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Quantum Chemical and Biochemical Study on Antioxidant Properties of Halogenated Boroxine $K_2[B_3O_3F_4OH]$

Maja Marasović,[1] Tea Marasović,[2,*] Borivoj Galić,[3] Mladen Miloš[1]

[1] Faculty of Chemistry and Technology, University of Split, Ruđera Boškovića 35, 21000 Split, Croatia

[2] Faculty of Electrical Engineering, Mechanical Engineering and Naval Architecture, University of Split, Ruđera Boškovića 32, 21000 Split, Croatia

[3] Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author's e-mail address: tmarasov@fesb.hr

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Abstract: The boron heterocyclic compound dipottasium trioxohydroxytetraflourotriborate, $K_2[B_3O_3F_4OH]$ has been listed as a promising new therapeutic for the epidermal changes treatment. In order to elucidate its free radical scavenging activity, several appropriate thermodynamic molecular descriptors were calculated with the help of quantum-chemistry methods and their values were compared with the data obtained for ascorbic acid, trimethlyboroxine and trimethoxyboroxine. Considering the results, it may be suggested that the single electron transfer followed by proton transfer (SET-PT) is more favourable reaction pathway than hydrogen atom transfer (HAT) for the halogenated boroxine $K_2[B_3O_3F_4OH]$. Experimental support is provided by evaluating the *in-vitro* antioxidant activity of the investigated compounds in terms of their ferric-reducing antioxidant power (FRAP). Our study reveals that all three examined boroxines are extremely weak antioxidants.

Keywords: halogenated boroxine, molecular modelling, antioxidant activity, reaction mechanism, FRAP.

INTRODUCTION

THE interest in boron-containing compounds has spurred over the last 20 years. Boron has an empty p-orbital, which makes it highly reactive toward nucleophiles, in the attempt to attain a stable octet configuration. Particularly, boronic acids have been studied due to their low molecular masses, their thermal stability, low toxicity, mild acidity and inertness to water and oxygen.[1] They act as excellent reactants and exhibit strong bioactivity. Boronic acids form several derivates that remain relatively unexplored. Boroxines are boronic acid anhydrides, consisting of 6-membered, heterocyclic compounds composed of alternating oxygen and singly hydrogenated boron atoms.[2] Due to their unique electron configurations, boroxines react readily with Lewis basis and are potentially selective enzyme inhibitors. They have the ability to bind to the active sites of enzymes and thus, prevent the catalytic reactions.[3] Previous *in vivo* and *in vitro* studies have reviled that boronic acids and their derivatives possess anticancer, antibacterial and antiviral properties.[4–6] It is known that modified dipeptidyl boronic acid is present as trimeric boroxine in a chemoterapeutic agent for treatment of multiple myeloma, bortezomib (Velacade®).

Recently, halogenated boroxine (dipotassium trioxohydroxytetrafluorotriborate, $K_2[B_3O_3F_4OH]$) has attacked much attention as a promising novel therapeutic for prevention and/or treatment of benign and malignant skin lesions.[7,8] Furthermore, this compound displays some interesting properties that open up the possibility of its application in future conventional, medical, dermatological and cosmetic formulations. Halogenated boroxine $K_2[B_3O_3F_4OH]$ is highly soluble in water, which could facilitate the production of pharmaceutical compositions. This solubility contributes to its high bioavailability, with effective absorption at the site of administration on the skin. Haverić *et al.* have examined the cytotoxic, genotoxic and

cytostatic effects of $K_2[B_3O_3F_4OH]$ on human lymphocyte cultures and antiproliferative effect on basal cell carcinoma culture.[9] A different group of authors analysed the *in vivo* genotoxic effects of $K_2[B_3O_3F_4OH]$ on BALB/c mice – an inbred albino research mouse strain[10] – by applying reticulocytes micronucleus assay.[11] Both of these studies have confirmed that exposure to $K_2[B_3O_3F_4OH]$ in tested concentrations is not harmful to human or mammalian health. Few other studies addressed the impact of $K_2[B_3O_3F_4OH]$ on inhibition of enzymes associated with hypothesized anticancer properties.[12] It has been reported that halogenated boroxine $K_2[B_3O_3F_4OH]$ inhibits catalase activity and human carbonic anhydrases.[13,14] Ivanković *et al.* have determined that $K_2[B_3O_3F_4OH]$ has a strong *in vitro* and *in vivo* antitumor activity, comparable to that of well-known anticancer drug, 5-fluorouracil.[15] In addition, Pojskić *et al.* observed that treatment with $K_2[B_3O_3F_4OH]$ induces a significant decrease of cell viability in melanoma cell line at concentrations of 0.1 and 1 mM and causes deregulation of more than 30 genes known as common antitumor drug targets.[16]

