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Original article

Experimental and theoretical study on structure-tautomerism among edaravone, isoxazolone, and their heterocycles derivatives as antioxidants

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ABSTRACT

Edaravone is a heterocyclic pyrazolone compound. It has pronounced effect against free radicals, however renal and hepatic disorders have been reported. Isoxazolones are considered bioisosteric analogues of pyrazolones and may have comparable properties. Thus, we investigated the structural and electronic influences for edaravone, isoxazolone, and their tautomers on antioxidant process. Structure and tautomerism study among edaravone, isoxazolone and their heterocycles derivatives were related to antioxidant mechanisms by using the hybrid DFT method B3LYP with the basis sets 6-31++G(2d,2p). The C—H tautomer was the most stable and energetically favored among them. Intramolecular N—H—N hydrogen bonds and polar medium were responsible for the low energy differences among all possible tautomers. N—H tautomers in both systems proved to be better antioxidant by SET (single electron transfer), while O—H tautomers were better antioxidant on HAT (homolytic hydrogen atom transfer) mechanism. Theoretical calculation showed that edaravone is more potent than phenylisoxazolone, however, both has similar antioxidant scavenging on experimental DPPH. The carbonyliminic system played a very important role in the antioxidant activity for both studied classes.

1. Introduction

Heterocyclic structures are present in >68% of the drugs therapeutically used today. They are available in pharmaceutical market and used in several therapeutic action, as well as in drug discovery and development (Parlapalli and Manda, 2015). They are increasingly responsible for many advances in this area (Pitt et al., 2009).

Pyrazoles and isoxazoles are heterocyclic compounds belonging to the azol family, containing nitrogen and oxygen arranged at position 1 and 2 of the five-membered ring, which, although rare in natural products, have significantly contributed to the development of effective and alternative bioactive agents, especially replacing pyrrol and imidazole in drug design and discovery approaches (Kumar and Kaur, 2014; Taylor et al., 2016). Their oxidized derivatives, i.e. pyrazolone and isoxazolone, have different molecular properties, especially due to keto-enol or imine-enamine tautomerization, which gives it a free radical scavenging capacity that may be greater than or equal to vitamin C with less toxicity when compared to phenols (Borges et al., 2012; Brígida et al., 2013; Queiroz et al., 2010).

Among their biological effects, pyrazolones and isoxazolones have antioxidant (Dinesha et al., 2015; Padmaja et al., 2009), anti-inflammatory (Bandgar et al., 2009; Joy et al., 2018; Shivkumar and Nargund, 1998) and anticancer activities (Lv et al., 2010; Sadashiva et al., 2012). Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186, Radicut) is the representative lead of this class and has shown effectiveness against the stroke effects and amyotrophic lateral sclerosis (Watanabe et al., 2018).

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Its antioxidant mechanism is due to its relative rates in the scavenging reaction of various free radical species such as hydroxyl radical, superoxide, alkoxyl, *tert*-butylperoxyl, methyl radical and singlet oxygen, very superior when compared to uric acid, glutathione and trolox, showing high elimination measurements in all these studied species (Kamogawa and Sueishi, 2014). In addition, the edaravone protection against oxidative stress has been demonstrated in other previous studies, however some negative aspects have been reported, including renal and hepatic disorders maybe due to toxic intermediates (Lapchak and Zivin, 2009; Wang et al., 2018; Watanabe et al., 2018; Wu et al., 2014). Carvalho et al., 2017 have demonstrated the existence of two metabolic hydroxylation mechanisms of edaravone using Density Functional Theory (DFT), which the generation of hydroxy pyrazole or hydroxy aniline intermediates, attributed as responsible for the toxic properties of edaravone by redox mechanism.

In this work, the antioxidant capacity and comparative study of derivative tautomerism between pyrazolones, isoxazolones, and their heterocycles derivatives by using DFT methods were approached to establish the influence on chemical stability of tautomers and preferential antioxidant mechanism, i.e. single electron transfer (SET) and hydrogen atom transfer (HAT). These theoretical calculations were supported by DPPH (2,2-Diphenyl-1-picrylhydrazyl radical) evaluation.

2. Materials and methods

2.1. Computational details

All the calculations were performed with Gaussian 09 molecular package (Frisch et al., 2009). A conformational search was performed by using PM3 semiempirical method (Patterson, 1989; Stewart, 1989). In addition, all geometries of the tautomers a, b, and c (DXH) for edaravone or pyrazolone **1**–**3** and isoxazolone **4**–**6** derivatives (Fig. 1) and their heterocycles derivatives **7** and **8** (Fig. 2) can be exploited. The torsion angles were chosen between both rings, i.e. pyrazolone or isoxazolone and phenyl or pyridyl rings. The structures of low energies were reoptimized at B3LYP hybrid density functional theory (Becke, 1988; Lee et al., 1988), with 6-31++G(2d,2p) bases (Hehre et al., 1986). These conformations of low energies are close to planar conformations and dihedral angles between 0 and 15° θ. The restricted B3LYP functional was used for the neutral tautomers, and the unrestricted (U) B3LYP functional was applied for all cation free radicals and semiquinones. The frequencies were calculated to prove that the neutral and charged structures are minima at the level of theory.

In order to understand the mechanisms involved in the scavenging of these compounds, electronic and theoretical parameters were obtained by theoretical values involved on redox process such as the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), neutral energies (*E*), adiabatic ionization potential (AIP), bond dissociation energies (BDE_{XH}) of hydroxyl (OH), amine (NH) or methylene (CH) moieties and $Gap^{L\text{-}H}$ (LUMO-HOMO) (Horton et al., 2019). AIP was calculated as the difference between the optimized neutral molecule energy and the optimized cation energy (Eq. (1)). It was assumed that the specific equilibrium geometry of each specie do not affect the AIP values due to the minimal structural deformation on dihedral angles between both rings with the loss of an electron due to the planar conformation and wide electronic delocalization of the evaluated compounds. The bond dissociation energies (BDE) were calculated as the energy differences between a neutral molecule and the respective semiquinone plus hydrogen radical (Eq. (2)). In order to examine the reactivity of the calculated compounds, $Gap^{L\text{-}H}$ was obtained by determining the difference in energy between the LUMO and HOMO (Eq. (3)).

