

# Structural and electronic effects of the C2' substituent in 1,4-benzodiazepines

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## ARTICLE INFORMATION

Received: 17 November 2010

Received in revised form: 08 January 2011

Accepted: 11 January 2011

Online: 31 March 2011

## KEYWORDS

Benzodiazepine

DFT

Structure characterization

X-ray

Fluoro

Isoelectronic potential surfaces

## ABSTRACT

Benzodiazepines are drugs used for treatment of several central nervous system disorders, such as anxiety and sleep. In spite of their wide and popular usage in clinics, the mechanism explaining why a certain pharmacological activity is superimposed onto another for a given benzodiazepine remains unclear. The knowledge of the conformation of benzodiazepines and their electronic charge distribution at molecular surfaces may give new insights into the pharmaco-benzodiazepine receptor interactions, contributing to the improvement of the existing models. In the present study, the solid state geometric and conformational parameters of the available X-ray benzodiazepine structures were analyzed and reviewed. The electronic features of two groups of benzodiazepines with different substituents at C<sub>7</sub> and C<sub>2</sub>' positions were studied by DFT quantum chemical calculations. The conformations of the molecules with optimized geometry were also analyzed. The relative charge distribution around the benzodiazepinic rings and electrostatic potential mapped on electronic density surfaces were obtained. The ring geometric parameters for the diazepine moiety in 1,4-benzodiazepines, do not vary significantly except for a few compounds in which steric and/or intermolecular interactions play a part. The benzodiazepine ring assumes a pseudo-symmetrical boat conformation and the torsion angle around the C<sub>5</sub>-Ph bond varies depending on the nature of the substituent on C<sub>2</sub>'. Also, the presence of the nitro or chloride substituent on the C<sub>7</sub> position and the presence of a fluorine atom on the C<sub>2</sub>' position significantly alter the relative charge distributions at the attached carbon atoms and the topology of the surface electrostatic potential.

## 1. Introduction

Benzodiazepines (BZs), a group of drugs widely used to treat anxiety and sleep disorders, are generally represented as in [Figure 1](#). Some of them also show remarkable activity as anticonvulsants and myorelaxants. Anxiety disorders (such as Generalized Anxiety Disorder – GAD, Social Phobia – SP, Social Anxiety Disorder – SAD, Panic Disorder – PD, Obsessive-Compulsive Disorder – OCD, Post-traumatic Stress Disorder – PTSD, and Specific Phobias) are the most common psychiatric diseases with incidence rates of around 16% to 25% [1-3]. In fact, epidemiological studies have shown that anxiety is the second leading cause of depression resulting in a large amount of work absenteeism and reduced productivity [2]. Despite their robust anxiolytic effect, most of the clinical trials with BZs were conducted before the current classification for the anxiety disease disorders came about. As a result, knowledge of their effectiveness in anxiety disorders may be incomplete and discrete. In clinical practice, BZs are used in generalized anxiety disorder with diazepam (**1.a**) as the most popular choice [1-2]. Response rates to therapy are high and the beginning of the therapeutic effect is immediate. Alprazolam and clonazepam (**2.b**) are the only BZs approved for the treatment of SP [4-6], although other high-potency BZs, such as lorazepam, also show

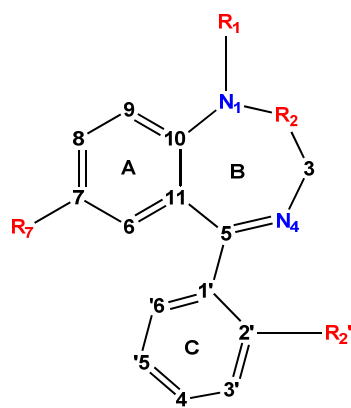
similar effects. There is little evidence of efficacy of BZs in OCD or PTSD and no BZ was approved specifically for these diseases [1].

Anxiety disorders may affect the quality of sleep. Thus, drugs such as BZs are also widely used to treat insomnia as hypnotics. In fact, they represent nowadays the most important group of drugs involved in the treatment of anxiety and sleep disorders [7]. BZs can also be used as anti-convulsive and myorelaxants. All the BZs exhibit similar pharmacological profiles but differ from each other in their selectivity and thus, may be used for various therapeutic purposes: this arises from the fact that they act as selective depressant on the central nervous system since they facilitate and increase the GABAergic transmission in all structures of the Central Nervous System (CNS). Also, their anxiolytic or hypnotic properties are difficult to differentiate since all BZs are anxiolytic and may act by modifying sleep, provided they achieve certain doses. This overlap of the pharmacological activity makes it difficult to distinguish between true sedatives and anxiolytics, and classification of BZs as anxiolytic or sedative agents is deeply based on pharmacokinetic considerations related to the half-life ( $t_{1/2}$ ) of these drugs [1].