This paper estimates the antioxidant activity of the halogen boroxine $K_2[B_3O_3F_4OH]$ (DPTFTB). To the best of our knowledge, this is the first study dealing with radical-scavenging potential of the specified compound. In the absence of knowledge about the action mechanism of $K_2[B_3O_3F_4OH]$ as free radical scavenger, we have decided to study it using both theoretical and experimental approach. The theoretical framework comprises several descriptors estimated with the help of quantum-chemical calculations. The computed values are compared with the data obtained for the well-known antioxidant - namely, the ascorbic acid (ASCACD) - and the two relatively simple, commercially available boroxines, trimethylboroxine (TMHYLB) and trimethoxyboroxine (TMOXYB). Based on the results of comparison, we draw conclusions about the radical scavenging / antioxidant activity of the halogenated boroxine $K_2[B_3O_3F_4OH]$ and relate them to the experimental results produced using feric-reducing antioxidant potential (FRAP) assay.

EXPERIMENTAL

Theoretical Approach

Antioxidants can deactivate free radicals through three main reaction mechanisms: direct hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT or ET-PT) and sequential proton loss electron transfer (SPLET).[17–19]

In the HAT mechanism, the hydrogen atom of OH group is transferred from antioxidant to active radical, which is neutralized, while the antioxidant itself becomes a radical:

$$A\cdots OH \rightarrow A\cdots O^{\bullet} + H^{\bullet} \quad (1)$$

The SET-PT mechanism is a two-step reaction. The first step is the electron transfer from antioxidant to active radical, resulting in the formation of a radical cation and an anion. The electron transfer is followed by a proton transfer from radical cation to anion, producing the corresponding neutral radical:

$$A\cdots OH \; -e^{-} \rightarrow A\cdots OH^{+} - H^{+} \rightarrow A\cdots O^{\bullet} \quad (2)$$

The probability of SET-PT mechanism depends on the antioxidant reduction capability, which cannot be unambiguously determined on purely chemical basis.

The SPLET mechanism occurs when the antioxidant anion is formed upon losing the proton from neutral moiety. The second step is an electron transfer from the resulting anion to the active radical, producing a neutral molecule and an antioxidant radical:

$$A\cdots OH \; -H^{+} \rightarrow A\cdots O^{-} - e^{-} \rightarrow A\cdots O^{\bullet} \quad (3)$$

The HAT and SPLET mechanism are only feasible when a dissociable hydrogen atom (or, respectively, a proton) is present. Note that all of the aforementioned mechanisms address only the formation of the stable radical intermediate, A–O• and do not account for its later fate that can involve any number of subsequent transformations.

The ideal chemical structures to scavenge free radicals combine the tendency to donate a hydrogen atom or an electron from the hydroxyl groups of the compound with the expanded ring system to delocalize an unpaired

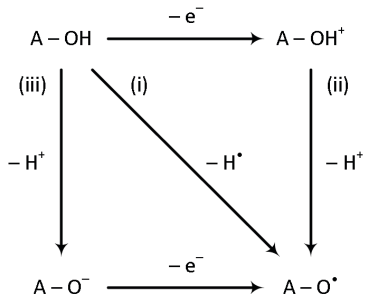

Figure 1. The main radical scavenging reaction mechanisms: (i) HAT, (ii) SET-PT and (iii) SPLET.

