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Experimental and density functional theory investigation of some biomedical compounds

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Spectroscopic methods (FT-IR, Raman, NMR EPR) and quantum chemical calculations based on density functional theory (DFT) were used for structural characterization of some biomedical compounds with antibiotic, cardiovascular and antimycobacterial activity. The investigated compounds are pyrazinamide (PZA), 5-para-bromo-benziliden-thiazolidin-2-thion-4-one (5pBBTT), atenolol (ATE), metoprolol (MET), pindolol (PIN) and verapamil (VER). The optimized structures of the two possible conformers and the dimer of PZA have been obtained at B3LYP/6-31G(d) level of theory and compared to X-ray data. The experimental vibrational bands (FT-IR and Raman) of PZA were confident assigned on the basis of calculations performed at the same level of theory. Moreover, theoretical and experimental NMR data suggest that only one of the two protons of NH_2 group is involved in intermolecular hydrogen bonds. All the possible conformers and tautomers of 5pBBTT have been investigated by DFT methods and their relative stability is discussed. The very good correlation between the experimental and B3LYP/6-31G(d) theoretical wave-numbers of 5pBBTT allow us to validate the calculated structure of this compound. Both, experimental and theoretical data regarding the NMR spectrum of this molecule suggest the coexistence of both thionic and thiolic tautomers in liquid phase. Powder EPR spectra of ATE, MET, PIN and VER Cu(II) complexes exhibit the absorption signals typical of randomly oriented singlet state (S=1/2) species with axial symmetry. The ground state for paramagnetic electron is $d_{x^2-y^2}$ orbital for ATE, MET and VER compounds and d_{z^2} orbital for PIN compound. Comparing the shape of these EPR spectra with those obtained for other copper complexes with nitrogen and oxygen ligands we have concluded that the local symmetry around metal ions is of square planar type with a CuN_2O_2 chromophore in the xOy plane.

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1. Introduction

Pyrazinamide (PZA) is a member of the Pyrazine family and it is known as a very effective antimycobacterial agent, with a well established role in tuberculosis treatment [1]. Molecular complexes having PZA (Fig. 1) as ligand are shown to have enhancement antimycobacterial properties [2].

Previous works are reported in the scientific literature, having as subject either the vibrational properties of PZA and its molecular complexes [2,3] or the magnetic properties of this molecule [4,5]. However, no theoretical work has been reported till now, having as subject the vibrational structure of PZA or its proton NMR spectrum.

The 5-para-bromo-benziliden-tiazolidin-2-tion-4-ona (5pBBTT) molecule was shown to be a very efficient antibiotic that has a superior activity to ampicilin on beta-hemolytic Streptococcus [6].

For a proper understanding of vibrational spectra of PZA and 5pBBTT, a reliable assignment of the experimental IR and Raman bands is essential. In this purpose, Density Functional Theory (DFT) approaches, esspecially those using hybrid functionals, have evolved to a powerful and very reliable tool, being now routinely used for the determination of the electronic structure and various properties of the molecules.

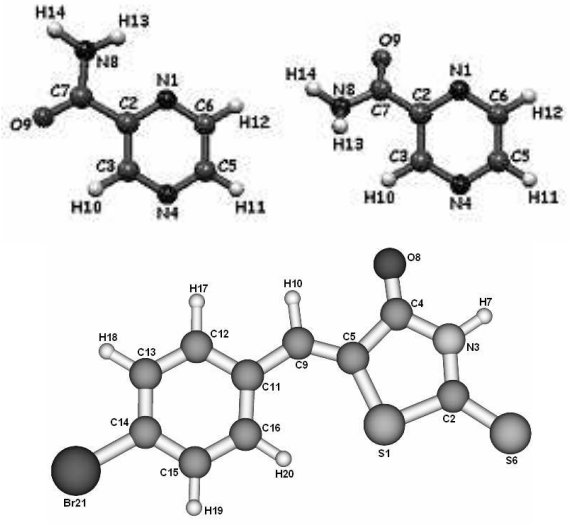

Fig.1. Molecular structure and atom numbering scheme for the two conformers of PZA (top) and 5pBBTT (bottom).