$$AIP = EDXH^{\cdot+} - EDXH, \quad (1)$$

$$BDE_{XH} = (EDX^{\cdot} + EH^{\cdot}) - EDXH, \quad (2)$$

$$Gap^{L-H} = ELUMO - EHOMO, \quad (3)$$

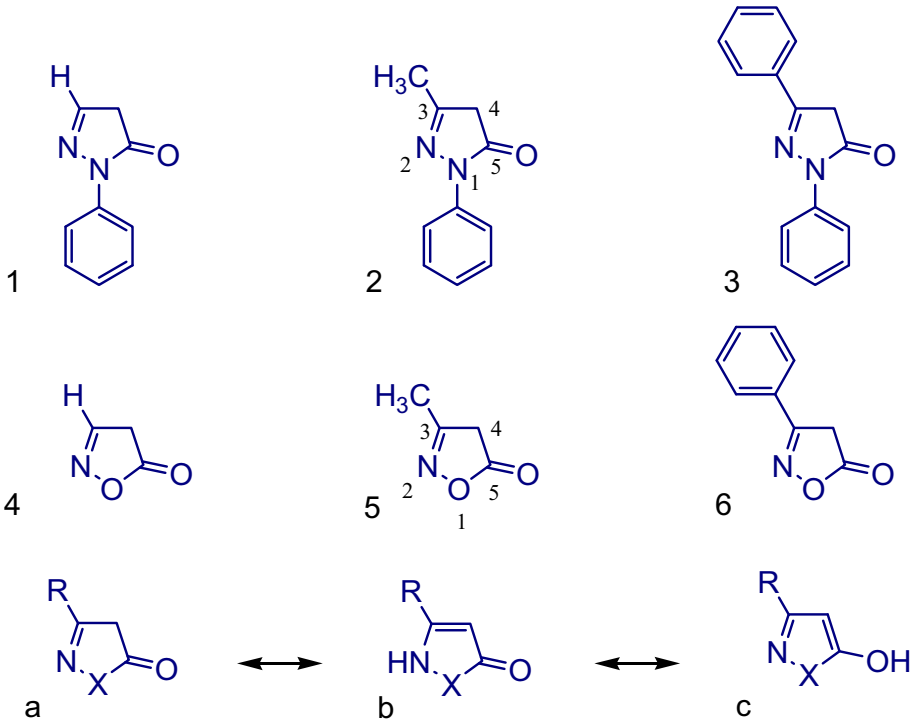

Fig. 1. Edaravone (**1**–**3**) and isoxazolone derivatives (**4**–**6**) and their tautomers (**a**-**c**), where R is **H**, **CH_3** or **C_6H_5** and X = **O** or **N**.

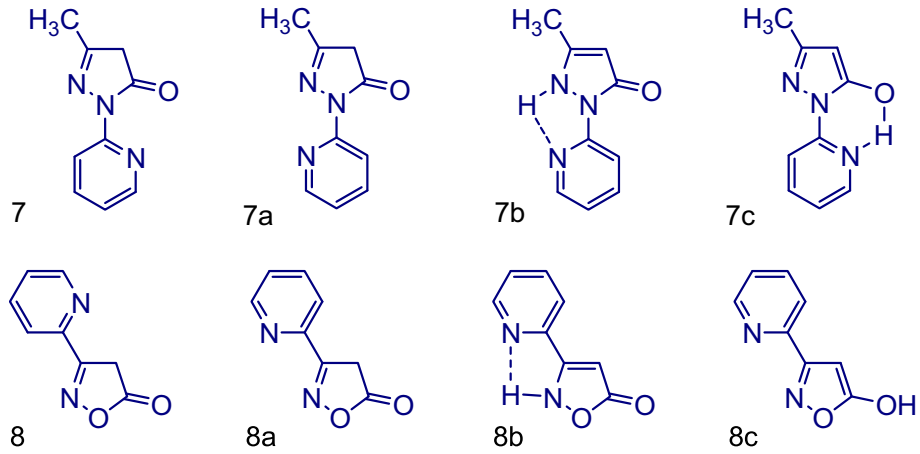

Fig. 2. Heterocycle derivatives of edaravone (**7**) and phenylisoxazolone (**8**) and their tautomers **a** (C—H), **b** (N—H) and **c** (O—H).

All calculations were performed in gas phase seeking the intrinsic properties of the studied tautomers. In addition, the solvent effect calculation was implemented in chloroform, ethanol and water by using polarizable continuum model (PCM) (Cossi and Barone, 1998; Miertuš et al., 1981) in the GAUSSIAN 09 W package.

2.2. Reagents

All chemicals, i.e. edaravone, phenylisoxazolone, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), and methanol were purchased from Sigma-Aldrich, St. Louise, MO, USA. The chemicals used were of analytical grades.

2.3. DPPH scavenging activity

A methanolic solution (25 μM) of the radical DPPH· was prepared and stored at 10 °C in the dark. All compounds were properly dissolved in methanol using the procedure described previously (Borges et al., 2013a, 2013b; Lôbo et al., 2009; Minnelli et al., 2019). Samples of different methanolic solutions of edaravone and related derivative were added to DPPH· methanolic solutions. Absorbance was recorded at 517 nm wavelength at different time intervals until the reaction reached an equilibrium. The DPPH radical absorbance without antioxidant was measured also as control and used 95% methanol as blank. All measurements were performed in triplicate. Nine different concentrations (250 at 1 μM) of each azolic derivative studied were assayed in order to check the linearity of response and to establish the values of antioxidant activity at proper linear range. Results were presented as inhibition % of the DPPH radicals.

3. Results

3.1. Tautomerism study

The relative energy differences between edaravone **1**–**3** or isoxazolone **4**–**6** tautomers and their corresponding heterocyclic analogs **7** and **8** in gas phase and under solvent effects (PCM method) were calculated and are presented in Table 1. The results demonstrated that the most stable tautomer in compounds **1**–**3** and **4**–**6** is C—H (a).

The energy differences for tautomerization was influenced by bulky substituents on position 3 on pyrazolone or isoxazolone rings, showing an increase of energy values as follows: **C_6H_5 > CH_3 > H**. The N—H form (b) is more favored than enolic (c) form (O—H). These differences decreased in polar medium (PCM), where edaravone **2** presented for N—H tautomers (b) and O—H (c) energy differences values in nonpolar solvent (chloroform) 3.44 and 6.68 kcal.mol^{-1}, in ethanol of 2.77 and 6.68 kcal.mol^{-1} and in polar solvent (water) values of 2.64 and 6.67 kcal.mol^{-1}, respectively.

Table 1
Energy differences for edaravone and isoxazolone tautomers in different media.

Tautomers	$\Delta E^{gas\ phase}$ (kcal.mol^{-1})	ΔE_3^{CHCl} (kcal.mol^{-1})	ΔE^{EtOH} (kcal.mol^{-1})	ΔE_2^{β} (kcal.mol^{-1})
1a	0	0	0	0
1b	4.14	2.43	1.70	1.56
1c	4.40	4.36	4.23	4.20
2a	0	0	0	0
2b	5.03	3.44	2.77	2.64
2c	6.30	6.68	6.68	6.67
3a	0	0	0	0
3b	5.19	3.61	2.95	2.82
3c	6.96	6.97	6.81	6.77
4a	0	0	0	0
4b	2.17	1.46	1.26	1.22
4c	6.02	6.47	6.59	6.61
5a	0	0	0	0
5b	2.96	2.31	2.16	2.14
5c	8.53	9.23	9.41	9.44
6a	0	0	0	0
6b	2.51	1.78	1.63	1.61
6c	8.08	8.36	8.33	8.32
7	0	0	0	0
7a	0	0	0	0
7b	−4.04	−3.37	−2.97	−2.88
7c	−9.04	−5.80	−4.44	−4.18
8	0	0	0	0
8a	0	0	0	0
8b	−0.88	−0.02	0.72	0.89
8c	7.73	7.79	7.66	7.63

The isoxazolonic derivatives **4**–**6** showed higher relative energy differences values when the substituents are in position 3. The following group in this order was: **CH_3 > C_6H_5 > H**. However, N—H (b) isoxazole tautomers **4**–**6** have the lowest average values when compared to the respective pyrazolone tautomers **1** to **3**. Regarding derivative **6**, the solvent effect reduced the differences of N—H or O—H tautomerization to 1.63 kcal.mol^{-1} in ethanol and 1.61 kcal.mol^{-1} in water, respectively, but the solvent effect did not show significant influence on the energy values among the O—H tautomers of the isoxazolone derivatives. These values show that N—H tautomers are favored, but their energy differences can decrease in polar medium.

