Struct Chem (2014) 25:1281–1287
DOI 10.1007/s11224-014-0403-9

ORIGINAL RESEARCH

Theoretical ^{14}N and ^{17}O nuclear quadrupole resonance parameters for tirapazamine and related metabolites

Mostafa Talebi Tari · Neda Ahmadinejad

Received: 27 November 2013 / Accepted: 17 January 2014 / Published online: 15 February 2014

Abstract Density functional theory (DFT) calculations were performed to realize the effects of the N–O group on the reactivity and electronic properties of 3-amino-1,2,4-benzotriazines. The electric field gradient, EFG, tensors of ^{14}N and ^{17}O nuclei and natural bond orbital (NBO) analysis in the tirapazamine (TPZ) and its four derivatives were calculated at the B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) method in the gas phase. The NBO analysis reveal that the bond strength, proton affinity and position of N–O group in the heterocyclic ring have major influence on the reactivity of considered molecules. Accordingly, we suggest that the TPZ and 4-oxide (**d**) structures due to having a weaker N–O bond with larger negative charge on the oxygen atom at the 4-position are more active than the other ones. Calculated ^{14}N and ^{17}O EFG tensors were used to evaluate nuclear quadrupole coupling tensors, χ, and asymmetry parameters, η_Q. Results showed that oxidation of a nitrogen atom at any position have significant influence on its ^{14}N nuclear quadrupole resonance (NQR) parameters. Also, the occupancy of nitrogen lone pair plays an important role in determination of the q_{zz} and χ values of mentioned nuclei. It is found that the η_Q and χ are appropriate parameters to study the contribution of lone pair electrons of nitrogen atom in the formation of chemical bond or conjugation with the aromatic system. Finally, a linear correlation is observed between the $\chi(^{14}N)$ and $\chi(^{17}O)$ values in the N–O bond which may be associated with the reactivity of these compounds.

Keywords Tirapazamine (TPZ) · DFT calculations · NBO analysis · NQR spectroscopy

M. Talebi Tari (✉) · N. Ahmadinejad
Young Researchers and Elite Club, Shahre-Rey Branch, Islamic Azad University, Tehran, Iran
e-mail: mustafa.talebi@yahoo.com

Introduction

A key strategy in cancer treatment is to try to exploit some intrinsic difference between normal and malignant tissues. One such difference is that a large proportion of solid tumors contain cells at lower levels of oxygenation than occurs in normal tissues, which this low oxygen condition is called hypoxia [1]. Heterocyclic aromatic *N*-oxides are being evaluated with some success as hypoxia-selective cytotoxins in the clinical treatment of solid tumors [2]. As classical aromatic di-*N*-oxide derivatives, tirapazamine (**a**; 3-amino-1,2,4-benzotriazine 1,4-dioxide, TPZ) is a promising new antitumor agent that selectively damages DNA in oxygen-poor (hypoxic) tumor cells and is currently undergoing phase III clinical trials for the treatment of various cancers [3]. Tirapazamine is relatively nontoxic to normally oxygenated cells because the activated form of the drug is rapidly quenched by reaction with O_2 [4, 5]. It has been suggested that in the cell, TPZ is enzymatically reduced to the corresponding radical anion ($TPZ^{\cdot -}$), and then protonation of the TPZ radical anion produces a neutral radical, $TPZH^{\cdot}$, which is believed to fragment to form the desoxytirapazamine (dTPZ, 3-amino-1,2,4-benzotriazine *N*-oxide) and $OH^{\cdot}$ [6]. The hydroxyl radical or the other radicals derived from tirapazamine can react with a DNA sugar moiety to form a DNA-centered radical [7]. And finally, the TPZ or dTPZs may oxygenate the deoxyribose radicals, and the resulting radicals could induce DNA strand breaks [8]. Tirapazamine and its analogs (**b**; 3-amino-1,2,4-benzotriazine 1-oxide, **c**; 3-amino-1,2,4-benzotriazine 2-oxide, **d**; 3-amino-1,2,4-benzotriazine 4-oxide, **e**; 3-amino-1,2,4 benzotriazine, nor-oxide) have been shown in Fig. 1.