In the framework of DFT approach the B3LYP hybrid functional [7,8] is one of the most preferred since it proved its ability in reproducing various molecular properties, including vibrational and NMR spectra. The combined use of B3LYP functional and standard split valence basis set 6-31G(d) has been previously shown to provide an excellent compromise between accuracy and computational efficiency of vibrational spectra for large and medium-size molecules [9-12].

Since the computed vibrational frequencies are typically larger than their experimental counterparts, empirical scaling factors are used in order to obtain a better agreement between experiment and theory. However, the Becke's exchange (B) functional have the advantage of standard frequency scaling factor very close to unity so that the B-based procedures can often be used without scaling [13-15]. For this reason we calculated the vibrational spectrum of PZA both by B3LYP and BLYP methods.

2. Computational details

The molecular geometry optimizations and vibrational frequencies calculations were performed with the Gaussian 98W software package [14] by using DFT methods with B3LYP and BLYP functionals, which have been previously shown to perform very well for vibrational spectra calculations [15]. The basis sets used in these calculations are: 6-31G(d) and cc-pVDZ for PZA, and 6-31G(d), 6-31+G(d,p) and 6-311+G(2df,p) for 5pBBTT. The geometries were fully optimized without any constraint with the help of analytical gradient procedure implemented within Gaussian 98W program [16]. The calculation of NMR spectrum of PZA and PZA dimer were performed using the GIAO (Gauge-Including Atomic Orbitals) method [17,18], implemented in the Gaussian package, with the B3LYP exchange-correlation functional, in conjunction with 6-31G(d) and cc-pVDZ basis sets. In order to express the chemical shifts in ppm, the geometry of the tetramethysilane (TMS) molecule has been optimized and then its NMR spectrum was calculated by using the same method and basis set as for the calculation on PZA molecule.

The contraction schemes for cc-pVDZ and 6-31G(d) basis sets are [19] (9s,4p,1d/4s,1p) → [3s,2p,1d/2s,1p] and (10s,4p,1d/4s) → [3s,2p,1d/2s], respectively. The former basis set includes by definition polarization functions on hydrogen atoms, while the latter includes polarization functions only for heavy atoms (C, N and O atoms). Moreover, for cc-pVDZ, the core 1s and 2s orbitals for heavy atoms are described by contractions consisting of 8 GTO. The corresponding number of basis functions for monomer and dimer are 151 and 302 for cc-pVDZ basis set and 145 and 272 for 6-31G(d).

3. Experimental

The experimental techniques used for the studied molecules are: FT-IR/ATR, FT-RAMAN, NMR and EPR. Powder FT-IR/ATR spectra for each molecule were recorded at room temperature on a conventional Equinox 55 FT-IR spectrometer equipped with an InGaAs detector, coupled with a Bruker Miracle ATR sampling device. The FT-Raman spectra were recorded in a backscattering geometry with a Bruker FRA 106/S Raman accessory attached to the FT-IR spectrometer. The 1064 nm Nd:YAg laser was used as excitation source, and the laser power was set to 400 mW. All spectra were recorded with a resolution of 4 cm^{-1} by co-adding 32 scans.

The ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker AVANCE NMR spectrometer (400.13 MHz for ^{1}H and 100.63 MHz for ^{13}C, internal standard TMS).

The samples were prepared by the dissolution of PZA in DMSO (signal for ^{1}H at 2.512 ppm and at 39.476 ppm for ^{13}C). The spectra were recorded using a single excitation pulse of 12 μs for ^{1}H and 9 μs for ^{13}C. The FID signal was acquired 100 times for ^{1}H and 400 times for ^{13}C. [^{1}H, ^{1}H] COSY NMR spectrum of PZA has been recorded by using a standard COSY45 $d_1 - \frac{\pi}{2} - d_2 - \frac{\pi}{4}$ pulse sequence.

EPR measurements were performed at room temperature with an ADANI-USA spectrometer in the X frequency band (9.4GHz).

