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Potent 1,2,4-Triazole-3-thione Radical Scavengers Derived from Phenolic Acids: Synthesis, Electrochemistry, and Theoretical Study

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Nine 1,2,4-triazole-3-thiones containing phenolic acid moiety have been synthesized and examined by scavenging of stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, measurement of reducing capacity, cyclic voltammetry experiments and density functional theory (DFT). The differences in DPPH-radical scavenging activity of the compounds **4a-i** are affected by the sta-

bility of the corresponding radicals or radical cations and possibility of delocalization of unpaired electron through benzene and triazole ring. Significantly, lower proton affinity (PA) values than bond dissociation enthalpy (BDE) indicate SPLET (sequential proton loss electron transfer) mechanism under these experimental conditions.

Introduction

Thione-substituted 1,2,4-triazoles represent an important class of heterocycles owing to their numerous features in various fields of medicinal and industrial chemistry. A large number of these derivatives exhibit diverse biological properties including anticonvulsant,^[1] antidepressant,^[2] anti-inflammatory,^[3] antibacterial,^[4] antifungal^[5] and anticancer activity.^[6] In the context of potential industrial applications, several 1,2,4-triazole-3-thiones have shown corrosion inhibition of copper and mild steel in chloride media and acidic solutions.^[7,8]

The antioxidant activity of 1,2,4-triazole-3-thione (thiol) compounds has attracted much attention in relation to their radical-scavenging potential. Most of these heterocycles with moderate to good scavenging activity toward DPPH radical were synthesized varying different substituents at 4- and 5-position of triazole ring.^[9] Ayhan-Kilcigil et al. designed a new 1,2,4-triazole-3-thione analogue (compound **A**, Figure 1) which

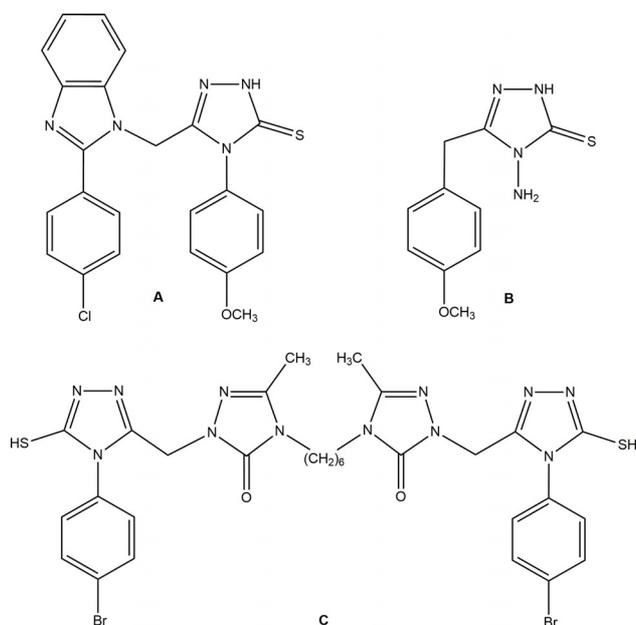


Figure 1. Some 1,2,4-triazole-3-thiones (thiols) as DPPH radical-scavengers.

is screened for its antioxidant properties using DPPH radical obtaining IC_{50} value of $54 \mu\text{M}$.^[10] An amino compound, 4-amino-5-aryl-3H-1,2,4-triazole-3-thione (**B**) displayed a significant decrease in the concentration of DPPH radical with $IC_{50} = 51.8 \mu\text{M}$.^[11] Düğdü et al. reported novel 1,2,4-triazole-3-thiol derivative (**C**) that exhibited good DPPH radical-scavenging activity ($IC_{50} = 10.0 \pm 0.7 \mu\text{M}$), better than using butylated hydroxytoluene (BHT) as a reference compound.^[12]

The antioxidant activity of phenolic compounds has been widely investigated in relation to prevention of heart disease, inflammation, cancer and food preservation.^[13–15] It is well

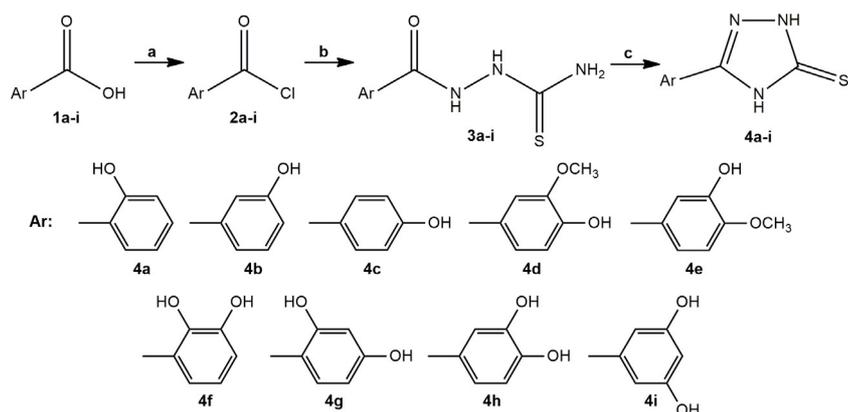
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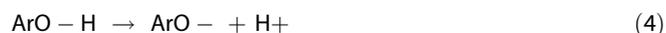
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Scheme 1. Reagents and conditions: a) SOCl_2 , DMF, CH_2Cl_2 , 2 h, r.t.; b) $\text{NH}_2\text{NHC(S)NH}_2$, THF; Yield for **3g**: 74%, other intermediates **3a–f**, **3h** and **3i** were not isolated; c) NaOH, 3 h, reflux; HCl; Yields for **4a–i** were in the range of 51 to 61%.

known that antioxidant capacity of phenolic compounds is directly related to their ability to release hydrogen atoms. The newly formed free radical should be more stable and less reactive than the initial one. There are several proposed mechanisms of antioxidant action, but three of them have been generally accepted:^[16] hydrogen atom transfer (HAT,^[17] eq. (1)), single electron transfer followed by proton transfer (SET-PT,^[16d,18] eqs (2) and (3)), and sequential proton loss electron transfer (SPLET,^[19] eqs (4) and (5)).