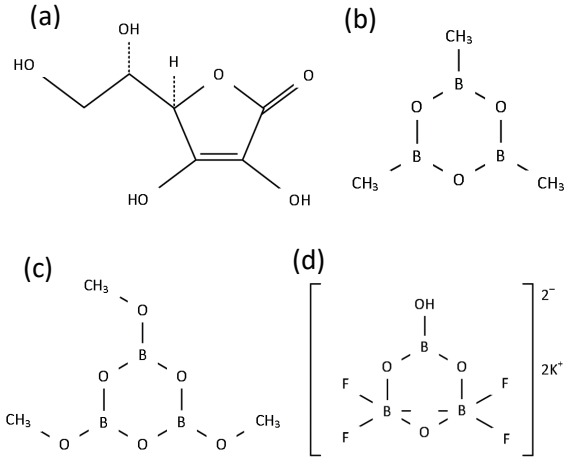

Figure 2. Molecular structures of tested compounds: (a) ascorbic acid, (b) trimethlyboroxine, (c) trimethoxyboroxine, (d) dipotassium trioxohydroxytetrafluorotriborate.

Table 1. Chemical and physical properties of the selected compounds.

Property	ASCACD	TMHYLB	tMOXYB	DPTFTB
Formula	$C_6H_8O_6$	$C_3H_9B_3O_3$	$C_3H_9B_3O_6$	$K_2[B_3O_3F_4OH]$
Form	powder	liquid	liquid	powder
Colour	white	colourless	colourless	white
Molar mass / g mol^{-1}	176.12	125.53	173.50	251.60
Density / g cm^{-3}	1.694	0.898	1.195	unknown
Melting point / °C	193	–38	10	unknown
Solubility	water, ethanol	water, ethanol	water, ethanol	water
Commercial available	yes	yes	yes	no

electron.[20] Based on its structure, as shown in Figure 2, it is obvious that $K_2[B_3O_3F_4OH]$ meets both of these demands, which leads us to assume that it might have a strong antioxidant potential. The other two boroxines included in this study - trimethylboroxine and trimethoxyboroxine - do not possess a hydroxyl group and in their case, providing that they do act as antioxidants, the reaction mechanism is most likely carried out through formation of the unstable carbanion intermediate. The boron atoms in these compounds form three single, stable, covalent bonds with three valence electrons and as such are not expected to participate in the electron transfer. Halogenated boroxine $K_2[B_3O_3F_4OH]$ molecule displays some unique behaviour due to the existence of 4 severely electronegative fluorine atoms bounded to the two boron atoms. To form these bonds, boron atoms presumably need to activate their valence electrons from the inner shell, close to the nucleus. This phenomenon affects the number and the delocalization of unpaired electrons over the entire molecule and results in the local bond configuration change from trigonal sp^2 to tetrahedral sp^3 hybridization.

Energy requirement computations for each mechanism may help us identify which radical scavenging mechanism, responsible for the antioxidant reaction, is preferable under certain conditions. The HAT mechanism is characterized by the homolytic bond dissociation enthalpy (BDE) of the OH group. A lower BDE values is usually attributed to a greater ability of the hydroxyl group to donate a hydrogen atom which indicates better antioxidant properties. BDE can be calculated using the following equation:

$$\mathrm{BDE} = H(\mathrm{A}\cdots\mathrm{O}^{\bullet}) + H(\mathrm{H}^{\bullet}) - H(\mathrm{A}\cdots\mathrm{OH}) \qquad (4)$$

where $H(\mathrm{A}\cdots\mathrm{O}^{\bullet})$ is the enthalpy of the radical, $H(\mathrm{H}^{\bullet})$ is the enthalpy of the hydrogen atom and $H(\mathrm{A}\cdots\mathrm{OH})$ is the enthalpy of the neutral compound.

Thermodynamical parameters that describe SET-PT mechanism are adiabatic ionisation potential (AIP) in the first step and proton dissociation enthalpy (PDE) in the second step. The molecules with low AIP and PDE values are more susceptible to ionization and have stronger antioxidant properties. These parameters can be computed as follows:

$$\mathrm{IP} = H(\mathrm{A}\cdots\mathrm{OH}^{+}) + H(\mathrm{e}^{-}) - H(\mathrm{A}\cdots\mathrm{OH}) \qquad (5)$$

where $H(\mathrm{A}\cdots\mathrm{OH}^{+})$ is the enthalpy of the radical cation, $H(\mathrm{e}^{-})$ is the enthalpy of the electron and $H(\mathrm{A}\cdots\mathrm{OH})$ is the enthalpy of the neutral compound.

$$\mathrm{PDE} = H(\mathrm{A}\cdots\mathrm{O}^{\bullet}) + H(\mathrm{H}^{+}) - H(\mathrm{A}\cdots\mathrm{OH}^{+}) \qquad (6)$$

where $H(\mathrm{A}\cdots\mathrm{O}^{\bullet})$ is the enthalpy of the radical, $H(\mathrm{H}^{+})$ is the enthalpy of the proton and $H(\mathrm{A}\cdots\mathrm{OH}^{+})$ is the enthalpy of the radical cation.