4. Results and discussion

a) Pyrazinamide (PZA)

The geometries of the two possible conformers of PZA (see Fig. 1) were optimized at B3LYP/6-31G(d) level of theory: one with NH_2 group in *cis* position relative to the N1 nitrogen atom from the pyrazinic ring (C1 conformer) and the other one with the NH_2 group in *trans* position (C2 conformer). In order to test the effect of intermolecular interactions, the PZA dimer formed by N-H...O intermolecular hydrogen bonds was also investigated by theoretical method B3LYP/6-31G(d). According to calculations, the C1 conformer has a planar structure while for the second conformer, the plane containing C2, C7, N8 and O9 atoms is 33.8° out of the pyrazinic ring and H13 and H14 atoms are 25.6° and 6.5° twisted from the C2C7N8O9 plane. C1 conformer is 8.33 Kcal/mol (8.23 Kcal/mol if ZPVE correction is considered) more stable than C2.

Even if in the gas phase the C1 conformer is more stabilized by intramolecular interactions, in solid phase, intermolecular interactions seems to play a dominant role, distorting the amide group out of the pyrazinic plane. In order to argue these observations the interaction energy for dimer has been calculated and we found a value of 16.14 kcal/mol (14.41 Kcal/mol if ZPVE correction is

considered). After basis set superposition error correction by counterpoise method [20] the interaction energy is further reduced to 13.82 kcal/mol, still significantly larger than the intramolecular hydrogen bond enthalpy of 8.23Kcal/mol. On the other hand, it is very close to the corresponding value of 12.6 kcal/mol for the adenine-thymine base pair [21] or to other strongly bound hydrogen bonded dimers formed as the result of the same type of N-H…O=C intermolecular hydrogen bonds [22], with interaction energies between 14 and 18 kcal/mol.

For the analysis of vibrational spectra of molecular complexes of PZA a very reliable assignment of all the vibrational bands of the molecule itself is neccesarry and computational methods proved to be a very useful tool in this sense. Thus, in this study the FT-IR/ATR and FT-Raman experimental techniques were supplemented with quantum chemical calculations of the vibrational spectra *via* DFT technique, using B3LYP/6-31G(d) and BLYP/6-31G(d) methods.

The FT-IR/ATR and FT-Raman spectra obtained for PZA powder at room temperature are given in Fig. 2. The highest discrepancies between the experimental and theoretical values were observed for vibrations related to the NH_2 group, which has a very important role in the biological activity and conformation of peptides [23] or Watson-Crick [24] type complexes. This group intermediates the hydrogen bonds (both intra- and intermolecular) responsible for the stabilization of a certain conformation of the peptides. For these reasons, a reliable assignment of these vibrations is very important. As compared to theoretical values of the monomer, the calculated values of these frequencies obtained for PZA dimer are in much better agreement with the experiment. Thus, the wavenumber corresponding to the $\nu_{as}(NH_2)$ vibration decreases from 3582 cm^{-1} for the monomer to 3503 cm^{-1} for dimer and also, the wavenumber corresponding to the symmetric stretch of the NH_2 group is predicted to be lower than for monomer but still apart from the experimental value.

The medium intensity bands at 3288 cm^{-1} and 3198 cm^{-1}, observed in the FT-IR/ATR spectrum are assigned to the symmetric stretching vibration of the NH_2 group. This assignment is well supported by the computed IR spectrum of dimer, where the corresponding wavenumbers are 3240 cm^{-1} and 3196 cm^{-1} respectively. In the study of Akyuz [2] the first of these bands appears at 3210 cm^{-1} and it was attributed to the overtone of the fundamental band corresponding to $\delta(NH_2)$ mode. Thus, the theoretical frequencies of the characteristic vibrational modes of NH_2 group suggest a significant influence of fairly strong intermolecular interactions.