Anxiolytic substances and BZs currently on the market have been developed as a result of serendipity allied to a

rational molecular design. Molecular design, whose main objective is the discovery of new active molecules, is based on provisional data concerning physical-chemical characterization, pharmacokinetics, pharmacodynamics, and studies of the structure/activity, even before synthesis of a compound in the laboratory. To facilitate this molecular design process, knowledge of the structural features and physical chemical characteristics of actual active drugs are essential. Also, the knowledge of the electronic and structural features of BZs may help on the understanding the affinity of BZs to specific subtypes of GABA<sub>A</sub> receptors [8-12], thus helping on the systematization of their main therapeutic activities.

This work presents a review of the structural characteristics of BZs. The conformational analysis of the molecules is based on structures of 1,4-benzodiazepines obtained by single crystal X-ray diffractometry that are available from the Cambridge Structural Database, CSD [13]. Knowledge of the conformation of these drugs is important since former studies [14] show that BZs induce changes in conformation in the  $\alpha_1$  subunit of GABA<sub>A</sub> receptors. In addition electronic characterization has been carried out for two sets of BZs that were grouped on the basis of their C<sub>7</sub> position substitution and C<sub>2'</sub> (refer to Figure 1), using quantum chemical calculations. By doing this, inferences can be made about charge density distribution in the molecule and the mapping of polar moments within the molecule. Although this work deals with the influence of substituents on the C<sub>7</sub> and C<sub>2'</sub> positions on the electronic distributions, the compounds chosen are limited to those with actual pharmacological action. The conformational and electronic aspects are the main requirements for BZs-receptor interactions and knowledge of these can contribute to the improvement of the available proposed models for the BZs-GABA<sub>A</sub> receptor interaction.



	R <sub>1</sub>	R <sub>2</sub>	R <sub>7</sub>	R <sub>2'</sub>
1.a	-Me	=O	-Cl	-H
1.b	-N(Et) <sub>3</sub>	=O	-Cl	-F
1.c	-H	=O	-Cl	-F
1.d	-OH	=O	-Cl	-F
2.a	-H	=O	-NO <sub>2</sub>	-H
2.b	-H	=O	-NO <sub>2</sub>	-Cl
2.c	-Me	=O	-NO <sub>2</sub>	-F
2.d	-H	=O	-NO <sub>2</sub>	-F
2.e	-N(Et) <sub>3</sub>	=O	-NO <sub>2</sub>	-F
2.f	-Et	-OH	-NO <sub>2</sub>	-F

Figure 1. Molecular scaffold of a 1,4-dibenzazepine, with the numbering of atoms and identification of the rings. Compounds studied on this work are:

## 2. Experimental

### 2.1. Structural analysis

A search of the CSD [13] was performed using the 5-phenyl-1,4-benzodiazepin-2-one moiety as a search fragment produced 68 organic structures with R-factors of 10% or less for which atomic co-ordinates were supplied. Polymeric and

powder diffraction structures were excluded from the search as were duplicate structures. Tables containing the CSD reference codes for the compounds are supplied as supplementary data.

### 2.2. Computational chemistry

The quantum chemical calculations were performed for 10 BZs derivatives (shown in Table 1) with the program Gaussian 03 [15]. Geometrical optimizations were carried at B3LYP/6-311G++ (d,p) level of theory. B3LYP refers to the combination of functional hybrid exchange of Becke [16] with the functional correlation gradient of Lee & Yang [17]. The notation 6-311G++ (d,p) refers to a set of split valence polarized bases [18]. Since all the benzodiazepines with pharmacological interest have a boat conformation for the diazepine ring in the solid state and all the models concerning the interaction with receptors are based on the boat conformation, this was assumed as the starting structure for optimization.

For the same level of theory, vibrational frequencies for all compounds were calculated to verify that the structure converged to a minimum using the zero imaginary frequencies criterion. Molecular orbitals, as well as the charges assigned to each atom, were made by Natural Population Analysis Phase (NPA) of the Natural Bond Orbital analysis (NBO) according to Carpenter [19]. Molecular representation and ESP mapped surfaces were made with the program GaussView 3.0 [20].

## 3. Results and discussion

### 3.1. Structures of BZs

The bond lengths in all 85 molecules for the 68 BZ structures, in some structures the numbers of molecules in the asymmetric unit is greater than 1, are in agreement with the canonical structure in Figure 1. Tables of bond lengths and angles and their statistical analysis are given as supplementary data. In all structures the diazepine ring adopts a boat conformation with a pseudo-reflection plane that cuts medially the C<sub>7</sub>-C<sub>8</sub> and C<sub>11</sub>-C<sub>10</sub> bonds of the diazepine and passes through the C<sub>3</sub> atom which has sp<sub>3</sub> hybridization (Figure 2).