In the last years, several theoretical and experimental studies using various techniques have been devoted toward investigation of the unambiguous synthesis and

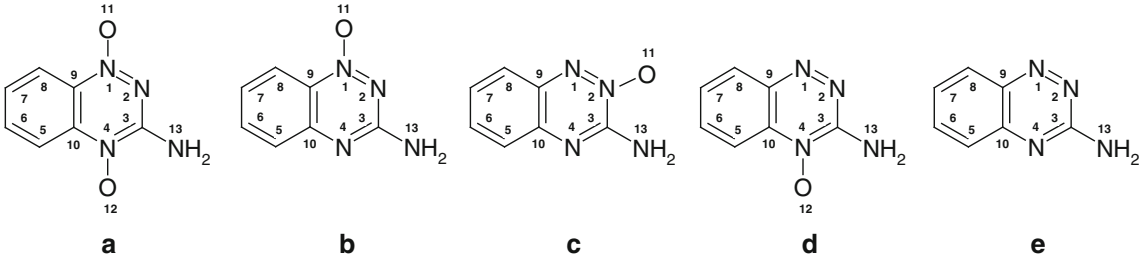

Fig. 1 The chemical structures of tirapazamine and its metabolites

characterization of tirapazamine and its known metabolites. In this letter, the structural properties and reactivity of *N*-oxides of 3-amino-1,2,4-benzotriazine are studied using density functional theory (DFT) method. Nuclear quadrupole resonance (NQR) spectroscopy along with natural bond orbital (NBO) analysis is performed to obtain useful information about the electronic structure and nuclear charge distribution around the nucleus of interest. Our results allow us to analyze the influence of oxidation at any nitrogen position of the heterocyclic ring on the reactivity of these molecules. However, we wish the physicochemical features obtained from present calculations would lead to better appreciate the mechanism of action of TPZ.

The NQR spectroscopy can serve as a useful method to study the details of electronic around the nitrogen and oxygen nuclei for tirapazamine and related analogs in solid state. Nuclei with spin angular momentum, I, greater than one half (such as ^{14}N with unit spin and ^{17}O with spin 5/2), have electric quadrupole moment, eQ, which interacts with electric field gradient tensor, EFG. The quadrupole coupling constant, QCC, calculated from NQR frequency, is proportional to charge on quadrupole nuclei and gives information on electron distribution in a molecule, whereas asymmetry parameter, η_Q, provides information on the direction and the order of chemical bonds [9].

There are two naturally occurring isotopes for nitrogen: ^{14}N with natural abundance of 99.635 % and nuclear spin $I = 1$ and ^{15}N with natural abundance of 0.365 % and $I = 1/2$. Thus, ^{14}N and ^{15}N are applied for NQR and nuclear magnetic resonance (NMR) studies, respectively [10]. Also, it has been suggested that more accurate determination of local distribution of electron density around nuclei is possible using QCCs, compared to NMR chemical shifts [11, 12]. Furthermore, the EFG at a nucleus in molecular environment is a one-electron property and can be obtained with a reasonable effort using theoretical calculations. Since, it involves only the ground state wave function, EFG calculation should be easier and faster than the calculation of the NMR chemical shifts. Therefore, theoretical efforts needed to devote to the interpretation of NQR spectroscopy are less than that of NMR spectroscopy [13, 14]. Accordingly, in order to study the effects of nitrogen atom on the structural features of TPZ and its derivatives, calculated ^{14}N NQR parameters in present theoretical work are comparable with experimental ^{15}N NMR parameters of Boyd and coworkers [15].

Computational details

The quantum chemical calculations at the level of DFT were performed on the five *N*-oxides of 3-amino-1,2,4-benzotriazine using the Gaussian 03 [16] package of program. The initial structures of them were adopted from X-ray diffraction structural data [6]. Geometry optimizations are carried out using B3LYP hybrid density functional and 6-31G(d) basis set [17, 18]. Then, NBO analysis and EFG calculations have been done at the B3LYP/6-311+G(d,p) level of theory on the optimized structures in gas phase. The natural hybrids (NHOs) and occupancies of the interested bonding and lone pair (LP) orbital, the atomic charges and also the two most important molecular orbitals, HOMO and LUMO, in these compounds were calculated using the NBO 3.1 program [19]. Also, the principal components of the EFG tensor, q_{ii}, are computed in atomic unit (1 a.u. = 9.717365×10^{21} Vm^{-2}), with $|q_{zz}| \geq |q_{yy}| \geq |q_{xx}|$ and $q_{xx} + q_{yy} + q_{zz} = 0$. These diagonal elements relate to each other by means of the asymmetry parameter: $\eta_Q = |(q_{yy} - q_{xx})/q_{zz}|$, $0 \leq \eta_Q \leq 1$, which measures the deviation of EFG tensor from axial symmetry. The computed q_{zz} component of EFG tensor is used to obtain the nuclear quadrupole coupling constant (χ_{zz}) from the equation; C_Q (MHZ) = $e^2 Q q_{zz}/h$, where "e" is the charge of electron, and "h" is the Planck's constant [20]. The standard values of Q reported by Pyykkö [21] are employed: $Q(^{17}\mathrm{O}) = 25.58$ mb and $Q(^{14}\mathrm{N}) = 20.56$ mb (1 mb = 1×10^{-31} m^2).