In eqs. (1) – (5) ArO^\bullet , ArO-H^\bullet , and ArO^- denote the radical, radical cation, and anion of the parent phenolic compound ArO-H , whereas H^\bullet , H^+ , and e^- stand for the hydrogen atom, proton, and electron. The HAT, SET-PT, and SPLET mechanisms are described with the following reaction enthalpies: BDE (bond dissociation enthalpy); IP (ionization potential) and PDE (proton dissociation enthalpy); and PA (proton affinity) and ETE (electron transfer enthalpy), respectively.

Some phenolic acids, especially protocatechuic (3,4-dihydroxybenzoic acid) and 2,3-dihydroxybenzoic acid are potent antioxidants and many papers have been published on their radical-scavenging activity.^[20,21] It is well known that the efficiency of phenolic acids very depends on number and arrangement of hydroxyl groups bonded to aromatic benzene ring.^[20,22] A detailed mechanism and kinetics of dihydroxybenzoic acids as free radical scavengers have been studied in nonpolar and aqueous solutions using Density Functional Theory.^[23] Unlike phenolic acids, few papers report on antioxidant activity of their derivatives. Protocatechuic acid alkyl esters demonstrated a higher scavenging potential than the parent phenolic acid.^[24] Some phenolic acid amides were found to be more effective DPPH radical scavengers when compared with standard α -tocopherol.^[25] To confirm whether 1,2,4-tria-

zole-3-thiones derived from phenolic acids show higher activity than starting acids or not, nine heterocyclic compounds are synthesized and the impact of triazole moiety and phenolic hydroxyl groups on their radical-scavenging activity is evaluated by different methods.

Results and discussion

Chemistry

The synthesis of the targeted 1,2,4-triazole-3-thione derivatives **4a–i** is presented in Scheme 1. The synthetic pathway started with a commercially available phenolic acid (**1a–i**) which was converted to the corresponding acid chloride (**2a–i**) in reaction with thionyl chloride in the presence of catalytic amounts of DMF. The next step was performed immediately due to the low stability of the obtained acid chloride. In the dry reaction with thiosemicarbazide, after 24 h of stirring at the room temperature, the 1-arylthiosemicarbazide derivatives **3a–d** and **3f–i** were obtained. The only exception was **3e** which preparation required 6 h of refluxing. The obtained solution was then evaporated under reduced pressure, and the crude residue (**3a–f,h,i**) was used in the next step without further purification. In order to obtain 1-arylthiosemicarbazide **3g** with satisfactory purity, we used more concentrated solution so that it would be possible to isolate it by filtration and dry it over CaCl_2 . In continuation, it was performed intramolecular cyclization by treating the crude residue of **3a–f,h,i** with 1.8 M aqueous solution of NaOH and refluxing for 3 h. For the compound **3g** it was used 0.9 M aqueous solution of NaOH, because more concentrated solutions resulted in significant amount of impurities. After refluxing and cooling, the solution was acidified using 2 M HCl (except for **4f** and **4g**, where concentrated HCl was used) until pH 1 was reached, enabling the formation of precipitate of the desired triazole compound **4a–i**. To obtain the higher yields of the final compound, the formed mixture was left to stand overnight at 4 °C and compound **4a–i** was then filtered off and dried. Compounds **4a** and **4e** needed further purification, which was performed by recrystallization from hot ethanol.

The exact structure of all compounds was confirmed by means of ^1H and ^{13}C NMR spectroscopy (see Supplementary information, Figure S1-S18).

DPPH free radical scavenging activity

The antioxidant capacity of nine 5-substituted-1,2,4-triazole-3-thiones was evaluated on the basis of the scavenging activity of the stable DPPH free radical. The radical-scavenging properties of the starting phenolic acids were also determined in the same conditions for the sake of comparison with triazole compounds. Among many available methods for determination of antioxidant activity including inhibition of lipid peroxidation, ethoxresofurin O-deethylase (EROD) activity, hydroxyl and superoxide radical scavenging, xanthine oxidase, reducing power etc., DPPH (2,2-diphenyl-1-picrylhydrazyl) method has been found to be the most frequent one. The degree of discoloration of DPPH solution indicates the radical-scavenging potential of the antioxidant.^[26] There are many modifications of this method and the results of the DPPH assay presented in many ways primarily depend on concentration of samples, the concentration of DPPH solution, time of incubation, applied solvent and temperature. The majority of studies express the results as the IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%.^[27] Unfortunately, the lack of standardization of the results makes much difficulties in comparison of scavenging strengths of the different compounds. Thus, numerous methods and antioxidant sources are not compatible, and the same antioxidant species can yield uncomparable results in different assay modifications.^[28]

The results of radical scavenging activity of the synthesized triazoles and corresponding phenolic acids expressed as concentration of tested compound that reduce 50% (IC_{50}) of the DPPH radicals are presented in Table 1. All synthesized triazoles

Table 1. DPPH scavenging activity of phenolic acids and their corresponding triazoles.			
Phenolic acids Compound	IC_{50} (μM)	Triazoles Compound	IC_{50} (μM) \pm SD
1a	> 1000	4a	106.48 \pm 2.30
1b	> 1000	4b	108.69 \pm 2.06
1c	> 1000	4c	122.98 \pm 2.27
1d	> 1000	4d	53.72 \pm 1.36
1e	> 1000	4e	130.81 \pm 1.59
1f	22.71 \pm 0.70	4f	14.63 \pm 0.36
1g	> 1000	4g	110.17 \pm 2.20
1h	35.17 \pm 0.81	4h	12.66 \pm 0.22
1i	> 1000	4i	118.53 \pm 2.24
Referent antioxidants			
Ascorbic acid	38.78 \pm 0.60		
NDGA	20.83 \pm 0.23		

Results are mean values \pm SD from three measurements.

showed moderate to excellent antioxidant activity, with IC_{50} values in the range of 12.66 to 130.81 μM , while IC_{50} values of

ascorbic acid and nordihydroguaiaretic acid (NDGA) were 38.78 and 20.83 μM , respectively. Triazoles **4f** and **4h** showed significantly higher ($p < 0.05$) radical scavenging activities compared to examined referent antioxidants, with IC_{50} values of 14.63 and 12.66 μM , respectively.