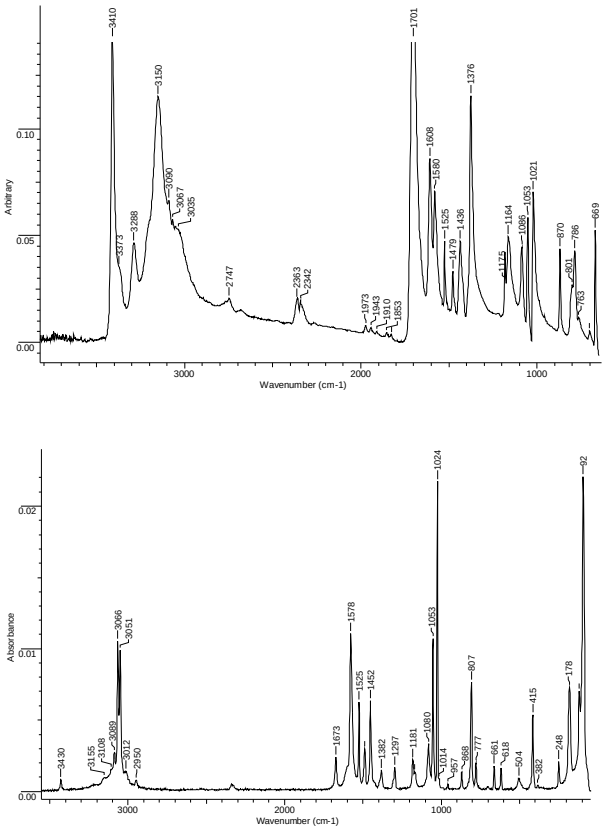

Fig. 2. FT-IR/ATR (top) and Raman (bottom) spectrum of PZA powder at room temperature.

For a reliable assignment of NMR spectra of organic molecules, the calculation of chemical shifts associated with their magnetic nuclei is essential [25]. Our ^{1}H and ^{13}C NMR experimental investigation is coupled with DFT calculations on the two monomers and the dimer of PZA. For NMR calculations purposes we used the GIAO (Gauge-Including Atomic Orbitals) ansatz [17,18], implemented in the Gaussian package, using the hybrid B3LYP functional, in conjunction with 6-31G(d) and cc-pVDZ basis sets.

As easily can be seen in Table 1, the main differences between our experimental values and those previously reported are noted for C2 and C7 nuclei and also minor differences for H11 and H12 protons. It is also evident that excepting the C7 nucleus, the ^{13}C calculated chemical shifts are essentially the same for C1 conformer and for dimer at B3LYP/6-31G(d) level of theory.

Table 1. Experimental and theoretical chemical shifts (in ppm) for pyrazinamide molecule.

Nucleus	Experimental			Calculated				
				B3LYP/6-31G(d)			B3LYP/cc-pVDZ	
	Ref. [4]	Ref. [37]	this work	Monomer C1	Dimer	Monomer C2	Monomer C1	Dimer
C2	143.9	n.a.	145.1	138.3	138.9	143.8	144.9	145.6
C3	143.4	n.a.	143.4	140.7	140.5	137.3	145.2	145.1
C5	143.4	n.a.	143.6	140.8	140.8	139.2	146.1	146.2
C6	148.3	n.a.	147.4	135.4	135.6	139.4	140.8	141.0
C7	167.1	n.a.	165.1	151.6	155.6	154.7	155.7	160.8
H10	n.a.	9.2	9.20	9.4	9.4	8.7	9.3	9.4
H11	n.a.	8.9	8.72	8.6	8.6	8.5	8.5	8.5
H12	n.a.	8.8	8.85	8.3	8.3	8.6	8.2	8.3
H13	n.a.	7.9	7.90	6.7	6.9	4.3	6.9	7.2
H14	n.a.	8.3	8.30	4.0	8.9	4.1	4.1	10.2

n.a. – not available

Moreover, for this nucleus, a significant difference between C1 conformer and dimer is noted: chemical shift increases from 151.6 ppm for C1 monomer to 155.6 ppm for dimer. Marked differences between the two conformers are noted, especially for C2, C6 and C7 nuclei and in a smaller manner for the others carbon nuclei. On the other hand, the much better performance of the correlation consistent cc-pVDZ basis set in predicting NMR shieldings for C2, C3 and C5 nuclei should be noted. Also, the chemical shifts associated with C6 and C7 nuclei are drastically improved by using this basis set.