Results and discussion

Geometrical properties and NBO analysis

It has been found that the formation of the HO$^{\cdot}$ radicals is directly dependent on the N–O bond strength, and the

Table 1 Some selected bond distances (Å), bond angles (°), and electronic energies, E_{el}, (Hartree) values for optimized structures of tirapazamine and its metabolites at the B3LYP/6-31G(d) level of theory

Parameters	TPZ (**a**)	1-oxide (**b**)	2-oxide (**c**)	4-oxide (**d**)	nor-oxide (**e**)
N1–N2	1.326	1.318	1.300	1.310	1.294
N1–C9	1.403	1.412	1.369	1.359	1.367
N1–O11	1.250	1.243	–	–	–
N2–C3	1.341	1.369	1.443	1.347	1.386
N2–O11	–	–	1.262	–	–
C3–N4	1.367	1.323	1.298	1.372	1.323
C3–N13	1.343	1.363	1.347	1.346	1.361
N4–C10	1.378	1.358	1.368	1.377	1.355
N4–O12	1.299	–	–	1.286	–
C9–C10	1.412	1.416	1.421	1.422	1.424
N1–N2–C3	118.789	116.642	121.266	120.838	118.400
N2–C3–N4	125.167	128.280	122.790	124.020	126.847
C3–N4–C10	116.946	114.672	117.298	116.785	114.806
N4–C10–C9	119.340	122.099	119.867	117.327	120.094
C10–C9–N1	118.992	117.123	121.701	122.565	120.442
C9–N1–N2	120.764	121.172	117.067	118.462	119.395
$-E_{el}$	639.6779	564.5235	564.5175	564.5025	489.3401

amount of DNA cleavage would be largely enhanced if a compound with weaker N–O bond is obtained [2]. Table 1 presents the values of some geometric parameters for five selected aromatic *N*-oxides. It is found that the N–O bond length of these molecules decrease in the order: TPZ (the N4–O12 bond), 4-oxide (**d**), 2-oxide (**c**), TPZ (the N1–O11 bond), and 1-oxide (**b**), which is in agreement with increasing trend of the occupancy value of N–O bond for considered molecules (see Table 2). The data reported in Table 1 also indicate that in each structure, the C3–N4–C10 has the smallest bond angle value in comparison with the other internal bond angles of the heterocyclic ring. Furthermore, the oxidation of a nitrogen atom in the nor-oxide (**e**) analog causes its internal bond angle in related *N*-oxide analog increase remarkably and be close to 120°. On the other hand, the study of natural hybrid orbitals in Table 2 reveals that the 2-oxide (**c**), TPZ (the N1 position), and 1-oxide (**b**) structures possess the smaller percent of *p* character for nitrogen atom in the N–O bond, as compared with the TPZ (the N4 position) and 4-oxide (**d**) structures. This is correlated with decreasing trend of the internal bond angle of oxidized nitrogen atom for mentioned compounds (see Table 1). We suggest that the increasing *p* character at these positions lead to the displacement of the bond angles from their idealized values (bond angles of 120° for sp^2 hybridized atoms) which this angle strain may make the molecule less stable and more prone to higher reactivity. Thus, the addition of an oxygen atom to the N4 position of 3-amino-1,2,4-benzotriazine may be created a structure with greater reactivity than the other ones.