The phenolic acids exhibited much lower antioxidant activities in comparison to triazole-3-thiones (Table 1). The obtained results of DPPH assay showed that among phenolic acids only **1f** and **1h** had $\text{IC}_{50} < 1000$ μM . These two phenolic acids showed good antioxidant activities in DPPH assay (22.71 and 35.17 μM , respectively), but antioxidant activity of their corresponding triazoles was significantly higher ($p < 0.05$). Literature data confirm high radical scavenging activities of **1f** and **1h**,^[20,29] while phenolic acids with one hydroxyl group, such as **1a**, **1b** and **1c** show negligible antioxidant capacities.^[20,29–31] Our results showed that phenolic acids with two hydroxyl groups, such as **1g** and **1i** are better antioxidants than monohydroxy acids, but their activity is also low. Vanillic and isovanillic acid **1d** and **1e**, having methoxy groups in *m*- and *p*-positions, also showed low scavenging activities ($\text{IC}_{50} > 1000$ μM), but higher than other phenolic acids except **1h** and **1f** (IC_{50} data not presented).

Compounds **4f** and **4h** showed considerable potential in neutralization of DPPH radicals among all synthesized triazoles; **4h** had the best activity with the lowest IC_{50} value (12.66 μM), which was not significantly different ($p > 0.05$) from IC_{50} of **4f** (14.63 μM).

In order to determine behavior of these two pairs of triazoles and phenolic acids which showed the highest antioxidant activity, we scanned their DPPH radical scavenging activity during 90 min and compared with their corresponding phenolic acids. The correlation between inhibition percent and time for **1f**, **4f**, **1h** and **4h** at a concentration of 5 $\mu\text{g}/\text{mL}$ is presented in Figure 2. The most important difference can be

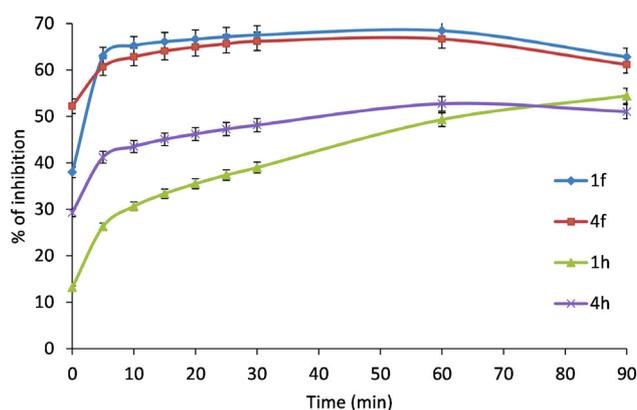


Figure 2. Correlation between inhibition percent and time for 2 pairs of acids and triazoles which showed the highest antioxidant activity measured by DPPH free radical scavenging activity method. Each value is the average of three measurements with error bars representing SD.

seen at the beginning of the reaction; at 0 minutes triazole **4f** obviously showed the highest inhibition of 52.22%, followed

by its corresponding phenolic acid **1f** with 37.98% of inhibition. Percent of inhibition at the same time for compounds **4h** and **1h** was 29.30 and 13.13%, respectively. At the time of 5 min, this order was changed – acid **1f** showed higher scavenging activity than its 1,2,4-triazole-3-thione, but it was not a significant difference ($p > 0.05$), and this ratio stays similar to the end of the reaction. Triazole **4h** displayed convincingly higher scavenging activity than its corresponding acid **1h** at 5 min, and this ratio also stays similar to the 90 min. Percent of inhibition of DPPH radicals for acids and triazoles, presented on Figure 2 and Figure 3, was obtained for same mass concen-

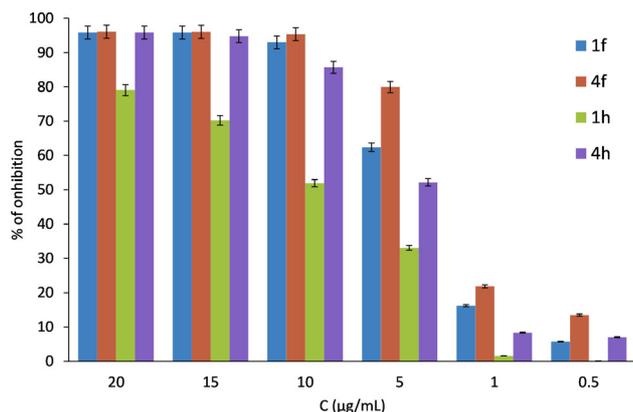


Figure 3. Percent of DPPH scavenging activity of **1f**, **4f**, **1h** and **4h** at different concentrations. Each value is the average of three measurements with error bars representing SD.

tration and could not be compared with the results of their IC_{50} values expressed as molar concentration, because they have not the same molar masses due to different number of bonded water molecules.