Finally, proton affinity (PA) and electron transfer enthalpy (ETE) are quantitative descriptors related to SPLET mechanism:

$$\mathrm{PA} = H(\mathrm{A}\cdots\mathrm{O}^{-}) + H(\mathrm{H}^{+}) - H(\mathrm{A}\cdots\mathrm{OH}) \qquad (7)$$

where $H(\mathrm{A}\cdots\mathrm{O}^{-})$ is the enthalpy of the anion, $H(\mathrm{H}^{+})$ is the enthalpy of the proton and $H(\mathrm{A}\cdots\mathrm{OH})$ is the enthalpy of the neutral compound.

$$\mathrm{ETE} = H(\mathrm{A}\cdots\mathrm{O}^{\bullet}) + H(\mathrm{e}^{-}) - H(\mathrm{A}\cdots\mathrm{O}^{-}) \quad (8)$$

where $H(\mathrm{A}\cdots\mathrm{O}^{\bullet})$ is the enthalpy of the radical, $H(\mathrm{e}^{-})$ is the enthalpy of the electron and $H(\mathrm{A}\cdots\mathrm{O}^{-})$ is the enthalpy of the anion.

Apart from these descriptors, the other informative quantities that are more closely associated with electron-donor capabilities of the molecule are HOMO and LUMO energies. E_{HOMO} is the energy of the highest occupied molecular orbital. It represents the initial energy required to release an electron from the compound. The compounds with higher HOMO energy values have better ability to donate an electron during interaction with free radicals.[21,22] In other words, the compound has a positive relation with the antioxidant activity. E_{LUMO} is the ionization energy of the lowest unoccupied molecular orbital, *i.e.* the amount of energy launched when an electron is absorbed by a molecule. The energy difference between the HOMO and LUMO orbital determines the chemical reactivity of the molecule. The larger the gap between HOMO and LUMO orbital, the more kinetically stable and less chemically active antioxidant the compound is.

The theoretical values were obtained using the Spartan program package.[23] All the examined compounds were drawn and pre-optimized by applying the molecular mechanic method using MM^{+} force field.[24] The optimization of the geometry was adopted using the semi-empiric method AM1 (Austin Model 1).[25] The options designated in the optimization process - such as total charge and multiplicity - were as follows: charge 0 and singlet for closed shell species, charge 0 and doublet for radical species, charge –1 and singlet for the anionic species and charge 1 and doublet for the cationic species.[26] Due to its unique structure, the $K_2[B_3O_3F_4OH]$ geometry was optimized using a special set of parameter values: charge –2 and triplet for closed shell species, charge –2 and quartet for radical species, charge –3 and triplet for the anionic species and charge –1 and quartet for the cationic species. The ground-state equilibrium geometries were optimized at the restricted Hartree-Fock level of theory and the optimization of the corresponding radical anion and radical cation geometries was performed with unrestricted open shell Hartree-Fock level of theory.[27] For the species having more conformers, all conformers were investigated. The conformer with the lowest electronic energy was used in this work.

Experimental Setup

The *in vitro* antioxidant activity of the three examined boroxine compounds was evaluated in terms of their ferric-reducing antioxidant power (FRAP), which were compared with a standard antioxidant. FRAP assay is a simple, quick and inexpensive test that is frequently used to measure total antioxidant capacity in the wide variety of biological samples and pure compounds. The principle is based on the reduction of ferric(III) tripyridytriazine (TPTZ) complex to ferrous form that can be monitored by measuring the change of absorption at 593 nm. The reaction is reproducible and linearly related to the molar concentration of the electron-donating antioxidant present in the reaction mixture.

All commercially available chemicals and reagents were supplied by Sigma-Aldrich, Merck, Germany. The halogenated boroxine $K_2[B_3O_3F_4OH]$ was synthesised in the Laboratory of Physical Chemistry, Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Hercegovina, through a simple reaction between potassium biflouride KHF_2 and boric acid in the 2 : 3 ratio, as reported in literature.[28] All the measurements were carried out on the Specord 200 Plus spectrophotometer.

