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ORIGINAL ARTICLE

Solvent Effects on Medicinal Structure and ^{15}N NMR Shielding of Medazepam

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KEYWORDS

1, 4-Benzodiazepines; Polarized Continuum Model (PCM); Chemical shielding; NBO analysis

ABSTRACT: The Density Functional Theory (DFT) and Tomasi's Polarized Continuum Model (PCM) were used to investigate the effects of solvent dielectric constant on the structural stability and ^{15}N NMR tensors of Medazepam (MDZ) drug. The results revealed that the structural stability of MDZ in polar protic solvents was higher than that in the polar aprotic and non-polar solvents; and its value depended on the solvent dielectric constant and its structure. so that in most cases, relative stability increased by increasing the solvent dielectric constant and the most stable structures were observed in water media at DFT level and in methanol at MP2 level. In this regard, natural bond orbital (NBO) interpretation showed that the tetravalent N_1 nucleus of diazepine ring in the MDZ structure had the highest value of negative charge and the resonance energy related to LP (1) $N_1 \rightarrow \sigma^*$ and π^* delocalizations among heteroatoms of MDZ structure in the tested solvents. The findings reported that with an increase in the solvent dielectric constant, the resonance energy related to LP (1) $N_1 \rightarrow \sigma^*$ and π^* delocalizations increased and the highest value of resonance energy was observed in water media. Furthermore, NMR results represented that the N_1 nucleus had a higher value of chemical shielding than the trivalent N_4 nucleus in all of the tested media. However, it may be concluded that by increasing the accumulation of negative charge and lone pair electrons participation of nitrogen nuclei in the resonance delocalizations, isotropic chemical shielding around them increase.

INTRODUCTION

Medazepam (Figure 1) is a member of 1, 4-benzodiazepine (BDZ) family [1]. Diazepam is one of its metabolites and its excretion product is called Oxazepam [2]. 1, 4-Benzodiazepines are a large group of chemical compounds with a wide range of pharmacological activities such as sedative-hypnotics, anxiolytics, muscle-relaxants and anticonvulsants [3–5]. BDZ consists of a seven-membered ring (diazepine ring), with nitrogen nuclei at positions 1 and 4, fused with the phenyl ring. Substitution at different positions of two rings makes a wide number of BDZ derivatives [6,7]. A substituent at position C_7 in BDZ system plays a significant role in improving the medicinal effects of benzodiazepine drugs [8]. It was reported that electron acceptor substituents increase the pharmacological activities. In all active benzodiazepine drugs, benzene or a substituted benzene ring is presented. The reported results indicate that the electron affinity of MDZ and the ionization potential of Nordazepam may be changed by substituting in both rings. The chemical hardness of Nordazepam is higher than MDZ and it has been found to follow the trend

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observed for the experimental singlet UV transition energies.

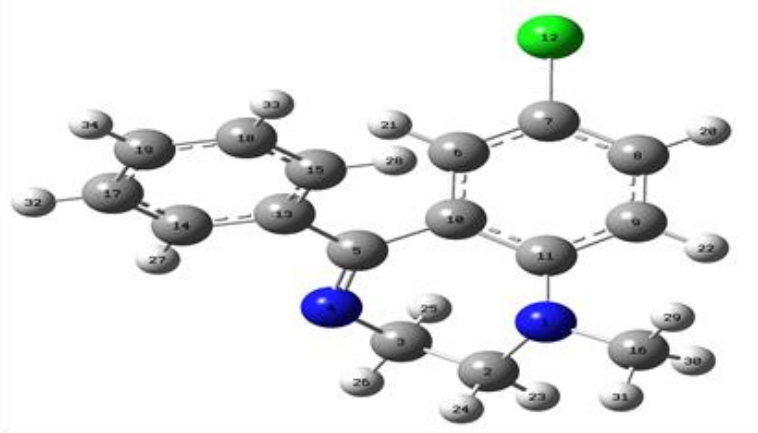

Figure 1. The optimized structure of Medazepam and atomic numbering used in this study.