Percents of inhibition of DPPH radicals for compounds **1f**, **4f**, **1h** and **4h**, tested in concentration range of 0.5 to 20 $\mu\text{g/mL}$ are presented in Figure 3. At high concentrations (20 $\mu\text{g/mL}$), percents of inhibition of triazole **4f** and phenolic acid **1f** were not significantly different ($p > 0.05$), while, at the same concentration, triazole **4h** has higher inhibition percent than its corresponding phenolic acid. According to the results for percent of inhibition of DPPH radicals at low concentrations of triazoles and phenolic acids (Figure 3), the differences between these compounds were more pronounced than at high concentrations. The obtained results for **4f** and **1f**, at concentrations lower than 10 $\mu\text{g/mL}$, showed that triazole **4f** had significantly higher ($p < 0.05$) percent of inhibition compared to **1f**, and this suggests that synthesized triazole possesses more powerful antioxidant properties in low concentrations than its parent phenolic acid. For compounds **4h** and **1h**, similar ratio of DPPH scavenging activities was observed in all applied concentrations.

Electrochemistry

The voltammetric investigations of four synthesized compounds (**4a**, **4d**, **4f** and **4h**) were performed in DMF in the potential range +1.7 V to –2.1 V. The obtained voltammograms were compared to those recorded under the same conditions for parent phenolic acids.

The acids which served as parent compounds for triazole derivatives behave similarly in DMF as already described in other media (DMSO, AN^[32] and EtOH-H₂O, pH 7^[33]). Basically, all four compounds (**1a**, **1d**, **1f** and **1h**) release 2 electrons in one complex peak combined of two partially overlapped peaks. The current functions (as compared to those for ferrocene) of the second (most prominent) peak for **1f** and **1h** correspond to successive release of 1e⁻ in each of slightly separated peaks. In contrast, for **1a** and especially for **1d** the peaks are very close and current enhanced that it seems to be 2e⁻ in a single step. In the same time, the potentials of these peaks are shifted in positive direction with increasing the scan rate. The function $\Delta E_p/\Delta \log v$ is about 50–60 mV dec⁻¹ at lower scan rates (< 200 mV s⁻¹), reaching about 100 mV dec⁻¹ at the higher ones (< 2000 mV s⁻¹). Judging by the appearance of the recorded voltammograms, the first value can mainly be ascribed to effects of chemical reactions coupled to oxidation process and the latter one must be due to irreversibility of electron transfer process.

The chosen triazoles (**4a**, **4d**, **4f** and **4h**) oxidize at potentials > 0.6 V in 2–3 one-electron peaks differing both in potential and in shape. However, their voltammetric patterns can be correlated to those of the corresponding phenolic acids revealing much better peak distinction. This fact can be ascribed to better stabilization of oxidation products of triazole derivatives prevailing by delocalization of unpaired electrons through triazole or both rings.

Thus, instead of two ill-defined peaks at +1.2 V and +1.5 V (**1a**), two well-shaped 1-e⁻ peaks at +0.68 V and +1.14 V are observed (**4a**). In addition, one apparently 2-e⁻ peak at +1.14 V (**1d**) is substituted by three 1-e⁻ peaks at +0.66 V, +0.83 V and +1.04 V in **4d** (Figure 4). Here the peaks at +0.83 V and +1.04 V are influenced by a chemical reaction following the process at +0.66 V. Since the peak at +0.83 V prevails at higher scan rates and rapidly diminishes at lower ones, but behavior of the most positive peak is reversed, it can be concluded that the first two peaks are connected by E–E process, while the first and third peak are chemically coupled. This probably means that at +1.04 V oxidizes a deprotonated form of **4d** as reported for similar compounds.^[32,33] Here also DMF could play a stabilizing role as a proton-accepting solvent, similarly to DMSO as already described.^[32]

The greatest likeness between the parent acids and their triazoles in voltammetric sense was established for **4f** and **4h** where two 1-e⁻ peaks at close potentials recorded for the acids are only moved in negative direction for 150–200 mV (Figure 5), indicating an easy oxidation process. The first pair of peaks is irreversible, with $\Delta E(a/c) \sim 450$ mV (at 100 mV s⁻¹).

In addition, after oxidation at voltammograms of all compounds 2–3 new small peaks appear in a subsequent cathodic

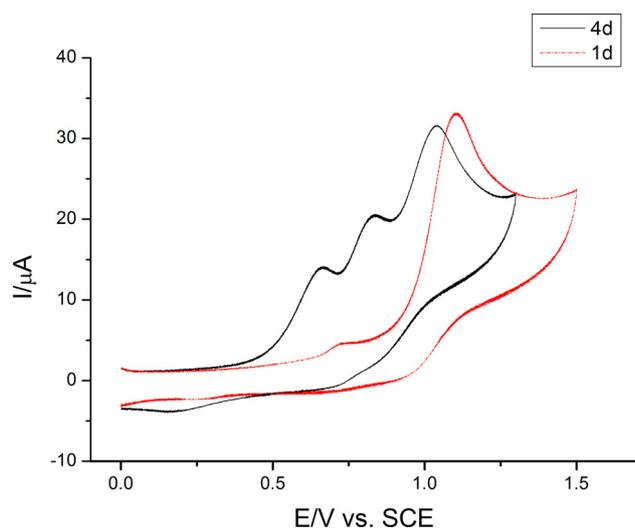


Figure 4. Cyclic voltammograms for **1d** (0.86 mM) and **4d** (1.11 mM) in DMF + 0.1 M TBAP on GC electrode, $v = 100 \text{ mV s}^{-1}$.