From the sixty eight structures analyzed, 9 of them had a -Br atom as substituent on the C<sub>7</sub> position, 4 of them were -NO<sub>2</sub> substituted and 8 substituted with -H. The remainder had -Cl as a substituent on the C<sub>7</sub> position. In the case of atom C<sub>2'</sub>, 16 of the BZs had -Cl as a substituent, 2 of them -F and the remainder an -H atom. Torsion angles *T* around the C<sub>5</sub>-Ph bond (Figure 3) show a variation within the [18-47°] interval range with mean value of 32° for compounds with C<sub>2'</sub>-H bond. Benzodiazepines with a chlorine atom on the C<sub>2'</sub> position exhibit a significant higher torsion angle lying within the [51-76°] range with mean of 63°. The only two structures with a C<sub>2'</sub>-F substituent have a torsion angle of 43°.

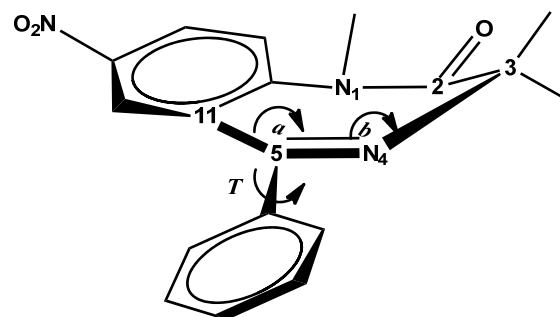
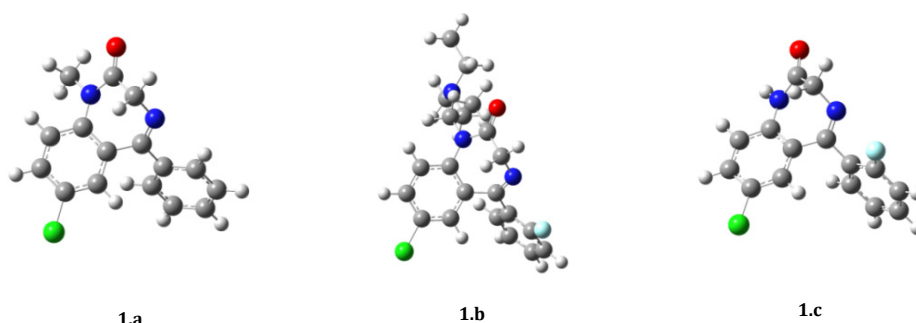


Figure 2. Schematic representation of the "boat" conformation for benzodiazepines. The parameters adopted for the conformational analysis are *a* (the C<sub>10</sub>-C<sub>5</sub>-N<sub>4</sub> angle); *b* (the C<sub>5</sub>-N<sub>4</sub>-C<sub>3</sub> angle) and *T* (torsion angle around bond C<sub>5</sub>-Ph).

**Table 1.** Common name, systematic name and adopted notation for benzodiazepines belonging to Group 1 and Group 2.

Group	Common name	Systematic name (International)	Adopted notation
1	Diazepam <b>1.a</b>	( <i>RS</i> )-7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2( <i>2H</i> )-one.	7-Cl-1-Me-2'H-BZ
	Flurazepam <b>1.b</b>	( <i>RS</i> )-7-chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one	7-Cl-1-N(Et) <sub>3</sub> -2'F-BZ
	Desalkylflurazepam <b>1.c</b>	( <i>RS</i> )-7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one	7-Cl-2'F-BZ
	Hydroxyethylflurazepam <b>1.d</b>	( <i>RS</i> )-7-chloro-1-[2-(hydroxy)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one	7-Cl-1-OH-2'F-BZ
2	Nitrazepam <b>2.a</b>	( <i>RS</i> )-9-nitro- 6-phenyl- 2,5-diazabicyclo [5.4.0] undeca-5,8,10,12- tetraen- 3-one	7-NO <sub>2</sub> -2'H-BZ
	Clonazepam <b>2.b</b>	( <i>RS</i> )- 5-(2-chlorophenyl)-7-nitro-2,3-dihydro-1,4-benzodiazepin-2( <i>2H</i> )-one	7-NO <sub>2</sub> -2'Cl-BZ
	Flunitrazepam <b>2.c</b>	6-(2-fluorophenyl)- 2-methyl- 9-nitro- 2,5-diazabicyclo [5.4.0] undeca- 5,8,10,12- tetraen- 3-one	7-NO <sub>2</sub> -1Me-2'F-BZ
	Desmethylflunitrazepam <b>2.d</b>	6-(2-fluorophenyl)- 9-nitro- 2,5-diazabicyclo [5.4.0] undeca- 5,8,10,12- tetraen- 3-one	7-NO <sub>2</sub> -2'F-BZ
	Nitroflurazepam <b>2.e</b>	( <i>RS</i> )-7-nitro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one	7-NO <sub>2</sub> -1-N(Et) <sub>3</sub> -2'F-BZ
	Desalkylnitraflurazepam <b>2.f</b>	( <i>RS</i> )-7-nitro-1-[2-ethyl]-5-(2-fluorophenyl)-1,3-dihydro-3hydroxy-2H-1,4-benzodiazepin-2-one	7-NO <sub>2</sub> -1-Et-3OH-2'F-BZ

**Figure 3.** Representation of the **1.a**, **1.b** and **1.c** obtained from gas phase geometry optimization carry out at B3LYP/6-311G++ (d, p) level of theory.