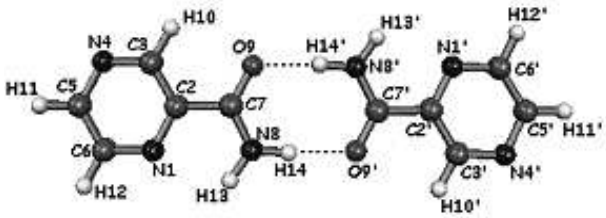

Fig. 3. Optimized geometry of PZA dimmer.

Regarding the proton chemical shifts, our experimental data are similar to those reported by Cox and Bothner-By [5], the only discrepancy being the different assignment for H11 and H12 protons. While they assigned the greater chemical shift to H11 and the lower one to H12, we made an opposite assignment, based on the bidimensional COSY ^{1}H-^{1}H NMR spectrum given in Fig. 4. We related the stronger off-diagonal peaks to the correlation between H11 and H12 protons (see Fig. 1 for atom numbering) which are only one C-C bond away. The weaker off-diagonal peaks are assigned to the correlation between H10 and H11 protons which are two C-C bonds away.

It is clear from our NMR study that for a complete characterization of NMR spectrum of PZA the intermolecular interactions must be taken into account. As seen in Table 1, this fact has a pronounced impact on the chemical shifts associated to H14 and C7 nuclei, which are significantly better reproduced for dimer than for monomer. It is also worth to mention that NMR spectra clearly show that only one of the two protons of NH_2 group is involved in intermolecular hydrogen bonds in liquid phase.

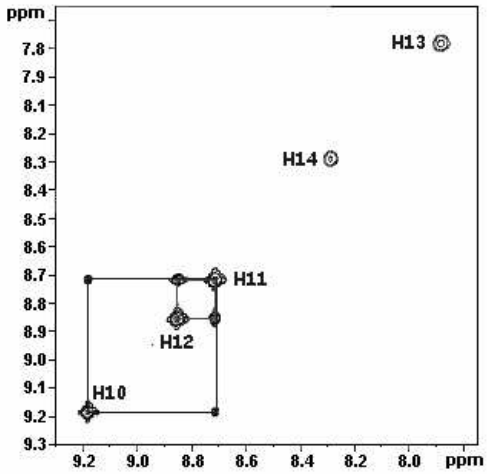

Fig. 4. [^{1}H, ^{1}H] COSY NMR spectrum of pyrazinamide in DMSO solution.

b) Antibiotic compound (5-pBBTT)

Two conformers are possible for 5-pBBTT molecule (see Fig. 1), differing by the relative orientation of the two rings. C1 is denoted the conformer with O8 in *cis* position

to H10 and C2 the corresponding *trans* conformer. Moreover, each conformer may exist in two tautomeric forms as thion (C1 and C2) and thiol (C1Thiol), respectively and for the thiolic tautomers there are also two possibilities for the orientation of the S6H bond relative to S1 (C1Thiol with H7 in trans position to S1 and C1Thiol1 in cis position). First we optimized the geometries for all the possible conformers and tautomers for 5-pBBTT molecule and in Fig. 5 are summarized their absolute and relative energies. The geometries were fully optimized without any constraint at B3LYP/6-31G(d) level of theory. No imaginary frequencies were obtained for optimized geometries and thus, all the optimized structures represent true minima on the potential energy surface. Comparing the calculated energies for each conformer we found that the lowest energy conformer is C1 conformer in its thionic form, as shown in Fig. 5. The small difference between the energy of the two thionic conformers of 5-pBBTT (C1 and C2) suggest that very possible, the two conformers coexist in liquid phase, so that for a careful analysis of the solvent effects, the two contributions must be taken into account [6].