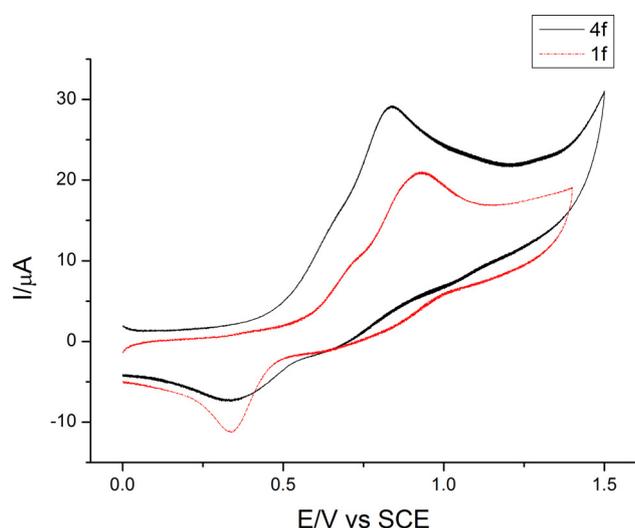


Figure 5. Cyclic voltammograms for **1f** (0.83 mM) and **4f** (1.05 mM) in DMF + 0.1 M TBAP on GC electrode, $v = 100 \text{ mV s}^{-1}$.

scan down to -2.1 V which are due to species formed in coupled chemical steps. In contrast, no well-defined reduction processes were recorded in this potential range when reactants were the starting triazoles or parent phenolic acids.

The overall oxidation processes at all triazoles are mix-controlled and have weak adsorption characteristics. The essential potentials are presented in Table 2.

Density Functional Theory

For each 1,2,4-triazole-3-thione all possible rotamers were constructed and optimized, and their energies calculated. The most stable conformers of all investigated compounds are presented in Figure S19 of Supplementary information. In addition, the thione-thiol tautomers of **4a** – **4h** were examined. It was

Compound	Ep(1) ^a	Ep(2)	Ep(3)
1a		$\sim +1.2$	$\sim +1.5$
4a	+0.68	+1.14	
1d		+1.14	
4d	+0.66	+0.83	+1.04
1f		+0.96	
4f	i.d. ^b	+0.84	i.d.
1h		+0.96	
4h	+0.75	+0.87	+1.11

[a] In V vs. SCE. [b] Ill-defined.

found that the thione tautomers are by more than 45 kJ mol^{-1} more stable than the corresponding thiol forms. Two examples of the thione-thiol tautomerism are presented in Figure S20. Taking this fact into account our further attention will be focused on the thione forms of the examined compounds.

The obtained geometrical parameters for all molecules are mutually very similar. The values of bond lengths, bond angles, and dihedral angles for compounds **4d**, **4e**, and **4h** are provided in Table S1, as an illustration. All investigated 1,2,4-triazole-3-thiones are planar, which indicates that they can use both rings in delocalization of charge and spin density. In addition, the C1–C7 bonds are of partial double character, which is demonstrated through their lengths of about 1.46 \AA and hybrid composition $0.70(\text{sp}^{2.24})\text{C1} + 0.71(\text{sp}^{1.51})\text{C7}$ obtained by NBO analysis. It is well known that antioxidant properties of compounds are in good correlation with their delocalization possibilities.

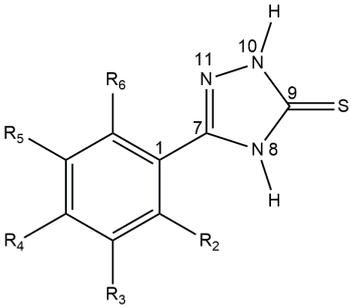
Except **4b**, **4c** and **4i**, all other compounds have internal hydrogen bonds (IHBs), which additionally stabilize corresponding compounds. For these IHBs the NBO analysis revealed the stabilizing donor–acceptor interactions between the lone pairs on oxygen or nitrogen and proximate antibonding O–H orbitals.

Free radicals, radical cations, and anions

Antioxidant activity of triazoles is associated to their ability to release hydrogen atoms, either from nitrogen or oxygen. Homolytic cleavage of the O–H and N–H bonds of 1,2,4-triazole-3-thiones leads to the formation of the corresponding radicals. The calculated BDE values are presented in Table 3.

It is clear that the stability of the radicals plays an important role in determining the antioxidant activity of the parent molecules. It is well-known that delocalization of spin density in a free radical significantly influences its stability.^[34] The spin density values for the most stable radicals are presented in Figure 6. Obviously, the better delocalization of the unpaired electron, the more stable the obtained radical. The radicals issued from p-hydroxyl groups (compounds **4c**, **4d**, and **4h**) are the most stable because their unpaired electrons are delocalized over both benzene and triazole rings. Somewhat weaker, but worth mentioning, is the stability of the radical obtained by a cleavage of the *ortho* O–H bond (compound **4f**) due to similar delocalization of unpaired electron. Very good delocalization of

Table 3. Calculated thermodynamical parameters (kJ mol^{-1}) for antioxidative activity of 1,2,4-triazole-3-thiones.