FRAP assay was performed according to the method of Benzie and Strain with slight modifications.[29] The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2,4,6-tripyridl-*s*-triazine) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The FRAP working solution was freshly prepared each time by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl_3 \cdot 6H_2O$. 0.075 mL of sample solutions at different concentrations (0.1, 0.5, 1, 5 and 10 mM) were added to 2.25 mL of FRAP reagent and mixed well. Each concentration was measured in three replicates and the mean values were calculated. The absorbance readings were taken at 595 nm at t = 0 and after 30 minute incubation at 37 °C in darkness. Aqueous solutions of known Fe(II) concentration ($FeSO_4 \cdot 7H_2O$) were utilized to generate a calibration curve using a similar procedure. The FRAP values were expressed as the concentration of the compound having a ferric-reducing ability equivalent to that of 1 mM of iron(II) sulphate and compared with ascorbic acid. FRAP working solution instead of a sample was used as a blank.

RESULTS AND DISCUSSION

Quantum Chemical Studies

All the computed enthalpies for the four investigated compounds are presented in Table 2. In the calculations, the following vacuum enthalpy values of proton, electron and hydrogen atom were employed: $H(\mathrm{H}^{+})$ = 0.00236 Ha (*i.e.* Hartree), $H(\mathrm{e}^{-})$ = 0.20043 Ha and $H(\mathrm{H}^{\bullet})$ = –0.49764 Ha.

The data in Table 2 reveals that BDE value of halogenated boroxine $K_2[B_3O_3F_4OH]$ is lower than the corresponding BDE values of trimethlyboroxine and

Table 2. Thermodynamical quantities relevant for antioxidant mechanisms of selected compounds, all in kJ mol^{-1}.

Compound	HAT	SET-PT		SPLET	
	BDE	IP	PDE	PA	ETE
ASCACD	304.30	924.33	–294.65	–213.44	762.61
TMHYLB	697.85	1139.49	–415-75	89.13	741.34
TMOXYB	628.35	1115.37	–408.24	116.75	731.22
DPTFTB	499.40	308.75	–367.56	762.36	1474.31

Calculated enthalpies regard to the most stable conformation of the corresponding radical.

trimethoxyboroxine by 198.45 and 128.95 kJ mol^{-1}, respectively. This can be explained by the fact that the C–H bonds present in trimethly- and trimetoxyboroxine are weaker than O–H bond in $K_2[B_3O_3F_4OH]$. Among the four examined compounds, ascorbic acid exhibited the lowest BDE value (304.3 kJ mol^{-1}), indicating that the HAT mechanism is the most favourable in this molecule. Generally, our results have shown that the BDE values of the currently studied species increase in the order ASCACD < DPTFTB < TMOXYB < TMHYLB.

The IP values, characterising the first step in the SET-PT mechanism, have been found to increase in the order DPTFTB < ASCACD < TMOXYB < TMHYLB. From this ranking, it can be concluded that halogenated boroxine $K_2[B_3O_3F_4OH]$ exhibits the greatest electron transfer capability, while trimethylboroxine shows the least electron transferability. The trend in the PDE values of the compounds studied is opposite to that of the IP values. Note that the PDE values for ascorbic acid (–294.65 kJ mol^{-1}), trimethlyboroxine (–415.75 kJ mol^{-1}) and trimethoxyboroxine (–408.24 kJ mol^{-1}) are all negative, which suggests that the proton transfer process of their cation radicals is exothermic.

The PA values, related to the SPLET mechanism, can be classified in the order ASCACD < TMHYLB < TMOXYB < DPTFTB. As a general observation, the PA values for the compounds studied are higher than their PDE values, which can be attributed to the high reactivity of the cationic radicals. The only exception to this trend is the ascorbic acid, which has roughly the same PA and PDE values. Table 2 also shows that, apart from the halogenated boroxine $K_2[B_3O_3F_4OH]$, the ETE values of the examined compounds are significantly lower than their corresponding IP values.