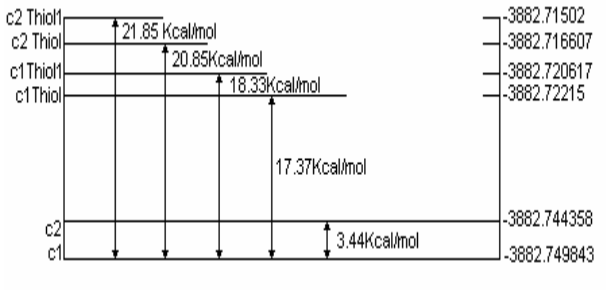

Fig. 5. Total and relative energies (not to scale) for the equilibrium geometries of the conformers and tautomers of 5-pBBTT molecule.

The ^{1}H and ^{13}C NMR measurements were made on liquid state samples, using DMSO as a deuterated solvent. The aromatic ring protons (H17, H18, H19 and H20) give rise to NMR signals as superimposed coupled doublets. The H10 signal appears as a singlet with a chemical shift at 7.604 ppm. The exact assignment of H10 and the ring protons (H17-H20) was made using the bidimensional ^{1}H-^{1}H COSY NMR spectrum.

The proton bonded to nitrogen atom in the thione conformers like C1 usually gives signals in 7-8 ppm range. When such a proton migrates to S, it becomes much more shielded and its chemical shift fall in the 3-4 ppm range. On the other hand, when hydrogen atoms are involved in hydrogen bonds their protons give chemical shifts in the 13-14 ppm range [25]. Therefore, H10 and the ring protons can be found at 7-8 ppm. In order to make a reliable assignment for H7 proton, we shall consider the coexistence of two conformers, the C1 thione conformer with the proton bonded to N and involved in a hydrogen bond with the oxygen atom from a molecule of solvent, and the C1 thiol conformer.

Indeed, as seen in the experimental ^{1}H NMR spectrum given in Fig. 6, the signal at 13.8 ppm is assigned to the hydrogen bonded proton of the thione conformer, while the signal at 3.4ppm is assigned to the SH proton in the thiol conformer. Theoretical spectrum is in very good agreement with experimental data: thus, C1 conformer with the lowest energy has the closest chemical shifts to the experimental ones, so that the values for the five protons (H10 and H17-H20) are well correlated to the experimental values. For the non-hydrogen bonded H7 proton a value of 7.13 ppm was calculated for its chemical shift. Modeling the hydrogen bond by adding a DMSO molecule in the vicinity of 5pBBTT, the B3LYP/6-31+G(d,p) calculated chemical shift the same proton is 14.14 ppm, in very good agreement with experiment. On the other hand, the SH proton in the thiol conformer has a calculated chemical shift of 3.5 ppm, again in very good agreement with experimental value. Analyzing the signal intensities in the ^{1}H NMR spectrum of 5-pBBTT a contribution of 66% and 33% to the NMR spectrum was obtained for the thiol and thione conformers, respectively.

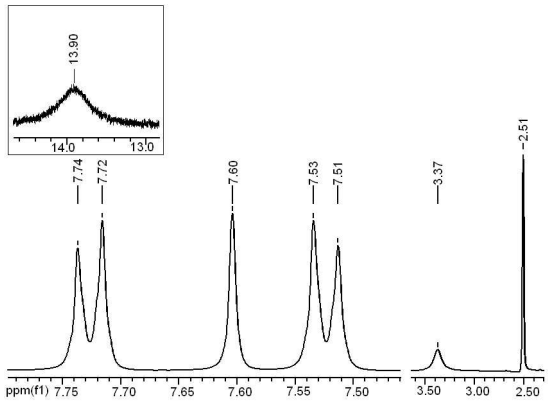

Fig. 6. ^{1}H NMR experimental spectrum for 5pBBTT molecule in DMSO solvent.

3. Cardiovascular compounds

The atenolol (ATE), metoprolol (MET), pindolol (PIN) and verapamil (VER) molecules are the most frequently used drugs in the treatment of cardiovascular diseases. In the last few years, some molecular copper complexes of different drugs (cardiovascular, anti-inflammation) are used because their activity is enhanced [26,27]. For a better understanding of their activity, structural investigation by different spectroscopic methods (FT-IR, Raman, EPR) was done.