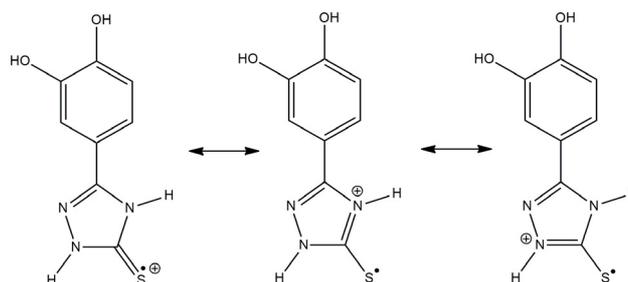
Comp.			BDE	IP	PDA	PA	ETE		
4a	$R_6 = \text{O}$	N_8H	N_{10}H	365.5	481.7	52.8	154.6	379.9	
	$R_6 = \text{OH}$	N_8	N_{10}H	361.5		48.7	128.8	401.7	
	$R_6 = \text{OH}$	N_8H	N_{10}	351.8		39.1	137.5	383.4	
4b	$R_3 = \text{O}$	N_8H	N_{10}H	356.8	473.7	52.1	145.9	379.9	
	$R_3 = \text{OH}$	N_8	N_{10}H	357.1		52.4	131.9	394.2	
	$R_3 = \text{OH}$	N_8H	N_{10}	357.4		52.7	149.0	377.4	
4c	$R_4 = \text{O}$	N_8H	N_{10}H	346.8	462.1	53.7	139.1	376.7	
	$R_4 = \text{OH}$	N_8	N_{10}H	355.0		61.9	139.4	384.6	
	$R_4 = \text{OH}$	N_8H	N_{10}	351.7		58.6	152.9	367.8	
4d	$R_3 = \text{OCH}_3$	$R_4 = \text{O}$	N_8H	N_{10}H	329.5	451.8	46.7	142.1	356.9
	$R_3 = \text{OCH}_3$	$R_4 = \text{OH}$	N_8	N_{10}H	349.5		66.6	138.4	385.4
	$R_3 = \text{OCH}_3$	$R_4 = \text{OH}$	N_8H	N_{10}	349.8		67.0	152.9	368.9
4e	$R_3 = \text{O}$	$R_4 = \text{OCH}_3$	N_8H	N_{10}H	336.8	452.1	53.7	148.9	356.9
	$R_3 = \text{OH}$	$R_4 = \text{OCH}_3$	N_8	N_{10}H	353.0		70.0	136.6	385.4
	$R_3 = \text{OH}$	$R_4 = \text{OCH}_3$	N_8H	N_{10}	350.1		67.1	150.3	368.9
4f	$R_6 = \text{O}$	$R_5 = \text{OH}$	N_8H	N_{10}H	339.4	481.4	26.9	138.4	370.0
	$R_6 = \text{OH}$	$R_5 = \text{O}$	N_8H	N_{10}H	341.3		28.9	150.2	360.1
	$R_2 = \text{OH}$	$R_3 = \text{OH}$	N_8	N_{10}H	361.6		49.1	127.6	403.0
4g	$R_2 = \text{OH}$	$R_3 = \text{OH}$	N_8H	N_{10}	352.1		39.7	136.1	385.1
	$R_6 = \text{O}$	$R_4 = \text{OH}$	N_8H	N_{10}H	364.6	466.6	67.8	153.0	380.8
	$R_6 = \text{OH}$	$R_4 = \text{O}$	N_8H	N_{10}H	352.8		55.9	137.4	384.5
4h	$R_6 = \text{OH}$	$R_4 = \text{OH}$	N_8	N_{10}H	377.6		60.6	131.3	395.3
	$R_6 = \text{OH}$	$R_4 = \text{OH}$	N_8H	N_{10}	346.5		49.5	140.2	375.3
	$R_3 = \text{O}$	$R_4 = \text{OH}$	N_8H	N_{10}H	327.9	456.2	40.8	131.1	365.9
4i	$R_3 = \text{OH}$	$R_4 = \text{O}$	N_8H	N_{10}H	320.4		33.3	125.0	364.4
	$R_3 = \text{OH}$	$R_4 = \text{OH}$	N_8	N_{10}H	353.9		66.7	138.7	384.2
	$R_3 = \text{OH}$	$R_4 = \text{OH}$	N_8H	N_{10}	351.1		63.9	152.4	367.7
4j	$R_3 = \text{O}$	$R_5 = \text{OH}$	N_8H	N_{10}H	357.3	477.3	49.0	144.9	381.4
	$R_3 = \text{OH}$	$R_5 = \text{O}$	N_8H	N_{10}H	359.3		51.0	145.8	382.5
	$R_3 = \text{OH}$	$R_5 = \text{OH}$	N_8	N_{10}H	358.2		49.9	135.3	391.9
4k	$R_3 = \text{OH}$	$R_5 = \text{OH}$	N_8H	N_{10}	357.4		49.1	146.1	380.4

the OH (**4d** and **4h**) or OCH_3 groups (**4e**) in the para position, which are in vicinal position with another OH or OCH_3 group. It is well-known that a structural detail of an antioxidant where an OH and OH/ OCH_3 groups are adjacent significantly contributes to its antioxidant activity.^[35]

Heterolytic cleavage of the O–H and N–H bonds of 1,2,4-triazole-3-thiones is the first step of the SPLET mechanism, which leads to the formation of the corresponding anions. The charge distribution in all anions, obtained by the NBO analysis, is presented in Figure S23. On the basis of the PA values (Table 3) it is apparent that the heterolytic cleavage of the N8–H bond is favourable. In these anions the negative charge is delocalized over the sulphur and nitrogens of the triazole ring. In the case of the compound **4h**, where the anion is formed by the cleavage of the O–H bond of the para phenolic group, the PA value is the lowest. In this anion the negative charge is delocalized over both rings, and they are additionally stabilized with the hydrogen bonds. The second step of the SPLET mechanism is

the unpaired electrons was observed in the case of the radicals obtained by a cleavage of the N8–H bond (compounds **4a** and **4g**). These radicals are mainly delocalized over the triazole ring including the thione moiety.

