

A THEORETICAL AND EXPERIMENTAL STUDY ON HYDROGEN-BONDING INTERACTIONS BETWEEN GUANAZOLE AND DMSO/WATER

Mustafa Tuğfan Bilkan

Department of Physics, Faculty of Science, Çankırı Karatekin University, 18100 Çankırı, Turkey
mtbilkan@gmail.com

In this paper, guanazole-DMSO/water complexes, formed by hydrogen bonding interactions, were investigated by a combined experimental and computational approach. Two conformations of guanazole molecule were considered. Seven hydrogen-bonded guanazole-DMSO/water (C_2H_6OS/H_2O) complexes were characterized in terms of geometries, energies and vibrational frequencies. The optimizations and calculations were performed for the complexes by Density Functional Theory. In the experimental part, the C_2H_6OS/H_2O (DMSO/water) solutions of guanazole were prepared and infrared spectra of the solutions were recorded. After the solvation process, significant shifts in the existing bands and appearance of new bands were observed in the experimental spectra of guanazole. The following results are found from this study: 1) guanazole (I) is more stable than guanazole (II). 2) Seven guanazole- C_2H_6OS and guanazole- H_2O complexes are investigated. All nitrogen atoms of guanazole are hydrogen bond acceptors and all hydrogen atoms are hydrogen bond donors. 3) Aqueous complexes of guanazole form stronger hydrogen bonds compared to C_2H_6OS complexes. 4) The complexes with the most stable structures have $lpO \cdots H-N$ and $lpN \cdots H-O$ type intermolecular interactions. For these interactions, hydrogen-bond lengths of 1.78 and 1.90 Å are calculated and interaction energies are -7.10 kJ/mol for guanazole- C_2H_6OS and -50.5 kJ/mol for guanazole- H_2O . Based on this energy difference between the complexes, guanazole forms more stable complexes with water molecules compared to C_2H_6OS molecules, and with this property, it is an ideal molecule for pharmacological purposes.

Keywords: guanazole; solvent effects; hydrogen bonding; DFT; FT-IR spectroscopy

ТЕОРИСКО И ЕКСПЕРИМЕНТАЛНО ИСТРАЖУВАЊЕ НА ИНТЕРАКЦИИТЕ НА ВОДОРОДНО СВРЗУВАЊЕ МЕЃУ ГВАЗАЗОЛ И DMSO/ВОДА

Во овој труд беа истражени водородните интеракции меѓу комплексите на гваназол–DMSO/вода со комбиниран експериментален и теориски пристап. Предвид беа земени два конформера на гваназолската молекула. Карактеризирани беа седум водородно сврзани комплекси на гваназол-DMSO/вода (C_2H_6OS/H_2O) во смисла на нивната геометрија, енергија и вибрациски фреквенции. Оптимизациите и пресметките на комплексите беа извршени со теоријата на густина на функционалот. Во експерименталниот дел беа приготвени раствори на C_2H_6OS/H_2O (DMSO/вода) на гваназол и беа снимени нивните инфрацрвени спектри. По процесот на растворање беше забележано значајно поместување на постојните ленти и појава на нови ленти во експерименталните спектри на гваназол. Од ова истражување се добиени следниве резултати: 1) гваназол (I) е постабилен од гваназол (II). 2) Испитани беа седум комплекси на гваназол- C_2H_6OS и гваназол- H_2O . Сите азотни атоми на гваназол се акцептори на врски и сите водородни атоми се донори на водородни врски. 3) Водни комплекси на гваназол образуваат посилни водородни врски во споредба со комплексите на C_2H_6OS . 4) Комплексите со најстабилни структури имаат тип на интеракции $lpO \cdots H-N$ и $lpN \cdots H-O$. За овие интеракции на водородните врски е пресметано дека должините им се 1,78 и 1,90 Å и енергијата на интеракциите $-7,10$ kJ/mol за гваназол- C_2H_6OS и $-50,5$ kJ/mol за гваназол- H_2O . Врз основа на овие разлики во енергиите меѓу комплексите,

гваназол образува постабилни комплекси со молекулите на вода одошто со молекулите на C_2H_6OS и поради ова својство е идеална молекула за фармаколошки цели.

Клучни зборови: гваназол; ефекти на растворувачи; водородно сврзување; DFT; FT-IR спектроскопија

1. INTRODUCTION

3,5-Diamino-1,2,4-triazole (guanazole) is a considerable biomolecule and with antitumor, anticancer and antimicrobial effects on living tissues [1–3]. Researches on the biological, medical and pharmacological properties of guanazole is still popular [4–7], and since the determination of the biological, chemical, and physical properties of such effective compounds is of great importance, many studies have been published on these properties of guanazole in the literature. Kumar *et al.* recorded the infrared (IR) spectrum of guanazole and calculated basic vibrational modes by using Density Functional Theory (DFT) [8]. Similarly, in another study, Guennoun *et al.* [9] calculated geometric parameters, energies and vibrational modes of guanazole using Hartree-Fock (HF) and DFT levels. In addition, there are many studies published on metal complexes of guanazole in the literature [10–13].

To the best of our knowledge, although there are many published studies on vibrational frequencies of guanazole, the studies on hydrogen-bonding interactions between guanazole and solvent molecules are very limited. Karpińska and Dobrowolski completed a theoretical study on the energetics of tautomers and conformers of guanazole in the gas phase and in water [14]. For this reason, a theoretical and experimental study of hydrogen-bonded guanazole- C_2H_6OS/H_2O complexes was carried out in this paper, in order to overcome this deficiency in the literature.

Determining of the effects of solvent media on the structural properties of any molecule is an important issue in computational quantum chemistry. Solvent media is one factor involved in the absorption, transport, and diffusion of drugs taken into living tissues and cells. Although many models and methods have been described for the investigation of solvent effects on molecular structures, few of them are close to explaining experimental results. Such calculations are very costly and difficult to make with high reliability. One of the reasonable models for analyzing the effects of solvent environments on molecular structures is the Polarizable Continuum Model (PCM) [15]. This model significantly reduces calculation costs. However, it is not enough to use this model alone to study sol-

vent-ligand interactions. Instead, using both implicit and explicit models is necessary to make calculations more acceptable.

In this work, C_2H_6OS and H_2O have been selected as solvents for the investigation of intermolecular interactions between solvent molecules and guanazole. Although C_2H_6OS may have some side effects on living cells, it is sometimes known to be used in a number of medical applications [16]. Similarly, H_2O is known as the most important solvent for chemical and biological processes, and it is essential for living organisms. Therefore, these liquids are very important for the investigation of intermolecular interactions. For the calculations, DFT chosen because, among electronic structure methods, it has given very successful results for intermolecular interactions. In many studies in the literature using this theory, efficient results have been obtained. Calculations with DFT are also known to be very successful in explaining experimental results. The investigations were done with both explicit and implicit approaches. In the experimental study section, Fourier Transform Infrared Spectroscopy was selected to reveal the hydrogen-bonding interactions from an experimental viewpoint. The obtained results from calculations were matched with experimental data, and the effects of the solvent environment on guanazole are discussed in detail.