When there is an H atom on N<sub>1</sub> these compounds form N<sub>1</sub>...O<sub>2</sub> dimers. In the presence of competitive donors or acceptors, this is rarely the case. In other cases, when there are hydrogen donors or acceptors in substituents or in solvent molecules, the hydrogen bonding can become more complicated. This may explain, in part, the divergence of bonds and angles from the norm for some of the compounds.

### 3.2. Geometric optimizations

Geometry optimizations (in gaseous state) were made using the Gaussian 03 program [15], which performs the optimization of the geometry of the molecule by Density Functional Theory (DFT) methods. The geometry is optimized as a criterion of the minimization of energy of the molecule, calculated by quantum chemical methods.

In order to evaluate the structural and electronic effects of -NO<sub>2</sub> and -Cl substituents at the C<sub>7</sub> position and the effect of -H, -Cl, and -F substituents at the C<sub>2'</sub> positions in BZs, the compounds were grouped into two sets as shown in Table 1: *i*) the first consists of the compounds with a C<sub>7</sub>-Cl substitution, the reference compound used being diazepam, *ii*) the second consists of compounds with a C<sub>7</sub>-NO<sub>2</sub> substitution, the reference compound being nitrazepam. The first group consists of diazepam **1.a**, flurazepam **1.b**, and the desalkylflurazepam **1.c**, hydroxyethylflurazepam **1.d**, (last two being the active

metabolites of flurazepam). The second group consists of nitrazepam **2.a**, clonazepam **2.b**, flunitrazepam **2.c** and its active metabolite desmethylflunitrazepam **2.d** as well as nitroflurazepam **2.e** and desalkylnitraflurazepam **2.f** (the latter two compounds are not used as commercial BZs).

Since the common names for benzodiazepines do not give any information about the nature of substitutions on the benzodiazepine ring, the discussion of the results will be made based on the notation shown in Table 1.

The optimized structures belonging to both groups, obtained for minimization of the total electronic energies, and their atom coordinates are supplied as supplementary information.

The optimized geometry for 7-Cl-1-Me-2'H-BZ (**1.a**), for 7-Cl-1-N(Et)<sub>3</sub>-2'F-BZ (**1.b**), and for 7-Cl-1-2'F-BZ (**1.c**) are given as examples in Figure 3. The geometries do not differ significantly, except for the conformation of the aromatic ring at C<sub>5</sub>. The values obtained for the lengths of bonds of the same nature do not differ significantly within the structures studied. In the aromatic ring, the BZ C<sub>ar</sub> bond lengths are typical of those found in aromatic rings. The imine and secondary amide bond distances of the diazepine ring are also within the normal range. Bond distances and angular values are also comparable to those obtained experimentally by X-ray structural analysis, although being slightly and systematically increased. This feature that can be attributed to the fact that

**Table 2.** Values obtained for *a*, *b* and *T* angles (refer to Figure 3), obtained at B3LYP/6-311G++ (d, p) level of theory as well as their dipole moments (Debye) in gas phase.

Compound		<i>a</i> (°)	<i>b</i> (°)	<i>T</i> (°)	<i>D</i> (D)	
G1	Diazepam <b>1.a</b>	7-Cl-1-Me-2'H-BZ	123.4(7)	118.7(7)	31(1)	2.30
	Flurazepam <b>1.b</b>	7-Cl-1-N(Et) <sub>3</sub> -2'F-BZ	124.3(7)	118.5(7)	49(1)	4.14
	Desalkylflurazepam <b>1.c</b>	7-Cl-2'F-BZ	136.8(7)	119.6(7)	50(1)	4.21
	Hydroxyethylflurazepam <b>1.d</b>	7-Cl-1-OH-2'F-BZ	124.1(7)	118.5(7)	46(1)	3.74
G2	Nitrozapam <b>2.a</b>	7-NO <sub>2</sub> -2'H-BZ	124.5(7)	119.7(7)	33(1)	1.35
	Clonazepam <b>2.b</b>	7-NO <sub>2</sub> -2'Cl-BZ	126.1(7)	119.7(7)	62(1)	2.47
	Flunitrazepam <b>2.c</b>	7-NO <sub>2</sub> -1Me-2'F-BZ	124.4(7)	118.5(7)	48(1)	3.20
	Desmethylflunitrazepam <b>2.d</b>	7-NO <sub>2</sub> -2'F-BZ	126.7(7)	119.6(7)	50(1)	2.35
	Nitroflurazepam <b>2.e</b>	7-NO <sub>2</sub> -1-N(Et) <sub>3</sub> -2'F-BZ	124.2(7)	118.2(7)	47(1)	3.95
	Desalkylnitroflurazepam <b>2.f</b>	7-NO <sub>2</sub> -1-Et-3OH-2'F-BZ	125.7(7)	119.5(7)	50(1)	2.74