Figure S21 clearly shows that the greatest contribution to the HOMOs of all investigated 1,2,4-triazole-3-thiones comes from the sulphur. In accordance with this finding is the NBO analysis which revealed that the HOMO of each compound is a lone electron pair on the sulphur. It is reasonable to conclude that a molecule will lose an electron from this orbital, thus yielding the corresponding radical cation (Scheme 2). Ionization potential (IP) is a measure of easiness to release an electron. The calculated IP values for the examined compounds are listed in Table 3. In the case of the radical cations of **4d**, **4e**, and **4h** the unpaired electron is well delocalized over triazole ring (Figure S22) which stabilizes these species, and, thus, the IP values are small. All three radical cations are characterized with



Scheme 2. Resonance stabilization of radical cation for triazole **4h**.

electron detachment from the intermediate anion. A comparison between the PA and ETE values shows that electron detachment is energetically more demanding than heterolytic cleavage. The obtained values for ETE are comparable with the

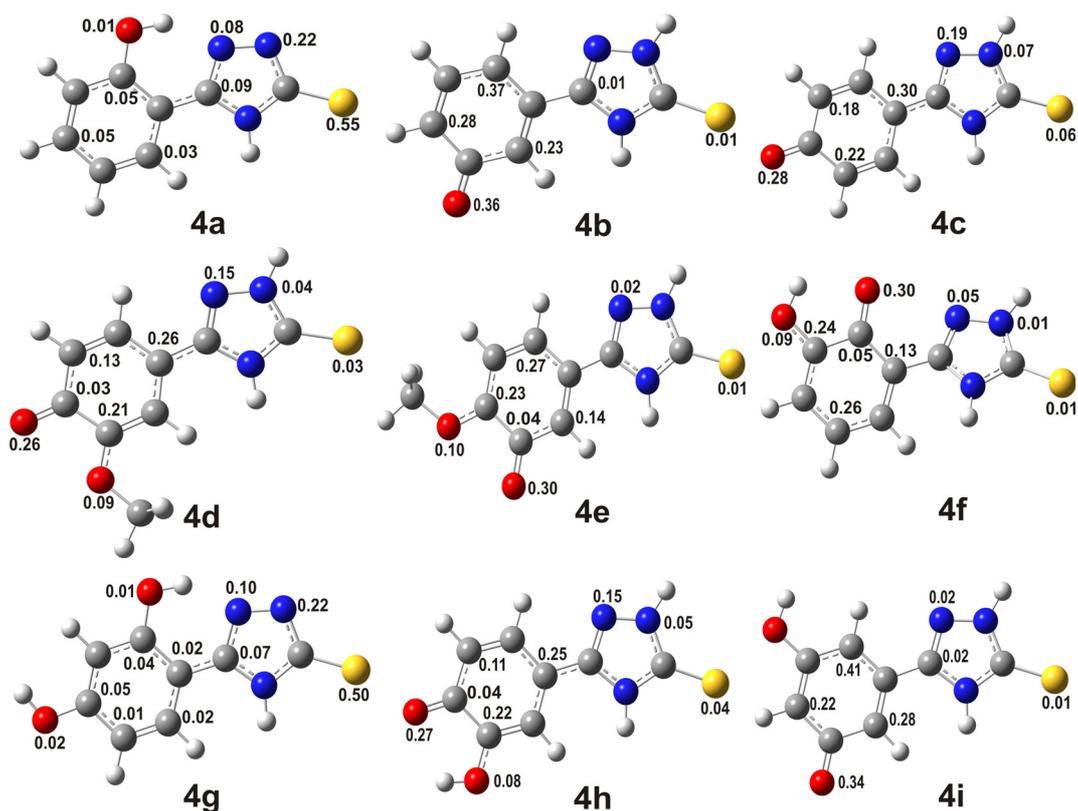
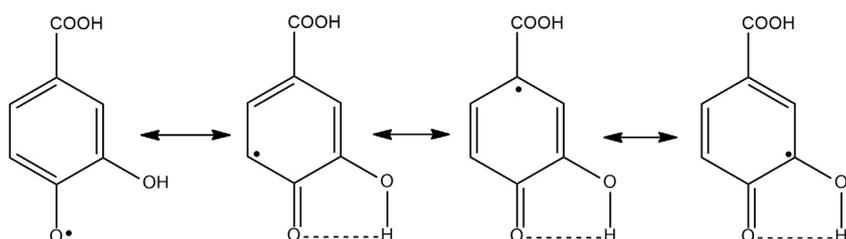


Figure 6. Spin density distribution in all radicals issued from the investigated 1,2,4-triazole-3-thiones in methanol.



Scheme 3. Resonance stabilization of protocatechuic acid radical.

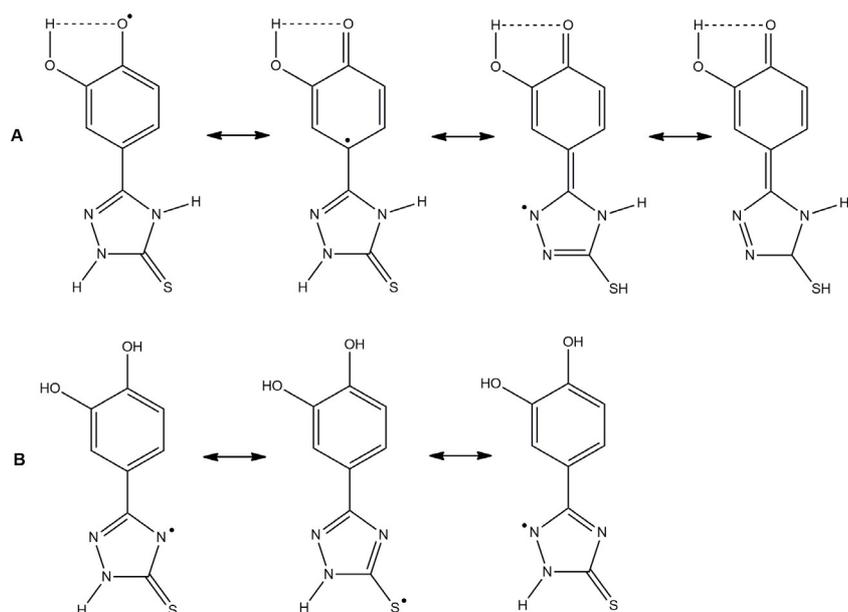
values for gallic acid,^[36a] caffeic acid, three caffeoylquinic acids^[36b] and hydroxybenzoic acids^[36c] obtained by the B3LYP–D2 method.