The molecular orbital (MO) theory is employed extensively to describe chemical behavior. The values for some calculated global reactivity descriptors such as HOMO–LUMO gap and the electrophilicity index of the studied molecules are tabulated in Table 3. The global electrophilicity, ω, has been calculated as $\omega = (\mu^2/2\eta)$, where μ is the chemical potential ($\mu = (E_{HOMO} + E_{LUMO})/2$), and η is the chemical hardness ($\eta = E_{LUMO} - E_{HOMO}$) [22]. It is well-known that molecules with large HOMO–LUMO gaps are generally stable and unreactive; while those with small gaps are generally reactive [23]. According to our results in this table, the value of HOMO–LUMO gap for these compounds decrease with increasing the electrophilicity value of them in the order of nor-oxide (**e**), 1-oxide (**b**), 2-oxide (**c**), 4-oxide (**d**), and TPZ. This result can be justified by the trend of electronic energy obtained from geometry optimization for mono-*N*-oxide analogs, see Table 1.

It has been proposed that the TPZ radical anion must be protonated at one of the oxygen atoms to form a species capable of generating hydroxyl radical [1, 6, 8]. The study of the atomic charges of some selected atoms in Table 4 reveals that the negative charge on the oxygen atom in 1-oxide (**b**), TPZ (the O11 atom), 2-oxide (**c**), 4-oxide (**d**), and TPZ (the O12 atom) molecules increase, respectively. This indicates that the oxygen atom at the 4-position of the heterocycle possess the highest proton affinity and,

Table 2 Calculated bonding and lone pair orbital occupancies and the natural hybrids (NHOs) for interested atoms and bonds in tirapazamine and its metabolites using NBO method at the B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) level of theory

Lewis-type NBOs	TPZ (**a**)		1-oxide (**b**)		2-oxide (**c**)		4-oxide (**d**)		nor-oxide (**e**)	
	Occu.	Hybrid	Occu.	Hybrid	Occu.	Hybrid	Occu.	Hybrid	Occu.	Hybrid
LP(1) N1	–	–	–	–	1.93011	$sp^{1.67}$	1.93866	$sp^{1.78}$	1.93946	$sp^{1.89}$
LP(1) N2	1.92044	$sp^{1.64}$	1.92704	$sp^{1.51}$	–	–	1.92682	$sp^{1.86}$	1.93666	$sp^{1.68}$
LP(1) N4	–	–	1.90913	$sp^{2.18}$	1.90241	$sp^{2.45}$	–	–	1.91357	$sp^{2.26}$
LP(1) N13	1.73372	p	1.77701	$sp^{17.81}$	1.73317	p	1.74132	p	1.77485	$sp^{19.28}$
LP(1) O11	1.98356	$sp^{0.30}$	1.98331	$sp^{0.32}$	1.98470	$sp^{0.29}$	–	–	–	–
LP(2) O11	1.90649	$sp^{99.99}$	1.91096	$sp^{99.99}$	1.90338	$sp^{99.99}$	–	–	–	–
LP(3) O11	1.54573	p	1.51434	p	1.59712	p	–	–	–	–
LP(1) O12	1.98463	$sp^{0.25}$	–	–	–	–	1.98457	$sp^{0.27}$	–	–
LP(2) O12	1.91666	$sp^{99.99}$	–	–	–	–	1.92036	$sp^{99.99}$	–	–
LP(3) O12	1.72089	p	–	–	–	–	1.66908	p	–	–
σ (N1–O11)	1.99338	51 %$sp^{2.28}$ 49 %$sp^{3.30}$	1.99366	50 %$sp^{2.33}$ 50 %$sp^{3.17}$	–	–	–	–	–	–
σ (N2–O11)	–	–	–	–	1.99319	51 %$sp^{2.23}$ 49 %$sp^{3.43}$	–	–	–	–
σ (N4–O12)	1.99123	52 %$sp^{2.42}$ 48 %$sp^{4.02}$	–	–	–	–	1.99227	51 %$sp^{2.45}$ 49 %$sp^{3.76}$	–	–

There are no data for the lone pair of oxidized nitrogens in the output files

Table 3 Calculated HOMO–LUMO gap and electrophilicity (ω) values (in eV) for tirapazamine and its metabolites at the B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) level of theory