Table 2
Theoretical properties of edaravone and isoxazolone tautomeric forms in gas phase calculated at the B3LYP 6-31++G(2d, 2p) level.

Tautomers	HOMO (eV)	LUMO (eV)	GAP (eV)	AIP (kcal.mol^{-1})	BDE_{XH} (kcal.mol^{-1})
1a	−6.19	−1.40	4.78	179.83	81.93
1b	−6.08	−1.20	4.88	176.48	77.93
1c	−6.32	−0.78	5.54	181.33	77.71
Average	*−6.19*	*−1.13*	*5.06*	*179.21*	*79.19*
2a	−6.03	−1.22	4.81	175.03	82.88
2b	−5.97	−1.09	4.88	172.70	78.17
2c	−6.12	−0.68	5.45	175.80	76.91
Average	*−6.04*	*−1.00*	*5.04*	*174.51*	*79.32*
3a	−5.95	−2.01	3.95	168.78	82.92
3b	−6.00	−1.88	4.12	170.25	77.96
3c	−5.90	−1.23	4.67	167.25	76.92
Average	*−5.95*	*−1.71*	*4.24*	*168.76*	*79.27*
4a	−8.05	−1.64	6.41	238.43	86.13
4b	−7.01	−1.38	5.63	205.62	84.29
4c	−6.91	−0.86	6.05	210.55	80.34
Average	*−7.33*	*−1.29*	*6.04*	*218.20*	*83.59*
5a	−7.87	−1.39	6.48	225.78	87.21
5b	−6.47	−0.74	5.73	199.20	84.77
5c	−6.73	−0.79	5.94	203.18	79.11
Average	*−7.03*	*−0.97*	*6.06*	*209.39*	*83.70*
6a	−7.11	−2.19	4.92	198.90	87.16
6b	−6.83	−2.09	4.75	193.25	84.93
6c	−6.65	−1.37	5.28	189.33	79.31
Average	*−6.86*	*−1.88*	*4.98*	*193.82*	*83.80*
7	−6.28	−1.18	5.09	181.65	83.39
7a	−6.22	−1.24	4.98	178.33	81.24
7b	−6.01	−1.32	4.68	173.32	85.95
7c	−6.06	−1.50	4.56	164.04	90.80
Average	*−6.14*	*−1.31*	*4.82*	*174.33*	*85.34*
8	−7.40	−2.42	4.98	205.84	86.60
8a	−7.35	−2.39	4.96	204.84	87.47
8b	−6.26	−2.30	3.96	189.38	87.88
8c	−6.48	−1.29	5.19	194.40	79.24
Average	*−6.87*	*−2.10*	*4.77*	*198.61*	*85.29*

Nevertheless, the changes of benzene ring to pyridinyl group at position 1 of edaravone and position 3 of the isoxazolone moiety to give **7** and **8** derivatives, respectively, and also their related isomers at **7a** and **8a** positions show different performance.

It was found that among edaravone analogues the most stable tautomers are **7b** (N—H) and **7c** (O—H) for compound **7**, due to the favoring of intramolecular hydrogen bond between the amine nitrogen at position 2 of the pyrazolone moiety and the nitrogen of the pyridinyl ring. Enolic oxygen at position 6 of the pyrazolone moiety with isopyridinil followed the same tendency. The relative energy differences are lower for enol form, favoring compound **7c** mainly in nonpolar solvents as chloroform (ΔE^{CHCl3} = −5.80 kcal.mol^{-1}) and disfavored on the phenyl ring analogue, the energy difference for formation of tautomers **7c** as well as **7b** increases in polar medium.

For the heterocyclic derivative of isoxazolone **8**, the pyridinyl ring showed an unclear tautomeric behavior. In nonpolar medium, the formation of the **8b** tautomer prevailed (N—H) (ΔE^{CHCl3} = −0.02 kcal.mol^{-1}), and with increasing solvent polarity, the most stable and favored tautomer was **8** (C—H), which is also the prevalent form in its corresponding position isomer **8a**.

These tautomers **7b**, **7c**, and **8b** are influenced mainly by intramolecular hydrogen bond on pyridyl ring followed by steric hindrance (Hatanaka, 2015). The interaction distances on tautomers **7b**, **7c**, and **8b** are 2.25, 1.76, and 2.50 Å, respectively. Therefore pyrazolone moieties are more favored than isoxazolone rings due to their fewer distances.

3.2. Electron abstraction

The antioxidant capacity by means of electron transfer among C—H (a), N—H (b) and O—H (c) tautomers for edaravone derivatives **1**–**3** and **7** and isoxazolones **4**–**6** and **8**, were theoretically predicted using HOMO and adiabatic ionization potential (AIP) values. HOMO and ionization potential values are important properties associated to nucleophilicity and single electron tansfer capacity.

Also, the HOMO topology for any molecule may show their active sites for qualitative quenching mechanism of free-radical elimination ability. The HOMO and AIP values for each tautomer studied here are shown in Table 2.

Substitution at position 3 of pyrazolone and isoxazolone rings have direct impact on electron donating capability of the **1**–**3** and **4**–**6** derivatives by increasing the HOMO values and decreasing the AIP values in the following order: **C_6H_5 > CH_3 > H**. In general, pyrazolone tautomers had higher HOMO values on gas phase than isoxazolone tautomers. The average values for the most stable tautomeric forms were: −6.19 eV for compound **1a**, −6.04 eV for **2a** and −5.95 eV for **3a**. However, on isoxazolone derivatives, compound **6** showed different behavior and its average HOMO value was −6.86 eV. This value is higher when compared to average values of **4** (−7.33 eV) and **5** (7.03 eV). The HOMO topologies (Fig. 3) of most stable tautomers show all nucleophilic positions for free radical scavenger reactions and it also highlights the influence in the electron donation capacity of heterocyclic moieties on pyrazolone and isoxazolone derivatives.

