1.345 g·cm^{-3}; Enraf–Nonius CAD-4 diffractometer, graphite monochromator, Cu Kα radiation (λ = 1.54178 Å); data collection temperature = 155 K, obtained by a cold gaseous nitrogen flux blowing on the brittle material; crystal lost during the recording of equivalent reflexions allowing no absorption corrections by azimuth scanning or face indexing, μ = 29.2 cm^{-1}; Lorentz and polarization corrections; ω-2θ scan; 2θ range = 4–144°, $-21 \leq h \leq 21$, $-14 \leq k \leq 16$, $0 \leq l \leq 9$; independent reflexions number, 1966, observed $\{I \geq 2.0\ \sigma(I)\}$ reflexions number, 1795.

Structure solution by direct methods using SHELX86 [23]; structure refinement on F by SHELX76 [24]; H(5), H(7), H(8), H(181), H(182), H(23) and H(26) localized in difference-Fourier maps, others calculated; full-matrix least-squares refinement of non hydrogen atom positional and anisotropic thermal parameters; hydrogen atoms fixed with isotropic temperature factors (U_{eq} of the carrier atom incremented by 0.02); $(\Delta/\sigma)_{max}$ in the final refinement step = 0.578 for the x/a value of N(4) atom; $-0.56 \leq \Delta\rho \leq 0.74$ e·Å^{-3}; final R = 0.059; X-RAY program [25] used for geometrical calculations.

Quantum theoretical molecular orbital calculations

Considering the crystalline conformation of such compounds involving several aromatic moieties, a stable conformation is certainly reliable enough. Exploring the conformational space would therefore be limited to allow rotations around the flexible single bonds (fig 1). Such calculations were performed using the semi-empirical quantum mechanical molecular orbital (MO) AM1 method developed by Dewar [26]. The good performance of this method for conformational analysis problems has been largely noted [26] and moreover, consideration of more sophisticated MO methods, such as non empirical approaches, would have been rather time-consuming. Internal coordinates for the heavy atoms were taken from X-ray data. For the hydrogens, only the torsion angles were retained from X-ray analysis; interatomic distances and valence angles were fixed at 1.08 Å and 1.09 Å, and 120° and 109.47°, respectively, depending on the hybridization of the carrier atom. Iso-contour energy maps were obtained by systematic variation of the torsion angles related to the single bonds (increment between 2 calculations: 10°) using the AM1 facilities with the standard parameters as available within GAUSSIAN88 [27].

The molecular orbital topology, *ie*, patterns of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), and the electronic properties, *ie*, π-overlap populations, atomic charges, and molecular electrostatic potential (MEP), have been obtained by non empirical RHF (Restricted Hartree–Fock) LCAO-MO-SCF (linear combination of atomic orbitals – molecular orbitals – self consistent field) calculations using the GAUSSIAN88 program [27]. Considering the relatively large dimensions of the current molecules (> 23 heavy atoms) and their relative composition (only atoms from the first 2 rows), the minimal STO-3G, or STO-3G* when containing chlorine atoms, with standard Slater exponents included in the program [28–30] was chosen as a cost–reliability compromise. Within this basic set, the computations included 136 and 176 contracted basic functions for Zolpidem and Alpidem, respectively. Atomic charges and interatomic overlap populations were calculated by the Mulliken population analysis [31] which has been commonly used for relative bonding studies. The imidazopyridine and the amide function were alternatively placed in a particular plane (for example xz) in order to directly deduce the π-overlap contribution (p_y orbital) with regard to the total one. The atomic charges were also evaluated by least-square fitting of the molecular electrostatic potential (MEP) as proposed by Kollman [32] and Williams [33], *ie*, by using viewpoints outside the molecule to define point charges inside the molecule. With the Connolly algorithm [34, 35], a great number of points in the vicinity of the molecule are generated on 4 envelopes using van der Waals radius of each atom (C, H, N, O, Cl: 1.65, 1.20, 1.55, 1.50, 1.80 Å, respectively) times scale factors from 1.4–2.0 (increment 0.2) and a density of points of 1.5 points Å^{-2} in order to obtain a sufficient points number and MEP values between –30 and +30 kcal·mol^{-1} to describe typical interaction distances and strong and weak interaction energies [36]. Starting from the *ab initio* STO-3G or STO-3G* wave function, MEP values were then calculated at each point using the electrostatic property capabilities implemented into GAUSSIAN88 and atomic point-charges were next least-squares derived by fitting to the expectation values of the envelope MEP values as implemented in the PDM88 program [37].

The generation of the electron charge density 2-dimensional iso-contour maps was performed with the MOPLOT (molecular orbital plot) subprogram [38] available within the MOTECC *(Modern Techniques in Computational Chemistry)* package [39]. This program, starting from the wave function computed with GAUSSIAN88, allows either 3- or 2- (within selected plane) dimensional charge density sampling. Beside the wave function, the input consists of the desired molecular orbitals (all of them, or a particular desired one as the HOMO or LUMO), and the iso-electron charge density surface or contour values.

All semi-empirical AM1 and *ab initio* MO computations were performed on the IBM 9377/90 and FPS 164 and 364 computers system of the Scientific Facility Center at the University of Namur. The generation of a 2-D iso-energy contour map taking into account 1369 conformations with AM1 takes ≈ 17 h for Zolpidem. For the *ab initio* computations, the bielectronic integral cut-off and convergence on the density matrix thresholds were fixed at 10^{-9} atomic units and 10^{-9}, respectively. A single geometry *ab initio* STO-3G (136 contracted basis functions) HF calculation takes ≈ 05 h for the non protonated form of Zolpidem, and the evaluation of the MEP on the Connolly envelopes (2630 points) requires ≈ 11 h. The least-squares fitting of atomic-centered charges takes < 1 minute, whereas a few minutes are required to compute the iso-electron density contour maps. Our experience has showed that within the STO-3G, or STO-3G* basic set the precision is: ≈ 1 kcal·mol^{-1} for total energy, 4 digits for the atomic charges and overlap populations (in electrons), 1–2 kcal·mol^{-1} for electrostatic potential values, ≈ 1 kcal·mol^{-1} for the root mean-square deviation of the MEP fitting, and maximum 0.05 electron for ESD values of the atom-centered point-charges.

Molecular graphics

The stereoscopic view drawings of the molecular conformation and crystal packing were generated by the ORTEP program [40]. The 2-D iso-contour maps, *ie*, iso-energy and iso-electron density maps generated by AM1 and MOPLOT, respectively, were drawn with an in-house device-independent contouring program CPS (contouring plotting system) [41]. ORTEP and CPS have been developed in Fortran with the IBM graPHIGS software [39] and adapted for IBM 5080 workstations and IBM 3179-G terminals. All other figures were drawn using the ChemDraw® software [42].

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