2. COMPUTATIONAL METHODS

Three-dimensional geometries of the structures were drawn with the help of the Gaussview visualization program [17]. By using drawn structures, all calculations on energies, molecular parameters and vibrational frequencies for guanazole were performed with the Gaussian09 program [18]. The calculations in vacuum, DMSO, and aqueous media were performed by the DFT/6-311++G(d,p) method on a high capacity computer. All vibrational modes were calculated from the optimized structures. The potential energy distribution (PED), obtained by using the VEDA4 program, was used to characterize fundamental vibrational modes [19]. The calculated vibrational frequencies were scaled by 0.9668 to correct the differences between the calculated and experimental results [20].

Molecular interaction energies between solvent molecules and guanazole can be obtained as the energetic difference between the sum of the monomers and the complexes. In this case, interaction energies for guanazole- C_2H_6OS and guanazole- H_2O complexes are $\Delta E_{interaction} = E_{complex} - (E_{guanazole} + E_{C_2H_6OS})$ and $\Delta E_{interaction} = E_{complex} - (E_{guanazole} + E_{H_2O})$, respectively. The counterpoise method [21], defined by Boys and Bernardi, was used to correct the basis set superposition error and better estimate the binding energies of the complexes. The PCM implicit solvation model calculations were named Model I and both implicit and explicit solvation model calculations were named Model II.

3. EXPERIMENTAL PROCEDURES

A commercial sample of guanazole (98 % purity) was purchased from Sigma-Aldrich and used without further purification. Two mmol of guanazole (about 200 mg) were dissolved in either 5 ml of C_2H_6OS and 5 ml of purified H_2O , and the solutions were stirred at 40 °C for 10 minutes, yielding fully saturated solutions. The solutions

were filtered before making spectroscopic measurements. The IR spectra of the solutions, free ligand, and pure solvents were recorded between 3750–550 cm^{-1} by Perkin Elmer Spectrum 100 FT-IR spectrometer with ATR (Attenuated Total Reflection) equipment. The spectra were recorded at room temperature with a resolution of 2 cm^{-1} . The IR spectra of pure solvents were used as reference while recording spectra of the solutions. Before the spectra of the solutions were recorded, the solvents were filtered to remove precipitates.

4. RESULTS AND DISCUSSION

4.1. Monomer geometry analysis and atomic charges

The two conformational structures of guanazole molecule considered in this work are given in Figure 1, as guanazole (I) and guanazole (II).

The distances between the 12H atom and the hydrogen atoms attached to the 6N and 7N atoms are greater in guanazole (I) than guanazole (II).

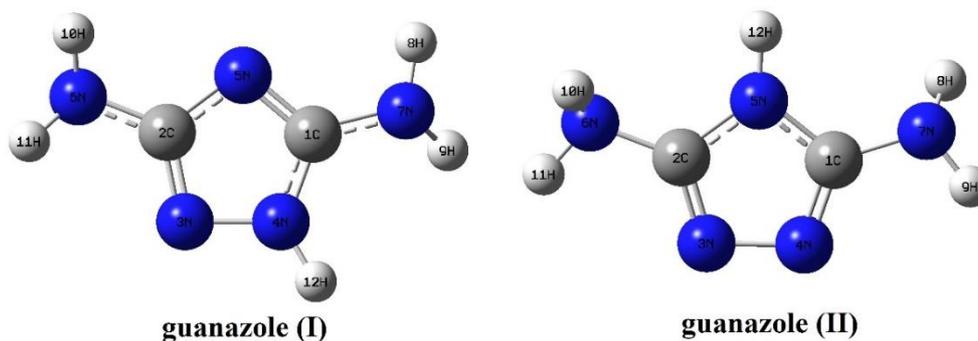


Fig. 1. Optimized conformational structures of guanazole (I) and guanazole (II) with labels for carbon, nitrogen and hydrogen atoms

Thus, the repulsion forces between hydrogen atoms are less, which allows guanazole (I) to be more stable (lower energy) than guanazole (II). The calculated energy difference between two structures is 9.95 Kcal/Mol, and this energy difference is quite large. According to the relative population analysis, it can be said that probability of guanazole (I) being found of is 100 %. The Boltzmann distribution (Eq. I) is used to obtain relative populations [22].

$$\frac{N_i}{N} = \frac{\rho_i e^{E_i/k_b T}}{Z(T)} \quad (I)$$

$$Z(T) = \sum_i \rho_i e^{E_i/k_b T} \quad (II)$$

Here, $Z(T)$ is the partition function, k_b is Boltzmann's constant, T (Kelvin) is absolute temperature, E_i is the i . energy level, and ρ_i is the number of states with energy E_i . According to the calculations, guanazole (I) is the most stable structure in both vacuum and solvent media and further calculations will be carried out only for this structure.

The optimized bond lengths, bond angles, and dihedral angles for guanazole, calculated in vacuum and solvent media using the DFT/6-311++G(d,p) method, are listed in Table 1 together with the experimental X-ray crystal data from the literature [23].

Table 1

Optimized geometrical parameters of guanazole monomer in vacuum, C₂H₆OS and H₂O at DFT/6-311++G(d,p) level with experimental X-ray crystal data from literature

Bond lengths (Å)	Vacuum	C ₂ H ₆ OS	H ₂ O	X-ray.*	Bond lengths (Å)	Vacuum	C ₂ H ₆ OS	H ₂ O	X-ray.*
1C-4N	1.349	1.343	1.343	1.339	3N-4N	1.386	1.389	1.389	1.398
1C-5N	1.320	1.334	1.335	1.363	4N-12H	1.006	1.023	1.024	0.870
1C-7N	1.380	1.367	1.367	1.353	6N-10H	1.009	1.017	1.018	0.830
2C-3N	1.319	1.324	1.324	1.319	6N-11H	1.009	1.018	1.018	0.930
2C-5N	1.371	1.368	1.368	1.363	7N-8H	1.011	1.018	1.018	0.890
2C-6N	1.380	1.381	1.381	1.376	7N-9H	1.010	1.018	1.018	0.860
Bond angles (°)									
4N-1C-5N	110.4	110.0	110.0	110.4	3N-4N-12H	119.7	120.8	120.9	118.0
4N-1C-7N	124.1	124.4	124.4	124.1	1C-5N-2C	102.8	103.1	103.1	102.7
5N-1C-7N	125.5	125.5	125.4	125.4	2C-6N-10H	113.9	113.8	113.7	115.0
3N-2C-5N	115.7	115.2	115.2	115.9	2C-6N-11H	114.5	114.3	114.2	114.0
3N-2C-6N	123.1	123.2	123.2	122.9	10H-6N-11H	113.9	112.4	112.3	114.0
5N-2C-6N	121.1	121.5	121.5	121.0	1C-7N-8H	112.2	114.5	114.5	115.0
2C-3N-4N	101.4	101.7	101.7	101.5	1C-7N-9H	115.9	116.4	116.3	119.0
1C-4N-3N	109.8	109.9	109.9	109.5	8H-7N-9H	112.5	113.5	113.5	115.0
1C-4N-12H	129.6	129.2	129.2	132.5					
Torsional angles (°)									
5N-2C-3N-4N	-0.7	-0.5	-0.4	-	4N-1C-7N-9H	38.4	24.9	24.8	-
5N-1C-4N-12H	-170.1	-0.7	-178.8	-	5N-1C-7N-8H	-13.6	-23.2	-23.6	-
7N-1C-4N-12H	7.3	-1.7	-2.1	-	5N-1C-7N-9H	-144.7	-158.9	-159.1	-
7N-1C-4N-3N	175.8	176.0	176.0	-	3N-2C-6N-11H	22.1	22.5	22.6	-
4N-1C-7N-8H	169.4	160.6	160.2	-	2C-3N-4N-12H	171.1	178.6	179.0	-

*Data were taken from Ref. [23].