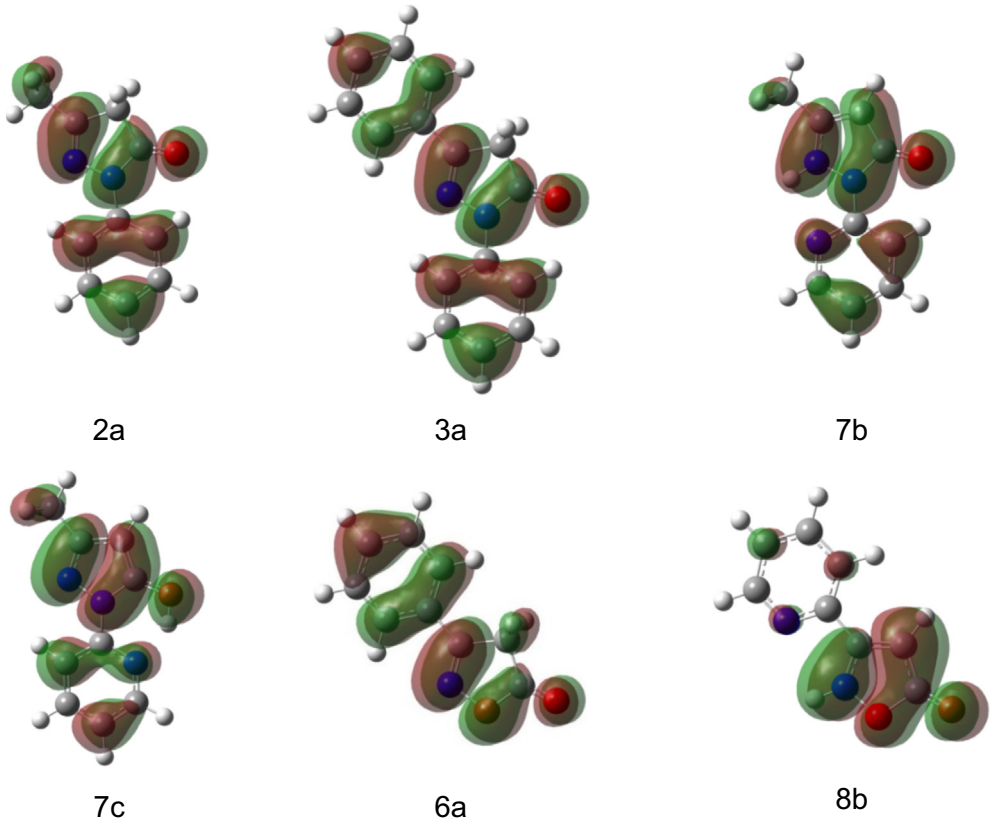

Fig. 3. HOMO topology of the most stable tautomers in gas phase calculated at the B3LYP 6-31++G(2d,2p) level.

Comparing the two rings, there was a difference in terms of hetreroatomic arrangement, between N_1-N_2 and O_1-N_2, whereby the contributions of free electron pairs of the two nitrogen increased the antioxidant capacity due to higher electron donation mechanism of the pyrazolone tautomers, as observed in edaravone **2a** and phenylisoxazolone **6a**.

From edaravone pyridinyl tautomers, the most stable forms **7b** (N—H) and **7c** (O—H), showed HOMO values of −6.01 and −6.06 eV, where hydrogen bonds amplified the resonance effect on pyrazolone moiety, increasing their electron donating ability when compared to the **7** and **7a** forms. On the contrary, the change of pyridinyl to phenyl moieties at position 3, in tautomers **6a** and **8b**, increased the resonance effects on the isoxazolone moiety.

Previous studies showed a good performance between HOMO and ionization potential (Antonczak, 2008; Lôbo et al., 2009), having similar results. The AIP values on gas phase for edaravone and isoxazolone tautomers matched the HOMO values.

Tautomers **3a** (C—H) and **7c** (O—H) had lower AIP values (168.78 and 164.04 kcal.mol^{-1}), when compared to edaravone **2** (174.51 kcal.mol^{-1}). The tautomers **6a** and **8b** are the most stable isoxazolone ones and had AIP values of 198.90 kcal.mol^{-1} and 189.38 kcal.mol^{-1}, respectively, and N—H tautomers of the compounds **1, 2, 4** and **5** showed better electron donation properties. Phenyl substitution was used in order to give the compounds the best theoretical electron donating capacity. Accordingly, in compound **3**, the most active tautomers are **3c** (O—H) and **3a** (C—H), and their AIP values are 167.25 and 168.78 kcal.mol^{-1}, respectively. On isoxazolones, the tautomer **6c** has the best electron donating capacity and the lower AIP value of 189.33 kcal.mol^{-1}.

The tautomers **7b** and **7c** are the most stable tautomeric forms when a pyridinyl ring is on position 1. They have more electron donating capacity than edaravone. Their HOMO and AIP values are −6.01 and −6.06 eV, 173.32 and 164.04 kcal.mol^{-1}, respectively. On the contrary, the pyridinyl on the isoxazolone, tautomer **8b** show the lesser capacity and the AIP value of 189.38 kcal.mol^{-1} is approximately 10 kcal.mol^{-1} less when compared to another tautomer **6a** of the same related compound.

HOMO and LUMO values are important parameters of the molecular structure related to the reactivity studied in this work. They show all nucleophilic and electrophilic regions around the molecule (Lôbo et al., 2009). The $GAP^{L\text{-}H}$ value, that is, the difference between the LUMO and HOMO values of these structures may indicate their chemical reactivity in terms of electron transfer, where the lowest values are related to the high chemical reactivity. In fact, studies show that, in general, it is possible to relate HOMO energy values and scavenging activities of compounds with antioxidant properties (Queiroz et al., 2009).

Thus, our results showed that the most reactive tautomers of edaravone derivatives **1** to **3** were the C—H and N—H forms. This result has a direct influence of the phenyl moiety on the tautomer **3a**, with the lowest GAP value. Similar tendency was observed in isoxazolone tautomers **6a** and **6b**, with the low GAP values and the phenyl group at position 3 of the heterocyclic ring. The replacement of the phenyl group by pyridinyl revealed more reactive tautomers by the low GAP values due to the available electrons in the nitrogen atom of the pyridinyl ring, especially in the isoxazolone tautomer **8b**, with a GAP value of 3.96 eV.