Many authors have so far attempted to correlate the molecular structure of BDZs with the results of their biological tests. In this regard, a quantitative structure-activity relationship (QSAR) study was performed on more than fifty BDZs with many substituents using the CNDO/2 method and data of several different tests in vivo conditions have been analyzed [9–13]. In addition, electronic and conformational properties of twenty-one benzodiazepines were investigated using empirical and semi-empirical methods [14–16]. Their findings revealed that compounds with the high activity or very weak activity in benzodiazepine receptor (BZR) binding (such as Medazepam and Diazepam) were found to have similar conformations, thus indicating that conformational indices are not significant for receptor recognition. On the other hand, electron density, tautomerism, and aromaticity of substituted benzodiazepinones, composed of six- and seven-membered rings were investigated using the B3LYP/6-31G method by Dobrowolski et al. [17]. Moreover, Ostafin et al. studied four derivatives of 1, 4-benzodiazepine (Temazepam, Lorazepam, Lormetazepam, and Oxazepam) by ^{35}Cl NQR technique to find the correlation between biological activity and electronic structure [18]. Contrary to lots of synthetic, medical, and pharmaceutical researches devoted to obtaining Medazepam and its analogues and characterizing their potency in the treatment of different mental disorders [19–24], kinds of research have been performed to understand the structural and inter-/intra-molecular factors influencing the stability, chemical reactivity, and pharmacological activities of Medazepam and its analogs [25–27].

However, following our researches on medicinal compounds [28–30], we reported the results of the performed calculations on Medazepam in the gas phase and different solvents. The main aim of the present work was to study the microscopic effects of implicit solvent molecules and their dielectric constants on the structural stability and NMR tensors of MDZ drug using DFT methods and NBO analysis.

Computational Details

The geometrical structure of Medazepam (7-chloro-1-methyl-5-phenyl-2, 3-dihydro-1, 4-benzodiazepine) was optimized by B3LYP/6-311++G** method. The nature of stationary points for the interested structures was fixed by the imaginary frequencies. Single-point energy calculations were performed on the optimized structure using MP2/6-311++G**, MP2/6-31G**, B3LYP/cc-PVDZ, and B3LYP/6-311++G** methods. Solvent effects were only modeled on the MDZ structure by the Self-Consistent Reaction Field (SCRF) method that was based on a continuum model with uniform dielectric constant (ε). Tomasi's Polarized Continuum Model (PCM) [31] defined the cavity as a union of a series of interlocking atomic

spheres. NBO analysis was then performed at the B3LYP/6-311++G** level of theory on the optimized structure using NBO 3.1 program in the gas phase and nine solvents [32, 33]. Relative solvent effects on ^{15}N NMR shielding of MDZ structure were calculated using the corresponding nuclear shielding in the cyclohexane as reference. Direct ($\Delta\sigma_{dir}$) and indirect ($\Delta\sigma_{ind}$) solvent effects were obtained with a slight modification of the method used by Cammi et al. [34]. Instead of deriving $\Delta\sigma_{ind}$ from the difference of PCM optimized shielding and PCM shielding of molecule held at the geometry optimized in vacuum, it was obtained from the shielding calculated in vacuum for a molecule that was geometry optimized in solution. Thus,

$$\Delta\sigma_{dir} = \sigma_{sol}(R_v) - \sigma_{cyc}(R_v) \quad (1)$$

$$\Delta\sigma_{ind} = \sigma_{vac}(R_s) - \sigma_{vac}(R_{cyc}) \quad (2)$$

Where σ_{sol} (R_v) was the value of nuclear shielding in solution but with the solute geometry optimized in vacuum, and σ_{vac} (R_s) was the value of nuclear shielding in vacuum but with solute geometry optimized in solution. σ_{cyc} (R_v) and σ_{vac} (R_{cyc}) were the corresponding parameters for calculations with cyclohexane. All calculations in this study were performed using the Gaussian 03 software [35].

RESULTS AND DISCUSSION

The relative energies values (ΔE_{rel}) of Medazepam were computed at different levels of theory and solvents (Table 1). Results showed that the structural stability of MDZ in polar protic solvents was higher than that in polar aprotic and non-polar solvents. In this regard, in most cases, relative stability (based on the reduction of electronic and relative energy values) increased by increasing solvent dielectric constant and the most stable structures were observed in water media at the B3LYP level and in methanol at the MP2 level of theory. The findings showed that solvation Gibbs free energy (ΔG_{sol}) of MDZ in polar protic solvents was higher than that in polar aprotic and non-polar solvents obviously and by increasing solvent dielectric constant, ΔG_{sol} increased in most cases and the highest value of ΔG_{sol} was released in alcoholic solvents. The mentioned results were consistent with the experimental results stating that Medazepam was freely soluble in alcohol [36]. Based on the ΔE_{rel} values in different solvent media and levels of theory, it can be indicated that the structural stability of MDZ depended on solvent dielectric constant and its structure. Moreover, the solvent dielectric constant effect on the relative stability was more obvious at MP2/6-311++G**// B3LYP/6-311++G** level than other used levels of theory.