The 2C-6N, 1C-4N and 1C-7N bonds exhibit single bond character, while 1C-5N and 2C-3N bonds show typical double bond character. In vacuum, single C-N bond lengths were calculated between 1.350–1.380 Å whereas double C-N bonds were calculated to be 1.320 Å. In C₂H₆OS and H₂O media, 2C-6N, 1C-4N and 1C-7N bonds were calculated to be 1.381, 1.343, and 1.367 Å respectively. As seen from the table, the measured experimental values of these bonds are 1.376, 1.339, and 1.353 Å. The lengths of the double C=C bonds were calculated to be shorter the single C-C bonds, and the average energies of C-C and C=C bonds are known to be about 350 and 600 kJ/mol, respectively. The bond lengths between the 1C-5N and 1C-7N atoms are the bond parameters most affected by the change of environment. When passed from vacuum to water, the bond length of 1C-5N increased while the bond length of 1C-7N decreased. The main reason for this change is that, when the PCM is included in the calculations for

H₂O, the 12H-9H interaction is relatively reduced because of a continuous dielectric environment. Thus, while the 1C-7N bond length is reduced in the solvent medium, the 1C-5N bond length increases. Though the 12H-9H distance was 2.756 Å in vacuum, this length decreased to 2.638 Å in H₂O medium.

When DFT calculated bond angles are examined, it can be seen that almost all of the angles changed in the H₂O environment. The alterations caused by the environment change are more serious at the angles between the atoms outside the ring and are small, as expected, at the angles between the atoms remaining in the plane of the triazole ring. Another important review here is related to dihedral angles. Dihedral angles can give very important information about how molecules are effected by changing physical conditions. In particular, planar structures can exhibit very precise changes for dihedral angles due to external physical effects.

In Table 1, some important dihedral angles of guanazole are presented for vacuum, C₂H₆OS and H₂O environments. The solvent-induced changes had considerable effects on the dihedral angles. The 5N-2C-3N-4N dihedral angle is very close to 180° and 0°. This result shows that the triazole portion is almost planar and this increases in the solvent environments. The 4N-1C-7N-8H and 4N-1C-7N-9H dihedral angles between the triazole ring and diamine portion of the molecule were calculated to be approximately 169°–160°, and 38°–25°, respectively, which indicates that the molecular structure of guanazole is non-planar. As seen from the table, others dihedral angles vary by

less than 1°, while some vary by more than 14°. If these changes are carefully examined, similar to the case of the bond angles, the dihedral angles in the triazole ring plane are effected less by the ambient change, while those outside the ring are much more effected. Nevertheless, all of the optimized geometric parameters, except for N-H bond lengths, are quite consistent with the experimental results from the literature, further verifying the calculated results of this work. The reason for the differences in N-H bond lengths between calculated and measured values are experimental errors that cannot be solved and they were caused by scattering factors in X-ray experiments.

Table 2

The calculated NBO atomic charges of guanazole in vacuum, C₂H₆OS and H₂O media (in *e*)

Atoms	Vacuum	C ₂ H ₆ OS	H ₂ O	Atoms	Vacuum	C ₂ H ₆ OS	H ₂ O
1C	0.560	0.572	0.573	7N	-0.803	-0.819	-0.819
2C	0.518	0.522	0.522	8H	0.395	0.420	0.420
3N	-0.403	-0.464	-0.465	9H	0.378	0.417	0.418
4N	-0.409	-0.417	-0.417	10H	0.384	0.407	0.407
5N	-0.601	-0.661	-0.663	11H	0.383	0.407	0.407
6N	-0.803	-0.836	-0.837	12H	0.402	0.453	0.454

The calculated atomic charges of guanazole are given in Table 2. The calculation of atomic charges is an important indication of possible molecular sites. Especially when solvent-ligand interactions are examined, the identification of the atomic charges belonging to the ligand can provide important information regarding the nature of the complex structures to be formed. All of the nitrogen atoms of guanazole have negative charges, while all of the carbon atoms have positive charges. The 6N and 7N atoms have the most negative charges in the molecule. These results show that all of the nitrogen and hydrogen atoms in guanazole have the potential to form hydrogen bonds with solvent molecules. When passed from vacuum to solvent environments, the negative charges of the nitrogen atoms increase, while the positive charges of hydrogen atoms increase more drastically. This indicates that the hydrogen-bonded complexes to be installed in the C₂H₆OS and aquatic environments may be even more stable.

Molecular electrostatic potential (MEP) maps are drawings that visualize the distribution of

charges on the molecule in three dimensions. These maps give the shape, size and charge distribution of a molecule. Generally, in the MEP maps, red colors represent electron-rich regions, while blue colors represent electron-poor regions. MEP maps also provide important information about the nature of molecular bonds and they say a lot about the difference in electronegativity.

To predict reactive sites for electrophilic and nucleophilic attacks of guanazole, the MEP was calculated, and in Figure 2, the MEP map is drawn as transparent.

Figure 2 shows that, for both structures of the molecule, the atoms with the greatest number of electrons are nitrogen atoms as expected, while the positive charges are concentrated on 9H and 12H atoms. Thus, hydrogen-bonded C₂H₆OS complexes, in which C₂H₆OS molecules are attached to 8H, 9H, and 12H atoms, are more stable than other cases. In aqueous complexes, hydrogen-bonded versions formed between hydrogen atoms of H₂O molecules and electron-rich nitrogen atoms of guanazole molecules are the most stable ones.