Compounds	HOMO (eV)	LUMO (eV)	Gap	ω
TPZ (**a**)	−0.2166	−0.1109	0.1057	0.1269
1-oxide (**b**)	−0.2391	−0.0976	0.1415	0.1001
2-oxide (**c**)	−0.2387	−0.1008	0.1379	0.1044
4-oxide (**d**)	−0.2280	−0.1048	0.1231	0.1125
nor-oxide (**e**)	−0.2399	−0.0946	0.1452	0.0963

therefore, is the most likely site of proton attachment as was commented in the previous work [24]. In contrast with this trend, the positive charge on the oxidized nitrogen atom in considered molecules decreases. Therefore, the charge difference across the N–O bond, q_N–q_O, for studied compounds increases in the order: TPZ (the N4–O12 bond) < 4-oxide (**d**) < 2-oxide (**c**) < TPZ (the N1–O11 bond) < 1-oxide (**b**), which is in fair agreement with our results for the occupancy of N–O bond in that smaller occupancy orders imply weaker bonds. So, the N–O bond cleavage in the 1- and 4-positions of the tirapazamine is much easier than that in the related positions of the 1-oxide (**b**) and 4-oxide (**d**) metabolites. Another interesting feature that one may obtain from the atomic charge analysis is the charge on the heterocyclic ring, which may be associated with the reactivity of these molecules. Therefore, we observe that the TPZ and 4-oxide (**d**) molecules have a larger positive charge and consequently a larger electrophilic capacity of the heterocyclic ring as compared with the 2-oxide (**c**), 1-oxide (**b**) and nor-oxide (**e**) metabolites. This is in line with the above trend of the global electrophilicity, wherein a molecule with larger global electrophilic capacity might be indicative of a more active one.

The last section of NBO analysis focused on the natural hybrids and occupancies of the oxygen lone pairs for TPZ and its metabolites. According to our results in Table 2, we note that in each structure the occupancy value of LP(3) O (with 100 % *p* character) is smaller than that of the other oxygen lone pairs. On the other hand, the oxygen atom in the TPZ (the O12 atom) and 4-oxide (**d**) molecules has a higher degree of occupancy for the third lone pair electrons compared to those of the 2-oxide (**c**), TPZ (the O11 atom) and 1-oxide (**b**) molecules, which accords with the observed increasing trend for the negative charge on the oxygen atom. Therefore, we suggest that the third lone pair of oxygen atom can play an important role in protonation and reactivity of these molecules.

Finally, for all the above reasons, we suggest that the reactivity of tirapazamine and its metabolites is dependent on several factors; the number and position of the NO group in the heterocyclic ring, the proton affinity of oxygen atom and the N–O bond strength. As a 3-amino-1,2,4-benzotriazine compound with a weaker N–O bond at the 4-position of the heterocycle, which possesses the largest negative charge on the oxygen atom should be more active than the other ones. Hence, we expect that the reactivity

Table 4 Calculated charges of some atoms for tirapazamine and its metabolites at the B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) level of theory

Compounds	N1	N2	C3	N4	C9	C10	O11	O12	Hetrocycle[a]
TPZ (**a**)	0.2871	−0.3168	0.5610	−0.0078	0.0824	0.1586	−0.4399	−0.5907	0.7645
1-oxide (**b**)	0.3130	−0.3291	0.5749	−0.5425	0.0621	0.1811	−0.4227	–	0.2595
2-oxide (**c**)	−0.2140	0.2254	0.5726	−0.5006	0.0952	0.1472	−0.4894	–	0.3258
4-oxide (**d**)	−0.1927	−0.2786	0.5409	0.0282	0.0934	0.1478	–	−0.5570	0.3390
nor-oxide (**e**)	−0.1549	−0.2776	0.5592	−0.5085	0.0700	0.1803	–	–	−0.1315

[a] The total charge of involved atoms in the heterocyclic ring (N1, N2, C3, N4, C9, C10)

order of these structures is as follows: TPZ > 4-oxide (**d**) > 2-oxide (**c**) > 1-oxide (**b**) > nor-oxide (**e**), which is in accordance with the facts mentioned in the previous studies, wherein the lifetime of TPZ and 4-oxide (**d**) is much shorter than 1-oxide (**b**) metabolite, and hence, compared with 1-oxide (**b**) metabolite, 4-oxide (**d**) metabolite more readily undergoes enzymatic reduction to produce the final product (nor-oxide (**e**)) [6, 24, 25].