In this regard, the NBO analysis demonstrated that the tetravalent N_1 nucleus of diazepine seven-membered ring in MDZ structure had the highest value of negative charge and the resonance energy related to LP (1) $N_1 \rightarrow \sigma^*$ and π^* delocalizations among heteroatoms of MDZ structure in tested solvents (Table 2). The findings showed that with an increase in the solvent dielectric constant, the resonance energy related to LP (1) $N_1 \rightarrow \sigma^*$ and π^* delocalizations increased; while, the LP N_1 occupancy decreased in most cases and the highest value of resonance energy and the lowest value of occupancy were observed in water. NBO results were in reasonable agreement with the energy data and they could be the probable structural reasons for MDZ stability in polar protic (water and alcoholic) solvents. It was observed that with an increase in the solvent dielectric constant, resonance energies related to LP (1) $N_4 \rightarrow \sigma^*$ and π^*, and LP $Cl_{12} \rightarrow \sigma^*$, and π^* delocalizations of MDZ structure decreased while the LP (N_4) occupancy increased, and the lowest value of resonance energies and the highest occupancies were observed in alcoholic solvents (ethanol and methanol solvents).

Table 1. Calculated electronic energies (in Hartree), relative energies values (in kcal/mol), and Gibbs free energies of solvation (ΔG_{sol}) for Medazepam structure at different computational levels and dielectric constants (ε)

ε*	MP2/6-31G**// B3LYP/6-311++G**		MP2/6-311++G** //B3LYP/6-311++G**		B3LYP/CC-PVDZ //B3LYP/6-311++G**		B3LYP/6-311++G**// B3LYP/6-311++G**		
	$-E_{el}$	ΔE_{rel}	$-E_{el}$	ΔE_{rel}	$-E_{rel}$	ΔE_{rel}	$-E_{rel}$	ΔE_{rel}	ΔG_{sol}
1.00									
	1185.60257	10.4982	1185.8967	14.3700	1188.4777	7.1536	1188.6289	10.4167	-
2.02									
	1185.6075	7.4046	1185.9022	10.9187	1188.48128	4.9071	1188.6345	6.90261	-3.52
2.38									
	1185.6028	10.3539	1185.8978	13.6797	1188.4823	4.2671	1188.6343	7.0281	-0.87
4.81									
	1185.6035	9.9147	1185.8988	13.0522	1188.4851	2.5100	1188.6313	8.9106	-1.45
7.58									
	1185.6041	9.5382	1185.8995	12.6130	1188.4861	1.8825	1188.6320	8.4714	-1.98
20.7									
	1185.6071	7.6556	1185.9029	10.4794	1188.4879	0.7530	1188.6354	6.3379	-4.11
24.55									
	1185.6123	4.3926	1185.9124	4.5181	1188.48841	0.4330	1188.6354	6.3379	-13.70
32.63									
	1185.6193	0.0000	1185.9196	0.0000	1188.4887	0.2510	1188.6419	2.2590	-9.29
46.7									
	1185.6026	10.4794	1185.9021	10.9814	1188.4883	0.5020	1188.6333	7.6556	-2.61
78.39									
	1185.61090	5.2083	1185.9121	4.7063	1188.4891	0.0000	1188.6455	0.0000	-10.41

* Above values are dielectric constant for vacuum, cyclohexane, toluene, chloroform, tetrahydrofuran (THF), acetone, ethanol, methanol, dimethyl sulfoxide (DMSO) and water solvents, respectively.