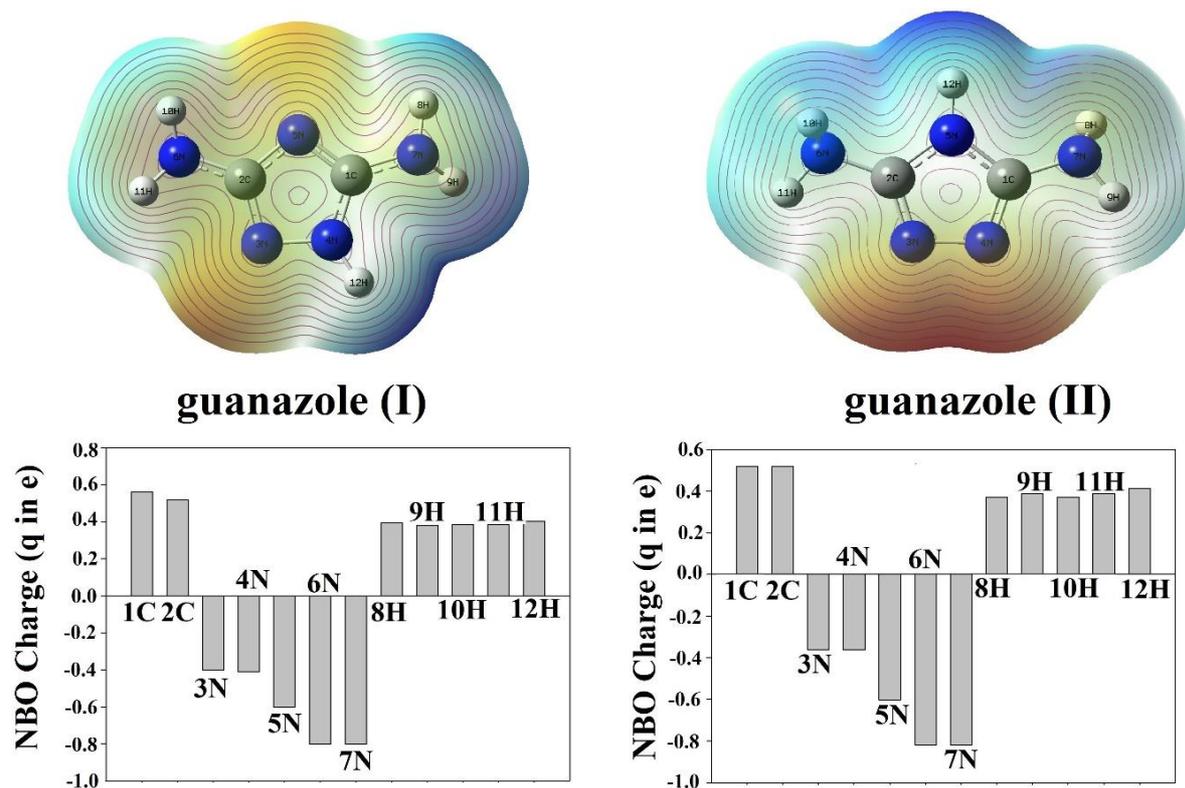


Fig. 2. MEP maps and histogram of the calculated NBO charges for guanazole (I) and guanazole (II)

4.2. Interaction energies and optimized geometries of guanazole- C_2H_6OS/H_2O complexes

Hydrogen bonds are important determining factors in the formation of physical and chemical properties of fluids and solutions. Ligand- H_2O complexes occur mostly in the form of non-covalent bonds. Such bindings contain some important parameters that provide useful information about the nature of the interaction. The interaction energy is one such important parameter which provides a lot of information about solvent-ligand complexes. Highly accurate determination of the interaction energy is possible by obtaining optimized structures of complexes. In this study, hydrogen-bonded complexes formed by guanazole molecules with C_2H_6OS and H_2O molecules are given in Figures 3 and 4, respectively.

As shown in the figures, the most stable optimized structures in both complexes are V1. Both guanazole- C_2H_6OS and guanazole- H_2O complexes have seven interaction structures. The binding distances of the structures are in the range of 1.78–2.58 Å and these values are less than the sum of the Van der Waals radii of hydrogen and oxygen (2.5–2.6 Å) [24]. This distance is very critical for

determining the existence of intermolecular hydrogen bonds. C_2H_6OS has an oxygen atom with lone pairs (Lp) of electrons. In the same way, the oxygen atom in H_2O molecule has two Lp of electrons and these electrons provide weak bonds with the hydrogen atoms. The guanazole molecule has five hydrogen atoms and two open-ended nitrogen atoms. The nitrogen atoms of guanazole can bind with hydrogen atoms on H_2O molecules. Intermolecular hydrogen bonds in the form of $lpN \cdots H-O$, $lpO \cdots H-N$ and $lpN \cdots H-C$ occur. The most stable complex structures have $lpN \cdots H-O$ and $lpO \cdots H-N$ type intermolecular H-bonds and the lengths of the bonds are 1.78 and 1.90 Å in C_2H_6OS complexes and 1.90 and 1.89 Å in H_2O complexes, respectively. In addition, while all of the H_2O complexes are singly connected, V3, V4, and V5 versions of guanazole- C_2H_6OS complexes are binary connected. The energy difference between the complex structures is quite small. The interaction energy is -7.10 kJ/mol for V1 guanazole- C_2H_6OS complex and -50.5 kJ/mol for V1 version of guanazole- H_2O complex. For a stable interaction complex, the interaction energy should be negative and the greater the energy value, the stronger the hydrogen bond strength.