**Table 3.** Charge distributions (e<sup>-</sup>) calculated by NPO at B3LYP/6-311++G(d,p) level of the BZs gas phase.

Compound		C7	C11	N1	C=O	N4	C5	C2'
G1	7-Cl-1-Me-2'H-BZ <b>1.a</b>	-0.066	+0.179	-0.491	-0.601	-0.428	+0.269	-0.195
	7-Cl-1-N(Et) <sub>3</sub> -2'F-BZ <b>1.b</b>	-0.063	+0.180	-0.495	-0.607	-0.398	+0.262	+0.464
	7-Cl-2'F-BZ <b>1.c</b>	-0.069	+0.168	-0.654	-0.590	-0.405	+0.266	+0.444
	7-Cl-1-OH-2'F-BZ <b>1.d</b>	-0.062	+0.178	-0.496	-0.604	-0.399	+0.263	+0.446
G2	7-NO <sub>2</sub> -2'H-BZ <b>2.a</b>	+0.045	+0.199	-0.650	-0.578	-0.430	+0.272	-0.194
	7-NO <sub>2</sub> -2'Cl-BZ <b>2.b</b>	+0.046	+0.201	-0.649	-0.577	-0.402	+0.275	-0.026
	7-NO <sub>2</sub> -1Me-2'F-BZ <b>2.c</b>	+0.048	+0.212	-0.486	+0.586	-0.398	+0.266	+0.447
	7-NO <sub>2</sub> -2'F-BZ <b>2.d</b>	+0.045	+0.200	-0.649	+0.576	-0.403	+0.268	+0.446
	7-NO <sub>2</sub> -1-N(Et) <sub>3</sub> -2'F-BZ <b>2.e</b>	+0.044	+0.213	-0.491	-0.590	-0.396	+0.264	+0.448
	7-NO <sub>2</sub> -1-Et-3OH-2'F-BZ <b>2.f</b>	+0.043	+0.234	+0.481	-0.589	-0.444	+0.292	+0.446

optimization in gaseous phase does not take the effects of intermolecular interactions due the crystal packing into account.

### 3.2.1. Conformation of the DFT optimized structures

The benzodiazepines in this study show the typical conformation for the diazepine ring. This conformation does not change significantly with the type of substituents. As mentioned above, Section 3.1, the diazepine ring adopts a boat conformation with a pseudo-reflection plane that cuts medially the C<sub>7</sub>-C<sub>8</sub> and C<sub>11</sub>-C<sub>10</sub> bonds of the diazepine and passes through the C<sub>3</sub> atom having the sp<sup>3</sup> hybridization (Figure 2). The valence angles involving the N<sub>4</sub> atom are close to 120°, the overall geometry of its neighborhood is flat and the bond length is typical of a double C=N bond. On the opposite side of the molecule, the carbonyl carbon atom of the set N1-(C=O) similarly has a flat triangular geometry. The conformation can be characterized by three angles, the angle *a* formed between the C<sub>11</sub>-C<sub>5</sub>-N<sub>4</sub> atoms, the angle *b* between the C<sub>5</sub>-N<sub>4</sub>-C<sub>3</sub> atoms, and the torsion angle *T* defined as the angle N<sub>4</sub>-C<sub>5</sub>-C<sub>1</sub>-C<sub>2</sub>. The general conformation adopted is represented in Figure 2 where 7-NO<sub>2</sub>-2'H-BZ, **2.a**, was chosen as an example. Table 2 summarizes the geometrical parameters *a*, *b*, and *T* for each compound. The angle *b* does not vary with the type of compound neither does angle *a* with the exception of 7-Cl-2'F-BZ, **1.c**, whose angle *a* of 136.6 (8)° is higher than that of the remaining BZs. On the other hand, the angle between the C<sub>5</sub> phenyl ring plane and the aromatic benzodiazepine residue plane varies from compound to compound. This variation is quantified by the torsion angle *T*, around C<sub>5</sub>-Ph bond (Figure 3). An analysis of this value suggests that it is dependent on the nature of the substituent present on the atom C2' of the phenyl ring.