3.3. Hydrogen elimination

Bond dissociation energies (BDE_{XH}) for edaravone, isoxazolone, their heterocycles derivatives, and their tautomers were calculated, being X substituted with C, N and O. This value represents the easiness of hydrogen donation among tautomer derivatives **1**

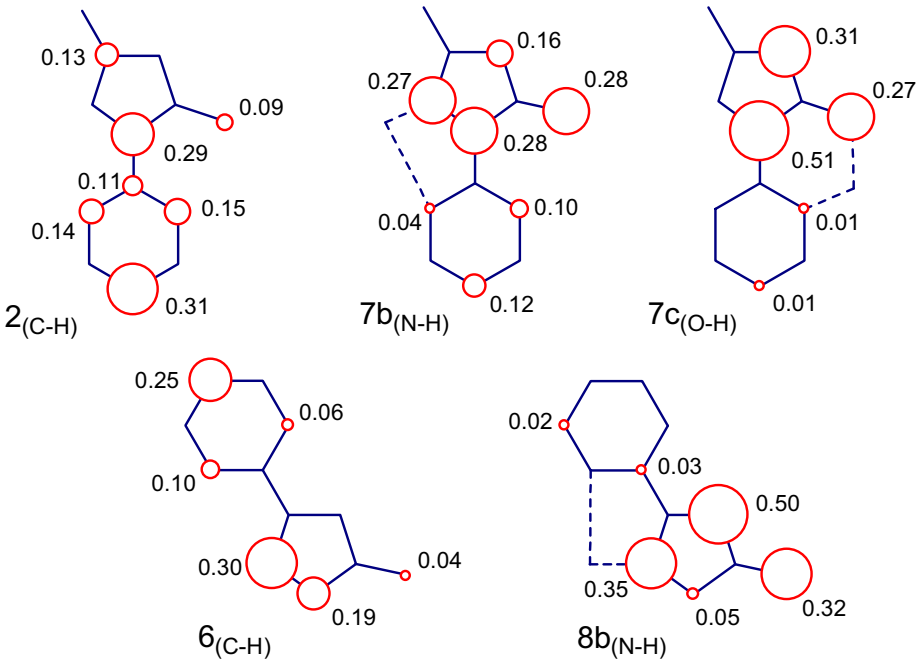

Fig. 4. Contribution of free radical cation spin density among the most stable tautomers of edaravone (**2a**), pyridinyl edaravone (**7b** and **7c**) and isoxazolones (**6a** and **8b**).

to **8** for formation of their semiquinone forms. Homolytic hydrogen atom transfer (HAT) is one of the main antioxidant mechanisms (Alves et al., 2006; Cao et al., 2003; Diniz et al., 2004; Sens et al., 2018). Therefore, molecules with lower BDE_{XH} values are more active.

According to Table 2, the best BDE_{XH} values for tautomers of edaravone derivatives **1, 2** and **3** are for O—H (enolic form), with values of 77.71, 76.91 and 76.92 kcal.mol^{-1}, respectively. For isoxazolone derivatives **4, 5** and **6**, the most active was enol tautomer in terms of hydrogen donation. Their respective values are 80.34, 79.11 and 79.31 kcal.mol^{-1}. In addition, the heterocyclic pyridinyl in edaravone tautomers **7b** and **7c** had the highest BDE_{XH} values of 85.95 and 90.80 kcal.mol^{-1}, due to hydrogen bonds, decreasing their HAT capacity.

Except for tautomer **7c**, O—H tautomers have better predisposition to perform a HAT mechanism, especially on C—H and N—H tautomers. The substitution of the phenyl ring by pyridinyl ring favor the formation of the tautomers **7b** and **7c** and increases their stability. This property is favored in polar medium. The formation of tautomer **8b** (N—H) in nonpolar medium is favored. However, tautomer **8a** (C—H) in polar medium is the most stable form. There may be some relationship between tautomers (**7** and **8**) with respect to their reactivity properties, antioxidant capacity or even an equilibrium which require more studies.

3.4. Chemical stability

Spin density contributions are related to chemical stability and depend on the number of resonance structures. It is an important parameter for the understanding of the preferential free radical scavenging mechanisms and it is often estimated as the stability of the free radicals produced after an electron or hydrogen transfer, which can be influenced by the molecular substituents that can shift their electron density to different intramolecular positions (Reis et al., 2007).

Therefore, the most stable tautomers of edaravone **2a** and isoxazolonic **6a**, as well as their respective pyridinyl tautomeric forms (**7b, 7c**, and **8b**) are shown in Fig. 4. They are generated after an electron abstraction with the respective cation radical forms. In Fig. 5, these tautomers are observed after the elimination of a hydrogen resulting in their respective semiquinone free radicals.

Spin density distributions for their cation free radical showed significant contributions from the heterocyclic rings. The spin density in **2a** (C—H) was particularly on N_1, with a value of 0.29 and a total contribution on pyrazolone ring of 0.51. A larger contribution was detected on phenyl ring at *ortho* (0.15) and *para* (0.31) positions. An increase was observed in pyridinyl, mainly on the cabonylimine system, especially in **7b** and **7c** tautomers and these results can be involved for chemical stability of cation free radical. Isoxazolone tautomer **6a**, showed a greater participation in heteroatoms O_1 and N_2. The total spin density contribution in isoxazolone moiety was 0.53, which is a lower contribution on phenyl ring at position 3, when compared to the pyridinyl ring of **8b** (N—H). The spin density contribution on isoxazolone moiety is 1.22 and a preeminent spin density contribution on carbon methylene at position 4 is 0.50.

Spin density calculations of the initial hydrogen elimination for edaravone tautomer **2a** and the isoxazolone tautomer **6a** showed a high spin density contribution on the carbonyliminic group. These values are 0.41, 0.22, 0.29 (0.92 total) and 0.53, 0.36, 0.26 (total of 1.15), respectively. These results show the important participation of these rings in the semiquinone stabilization. Isoxazolone moiety has a more spin density contribution than the pyrazolone ring. The replacement of the phenyl ring to pyridinyl ring shows that the contribution of position 4 of the heterocyclic rings had expressive impact on BDE_{CH} values for tautomeric forms **2a**, **7b**, and **7c**.

The contribution values were 0.22, 0.24 and 0.27, and the BDE_{CH} values were 82.88, 85.95, and 90.80 kcal.mol^{-1}, respectively. These values are similar to those of isoxazolone tautomers **6a** and **8b**. For these compounds, the spin density contributions were 0.36 and 0.38, and the BDE_{XH} values were 87.16 (BDE_{CH}) and 87.88 (BDE_{NH}) kcal.mol^{-1}, respectively.

3.5. Scavenging activity against DPPH

The antioxidant capacity of isoxazolone **6** was compared with edaravone **2** using the reaction with the 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) at the same concentrations in

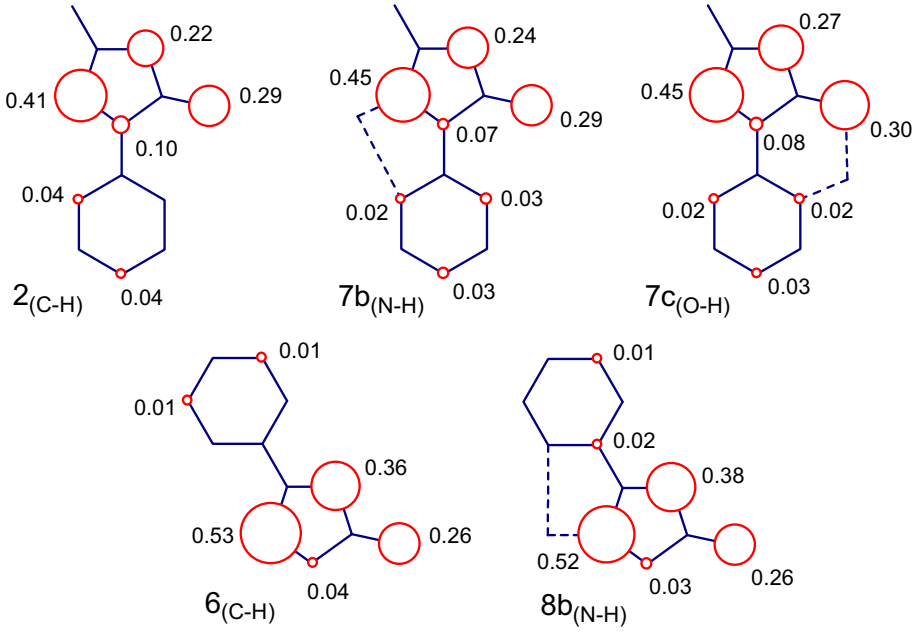

Fig. 5. Contribution of spin density of semiquinones among the most stable tautomers of edaravone (**2a**), pyridinyl edaravone (**7b** and **7c**) and isoxazolones (**6a** and **8b**).