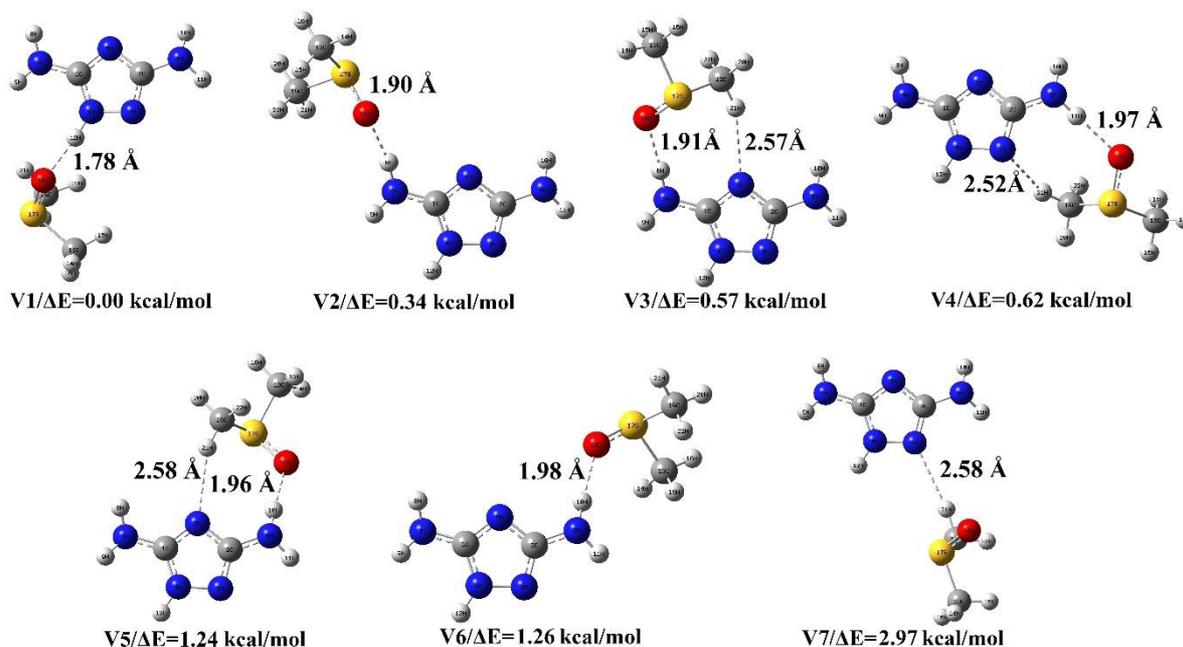


Fig. 3. Optimized geometric structures of guanazole-C₂H₆OS complexes

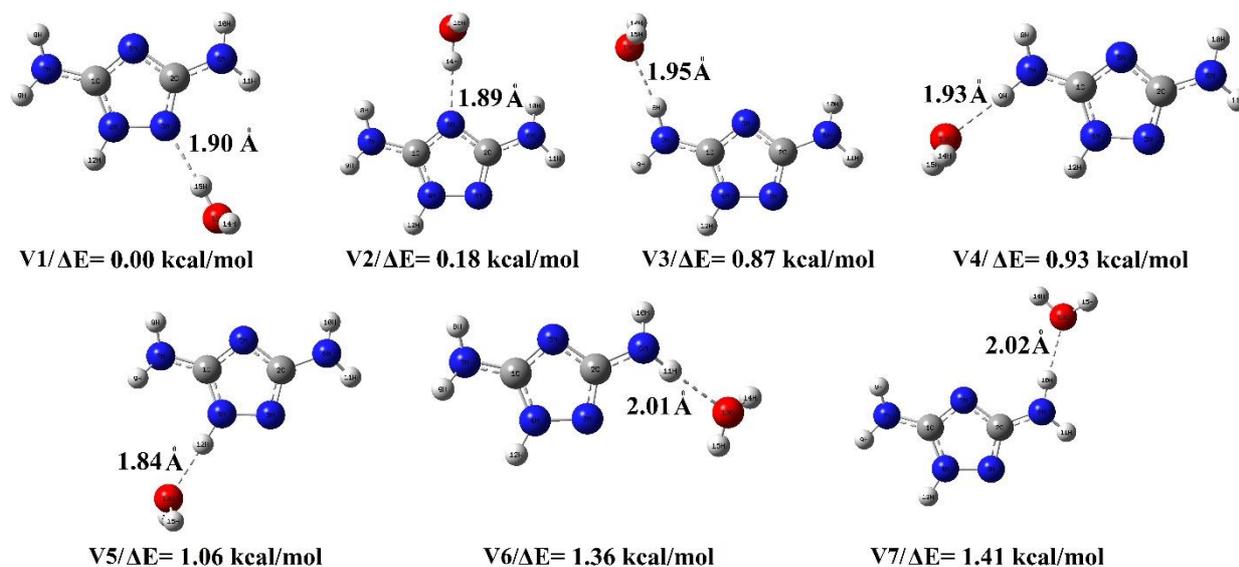


Fig. 4. Optimized geometric structures of guanazole-H₂O complexes

In Figure 5, the interaction energies of the C₂H₆OS/H₂O complexes of guanazole are given as histograms. In particular, H₂O complexes have much stronger hydrogen bonds than C₂H₆OS complexes. For guanazole-C₂H₆OS, the energies of V1, V2, V3 and V4 are close to each other and

significantly higher than V5, V6 and V7 structures. For guanazole-H₂O, the energies of the V1 and V2 are close to each other and significantly higher than other versions. This can also be attributed to the excellent solubility of guanazole in H₂O.

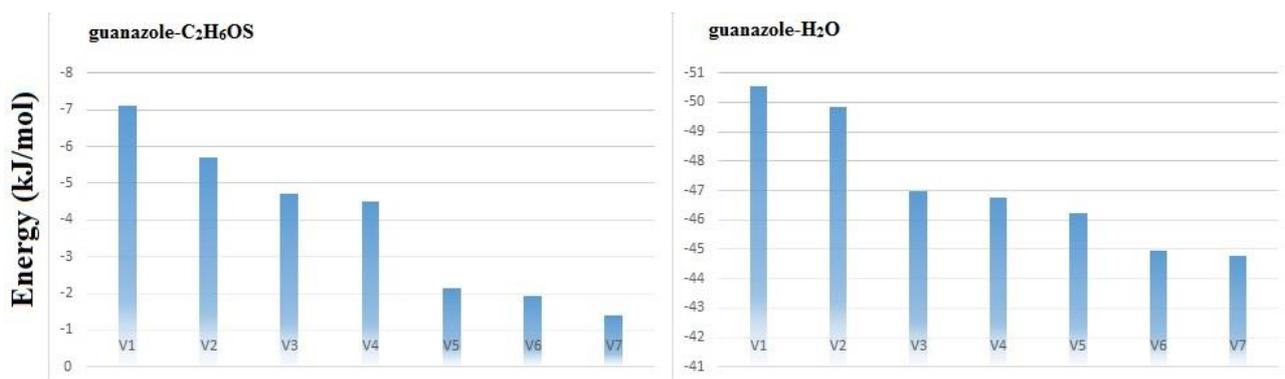


Fig. 5. Histogram for the interaction energies of guanazole-C₂H₆OS/H₂O complexes

4.3. Vibrational modes of guanazole-C₂H₆OS/H₂O complexes

It is known that external physical influences, such as temperature-pressure changes, hydrogen bonds, and solvent effects cause variation in molecular geometries and therefore shifts in vibrational modes. Such external influences also significantly affect intra-molecular and intermolecular hydrogen bonds. In particular, the solvent media effects both intramolecular bonds and intermolecular hydrogen bonds directly. Moreover, hydrogen-bonding interactions can occur between the solvent and ligand molecules and this can cause new bands rising in the experimental IR spectrum. For determination of all these effects the existence of guanazole-C₂H₆OS/H₂O complexes were investigated experimentally and theoretically. In theoretical calculations, all of the interactions have been studied in solvent environments.

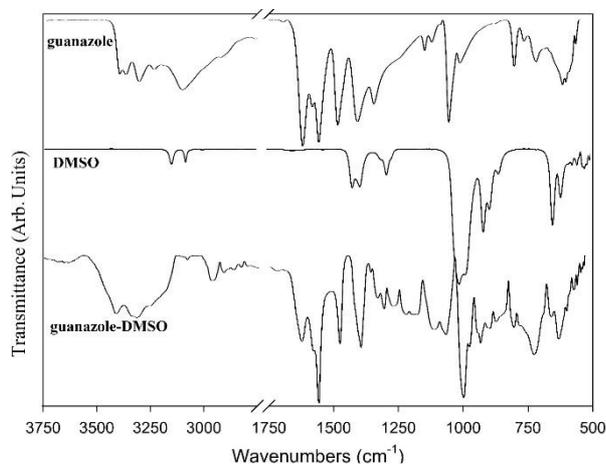


Fig. 6. Experimental IR spectra of guanazole, C₂H₆OS and guanazole-C₂H₆OS solution

The experimental IR spectra of the guanazole-C₂H₆OS/H₂O solutions were plotted in Figures 6 and 7 together with free ligand and solvent spectra. While the spectra of the solutions are recorded, the solvent spectra were used as backgrounds. The reason for this is that the vibrations of solvent-ligand interactions can be observed by eliminating the internal vibrations of the solvent molecules as much as possible. When the recorded spectra of solutions were compared with those of free ligand, it was seen that the spectral bands shifted and/or new bands arose. This shows that hydrogen-bonded complexes may form between solvent molecules and ligand molecules.