Table 2. Calculated natural charges, sum of resonance energies (∑E (2) in kcal/mol) and lone pairs occupancies of Medazepam structure heteroatoms using NBO analysis and B3LYP/6-311++G** method in different dielectric constants (**ε**)

ε*	Type	NBO Parameters	charge	∑E(2)	Occupancy	ε*	Type	NBO Parameters	charge	∑E(2)	Occupancy
1.00	CL_{12}	$LPCL_{12}$	-0.00419	23.08	1.99214	20.7	CL_{12}	$LPCL_{12}$	-0.02425	23.91	1.99235
	N_1	$LP(1)N_1$	-0.52331	50.2	1.78853		N_1	$LP(1)N_1$	-0.51790	50.83	1.77906
	N_4	$LP(1)N_4$	-0.44598	23.01	1.90840		N_4	$LP(1)N_4$	-0.48317	22.38	1.91460
2.02	CL_{12}	$LP(1)CL_{12}$	-0.01223	22.66	1.99220	24.55	CL_{12}	$LP(1)CL_{12}$	-0.02670	21.6	1.99238
	N_1	$LP(1)N_1$	-0.52073	50.3	1.78541		N_1	$LP(1)N_1$	-0.52179	49.81	1.78411
	N_4	$LP(1)N_4$	-0.45545	22.86	1.90956		N_4	$LP(1)N_4$	-0.48151	22.16	1.91571
2.38	CL_{12}	$LP(1)CL_{12}$	-0.01564	22.35	1.99226	32.63	CL_{12}	$LP(1)CL_{12}$	-0.02699	21.61	1.99239
	N_1	$LP(1)N_1$	-0.5212	50.57	1.78414		N_1	$LP(1)N_1$	-0.52237	45.97	1.78454
	N_4	$LP(1)N_4$	-0.45680	22.66	1.91056		N_4	$LP(1)N_4$	-0.48299	22.12	1.91598
4.9	CL_{12}	$LP(1)CL_{12}$	-0.01947	22.22	1.99227	46.7	CL_{12}	$LP(1)CL_{12}$	-0.02452	21.97	1.99232
	N_1	$LP(1)N_1$	-0.51959	50.61	1.78199		N_1	$LP(1)N_1$	-0.51987	49.24	1.78155
	N_4	$LP(1)N_4$	-0.46617	22.61	1.91104		N_4	$LP(1)N_4$	-0.48306	22.38	1.91441
7.58	CL_{12}	$LP(1)CL_{12}$	-0.02128	23.51	1.99229	78.39	CL_{12}	$LP(1)CL_{12}$	-0.03050	21.28	1.99247
	N_1	$LP(1)N_1$	-0.51924	50.65	1.78121		N_1	$LP(1)N_1$	-0.51556	52.66	1.76932
	N_4	$LP(1)N_4$	-0.46980	22.76	1.91144		N_4	$LP(1)N_4$	-0.48740	22.30	1.91543

*Above values are dielectric constant for vacuum, cyclohexane, toluene, chloroform, tetrahydrofuran (THF), acetone, ethanol, methanol, dimethyl sulfoxide (DMSO) and water solvents, respectively.

In addition, the results indicated that with increasing solvent dielectric constant, the dipole moment of MDZ increased in most cases (Table 3) due to the incrementing solvent-solute interactions. Dipole moment was a measure of asymmetry in molecular charge distribution. Hence, MDZ had the most asymmetric charge distribution in water solution. The lack of C=O group in the molecular structure of MDZ is the reason for the low dipole moment and a very low receptor affinity [16]. In this paper, the effects of solvent dielectric constant, intramolecular interactions such as resonance ones on ^{15}N NMR shielding tensors of MDZ were investigated by B3LYP/6-311++G** method (Tables 2 and 3). The obtained results showed that ^{15}N NMR shielding tensors were drastically affected by the chemical environment, solvent dielectric constant, and its structure. Hence, their values were different for the nitrogen of diazepine ring (N_1 and N_4) in the same media so that the chemical shielding (σ_{iso}) around Cl_{12} nucleus was higher than that around nitrogen nuclei in the polar solvents and σ_{iso} around N_1 nucleus was higher than that around other considered nuclei in non-polar solvents. Furthermore, N_1 had a higher value of chemical shielding than N_4 in all tested media. However, it could be concluded that the accumulation of negative charge on N_1 and lone pair electrons participation of N_1 in resonance interactions produced the strong chemical shielding around it. Electron-donor substitution of methyl on the tetravalent nitrogen N_1 could be the probable structural reason of accumulation of negative charge at this position. The interesting thing was that by increasing solvent dielectric constant, chemical shielding around Cl_{12} increased, while it decreased around N_1 and N_4 by passing from non-polar to polar solvents.