^{14}N and ^{17}O NQR parameters

The ^{14}N and ^{17}O EFG tensors and related NQR parameters (η_Q and C_Q) of the investigated heterocyclic aromatic *N*-oxides are listed in Table 5. Since homonuclear diatomic molecules (such as N_2 and O_2) have axial symmetry, the EFG tensors for these molecules are $q_{xx} = q_{yy} = -q_{zz}/2$ and so $\eta_Q = 0$. Inspection of η_Q data reported in this table indicates that the distribution of electric charge around the nitrogen nucleus is significantly deviated from cylindrical symmetry compared to that of N_2 molecule. Also, a quick look at the results reveals that oxidation at the N positions of the nor-oxide (**e**) metabolite caused the electron density distribution around the oxidized nitrogen atoms in the related *N*-oxide metabolites deviate remarkably from axial symmetry. In contrast, the asymmetry parameter of N4 in the considered molecules is approximately cylindrical, except for the TPZ and 4-oxide (**d**) molecules, which possess the oxygen atom in this position. These results can be attributed to the influence of chemical bonds or the electric field of neighboring nucleus on the quality of the charge distribution in molecule. Also, the conjugation of nitrogen lone pair with the π-system of the ring is another important factor which may change the symmetry of the EFG around a nitrogen atom. On the other hand, we noticed that the addition of an oxygen atom to the nitrogens of nor-oxide (**e**) metabolite leads to the creation of *N*-oxide derivatives with a smaller q_{zz} and χ values of nitrogen at the oxidized positions; such that in each structure, the q_{zz} and χ values of oxidized nitrogen are smaller than those of the other nitrogens. To better appreciate the electronic properties of studied structures, NBO analysis was applied to rationalize the ^{14}N NQR parameters. In this regard, we initially focused on the three nitrogen atoms (N1, N2, and N4) involved in the π-system of the heterocyclic ring. The χ value of these nitrogens in the nor-oxide (**e**) molecule decreases with decreasing the occupancy value of LP(N). This means that with the increasing contribution of the lone pair electrons of nitrogen in the conjugation with the π-system of the ring, the q_{zz} and consequently its χ value of ^{14}N nuclei decreases. Furthermore, with the exception of the oxidized nitrogens, N1 and N4 atoms possess, respectively, the largest and the smallest quadrupole coupling constant in tirapazamine and related analogs, which accords with the increasing order of occupancy values for lone pair electrons of N4, N2, and N1. Hence, the lone pair electrons of N4 are more disposed to participate in the aromatic system, and this can be attributed to the high measure of delocalization of LP(N4) in comparison with the other nitrogens of the ring. In contrast, it seems that the lone pair electrons of oxidized nitrogens in the TPZ and dTPZ molecules have insignificant contribution in the π-system of the heterocyclic ring. On the other hand, the amino group attached to the C3 position can exert some influence on the electric field of the other nucleus and also, the resonance effects between this group and the adjacent N–O bond may be effective to better understand the activity of these molecules. Analyses of the data in Tables 2 and 5 illustrate that in the investigated compounds, the q_{zz} and χ values of the amino nitrogen atom (N13) increase with increasing the occupancy value of LP(N13) in the order: 2-oxide (**c**) < TPZ < 4-oxide (**d**) < nor-oxide (**e**) < 1-oxide (**b**). Also, the percent of *p* character for lone pair electrons of N13 in 2-oxide (**c**), TPZ and 4-oxide (**d**) molecules is higher (∼100 %) than that of the other ones. The reason is due to the presence of an intramolecular hydrogen bond between a hydrogen atom of amino group and the neighboring oxygen atom, which is expressed by means of the second-order perturbation stabilization energy (E_2) for the interaction between the filled (bonding or lone pair) and empty (antibonding and Rydberg) orbitals [26]. The study of other results obtained from NBO analysis revealed that the stabilization energy of LP(2) O11 → σ^*_{N13-H} delocalization in 2-oxide (**c**) and that of LP(2) O12 → σ^*_{N13-H} delocalization in TPZ and 4-oxide (**d**) are 1.90, 1.84 and 1.40 kcal mol^{-1}, respectively. By a quick look at the present findings, we found a close relationship between the ^{14}N nuclear

Table 5 Calculated EFG tensor and NQR parameters of ^{14}N and ^{17}O nuclei for tirapazamine and its metabolites at the B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) level of theory