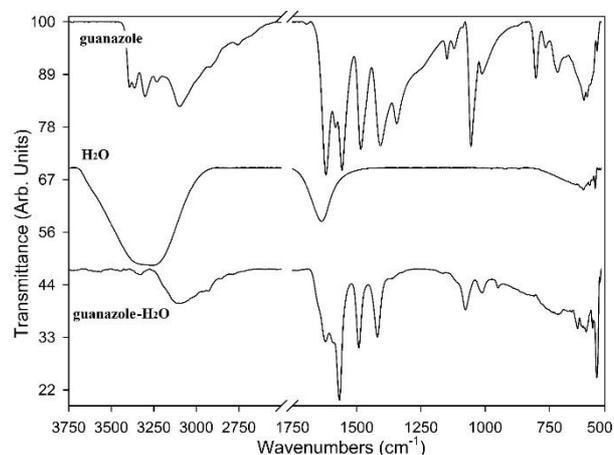


Fig. 7. Experimental IR spectra of guanazole, H₂O and guanazole-H₂O solution

For the most stable complex structures, vibrational frequencies and their relative intensities calculated by Model II (guanazole-C₂H₆OS/H₂O complex in solvent medium) and Model I (only guanazole in solvent medium) were given in Tables 3 and 4 alongside experimental values.

Table 3

Vibrational modes of the most stable guanazole-C₂H₆OS complex (for VI)

Mode	Model II		Model I		Exp.	PED (%)*
	Freq.	IIR	Freq.	IIR		
18	548	24.20	515	94.29	565 w	$\Gamma_{\text{HNCN}}(75)+\delta_{\text{HNNH}}(11)$
19	603	23.30	590	60.55	605 w	$\Gamma_{\text{HNCN}}(60)$
20	621	0.27	–	–	618 w	V_{sc}(94)
21	640	0.78	644	0.89	638sh	$V_{\text{NC}}(32)+\delta_{\text{CNC}}(24)$
22	655	0.75	–	–	648 m	V_{sc}(92)
23	673	2.12	689	2.79	678 w	$\Gamma_{\text{NNNC}}(36)+\Gamma_{\text{CNCN}}(14)$
24	740	8.40	746	9.27	743 s	$\Gamma_{\text{NCNC}}(36)+\Gamma_{\text{NNNC}}(31)$
25	775	1.57	778	2.80	772 m	$\delta_{\text{NCN}}(60)+V_{\text{NC}}(23)$
26	793	4.37	–	–	820 m	$\Gamma_{\text{HNNC}}(28)+\Gamma_{\text{CNCN}}(17)+\Gamma_{\text{OHNC}}(15)$
27	885	0.24	–	–	888 m	$\Gamma_{\text{HCSC}}(66)$
28	908	11.63	–	–	911 m	V_{so}(74)
29	921	0.75	–	–	946 m	$\Gamma_{\text{HCSC}}(50)$
30	975	8.98	–	–	990 m	$\Gamma_{\text{HCSC}}(37)+V_{\text{so}}(22)$
31	997	0.07	988	3.25	–	$\delta_{\text{NCN}}(48)+V_{\text{NC}}(22)+\delta_{\text{CNC}}(13)$
32	1004	1.64	–	–	1011 vs	$\Gamma_{\text{HCSC}}(62)$
33	1029	2.29	–	–	–	$\delta_{\text{HNC}}(52)+\delta_{\text{CNC}}(28)$
34	1045	1.51	1030	1.76	1064sh	$V_{\text{NC}}(42)+\delta_{\text{HNC}}(12)$
35	1084	1.09	1085	2.20	1078 m	$V_{\text{NC}}(45)+\delta_{\text{HNC}}(28)$
36	1123	0.15	1024	10.74	1123 m	$\delta_{\text{HNC}}(25)+V_{\text{NC}}(21)+\delta_{\text{NCN}}(12)$
37	1287	0.06	–	–	1280 w	$\delta_{\text{HCH}}(98)$
38	1308	0.44	–	–	1314 w	$\delta_{\text{HCH}}(97)$
39	1338	2.09	1122	0.64	1338 w	$V_{\text{NC}}(32)+\delta_{\text{HNN}}(17)+\delta_{\text{HNC}}(11)$
40	1389	2.74	–	–	–	$\delta_{\text{HCH}}(58)$
41	1391	17.90	1282	0.74	1365 w	$V_{\text{NC}}(27)+\delta_{\text{HNN}}(26)+\delta_{\text{NCN}}(11)$
42	1399	1.55	–	–	1402 m	$\delta_{\text{HCH}}(72)$
43	1400	0.12	–	–	–	$\delta_{\text{HCH}}(64)$
44	1414	2.00	–	–	1403 m	$\delta_{\text{HCH}}(74)$
45	1442	8.65	1369	47.03	1416 sh	$V_{\text{NC}}(58)$
46	1524	10.94	1437	43.18	1483 m	$V_{\text{NC}}(29)+\delta_{\text{HNN}}(15)+\delta_{\text{HNC}}(12)$
47	1547	8.79	1526	12.31	1563 vs	$\delta_{\text{HNNH}}(43)+\delta_{\text{HNN}}(18)$
48	1561	23.94	1558	100.00	1584 s	$\delta_{\text{HNNH}}(67)$
49	1590	14.92	1585	43.96	1628 m	$V_{\text{NC}}(35)+\delta_{\text{HNNH}}(20)$
50	2934	0.01	–	–	2700 vw	V_{ch}(99)
51	2936	0.04	–	–	2709 vw	V_{ch}(100)
52	3030	0.01	–	–	2756 vw	V_{ch}(94)
53	3034	0.04	–	–	2775 vw	V_{ch}(93)
54	3041	0.00	–	–	2797 vw	V_{ch}(99)
55	3041	0.01	–	–	2910 w	V_{ch}(98)
56	3176	100.00	3225	74.84	3122 m	$V_{\text{NH}}(97)$
57	3307	13.52	3300	40.73	3191 m	$V_{\text{NH}}(96)$
58	3311	3.02	3311	15.77	3309 w	$V_{\text{NH}}(96)$
59	3391	8.67	3388	28.95	3322 w	$V_{\text{NH}}(100)$
60	3396	6.76	3396	22.14	3368 w	$V_{\text{NH}}(100)$

vs: very strong; s: strong; m: medium; w: weak; vw: very weak

*V: stretching modes; δ : bending modes; Γ : torsional modes.

Table 4

Vibrational modes of the most stable guanazole-H₂O complex (for VI)