methanolic medium. Nine dilutions at doses of 250 to 1 μM were used. The test was performed in triplicate and the results presented as the mean and standard deviations as shown in Fig. 6.

Despite the results of DPPH inhibition between the compounds edaravone (**2**) and isoxazolone (**6**) did not demonstrate statistical significance in the highest concentrations from 250 to 62.5 μM there was a close relationship in the free-radical inhibition where edaravone achieved values from 88 to 90% and isoxazolone from 78 to 82%. However, in the lowest concentrations of 16 to 4 μM, the radical inhibition was greater for the isoxazolone. Therefore, the EC_{50} was inferior to 16 μM for both edaravone (**2**) and isoxazolone (**6**). This result shows a similarity in the DPPH free-radical scavenging behavior between the phenylisoxazolone derivative and edaravone.

4. Discussion

Studies of chemical structure-anti-ischemic activity relationship showed that edaravone biological effects can be caused by its tautomeric forms (Queiroz et al., 2010). In spite of the balance between neutral and anionic forms, as well as their respective keto-enolic and imine-enamine tautomers, the tautomerization energy barriers of these molecules are reduced by the solvent effect (Queiroz et al., 2010; Watanabe et al., 2018; Zhao and Truhlar, 2008). Our study, show that edaravone derivatives with two methyl on 4-position did not show inhibitory activity on lipid peroxidation, presumably because keto-enolic and imine-enamine tautomerization was blocked (Watanabe et al., 2018).

Our theoretical study among tautomers of pyrazolone **1**–**3** or isoxazolone **4**–**6**, shows the keto or CH (a) is the most thermodynamically stable form between these two classes. Substitutions in position 3 of these heterocycles and chemical similarity have great influence on the HOMO, AIP, and BDE_{XH} energy differences. Changes in polar medium have no significant impact, even so PCM medium decreases the energy differences for the formation of the NH (b) tautometer. The tautomeric forms presented the lower values of the HOMO, AIP, and BDE_{XH} energy differences and are formed more easily in the compounds of **4**–**6** especially due to their electron transfer mechanisms in isoxazolone compounds.

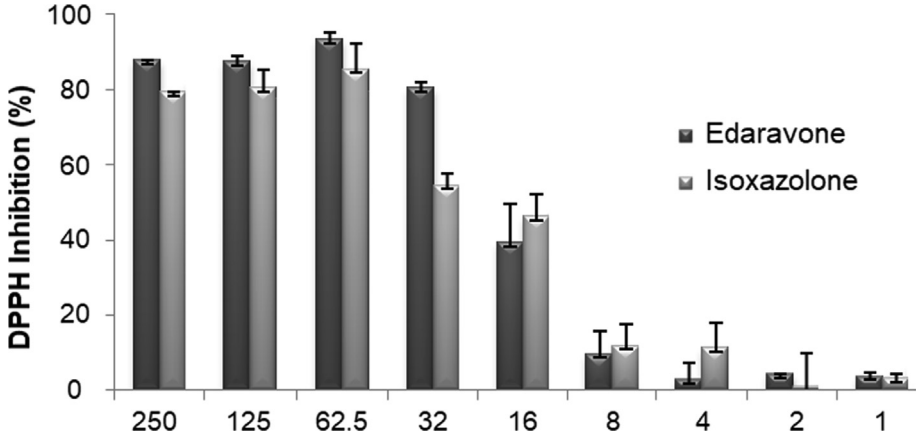

Fig. 6. Evaluation of scavenging capacity of edaravone (2) and isoxazolone (**6**) at different concentrations (250-1 μM) in methanolic solution against DPPH radical (25 μM).

A thermodynamic equilibrium between tautomers of isoxazolone **8** and edaravone **7** can be shown when the substitution on position 3 (Queiroz et al., 2010) favored intramolecular interactions. The pyridinyl ring in position 1 of the pyrazolone moiety favored hydrogen bond on N_2 when compared with the isoxazolone moiety. In fact, intramolecular bonds between heteroatoms of the pyridinyl ring and their tautomeric forms **7b** and **7c** are favored in pyrazolone, thus, the predominance of these forms is energetically favored. Tautomerism of these compounds may contribute significantly to the understanding of pharmacological and biological activities related to their antioxidant properties.

All compounds with the highest energy of HOMO and lower AIP values have potent electron donating ability. These values are in agreement with antioxidant activities (Antonczak, 2008; Lôbo et al., 2009; Queiroz et al., 2009). Any electron donating group (EDG) on position 3 of pyrazolone or isoxazolone tautomers increases HOMO values and decreases AIP values for tautomers of low energies, following the order **7c** > **3a** > **2a** for pyrazolones and **8b** > **6a** > **5a** for isoxazolones. Therefore, EDG and resonance effects can be linked to heterocyclic rings, increasing the nucleophilicity and the carbonyliminic system involvement, favoring a greater stabilization of the cation free-radical related to the HOMO topology. These properties have great impact on the antioxidant process by SET mechanism for these molecules.

All molecular substitutions on 3-position of pirazolone or isoxazolone rings by hydrogen, methyl, phenyl group and pyridinyl ring did not show significant change on BDE_{XH} values. The BDE_{CH} for edaravone **2a** of 82.88 kcal.mol^{-1} shows a difference of 4.28 kcal.mol^{-1} when compared to the more stable isoxazolone tautomer **6a** (pheniyisoxazolone) with BDE_{CH} of 87.21 kcal.mol^{-1}, showing consequently low influence on antioxidant activity by HAT mechanism. These values were confirmed by the chemical similarity and stability of the semiquinone free-radical generated after hydrogen elimination in the most stable tautomers of both classes and high contribution on carbon methylene at 4 position. Thus, the high spin density contribution on methylenic carbon at position 4 is in accordance with the lowest average BDE_{XH} values. In fact, the differences between edaravone **2** and **6** can be an important strategy for new derivatives with better antioxidant activity by HAT mechanism.