When the substituent is a hydrogen atom, as is the case of 7-Cl-1-Me-2'H-BZ, **1.a**, and 7-NO<sub>2</sub>-2'H-BZ, **2.a**, the torsion angle is about 30°. When the substituent is a fluorine atom, this angle increases to lie in the 46–50° range. BZ 7-NO<sub>2</sub>-2'Cl-BZ, **2.b**, presents the highest value (60°) of twist angle around the C<sub>5</sub>-Ph bond. These variations, which probably reflect the electrostatic repulsions that exist between the electronic clouds of halogens and the unoccupied orbital of the imine nitrogen atom, are also observed in the set of crystalline structures that were analyzed. It is worth noting that the modification of the conformation of the molecule may affect the extent of its

linking to a pharmacologically active site, therefore affecting the pharmacological activity of the drug.

### 3.3. Polarity and charge distributions

The dipole moments obtained for the studied compounds are given in Table 2. It appears that the compounds containing a chlorine atom in position C<sub>7</sub> have a higher dipole moment than those with a nitro group in the same position. Also, the existence of a fluorine atom at position C<sub>2</sub>' and the introduction of a polar substituent at the nitrogen atom N<sub>1</sub> increase the polarity of the BZ.

In addition to the dipole moment, it is also of interest to analyze the relative charge distribution among the atoms of the molecule. These relative distributions may indicate preferential binding sites on the hydrophilic or lipophilic regions of the receptor. Moreover, they can determine sites of the receptor regions at which polarity could be more easily induced.

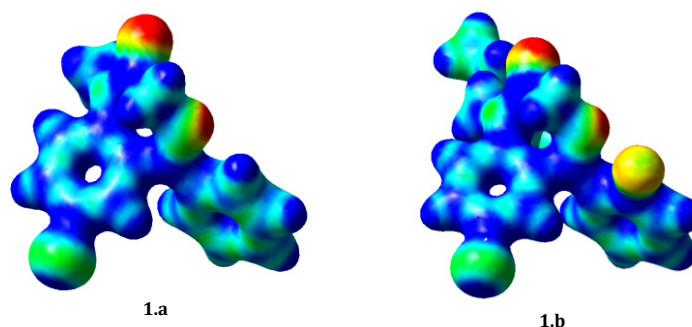
The atomic charges were calculated by Natural Population Analysis (NPA) [19] using the same level of theory as for geometrical optimizations. The values obtained for selected atoms in compounds **1.a-e** and **2.a-f** are summarized in Table 3. Since such charge distributions were calculated for the gas phase geometries care must be taken in the analysis of their absolute values. However, the resultant simplifications introduced by the model do not preclude the validity of the discussion based on relative comparisons.

#### 3.3.1. Charge distribution at N<sub>1</sub>

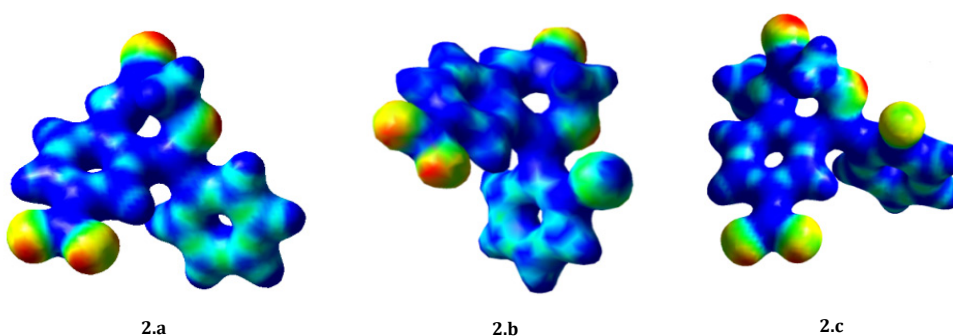
The most negative atomic areas on the molecule become more sensitive to acidic coordination. The negative charge distributions are often due to inductive effects of substituents such as alkyl chains or other sigma electronic density donors. This seems to be the case for the nitrogen atom N<sub>1</sub> where the presence of an alkyl substituent significantly alters the charge distribution in the atom, decreasing its polarity thereby making a molecule with lower donor ability in this region.

#### 3.3.2. Charge distribution at C<sub>7</sub>

The variation of the charge distribution at carbon atom C<sub>7</sub> shows that the electron density withdrawing effect of nitro group is superior to chlorine. Thus, the charges on C<sub>7</sub> for compounds belonging to the G1 group are slightly negative or



**Figure 4.** The electrostatic potential (ESP), mapped on an electron density surface (that was given by SCF) for **1.a** (7-Cl-1-Me-2'H-BZ) and for **1.b** (7-Cl-1-N (Et) 3-2'F-BZ). The positively charged regions are blue and green while regions with higher electron density are mapped from red to yellow. The isovalue taken was  $0.04 \text{ e}/\text{\AA}^3$ .