Compounds	Nuclei	q_{xx} (10^{21}Vm^{-2})	q_{yy} (10^{21}Vm^{-2})	q_{zz} (10^{21}Vm^{-2})	e^2Qq_{zz}/h (MHZ)	η_Q
TPZ (**a**)	N1	−0.32106	−1.88140	2.20247	1.08854	0.70845
	N2	−0.07979	−8.50512	8.58491	4.24298	0.98141
	N4	−0.93387	−4.14990	5.08376	2.51258	0.63260
	N13	−3.12561	−5.85924	8.98485	4.44064	0.30424
	O11	1.16870	21.39360	−22.56231	−13.95526	0.89640
	O12	8.01704	18.77820	−26.79520	−16.57340	0.40160
1-oxide (**b**)	N1	−0.27449	−1.13168	1.40617	0.69498	0.60959
	N2	−0.21102	−8.28033	8.49135	4.19674	0.95029
	N4	−2.41739	−4.98903	7.40642	3.66052	0.34721
	N13	−3.90332	−5.51840	9.42172	4.65656	0.17142
	O11	0.60039	21.79582	−22.39620	−13.85250	0.94638
2-oxide (**c**)	N1	−3.35006	−7.05142	10.40148	5.14079	0.35584
	N2	−0.44539	−1.88495	2.33033	1.15173	0.61774
	N4	−4.08686	−4.52653	8.61339	4.25705	0.05104
	N13	−3.08054	−5.74292	8.82345	4.36087	0.30173
	O11	2.44999	20.55162	−23.00160	−14.2270	0.78697
4-oxide (**d**)	N1	−5.30686	−6.23686	11.54371	5.70533	0.08056
	N2	−4.49642	−6.09082	10.58722	5.23259	0.15059
	N4	−0.31838	−3.23200	3.55039	1.75474	0.82064
	N13	−3.31461	−5.77523	9.08984	4.49253	0.27070
	O12	5.43224	19.80748	−25.23970	−15.61130	0.56954
nor-oxide (**e**)	N1	−4.05677	−8.75097	12.80775	6.33006	0.36651
	N2	−4.98986	−5.50167	10.49153	5.18530	0.04878
	N4	−4.17681	−4.27356	8.45037	4.17648	0.01144
	N13	−3.95353	−5.38268	9.33622	4.61430	0.15307

quadrupole coupling constant, QCC, and hydrogen bond length; such that the q_{zz} and χ values of N13 in these three molecules increase with the increasing related hydrogen bond length, N13–H···O (2.12, 2.16 and 2.20 Å for 2-oxide, TPZ and 4-oxide molecules, respectively).

In the last section of our investigation, the calculated NQR parameters of oxygen nuclei are discussed (Table 5). We noted that among the studied molecules, the highest and the lowest degree of asymmetry parameter for oxygen atom belongs to 1- and 4-positions, respectively. Also, the ^{17}O EFG calculation results illustrated that the order of negative values of both q_{zz} and χ for these compounds is as follows: 1-oxide (**b**), TPZ (the O11 atom), 2-oxide (**c**), 4-oxide (**d**), and TPZ (the O12 atom). This trend is in accordance with the observations discussed above about the atomic charge and the occupancy value of oxygen atoms. Finally, it was seen that there is an acceptable linear correlation between the $\chi(^{17}O)$ and related $\chi(^{14}N)$ values in the N–O bond. In our view, these findings may be associated with the electron attraction character and the reactivity of the oxygen position. As a main result, since the magnitude and orientation of the quadrupole coupling constant are fundamentally sensitive to the environment around the nuclei [27], this tensor along with the asymmetry parameter seems to be a helpful probe to determine the N–O position and the other interested sites of this type of aromatic *N*-oxide molecules.

Conclusions

Theoretical calculations are performed to investigate the reactivity in the tirapazamine and some of its derivatives. Results indicate that the position of N–O group in the heterocyclic ring is one of the most important factors to determine the structural features of considered structures. The NBO analysis in a good agreement with geometric, energetic, and structural properties indicates that the trend of reactivity for these compounds is: TPZ > 4-oxide (**d**) > 2-oxide (**c**) > 1-oxide (**b**) > nor-oxide (**e**). Also, we have demonstrated that the η_Q and C_Q measures of nitrogen and oxygen nucleus are appropriate parameters to study of