Thermodynamical favourableness of the particular antioxidant mechanism can be estimated on the basis of enthalpy values describing the first step of each mechanism. More specifically, the lowest enthalpy value indicates the most probable reaction pathway. To facilitate the comparisons, BDE, IP and PA values for all the compounds studied in this paper were plotted on the same axes, as illustrated in Figure 3. From this figure, it is evident that the preferred mechanism for the ascorbic acid, trimethly- and trimethoxyboroxine is the SPLET, since each of these compounds exhibits notably lower PA than the BDE and IP values. These results are in agreement with those reported in literature, confirming that SPLET is the predominant mechanism of antioxidant activity for ascorbic acid.[30] On the other hand, in case of the halogenated boroxine $K_2[B_3O_3F_4OH]$, the most plausible mechanism of radical scavenging reaction is SET-PT, since its BDE, and particularly PA value, is considerably higher than IP, which undoubtedly rules out HAT and SPLET mechanisms. The second most favourable antioxidant mechanism for all the molecules studied is HAT.

Table 3. Energy levels (in eV) of the examined compounds.

Compound	E_{HOMO}	E_{LUMO}	ΔE
ASCACD	–10.54	–5.48	5.06
TMHYLB	–10.09	–0.76	9.33
TMOXYB	–9.58	5.39	14.97
DPTFTB	–3.11	7.78	10.45

The HOMO and LUMO energies are not antioxidant descriptors, but they can be connected to the antioxidant activity of the molecule. The molecular electron-donating ability is strictly correlated with the HOMO energy. Analysis of the computed HOMO orbital energy values in Table 3 reveals that halogenated boroxine $K_2[B_3O_3F_4OH]$ is characterized with the highest value of this parameter in vacuum. This result is highly compatible with the computed IP values and proves that $K_2[B_3O_3F_4OH]$ has a higher ability to interact with free radicals and scavenge them via SET-PT mechanism. However, all three examined boroxine derivatives exhibit very large HOMO-LUMO gaps (9.33 eV,

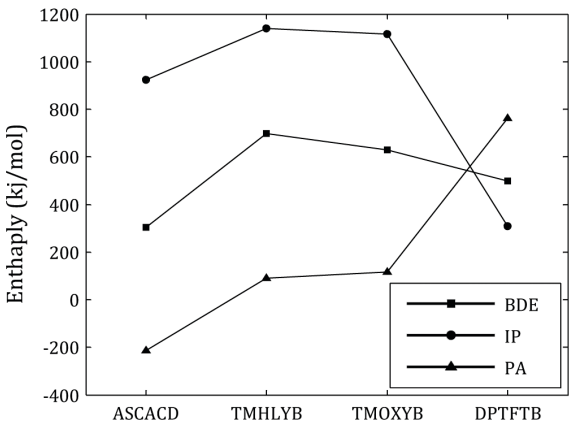

Figure 3. Superposition of BDE, IP and PA values for ascorbic acid, trimethlyboroxine, trimethoxyboroxine and halogenated boroxine $K_2[B_3O_3F_4OH]$.

Table 4. Energy levels (in eV) of the examined compounds.

Compound	Inhibition	
	Mean	Std.
ASCACD	201.5646	16.5117
TMHYLB	0.1130	0.0041
TMOXYB	0.0277	0.0007
DPTFTB	0.0680	0.0024

14.97 eV and 10.45 eV, respectively). According to this descriptor, all of these compounds show far less chemical activity against free radical scavenging than ascorbic acid.

Ferric-Reducing Antioxidant Power Assay

The FRAP assay was employed to estimate the reducing ability of the selected compounds *in vitro*. In this test, the results revealed that a good linearity of ferrous sulphate ($FeSO_4$) was obtained within the range of 0.15–1 mM (R^2 = 0.9971). The regression equation expressing the absorbance of ferrous sulphate standard solution as a function of concentration is:

$$y = 0.4493x + 0.0773 \quad (9)$$

Linearity of FRAP (dose-response curve) for $FeSO_4$ standard solution is shown in Figure 4. The respective FRAP assay values, calculated from the standard calibration curve using [Eq. (9)], are reported in Table 4.

As it can be seen, three boroxines studied exhibit negligible antioxidant activity, since their FRAP values are few orders of a magnitude lower than FRAP value for ascorbic acid and iron(II) sulphate. Among the three, trimethlyboroxine is showing the highest antioxidant power (0.113 %), while trimethoxyboroxine and halogenated boroxine $K_2[B_3O_3F_4OH]$ are having considerably lesser extent of free radical scavenging power (0.0277 and 0.068 %, respectively). One-way analysis of variance (ANOVA), followed by Tukey test, at α = 0.05 level of significance, confirms that there are statistically significant differences between antioxidant powers of the three boroxine compounds studied.