Previous studies (Lôbo et al., 2009; Borges et al., 2013a; Borges et al., 2013b) have demonstrated a direct correlation between theoretical and experimental evaluation in scavenger of free radicals. The antioxidant activity against DPPH free-radical can be directly related to the hydrogen atom transfer mechanism (HAT). Indeed, HAT calculation is a theoretical parameter to predict the antioxidant capacity for many phenolic and heterocyclic derivatives (Al-Salahi et al., 2018; Almehizia et al., 2019; Borges et al., 2013b; Xue et al., 2014). The experimental results in the DPPH free-radical scavenging showed that edaravone **2** had a higher activity at almost all evaluated concentrations and the DPPH activity was accompanied by the isoxazolone **6** mainly from the concentration of 16 μM.

Our results have showed that derivatives containing isoxazolone ring have similar antioxidant activity to edaravone. This result is confirmed to their BDE_{CH} of 87.21 and 82.88 kcal.mol^{-1}. These results match the values related of electron and hydrogen donations, since edaravone presented the lowest values for these properties. Further studies are in progress in our research group looking for a possible prediction of the structure development of new analogous of isoxazolone derivative from the edaravone.

5. Conclusions

The association between DFT methods and DPPH evaluation showed the presence of similar electronic performance between pyrazolone and isoxazolone derivatives. The tautomerism is essentially important for the choise of more related stable compounds. Keto or C—H forms were the most related stable and thermodynamically favored. The polar medium values decrease the energy differences forN—H and O—H tautomer formations. A hydrogen bond changes the preferential tautomers. The electron donating capacity was influenced by the modification in position-3. Phenyl and pyridinyl groups can provide the most potent derivatives for free-radical scavenging. N—H tautomers showed the best HOMO and AIP values by the SET mechanism. BDE_{XH} values of O—H tautomers were the forms with the best antioxidant capacity through HAT mechanism. A structural similarity was observed between pyrazolone and isoxazolone systems for SET and HAT as antioxidant mechanisms. The carbonylinic system is the essential chemical structure for the antioxidant activity of these compounds. Theoretical calculation showed that edaravone (2) is more potent than phenylisoxazolone (**6**), however, both has similar antioxidant scavenging on experimental DPPH. Thus, isoxazolone derivatives may be promising candidates as new analogue drugs of the edaravone to act as antioxidant, and perhaps anti-inflammatory, anticancer and neuroprotective agents.

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Declaration of Competing Interest

The authors declare no conflict of interest.

References

Al-Salahi, R., Anouar, E.-H., Marzouk, M., Taie, H.A., Abuelizz, H.A., 2018. Screening and evaluation of antioxidant activity of some 1, 2, 4-triazolo [1, 5-a] quinazoline derivatives. Future Med. Chem. 10, 379–390.

Almehizia, A.A., Abuelizz, H.A., Taie, H.A., ElHassane, A., Marzouk, M., Al-Salahi, R., 2019. Investigation the antioxidant activity of benzo [g] triazoloquinazolines correlated with a DFT study. Saudi Pharmac. J. 27, 133–137.

Alves, C.N., Borges, R.S., Da Silva, A.B.F., 2006. Density functional theory study of metabolic derivatives of the oxidation of paracetamol. Int. J. Quantum Chem. 106, 2617–2623.

Antonczak, S., 2008. Electronic description of four flavonoids revisited by DFT method. J. Mol. Struct. 856, 38–45.

Bandgar, B.P., Gawande, S.S., Bodade, R.G., Gawande, N.M., Khobragade, C.N., 2009. Synthesis and biological evaluation of a novel series of pyrazole chalcones as anti-inflammatory, antioxidant and antimicrobial agents. Bioorg. Med. Chem. 17, 8168–8173.

Becke, A.D., 1988. Density-functional exchange-energy approximation with correct asymptotic behavior. Phys. Rev. A. 38, 3098.

Borges, R.S., Pereira, G.A., Vale, J.K., França, L.C., Monteiro, M.C., Alves, C.N., da Silva, A.B.F., 2013a. Design and evaluation of 4-aminophenol and salicylate derivatives as free-radical scavenger. Chem. Biol. Drug Des. 81, 414–419.

Borges, R.S., Queiroz, A.N., Mendes, A.P., Araújo, S.C., França, L., Franco, E., Leal, W.G., da Silva, A.B.F., 2012. Density functional theory (DFT) study of edaravone derivatives as antioxidants. Int. J. Mol. Sci. 13, 7594–7606.

Borges, R.S., Queiroz, A.N., Silva, J.R., Mendes, A.P., Herculano, A.M., Neto, A.M.C., da Silva, A.B.F., 2013b. A combined experimental and theoretical approach for radical-scavenging activity of edaravone and its related derivatives. Struct. Chem. 24, 349–355.

Brígida, S.A.S., Orestes, E., Barros, T.G., Barros, C.A.L., Carneiro, A.S., da Silva, A.B.F., Borges, R.S., 2013. The tautomerism influence on the antioxidant prediction of oxederavone. Med. Chem. Res. 22, 5617–5623.

Cao, H., Pan, X., Li, C., Zhou, C., Deng, F., Li, T., 2003. Density functional theory calculations for resveratrol. Bioorg. Med. Chem. lett. 13, 1869–1871.

Carvalho, E.S., Santa-Brígida, S.A., Queiroz, A.N., Silva, J.R., Silva, O.P., Barros, C.A., Borges, R.S., 2017. Edaravone toxicity can be related to redox properties of their oxidized derivatives. Chem. Data Collections. 7, 51–57.

Cossi, M., Barone, V., 1998. Analytical second derivatives of the free energy in solution by polarizable continuum models. J. Chem. Phys. 109, 6246–6254.

Dinesha, Viveka, S., Priya, B.K., Pai, K.S., Naveen, S., Lokanath, N.K., Nagaraja, G.K., 2015. Synthesis and pharmacological evaluation of some new fluorine containing hydroxypyrazolines as potential anticancer and antioxidant agents. Eur. J. Med. Chem. 104, 25–32.

Diniz, J.E.M., Borges, R.S., Alves, C.N., 2004. A DFT study for paracetamol and 3, 5-disubstituted analogues. J. Mol. Struct. 673, 93–97.

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G.V., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Marenich, M., Bloino, J., Janesko, B.G., Gomperts, R., Li X, Hratchian, H.P., Ortiz, J.V., Izmaylov, A.F., Sonnenberg, J.L., Williams-Young, D., Ding, F., Lipparini, F., Egidi, F., Goings, J., Peng, B., Petrone, A., Henderson, T., Ranasinghe, D., Zakrzewski, W.G., Gao, J., Rega, N., Zheng, G., Liang, W., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, J.A.J., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E.E., Kudin, K.N., Staroverov, V.N., Keith, T., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Millam, J.M., Klene, M., Adamo, C., Cammi, R., Ochterski, J.W., Martin, R.L., Morokuma, K., Farkas, O., Foresman, J.B., Fox, D.J., Li, X., 2009. Gaussian 09, Revision A. 02, gaussian. Gaussian, Inc., Wallingford CT, 2009 200, 28.

Hatanaka, M., 2015. Role of topological charge stabilization in protomeric tautomerism. J. Phys. Chem. A 119 (6), 1074–1086.