Molecular complexes with Cu (II) were prepared on going from the starting salts (sodium benzoate and copper sulphate) by coprecipitation procedure.

A comparative study of the FT-IR and Raman spectra of ligands (ATE, MET, PIN and VER) and the corresponding metal complex allowed us to establish the molecular groups involved in the coordination and the local symmetry around the Cu(II) ions.

The assignment of the atenolol and metoprolol absorption bands from IR spectra are given in Table 2 and 3.

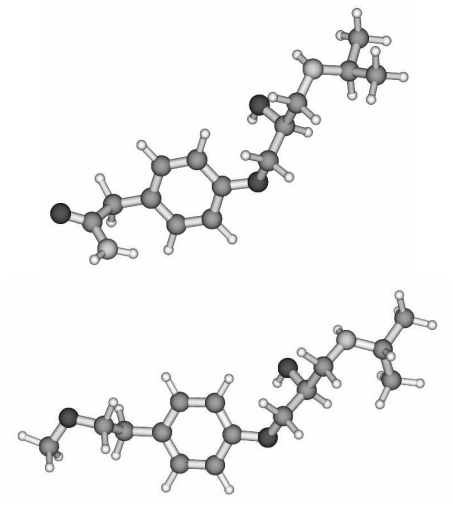

Fig. 7. Optimized geometries of atenolol (left) and metoprolol (right).

Table 2. Assignment of the atenolol FTIR bands.

Wavenumber (cm^{-1})	Assignment
1038	ν (C-N)
1093	ν (ring)
1243	ν (C-N)
1339	δ (CH_3)
1418	δ (ring)
1637	δ (H-O_H)
3125	ν_{sim}(N-H)+ ν_{sim}(C-H)
3300	ν (O-H)
3500	ν (N-H)

Table 3. Assignment of the metoprolol FTIR bands.

Wavenumber (cm^{-1})	Assignment
1038	ν (C-N)
1093	ν (ring)
1243	ν (C-N)
1339	δ (CH_3)
1418	δ (ring)
1637	δ (H-O_H)
3125	ν_{sim}(N-H)+ ν_{sim}(C-H)
3300	ν (O-H)
3500	ν (N-H)

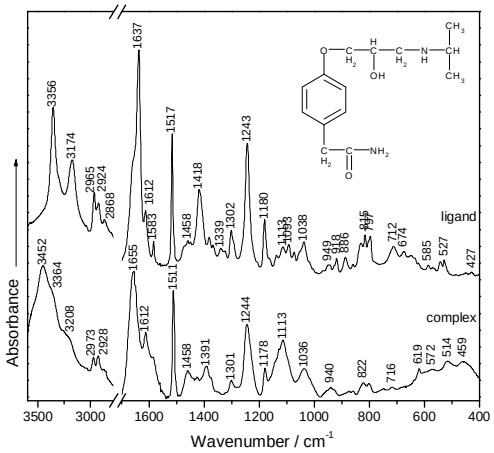

Fig. 8. FT-IR spectrum of atenolol (top) and Cu^{2+}-complex (bottom).

The 3356 cm^{-1} band, attributed to the NH_2 group of atenolol, is shifted to 3452 cm^{-1} after the complexation with copper (Fig. 8). Also, the 1637 cm^{-1} band (C=O) from the ATE spectrum is shifted to 1655 cm^{-1} in the Cu-ATE complex spectrum showing the involvement of the oxygen atom in the coordination.

Another ν_{sym} band characteristic for NH_2 group involved in hydrogen bonds appears at 3174 cm^{-1}. For Cu-ATE complex these vibrations are localized in the 3452-3208 cm^{-1} region suggesting the cleavage of hydrogen bonds during the complexation.

The ring characteristic bands (1614 cm^{-1} and 1448 cm^{-1}) from the Raman spectrum of MET (Fig. 9) are not meaningfully shifted by the complexation with copper (1610 cm^{-1} and 1446 cm^{-1}). This fact shows that oxygen atom directly bonded to the MET ring does not participate to the Cu coordination. On the other hand, bands which appear at 431 cm^{-1}, 236 cm^{-1} and 204 cm^{-1} in the spectrum of Cu-MET compound assigned to Cu-O bands clearly show the participation of the other MET and tartrate oxygens to copper coordination.