Mode	Model II		Model I		Exp.	PED (%)*
	Freq.	I _{IR}	Freq.	I _{IR}		
13	502	89.67	516	94.16	566 w	Γ _{HNCN} (80)+δ _{HNH} (10)
14	570	60.51	592	60.30	605 m	Γ _{HNCN} (70)
15	645	0.12	644	0.93	617 m	δ _{CNC} (31)+V _{NC} (25)
16	686	2.18	689	2.88	722 w	Γ _{NNNC} (60)+Γ _{NCNC} (20)
17	745	7.82	746	9.56	767 vw	Γ _{NNNC} (49)+Γ _{NCNC} (30)
18	783	1.91	778	2.86	805 m	δ _{NCN} (59)+V _{NC} (26)
19	967	19.61	–	–	951 w	δ_{OHN}(51)+δ_{HNC}(18)+Γ_{OHNN}(13)
20	998	5.75	988	3.46	1015 m	δ _{NCN} (40)+V _{NC} (17)+δ _{CNC} (15)
21	1025	9.10	1024	10.35	1047 sh	δ _{HNC} (50)+δ _{CNC} (27)
22	1032	4.27	1030	1.83	1056 s	V _{NC} (38)+δ _{HNC} (35)
23	1086	2.23	1084	2.23	1120 vw	V _{NC} (61)+δ _{HNC} (17)
24	1126	0.64	1121	0.69	1149 vw	δ _{HNC} (26)+V _{NC} (23)+δ _{NCN} (13)
25	1283	0.70	1281	0.75	1345 m	δ _{HNC} (53)+V _{NC} (22)
26	1371	45.49	1369	47.01	1409 s	V _{NC} (38)+δ _{NCN} (10)
27	1442	43.15	1437	43.07	1486 s	V _{NC} (56)
28	1516	50.56	1516	47.02	1558 vs	V _{NC} (35)+δ _{HNH} (16)+δ _{HNC} (10)
29	1528	12.17	1525	11.95	1582 s	δ _{HNH} (38)+V _{NC} (14)
30	1556	13.92	1557	44.69	–	δ _{HNH} (29)
31	1563	100.00	1585	100.00	1621 vs	δ _{HNH} (66)
32	1589	41.56	–	–	1640sh	V_{NC}(35)+δ_{HOH}(87)
33	3206	72.47	3218	75.75	3099 m	V _{NH} (99)
34	3297	45.55	3297	40.87	3236 w	V _{NH} (98)
35	3309	21.02	3308	15.99	3304 w	V _{NH} (98)
36	3344	202.89	–	–	3419 vw	V_{OH}(94)
37	3385	28.03	3384	29.33	3361 w	V _{NH} (100)
38	3394	22.88	3392	22.30	3393 w	V _{NH} (99)
39	3551	33.70	–	–	3565w	V_{OH}(98)

vs: very strong; s: strong; m: medium; w: weak; vw: very weak

*V: stretching modes; δ: bending modes; Γ: torsional modes

Tables 3 and 4 show vibrational modes of VI structures, the most stable complex structures of the a 1:1 binding ratio in both solvents. Modes, bolded in the tables, are vibrations resulting from guanazole-C₂H₆OS/H₂O interactions. When Table 3 is examined, carbon-hydrogen stretching modes were calculated in the range of 2900–3040 cm⁻¹ at weak intensity. In the recorded solution spectrum, C-H vibrations are seen at 2700–2900 cm⁻¹. In the experimental spectrum of guanazole/C₂H₆OS solution, bands at 1404 and 1402 cm⁻¹ are from C₂H₆OS vibrations. In the experimental part of Table 3, 1011vs, 990m and 888m modes belong to H-C-S-C torsional vibrations and these modes are calculated at 1004, 975, and 885 cm⁻¹. The medium intensity band at 911 cm⁻¹ belongs to a S-O stretching action, whereas the 648m and 618w bands belong to S-C stretching vibrations. These

modes were calculated at 908, 655 and 621 cm⁻¹, respectively. It is surprising in Table 3 that the mode, which is the combination of H-N-C, N-C-N bending, and NC stretching vibrations, is calculated at 1024 cm⁻¹ in implicit (Model I) calculations and 1123 cm⁻¹ in explicit (Model II) calculations. Although this mode belongs to the internal vibration of guanazole, hydrogen bonding between the 12H atom of guanazole and the 18O atom of C₂H₆OS caused this shift.

In Table 4, strong O-H stretching vibrations are calculated at 3551 and 3344 cm⁻¹. These modes correspond to the bands seen at 3565 and 3419 cm⁻¹ in the experimental spectrum of the guanazole-H₂O solution. The shoulder band at 1640 cm⁻¹, which is not present in the spectrum of solid guanazole, was determined as the H-O-H angle-bending mode and this was calculated at 1589 cm⁻¹. Another band not

present in the spectrum of free guanazole is 951w. This band is in the spectrum of the solution and it was calculated at 967 cm^{-1} with medium intensity. All of these calculated modes are quite compatible with experimental values and results from the literature [25].

5. CONCLUSIONS

In this study, solvent effects and hydrogen bonding interactions on the guanazole molecule were investigated through experimental and theoretical approaches. Two conformations of guanazole were taken into account. Geometry optimizations were done using the DFT/6-311++G(d,p) method and geometric parameters of stable geometries, total energies, NBO charges and vibrational frequencies were calculated by the same level of theory. In the experimental part, $\text{C}_2\text{H}_6\text{OS}$ and aqueous solutions of the guanazole were prepared and experimental IR spectra were recorded in the mid-region. In order to describe the interaction between guanazole and solvent molecules in solutions, various models were made from experimental data, and the nature of interaction was determined. The results of the study can be summarized as follows:

- Guanazole may theoretically exist in two tautomeric structures guanazole (I) and guanazole (II). The probability of the presence of guanazole (I) is 100%.
- Seven guanazole- $\text{C}_2\text{H}_6\text{OS}$ and seven guanazole- H_2O complexes were obtained. All nitrogen atoms of guanazole were determined to be hydrogen bond acceptors and all hydrogen atoms are hydrogen bond donors. In addition, aqueous complexes of guanazole were found to form stronger hydrogen bonds compared to $\text{C}_2\text{H}_6\text{OS}$ complexes.
- Guanazole is dissolves well in $\text{C}_2\text{H}_6\text{OS}$ and H_2O and homogeneous mixtures can be obtained in both solvents, but more stable complexes form with H_2O molecules. With this property, guanazole is thought to be an ideal molecule for pharmacological purposes.
- According to the results of the spectral analysis, it was observed that new bands, which were not present in the solid phase spectrum, appeared in the IR spectrum of the aqueous solution of guanazole, and this was attributed to the formation of guanazole- $\text{C}_2\text{H}_6\text{OS}/\text{H}_2\text{O}$ complexes. Among the 1:1 interactions, intermolecular $\text{lpO}\cdots\text{H-N}$ and $\text{lpN}\cdots\text{H-O}$ interactions were determined to be the most stable structures for the complexes.

For these interactions, h-bond lengths were calculated to be about 1.78 and 1.90 Å, and interaction energies were -7.10 kJ/mol for the guanazole- $\text{C}_2\text{H}_6\text{OS}$ (V1) complex and -50.5 kJ/mol for the guanazole- H_2O (V1) complex.

- 8H, 9H and 12H atoms were the most positive atoms of the molecule and the hydrogen-bonded complexes of the $\text{O}\cdots\text{H-N}$ type formed with these atoms are more stable than the ones formed with other hydrogen atoms of the molecule.
- As expected, all physical and chemical properties of guanazole changed when passed from vacuum to solvent environment and important shifts occurred in the vibrational frequencies of guanazole.

Acknowledgements. The author would like to thank the Çankırı Karatekin University BAP department (GrantNo: FF150219B26) for financial support of this projects. A small portion of the work in this article was presented as an oral presentation at the “International Conference on Advances in Natural and Applied Sciences (ICANAS 2017)”. The authors would like to thank the reviewers for their comments that helped to improve the manuscript.