Hehre, W., Radom, L., Schleyer, P., Pople, J., 1986. Ab Initio Molecular Orbital Theory. John Wiley and Sons.

Horton, W., Peerannawar, S., Török, B., Török, M., 2019. Theoretical and experimental analysis of the antioxidant features of substituted phenol and aniline model compounds. Struct. Chem. 30, 23–35.

Joy, M., Elrashedy, A.A., Mathew, B., Pillay, A.S., Mathews, A., Dev, S., Soliman, M.E.S., Sudarsanakumar, C., 2018. Discovery of new class of methoxy carrying isoxazole derivatives as COX-II inhibitors: Investigation of a detailed molecular dynamics study. J. Mol. Struct. 1157, 19–28.

Kamogawa, E., Sueishi, Y., 2014. A multiple free-radical scavenging (MULTIS) study on the antioxidant capacity of a neuroprotective drug, edaravone as compared with uric acid, glutathione, and trolox. Bioorg. Med. Chem. Lett. 24, 1376–1379.

Kumar, V., Kaur, K., 2014. Triazole and oxadiazole containing natural products: a review. The Nat. Prod. J. 4, 115–130.

Lapchak, P.A., Zivin, J.A., 2009. The lipophilic multifunctional antioxidant edaravone (radicut) improves behavior following embolic strokes in rabbits: a combination therapy study with tissue plasminogen activator. Exp. Neurol. 215, 95–100.

Lee, C., Yang, W., Parr, R.G., 1988. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys. Rev. B. 37, 785.

Lôbo, L.T., Silva, G.A.d., Ferreira, M., Silva, M.N.d., Santos, A.S., Arruda, A.C., Guilhon, G., Santos, L.S., Borges, R.d.S., Arruda, M.S.P., 2009. Dihydroflavonols from the leaves of Derris urucu (Leguminosae): structural elucidation and DPPH radical-scavenging activity. J. Braz. Chem. Soc. 20, 1077–1081.

Lv, P.-C., Li, H.-Q., Sun, J., Zhou, Y., Zhu, H.-L., 2010. Synthesis and biological evaluation of pyrazole derivatives containing thiourea skeleton as anticancer agents. Bioorg. Med. Chem. 18, 4606–4614.

Miertuš, S., Scrocco, E., Tomasi, J., 1981. Electrostatic interaction of a solute with a continuum. A direct utilizaion of AB initio molecular potentials for the prevision of solvent effects. Chem. Phys. 55, 117–129.

Minnelli, C., Laudadio, E., Galeazzi, R., Rusciano, D., Armeni, T., Stipa, P., Cantarini, M., Mobbili, G., 2019. Synthesis, characterization and antioxidant properties of a new lipophilic derivative of edaravone. Antioxidants 8, 258.

Padmaja, A., Payani, T., Reddy, G.D., Padmavathi, V., 2009. Synthesis, antimicrobial and antioxidant activities of substituted pyrazoles, isoxazoles, pyrimidine and thioxopyrimidine derivatives. Eur. J. Med. Chem. 44, 4557–4566.

Parlapalli, A., Manda, S., 2015. In vitro antioxidant activity of novel 4-hydroxy-1-methyl-3-(3-substituted)-4, 5-dihydroisoxazol-5-yl) quinolin-2 (1H)-One derivatives. Pharmacophore Int. Res. J. 6, 243–248.

Patterson, J.D., 1989. Density-functional theory of atoms and molecules: Robert G. Parr and Weitao Yang. Oxford University Press, New York, and Clarendon Press, Oxford (1989). Pergamon.

Pitt, W.R., Parry, D.M., Perry, B.G., Groom, C.R., 2009. Heteroaromatic rings of the future. J. Med. Chem. 52, 2952–2963.

Queiroz, A.N., Gomes, B.A., Moraes Jr, W.M., Borges, R.S., 2009. A theoretical antioxidant pharmacophore for resveratrol. Eur. J. Med. Chem. 44, 1644–1649.

Queiroz, A.N., Mendes, A.P., Leal, M.S., Chaves Neto, A.M., Borges, R.S., 2010. Tautomerism and radical-scavenging activity of edaravone by DFT methods. J. Comput. Theor. Nanosci. 7, 153–156.

Reis, M., Lobato, B., Lameira, J., Santos, A.S., Alves, C.N., 2007. A theoretical study of phenolic compounds with antioxidant properties. Eur. J. Med. Chem. 42, 440–446.

Sadashiva, M.P., Basappa, NanjundaSwamy, S., Li, F., Manu, K.A., Sengottuvelan, M., Prasanna, D.S., Anilkumar, N.C., Sethi, G., Sugahara, K., Rangappa, K.S., 2012. Anti-cancer activity of novel dibenzo[b,f]azepine tethered isoxazoline derivatives. BMC Chem. Biol. 12, 5.

Sens, L., Oliveira, A.S.d., Mascarello, A., Brighente, I., Yunes, R.A., Nunes, R.J., 2018. Synthesis, Antioxidant activity, acetylcholinesterase inhibition and quantum studies of thiosemicarbazones. J. Braz. Chem. Soc. 29, 343–352.

Shivkumar, B., Nargund, L., 1998. Synthesis, characterization and anti-inflammatory activity of some isoxazoline derivatives. Ind. J. Het. Chem. 8, 27.

Stewart, J.J., 1989. Optimization of parameters for semiempirical methods II. Applications. J. Comput. Chem. 10, 221–264.

Taylor, A.P., Robinson, R.P., Fobian, Y.M., Blakemore, D.C., Jones, L.H., Fadeyi, O., 2016. Modern advances in heterocyclic chemistry in drug discovery. Org Biomol Chem. 14, 6611–6637.

Wang, J., Chen, X., Yuan, B., Wang, W., Xu, C., Zhao, W., Zhao, P., Wang, Y., Zhao, X., Wang, Y., 2018. Bioavailability of Edaravone Sublingual Tablet Versus Intravenous Infusion in Healthy Male Volunteers. Clin. Ther. 40, 1683–1691.

Watanabe, K., Tanaka, M., Yuki, S., Hirai, M., Yamamoto, Y., 2018. How is edaravone effective against acute ischemic stroke and amyotrophic lateral sclerosis?. J. Clin. Biochem. Nutr., 17–62

Wu, H.-Y., Tang, Y., Gao, L.-Y., Sun, W.-X., Hua, Y., Yang, S.-B., Zhang, Z.-P., Liao, G.-Y., Zhou, Q.-G., Luo, C.-X., 2014. The synergetic effect of edaravone and borneol in the rat model of ischemic stroke. Eur. J. Pharmacol. 740, 522–531.

Xue, Y., Zheng, Y., An, L., Dou, Y., Liu, Y., 2014. Density functional theory study of the structure–antioxidant activity of polyphenolic deoxybenzoins. Food Chem. 151, 198–206.

Zhao, Y., Truhlar, D.G., 2008. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. Theor. Chem. Acc. 120, 215–241.