**Figure 5.** The electrostatic potential (ESP), mapped on an electron density surface (that was given by SCF) for **2.a** (7-Cl-1-Me-2'H-BZ), for **2.b** 7-NO<sub>2</sub>-2'Cl-BZ and **2.c**, 7-NO<sub>2</sub>-2'F-BZ. The positively charged regions are blue and green while regions with higher electron density are mapped from red to yellow. The isovalue taken was  $0.04 \text{ e}/\text{\AA}^3$ .

very close to neutrality. However, C<sub>7</sub> in G2 group compounds is slightly electropositive due to the negative inductive effect of the nitro group, since the *sp*<sup>2</sup> hybridization of this group allows the flow of the electron cloud to the oxygen atoms.

### 3.3.3. Charge distribution at C<sub>2</sub>'

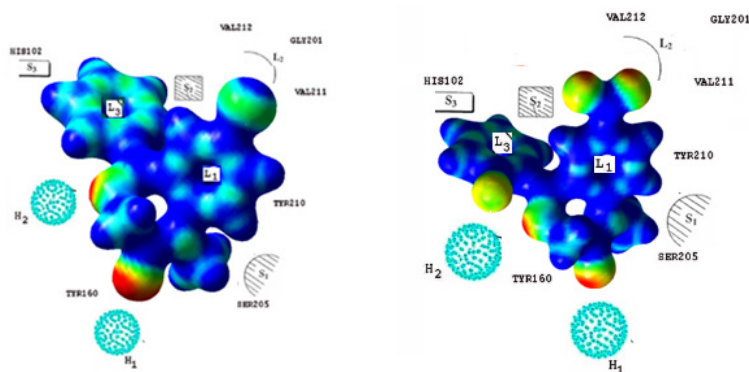
The effect of variation in charge on the C<sub>2</sub>' carbon atom seems to be more pronounced for the C–F bond as compared with C–H and C–Cl bonds. In fact, by comparing the atomic charges at carbon atom C<sub>2</sub>' in the presence and absence of the fluorine atom, it appears that the C–F bond reverses the polarity of the carbon atom C<sub>2</sub>' making it quite electropositive. The replacement of a hydrogen atom of a hydrocarbon by a fluorine atom significantly influences the physicochemical properties of this compound. The fluorine atom is more electronegative than the hydrogen atom and its atomic volume is also much higher: the van der Waals radius for hydrogen atom is 1.20 Å, while for fluoride is 1.47 Å. The high electronegativity of fluorine atom and the similarity of its orbital, in terms of size and energy on the carbon atom, allows for a very strong bond. Thus, the carbon–fluorine bond is the highest energy at which a carbon atom can participate [21,22]. The difference in electronegativity between the carbon and fluorine atom generates a large dipole moment which, if combined with the electrostatic distribution of a specific molecule, may contribute to the ability of the molecule to participate in intermolecular interactions. This is particularly true in aromatic systems, examples of which are the BZs, where the introduction of a fluorine atom modifies the distribution of electrostatic surface of the molecule and may also induce new potential binding sites on the molecule in question located close of fluorine atoms.

### 3.4. Isoelectronic potential surfaces

Concerning ligand-receptor binding (drug-receptor) not only the polarity of the molecule or its electronic charge distribution should be envisaged. The approach of the drug to the receptor for linking is, possibly, carried out by a process that involves the presence of a cavity in the active site, the establishment of intermolecular connections (that can be of electrostatic nature or hydrophobic/hydrophilic interactions), and a possible rearrangement of the active site due to the above mentioned interactions. Since this is a dynamic process, the charge distribution on the molecular surface could probably play an important role in the establishing of the drug-receptor interaction. Molecules can exhibit different molecular electronic charge distributions depending on the radial distance to the atoms nuclei. Thus, a group that is "a strong attractor of electron density" can localize the negative charge over a distance closer to the core than a weaker attractor of that density. Consequently, the effect of the less electron attractor group density on the approach to a receptor can prevail over the former.

The electrostatic potential surfaces obtained for the representative compounds are depicted in Figure 4 and 5.

The large red region around the carbonyl oxygen is indicative of the existence of higher negative electrostatic potential indicating favorable interaction energy with electronic density acceptors. It should be noted that, due to differences in electronegativity between carbon and fluorine atom, the C<sub>2</sub>' carbon atoms assume a "partial positive charge" that contrasts with the partial negative charge distribution on compounds that do not have fluorinated substitution at that site.