REFERENCES

- [1] J. A. Vick, E. H. Herman, Cardiovascular effects of guanazole, *Toxic. App. Pharm.*, **16**, 108–119 (1970). DOI: [https://doi.org/10.1016/0041-008X\(70\)90167-5](https://doi.org/10.1016/0041-008X(70)90167-5)
- [2] M. A. Hahn, H. R. Adamson, The disposition of the anti-tumour agent 3,5-diamino-1,2,4-triazole (guanazole) in mice, rats and dogs, *Xenobiotica*, **3**, 247–255 (1973). DOI: <https://doi.org/10.3109/00498257309151520>
- [3] C. Dave, M. A. Paul, Y. M. Rustum, Studies on the selective toxicity of guanazole in mice, *Eur. J. Cancer.*, **14**, 33–40 (1978).
- [4] C. D. Selassie, E. J. Lie, T. A. Khwaja, Synthesis and evaluation of guanazole prodrugs as antineoplastic agents, *J. Pharm. Sci.*, **70**, 1281–1283 (1981). DOI: <https://doi.org/10.1002/jps.2600701126>
- [5] W. Suter, F. Romagna, DNA repair induced by various mutagens in rat hepatocyte primary cultures measured in the presence of hydroxyurea, guanazole or aphidicolin, *Mutat. Res-fund. Mol. M.* **231**, 251–264 (1990). DOI: [https://doi.org/10.1016/0027-5107\(90\)90031-X](https://doi.org/10.1016/0027-5107(90)90031-X)
- [6] J. A. Ho, C. V. Pickens, M. P. Gamseik, O. M. Colvin, R. E. Ware, In vitro induction of fetal hemoglobin in human erythroid progenitor cells, *Exp. Hematol.*, **31**, 586–591 (2003). DOI: [https://doi.org/10.1016/S0301-472X\(03\)00086-9](https://doi.org/10.1016/S0301-472X(03)00086-9)
- [7] X. Zhao, M. R. Rosenberg, J. Wan, S. Zeng, W. Cui, Y. Xiao, Z. Li, Z. Tu, M. G. Casarotto, W. Hu, Design and synthesis of pinanamine derivatives as anti-influenza A M2 ion channel inhibitors, *Antivir. Res.*, **96**, 91–99 (2012). DOI: <https://doi.org/10.1016/j.antiviral.2012.09.001>

- [8] V. K. Kumar, G. Keresztury, T. Sundius, R. J. Xavier, Hydrogen bonding and molecular vibrations of 3,5-diamino-1,2,4-triazole, *Spectrochim. Acta*, Part A. **61**, 261–267 (2005). DOI: <https://doi.org/10.1016/j.saa.2004.03.039>
- [9] L. Guennoun, J. El Jastimi, F. Guédira, K. Marakchi, O.K. Kabbaj, A. El Hajji, S. Zaydoun, Molecular geometry and vibrational studies of 3,5-diamino-1,2,4-triazole using quantum chemical calculations and FT-IR and FT-Raman spectroscopies, *Spectrochim. Acta*, Part A. **78**, 347–353 (2011). DOI: <https://doi.org/10.1016/j.saa.2010.10.019>
- [10] A. Ray, M. Samiran, M. R. Georgina, A novel hydroxobridged cyclic tetranuclear copper(II) complex using the guanazole ligand: Synthesis, crystal structure and thermal analysis, *Inorg. Chem. Com.*, **11**, 1256–1259 (2008). DOI: <https://doi.org/10.1016/j.inoche.2008.07.010>
- [11] I. Matulková, I. Císařová, P. Němec, J. Kroupa, P. Vaněk, N. Tesařová, Organic salts of guanazole – Seeking for new materials for second harmonic generation, *J. Mol. Struct.*, **1044**, 239–247 (2013). DOI: <https://doi.org/10.1016/j.molstruc.2012.11.011>
- [12] P. Cui, L. Xiaohui, Construction of two new Zn(II)-guanazole frameworks via varying organic carboxylate ligands, *J. Mol. Struct.*, **1081**, 182–186 (2015). DOI: <https://doi.org/10.1016/j.molstruc.2014.09.081>
- [13] H. Zhao, D. Yanli, L. Haiping, Two new luminescent Zn(II) compounds constructed from guanazole and aromatic polycarboxylate ligands, *J. Mol. Struct.*, **1105**, 112–117 (2016). DOI: <https://doi.org/10.1016/j.molstruc.2015.10.046>
- [14] G. Karpińska, J. C. Dobrowolski, On tautomeric equilibria in the guanazole molecule. A DFT study, *J. Mol. Struct. THEOCHEM.*, **853**, 7–17 (2008). DOI: <https://doi.org/10.1016/j.theochem.2007.11.036>
- [15] S. Miertuš, E. Scrocco, J. Tomasi, Electrostatic interaction of a solute with a continuum. A direct utilization of AB initio molecular potentials for the prevision of solvent effects, *Chem. Phys.*, **55**, 117–129 (1981). DOI: [https://doi.org/10.1016/0301-0104\(81\)85090-2](https://doi.org/10.1016/0301-0104(81)85090-2)
- [16] B. N. Swanson, Medical use of dimethyl sulfoxide (C₂H₆OS). *Reviews in Clinical & Basic Pharmacology*, **5**, 1–33 (1985).
- [17] R. D. Dennington, T. A. Keith, J. M. Millam, *GaussView 5*, Gaussian, Inc. 2008.
- [18] Frisch, M. J. et al. *Gaussian 09*. Gaussian, Inc.: Wallingford, CT, 2009.
- [19] M. H. Jamróz, *Vibrational Energy Distribution Analysis VEDA 4*, Warsaw 2004.
- [20] N. Sundaraganesan, G. Elango, S. Sebastian, P. Subramani, Molecular structure, vibrational spectroscopic studies and analysis of 2-fluoro-5-methylbenzonitrile, *Indian J. Pure Ap. Phys.*, **47**, 481–490 (2009). DOI: <http://hdl.handle.net/123456789/5056>
- [21] S. F. Boys, F. Bernardi, The calculation of small molecular interactions by the differences of separate total energies. Some procedures with reduced errors, *Mol. Phys.*, **19**, 553–559 (1970).
- [22] M. T. Bilkan, Quantum chemical studies on solvent effects, ligand–water complexes and dimer structure of 2,2'-dipyridylamine, *Phys. Chem. Liq.*, **57**, 100–116 (2019). DOI: <https://doi.org/10.1080/00319104.2018.1423564>
- [23] G. L. Starova, O. V. Frank-Kamanetskaya, E. F. Shibanova, V. A. Lopyrev, M. G. Voronkov, V. V. Makarskii, X-ray diffraction examination of the molecular structure of guanazole (3,5-diamino-1*h*-1,2,4-triazole), *Khimiya Geterotsiklicheskikh Soedinenii*. **10**, 1422–1423 (1979).
- [24] Y. Z. Zheng, Y. Zhou, Q. Liang, D. F. Chen, R. Guo, R. C. Lai, *Hydrogen-bonding Interactions between Apigenin and Ethanol/Water: A Theoretical Study* SCI. REP-UK, **6**, 34647–34660 (2016). DOI: <https://doi.org/10.1038/srep34647>
- [25] M. T. Bilkan, Structural and spectroscopic studies on dimerization and solvent-ligand complexes of theobromine, *J. Mol. Liq.*, **238**, 523–532 (2017). DOI: <https://doi.org/10.1016/j.molliq.2017.05.051>