These results are in accordance with the findings of a previous study, conducted using DPPH methodology, which revealed that trimethyl- and trimethoxyboroxine do not scavenge free radicals via electron donation. The same study reported similar results for halogenated boroxine $K_2[B_3O_3F_4OH]$, providing that at lower concentrations it exhibited slight antioxidant activity (from 0.38 to 0.92 %); whereas at higher concentrations, it showed no antioxidant activity whatsoever.[31]

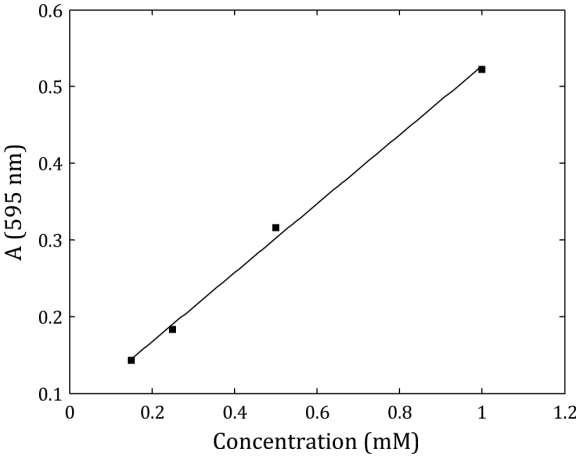

Figure 4. Ferrous sulphate ($FeSO_4$) standard curve].

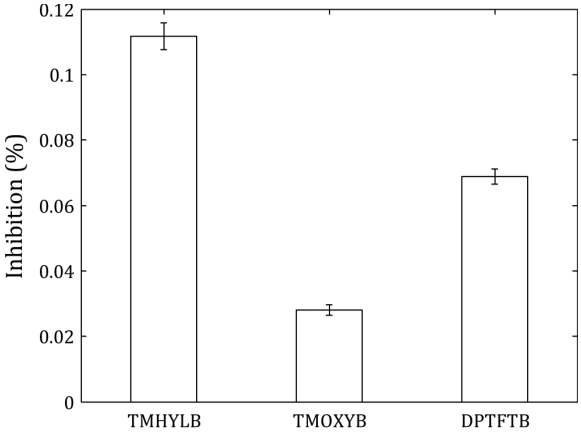

Figure 5. A comparison of selected boroxine compounds ferric-reducing ability power.

CONCLUSION

Radical scavenging potential is based on the capability of a neutral molecule to generate stable radicals. Quantum-chemical study based on the AM1 / Hartree-Fock method has been performed herein in order to determine the antioxidant activity of three different boroxine derivatives - halogenated boroxine $K_2[B_3O_3F_4OH]$, trimethylboroxine and trimethoxyboroxine. The usual molecular descriptors BDE, IP, PDE, PA and ETE were used to assess thermodynamical favourableness of forming radicals via certain reaction pathway. Analysis of their values reveals that SPLET is the preferred mechanism of radical scavenging activity for trimethyl- and trimethoxyboroxine. However, in case of the halogenated boroxine $K_2[B_3O_3F_4OH]$, the low IP values - considerably lower than BDE and PA values - indicate that the interaction of this compound with active radical most likely follows the SET-

PT mechanism. The BDE values for all three investigated boroxines have been found to be mutually very similar, which shows that all of these compounds have similar ability to react via the HAT mechanism.

On the other hand, large HOMO-LUMO gap values speak in favour of distinctly low chemical reactiveness of examined compounds. It should be pointed out that these results are in strong agreement with the corresponding experimental FRAP values, which clearly predict that these compounds are inactive antioxidants. Furthermore, the FRAP values of all three boroxine derivatives studied are few orders of a magnitude smaller than those for ascorbic acid.