**Figure 6.** The electrostatic potential (ESP), mapped on an electron density surface (that was given by SCF) for **2.a** (7-Cl-1-Me-2'H-BZ) and for **2.c**, 7-NO<sub>2</sub>-2'F-BZ combined with the receptor locals of interaction of the  $\alpha_1\beta_2\gamma_2$  GABA<sub>A</sub> subtype as proposed by Clayton and co-workers [23]. The model presents two areas with two electronic acceptor interactions (H1 and H2) three lipophilic interaction regions (L1, L2, L3) as well as regions of negative steric repulsion (S1, S2 and S3).

In recent years, a unified model of receptor/pharmacophore for agonists, antagonists, and inverse agonists of BZs site on GABA<sub>A</sub> receptor has been developed. This can be achieved by the combination of several techniques such as synthesis of drugs with radioisotopic markers and techniques for mapping the receptor. Clayton *et al.* [23] in 2007 proposed a base model for the binding receptor/ pharmacophore at the  $\alpha_1\beta_2\gamma_2$  GABA<sub>A</sub> receptor subtype that is depicted in Figure 6. According to the author the model consists of two domains with acceptor (H1 and H2). In addition to these areas of connection, there are three regions of lipophilic interaction (L1, L2 and L3) as well as regions of negative steric repulsion (S1, S2 and S3). In the same figure the receptor/ pharmacophore model is overlapped with the benzodiazepines studied in this work shown as ESP mapped density surfaces. As mentioned before, the main structural differences between the BZs presented in this study concerns C<sub>7</sub> (either -NO<sub>2</sub> or -Cl) and C<sub>2</sub> substituents (which may be -H, -Cl or -F).

The substituent at C<sub>7</sub> position interacts with a lipophilic region of the receptor by the proximity of residues of valine and glycine. The -Cl substituent is reasonably lipophilic and more lipophilic than -NO<sub>2</sub> (judging by the  $\pi$  (aromatic) hydrophobic descriptor which is 0.71 for -Cl and -0.28 for -NO<sub>2</sub>), the latter being more hydrophilic [24]. This suggests that benzodiazepines belonging to G1 present a more favorable interaction at this site than those from G2.

The electron acceptor interaction in the H<sub>2</sub> region is well illustrated by the quantitative study made here. As shown in Figure 6, the presence of the highly polarized C2'-F aromatic residue may provide another binding to H<sub>2</sub> local besides the unpaired electrons of imine N<sub>4</sub>. In fact, monosubstitution of fluorine in aromatic groups in drugs can lead to increased protein binding. Razgulín and Mecozzi, have highlighted, through quantitative computational chemistry calculations, the role of carbon bonding to the aromatic substituent fluorine and its influence on intermolecular interactions. In their study, a series of representative compounds with medical interest were used in which several aromatic C-H bonds were replaced by their fluoro aromatic like compounds. These studies demonstrate that the binding of aromatic carbon to fluorine can participate in the formation of hydrogen bonds and can also interact strongly with positively charged molecules [22].

#### 4. Conclusion

The analysis of the results obtained in this work lead to the conclusion that, of the set of compounds studied, **1.a** (and their active metabolites **1.c** and **1.d**) are the BZs with the best electronic characteristics for interaction with the receptor presented here. Since the model refers to the  $\alpha_1\beta_2\gamma_2$  sub-type

whose main effects are sedation, ataxia and anterograde amnesia [11,12] drugs belonging to G1, with exception of **1.a**, presents more pronounced effects on sedation [9] and side-effects as hypnotic, anxiolytic and myorelaxants. In fact, with regard to their primary pharmacological action diazepam **1.a** has a markedly anxiolytic action, being a full agonist of BZs receptors, while flurazepam **1.b** has a hypnotic action [25,26]. In the group of 7-NO<sub>2</sub>-benzodiazepines the pharmacological effects are more dispersed: nitrazepam acts as the anti-convulsant, clonazepam as antiepileptic [27] and is used as an aid on treatment of depressive disorders [28]. Nevertheless compounds with C<sub>2</sub>-F substitution such as flurazepam [26] **1.b** and flunitrazepam [29] **2.b** are mainly hypnotic-sedatives. The compounds with -NO<sub>2</sub> groups on C<sub>7</sub> and -Cl at C<sub>2</sub> present anticonvulsant activities and may have more affinity for another subtype receptor, such as the  $\alpha_3\beta_2\gamma_2$  receptor [8,10,30]. This study contributes, with relevant data, to the systematic three-dimensional quantitative relations between molecular properties and their biological activity (3D-QSAR relationships) and aids the understanding of structural mechanisms of benzodiazepine modulation at the GABA receptors [31].

#### Acknowledgement

Thanks are due to Fundação para o Ensino e Cultura Fernando Pessoa for financial support.

#### Supplementary material

Diagrams of the optimized structures, their atomic coordinates, tables of bond lengths and angles for the structures of benzodiazepines and their references are given in supporting information.

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