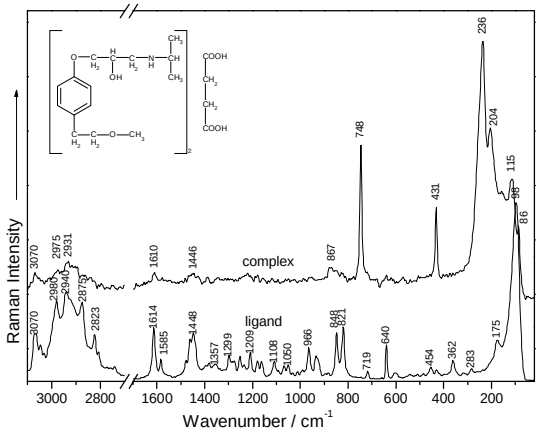

Fig. 9. Raman spectrum of metoprolol (bottom) and Cu^{2+}-MET complex (top).

Powder EPR spectra of the Cu(II)-complexes obtained at room temperature exhibit the absorption signals typical of randomly oriented single state (S=1/2) species having axial symmetry (Fig. 10). The ground state for the paramagnetic electron is $d_{x^2-y^2}$ for ATE, MET and VER compounds and d_{z^2} for the PIN compound. By comparing the shape of these EPR spectra and the value of the metallic hyperfine splitting for the complex with ATE with those obtained for the Cu(II) complexes with nitrogen and oxygen ligands we have concluded that the local symmetry around the metal ions is of square-planar [26,27] type with a CuN_2O_2 chromofore in the xOy plane.

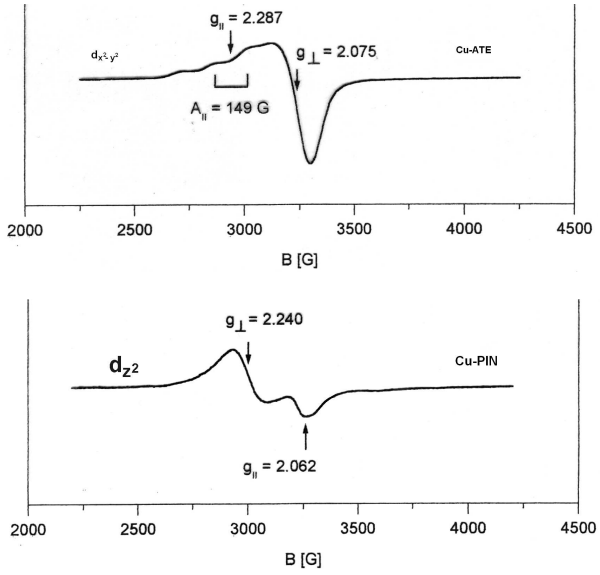

Fig.10. EPR spectra of Cu-ATE (top) and Cu-PIN (bottom) complexes.

4. Conclusions

Experimental data and DFT calculations of Pyrazinamide molecule evidenced that the dimeric form prevail in solution and the intermolecular interactions must be considered for a proper assignment of its vibrational spectra. NMR spectra clearly show that only one of the two protons of NH_2 group is involved in intermolecular hydrogen bonds in liquid phase.

In the case of 5-pBBTT molecule we found that the lowest energy conformer is C1 in its thionic form. Also, in deuterated DMSO solution both, the thiolic and thione conformers coexist with about 66% and 33% contribution, respectively.

The comparative analysis of FT-IR and Raman spectra of the cardiovascular drugs (ATE, MET, PIN and VER) and the corresponding metal complexes allowed us to establish the molecular groups involved in the complexation and the local symmetry around the Cu(II) ions. The ground state of the paramagnetic electron is $d_{x^2-y^2}$ orbital for ATE, MET and VER compounds and d_{z^2} orbital for PIN compound.

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