As we know, the total solvation effect was composed of two distinct components: $\Delta\sigma_{dir}$ and $\Delta\sigma_{ind}$. The first component was directly related to the intensity of solvent reaction field used in PCM calculation; whereas the second was due to the relaxation of molecular geometry of solute brought about by solvent. The results showed that the direct solvent effect ($\Delta\sigma_{dir}$) on Cl_{12} was stronger than other considered nuclei and it was stronger on N_1 than on N_4. Besides, the findings demonstrated that as solvent dielectric constant increased, the direct solvent effect on Cl_{12} and N_1 increased and the highest values of $\Delta\sigma_{dir}$ were observed in water, while it decreased on N_4 and its lowest value was observed in water. Moreover, NMR data showed that with increasing solvent dielectric constant, the indirect solvent

Table 2. Continued.

e and nitrogen nuclei of the diazepine ring had not regular trend in most cases.

Table 3. Dipole moments (μ in Debye) and NMR parameters of ^{15}N and ^{35}Cl nuclei (isotropic chemical shielding, σ_{iso}, values of $\Delta\sigma_{dir}$ and $\Delta\sigma_{ind}$ (in ppm)) involving in Medazepam structure using the B3LYP/6-311++G** method in different dielectric constants (ε)

ε[a]	**1.00**	**2.02**	**2.38**	**4.81**	**7.58**	**20.7**	**24.55**	**32.63**	**46.7**	**78.39**
μ	2.6127 2.26[b]	3.0379	3.0175	3.4122	3.5165	3.6971	3.6971	3.3976	3.6909	4.7844
NMR tensors										
Cl_{12}										
σiso	142.646	161.884	164.955	179.064	184.518	190.647	196.506	197.145	212.754	200.522
$\Delta\sigma_{dir}$	-	0.000	3.412	15.017	19.5526	25.329	46.192	47.285	27.319	48.829
$\Delta\sigma_{ind}$	-	0.000	-0.040	-0.208	-0.2193	-0.437	-0.471	-0.567	-0.443	-0.986
N_1										
σiso	181.664	185.245	185.167	185.620	185.808	186.495	163.318	163.028	192.614	158.226
$\Delta\sigma_{dir}$	-	0.000	0.625	2.417	2.9697	3.541	-15.592	-15.591	8.148	16.264
$\Delta\sigma_{ind}$	-	0.000	-0.398	-0.925	-0.9791	-1.592	-1.147	-1.094	-1.478	-63.810
N_4										
σiso	-109.464	-122.723	-128.468	-137.492	-142.411	-151.401	-145.740	-147.566	-191.615	-149.005
$\Delta\sigma_{dir}$	-	0.000	-2.995	-14.194	-19.0289	-25.596	-21.020	-21.543	-27.970	-22.499
$\Delta\sigma_{ind}$	-	0.000	-1.827	-0.238	-0.0823	-1.499	-4.534	-4.906	-0.772	-5.430

[a] Above values are dielectric constant for vacuum, cyclohexane, toluene, chloroform, tetrahydrofuran (THF), acetone, ethanol, methanol, dimethyl sulfoxide (DMSO) and water solvents, respectively. [b] The reported value from Ref.(16).

CONCLUSIONS

This study provided a detailed scheme of performance of Tomasi's polarizable continuum model (PCM) and DFT methods in exploring effects of solvent on the structural stability and NMR tensors of MDZ structure. The results revealed a periodic and reasonable correlation among the values of electronic energies, NMR tensors, and structural parameters from NBO analysis for MDZ in different media. According to the obtained results, it could be deduced that NMR tensors of nitrogen nuclei in the diazepine ring were mainly dependent on their positions in molecular structure, solvent media, and intramolecular interactions such as resonance ones. Our findings showed that PCM could differentiate between structure, energy, and NMR tensors of a heterocyclic compound in polar and non-polar as well as aprotic and protic solvents. Nevertheless, it could not exactly recognize differences of the above parameters in the solvents with a similar structure, such as methanol and ethanol. In addition, it was observed that the direct and indirect solvent effects on heteroatoms of MDZ structure were not the same and values of $\Delta\sigma_{dir}$ and $\Delta\sigma_{ind}$ depended on the type of nuclei and their chemical positions.

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Conflict of interests

The authors declare that they have no conflict of interest.

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