the electronic structure of 3-amino-1,2,4-benzotriazine *N*-oxides. It was found that the oxidation of nitrogen atom at any position of the ring caused its asymmetry parameter significantly increases. In contrast, we noticed that in each structure, the q_{zz} and χ values of oxidized nitrogen are smaller than those of the other nitrogens. Inspection of the present results illustrates that there is a logical relationship between the occupancy and *p* character of nitrogen lone pair with the ^{14}N NQR parameters. Furthermore, it was seen that the quadrupole coupling constant of ^{14}N nuclei is sensitive to the related intramolecular hydrogen bond which is expressed as the resonance energy of LP(O) → σ^*_{N-H} delocalization. Also, there is a linear correlation between the ^{17}O quadrupole coupling tensor and some of the electronic features of oxygen atom for studied molecules that might be linked to their activity. Finally, since the EFG around a nitrogen or oxygen nuclei in each position of the ring is largely different, the NQR spectroscopy can be employed as a very sensitive technique to characterize: the active sites, the resonance interactions and the N–O position in the 3-amino-1,2,4-benzotriazines. However, the theoretical methods used in this investigation are useful to supplement the information obtained from experimental measurements.

Acknowledgments The authors are highly grateful to Dr. Arezoo Tahan (Semnan Branch, Islamic Azad University) for fruitful discussions and help in initial calculations.

References

1. Brown JM (1993) Br J Cancer 67:1163–1170
2. Gomes JRB, da Silva MDMCR, da Silva MAVR (2006) Chem Phys Lett 429:18–22
3. Brown JM (1999) Cancer Res 59:5863–5870
4. Wardman P, Priyadarsini KI, Dennis MF, Everett SA, Naylor MA, Patel KB, Stratford IJ, Stratford MRL, Tracy M (1996) Br J Cancer 74:S70–S74
5. Lloyd RV, Duling DR, Rumyantseva GV, Mason RP, Bridson PK (1991) Mol Pharmacol 40:440–445
6. Fuchs T, Chowdhury G, Barnes CL, Gates KS (2001) J Org Chem 66:107–114
7. Daniels JS, Gates KS, Tronche C, Greenberg MM (1998) Chem Res Toxicol 11:1254–1257
8. Daniels JS, Gates KS (1996) J Am Chem Soc 118:3380–3385
9. Duer MJ (2002) Solid state NMR spectroscopy. Blackwell, London
10. Shirvani BB, Beheshtian J, Esrafili MD, Hadipour NL (2010) Phys B 405:1455–1460
11. Latosińska JN (2004) Chem Phys Lett 398:324–329
12. Latosińska JN (2005) J Pharm Biomed 38:577–587
13. Hemmingsen L, Ryde U (1996) J Phys Chem 100:4803–4809
14. Ha TK, Kellr MJ, Gunde R, Gunthard HH (1996) J Mol Struct (THEOCHEM) 364:161–181
15. Boyd M, Hay MP, Boyd PDW (2006) Magn Reson Chem 44:948–954
16. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Zakrzewfki VG, Montgomery JA, Stratmann RE, Burant JC, Dapprich S, Millam JM, Daniels AD, Kudin KN, Strain MC, Farkas O, Tomasi J, Barone V, Cossi M, Cammi R, Mennucci B, Pomelli C, Adamo C, Clifford F, Ochterski J, Petersson GA, Ayala PY, Cui Q, Morokuma K, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Cioslowski J, Ortiz JV, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Gomperts R, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Gonzalez C, Challacombe M, Gill PMW, Johnson BG, Chen W, Wong MW, Andres JL, Head-Gordon M, Replogle ES, Pople JA (2004) Gaussian 03, revision D.01. Gaussian, Inc., Wallingford
17. Becke AD (1988) Phys Rev A 38:3098–3100
18. Lee C, Yang W, Parr RG (1988) Phys Rev B 37:785–789
19. Glendening ED, Reed AE, Carpenter JE, Weinhold F (1998) NBO version 3.1. University of Wisconsin, Wisconsin
20. Bersohn R (1952) J Chem Phys 20:1505–1509
21. Pyykkö P (2001) Mol Phys 99:1617–1629
22. Parr RG, Szentpaly LV, Liu S (1999) J Am Chem Soc 121:1922–1924
23. Zhang G, Musgrave CB (2007) J Phys Chem A 111:1554–1561
24. Zagorevskii D, Song M, Breneman C, Yuan Y, Fuchs T, Gates KS, Greenlief CM (2003) J Am Soc Mass Spectrom 14:881–892
25. Shi X, Poole JS, Emenike I, Burdzinski G, Platz MS (2005) J Phys Chem A 109:1491–1496
26. Carpenter JE, Weinhold F (1988) J Mol Struct (THEOCHEM) 169:41–62
27. Esrafili MD, Behzadi H, Hadipour NL (2008) Biophys Chem 133:11–18