Mechanism of antioxidant action and SAR studies

On the basis of the thermodynamic values: BDE, IP, PDE, PA, and ETE, one can assume which antioxidant mechanism will prevail under certain conditions. Namely, it can be supposed that a pathway characterized with the lowest value of reaction enthalpy may be the dominant antioxidant mechanism for investigated compounds. The calculated values in Table 3 show that the IP values for all examined compounds are significantly higher than the corresponding BDE, and particularly PA values. This finding indicates that the SET-PT mechanism is not favourable reaction pathway for the investigated 1,2,4-triazole-3-thiones in methanol. On the other hand, the PA values are notably lower than the BDE values, which implies that SPLET is the

dominant mechanism under these conditions. These thermodynamic results are in good agreement with the experimental IC_{50} values (Table 1). Namely, the compounds **4f** and **4h** are characterized with the lowest PA and IC_{50} values. This result is not surprising, because previous investigations of the antioxidant activity of Schiff bases showed that polar protic solvents, such as methanol and ethanol, favour the SPLET mechanistic pathway.^[36d]

As we can see from DFT calculations, the presence of triazole-3-thione moiety substantially increases radical scavenging activity of the compounds **4a–i** in comparison with parent phenolic acids. As a result of a SPLET mechanism, the phenoxyl radical formed from phenolic acid can be converted into quinone structure as shown in Scheme 3. However, the formation of quinone is much slower than that of triazole derivative due to electron-withdrawing properties of carboxylic function. According to SPLET mechanism, in polar media carboxylic group tends to dissociate to the electron-donating carboxylate anion



Scheme 4. Resonance stabilization of radicals for triazole **4h** after heterolytic: A) O–H, B) N–H cleavage, and loss of one electron according to SPLET mechanism.

which decreases electrophilicity of quinone lowering reactivity towards a nucleophilic attack of methanol. It is well known that quinone readily reacts with methanol leading to a regeneration of phenolic structure which can scavenge additional equivalents of DPPH radicals.^[21]

In the triazole compounds phenoxyl radicals can be highly stabilized through resonance since the unpaired electron may be present not only on the oxygen and benzene ring, but it can be delocalized across triazole part of molecule (Scheme 4). The resonance stabilization causes an easy formation of phenoxyl radical with lower reactivity and higher stability. In other words, this radical is unable to be included in initiation or propagation of chain process and its probable fate leads to neutral quinone structures or, to a lesser extent, radical coupling to yield dimeric structures, all of which still contain phenolic hydroxyl groups capable of further scavenging.^[37] Better possibilities for delocalization outside of benzene ring decrease chance for dimerization and eventual complexation with DPPH radical.^[38]

The most active compounds **4f** and **4h** contain two neighboring hydroxyl groups having the possibility to form intramolecular hydrogen bonding. After a dissociation of the proton which is not involved in this bond and loss of one electron a very stable phenoxyl radical was formed.^[39,40] Comparing the results of the DPPH radical scavenging capacity of **4d** and **4f** or **4h**, a large difference was observed. Introduction of additional methoxy group into aromatic ring will increase the nucleophilicity of the phenoxyl radical formed from **4d** enhancing its DPPH-scavenging activity. However, substitution of the methoxy group instead of hydroxyl one will reduce activity due to less effective radical stabilization by intramolecular hydrogen bonding. Again, comparison of **4d** and **4e** shows that **4d** structure is more effective than **4e** because meta-position of hydroxyl group in **4e** does not allow extended resonance stabilization of the phenoxyl radical throughout the whole mole-

cule.^[41] The compounds **4g** and **4i** having two hydroxyl groups in meta-position exhibit significantly lower antiradical effect in comparison with **4f** and **4h**. Thus, it seems that the position of the hydroxyl group is far more important than the number of them. It is clear that ortho- or para-hydroxyl group in combination with meta-hydroxyl in benzene ring is essential for a good antioxidant activity.^[42] Interestingly, there are small differences in antiradical activities between **4a**, **4b** and **4c**. These results suggest that intramolecular hydrogen bond is the main condition for stabilization of phenoxyl radical and high antioxidant power, but not position of hydroxyl group. Lower scavenging activity of **4g** is in accordance with this hypothesis.

Conclusion

Phenolic 1,2,4-triazole-3-thiones are much more potent DPPH radical scavengers in comparison with parent phenolic acids as a result of participation of triazole moiety in resonance stabilization of phenoxyl radical. Intramolecular hydrogen bonding, possibility of resonance stabilization outside of aromatic ring, position of hydroxyl group and nucleophilicity of phenoxyl radical are the crucial conditions for good antioxidant activity.

High antioxidant activity of 1,2,4-triazole-3-thiones **4f** and **4h** was confirmed by both theoretical and experimental methods. It was found that OH groups in the *p*- and *o*-positions of the benzene ring are responsible for good antioxidative activity of these compounds, especially because there is another hydroxyl group in the adjacent position. The phenolic acids **1f** and **1h** also possess good antioxidant activity but significantly better antioxidant capacity of the corresponding 1,2,4-triazole-3-thiones **4f** and **4h** is a consequence of the possibility of additional stabilization of phenoxyl radical across triazole moiety. In the same manner, we can explain much better activity of other 1,2,4-triazole-3-thiones in comparison with starting phenolic acids.

On the basis of the collected voltammetric data in DMF, which suggest mixed kinetics and adsorption in practically all processes, it is not possible to distinguish between the mechanisms with precision. However, based on the thermodynamic parameters, it can be supposed that SPLET mechanism is dominant in methanol. This occurrence can be attributed to the resonance stabilization of the putative anions. The anions obtained by heterolytic cleavage of the N₃-H bonds in the triazole rings are particularly well stabilized, which is demonstrated through small PA values (Table 3).

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Keywords: Antioxidant · DFT · electrochemistry · triazole

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