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REFERENCES

[1] D. G. Hall, *Boronic Acids: Preparation and Applications in Organic Synthesis, Medicine and Materials*, Wiley, Hoboken, NJ, **2011**, pp. 1–2.
[2] S. A. Westcott, *Angew. Chem. Int. Ed. Engl*. **2010**, *49*, 9045.
[3] A. L. Korich, P. M. Iovine, *Dalton Trans*. **2010**, *39*, 1423.
[4] O. Eidam, C. Romagnoli, E. Caselli, K. Babaoglu, D. T. Pohlhaus, J. Karpiak, R. Bonnet, B. K. Shoichef, F. Prati F, *J. Med. Chem*. **2010**, *53*, 7852.
[5] P. C. Trippier, C. McGuigan, J. Balzarini, *Antivir. Chem. Chemother*. **2010**, *20*, 249.
[6] P. C. Trippier, C. McGuigan, *MedChemComm*. **2010**, *1*, 183.
[7] B. Galić, *Boroxine composition for removal of skin changes*. Patent US8278289 B2, Issued 2 October **2012**.
[8] B. Galić, *Removal of skin changes*. Patent EP1996514 B1, Issued 31 July **2013**
[9] S. Haverić, A. Haverić, K. Bajrović, B. Galić, M. Maksimović, *Drug Chem. Toxicol*. **2011**, *34*, 250.
[10] M. Potter in *The Balb/c Mouse: Genetics and Immunology*. Springer, New York, NY, **1985**, pp. 1–5.
[11] S. Haverić, M. Hadžić, A. Haverić, M. Mijanović, R. Hadžiselimović, B. Galić, *Braz. Arch. Biol. Technol*. **2016**, *59*, 1.
[12] M. Hadžić, S. Haverić, A. Haverić, B. Galić, *Biologia* **2015**, *70*, 553.
[13] S. Islamović, B. Galić, M. Miloš, *J Enzyme Inhib. Med. Chem*. **2013**, *29*, 744.
[14] D. Vullo, M. Miloš, B. Galić, A. Scozzafava, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 341.
[15] S. Ivanković, R. Stojković, Z. Galić, B. Galić, J. Ostojić, M. Marasović, M. Miloš, *J. Enzyme Inhib. Med. Chem*. **2014**, *30*, 354.
[16] L. Pojskić Kapur, S. Haverić, N. Lojo-Kadrić, M. Hadžić, A. Haverić, Z. Galić, B. Galić, D. Vullo, C. T. Supuran, M. Miloš, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 999.
[17] J. S. Wright, E. R. Johnson, G. A. DiLabio, *J. Am. Chem. Soc*. **2001**, *123*, 1173.
[18] E. Klein, V. Lukeš, M. Ilčin, *Chem. Phys*. **2007**, *336*, 51.
[19] G. Litwinienko, K. U. Ingold, *Acc. Chem. Res*. **2007**, *40*, 222.
[20] J. Dai, R. J. Mumper, *Molecules* **2010**, *15*, 7313.
[21] K. M. Honório, A. B. F. Da Silva, *Int. J. Quant. Chem*. **2003**, *95*, 126.
[22] A. N. Quieroz, B. A. Gomes, W. M. Moraes Jr, R. S. Borges, *Eur. J. Med. Chem*. **2009**, *44*, 1644.
[23] W. Hehre, S. Ohlinger, P. Klunzinger, B. Deppmeier, A. Driessen, J. Johnson, P. Ohsan, *Spartan'14 for Windows, Macintosh and Linux: Tutorial and User's Guide*. Wavefunction Inc, Irvine, CA, **2014**.
[24] N. Allinger, *J. Am. Chem. Soc*. **1977**, *99*, 8127
[25] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, J. J. P. Stewart, *J. Am. Chem. Soc*. **1985**, *107*, 3902.
[26] L. Scotti, M. T. Scotti, K. F. M. Pasqualoto, V. Bolzani, E. I. Ferreira, *Rev. Bras. Farmacogn*. **2009**, *19*, 908.
[27] C. J. Cramer, *Essentials of Computational Chemistry: Theories and Models,* Wiley, Hoboken, NJ, **2004**.
[28] I. G. Ryss, M. M. Slutskaya, *Zhur. Fiz. Khim*. **1951**, *22*, 1327.
[29] I. F. Benzie, J. J. Strain, *Methods Enzymol*. **1999**, *299*, 15.
[30] S. B. Nimse, D. Pal, *RSC Adv*. **2015**, *5*, 27986.
[31] M. Marasović, A. Roščić, B. Galić, M. Miloš, *CBUIC Int. Conf. Proc*. Prague, Czech, **2017**.