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Pro(anti)oxidant Properties of Amino Acids and Their Derivatives in The Presence of Fe^{2+} and Cu^{2+} Ions

O. A. Milach[a], I. V. Mel'sitova[a], and I. L. Yurkova[a,b,*]

[a] *Belarussian State University, Minsk, 220030 Belarus*
[b] *Research Institute of Physicochemical Problems, Belarussian State University, Minsk, 220030 Belarus*
**e-mail: yurkovail@tut.by*

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Abstract—A fluorescent probe (terephthalic acid) has been used for rapid assessment of the ability of amino acids and their derivatives to accept and/or promote the formation of HO$^{\bullet}$ radicals in the Cu^{2+}–H_2O_2, Fe^{2+}/EDTA–H_2O_2 systems. In the concentration range of 0.005-30 mM, the amino acids are arranged in the following series according to the increase in IC_{50}: Trp < Phe ≤ Met ≤ His < Gly ≤ Glu < α-Ala < Cys << β-Ala << Tau (system with Cu^{2+}); His ≤ Met < Trp ≤ Cys << β-Ala < Tau ≤ Glu < α-Ala < Gly (system with free Fe^{2+}). In the presence of copper(II), Cys and its derivatives, Glu, α-Ala, His (S) favored the HO$^{\bullet}$ formation and acted as antioxidants at the S : Cu^{2+} molar ratio > 2 : 1 (≥1 : 1 for His). Cys is also a prooxidant in the Fe^{2+}/EDTA system.

Keywords: amino acid, hydroxyl radical, prooxidant, Fe^{2+} ion, Cu^{2+} ion, fluorescent probe, radical scavenging activity

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Amino acids and their derivatives form a part of the antioxidant system of an organism protective the cells from oxidative stress [1, 2]. The latter is developed due to the disturbance of the balance between the oxidants and antioxidants and is characterized by excessive accumulation of reactive oxygen species ($O_2^{\bullet-}$, H_2O_2, HClO, HO$^{\bullet}$) [2]. These can induce the damage of cellular components and the organs. Oxidative stress plays a key part in the development of numerous pathologies (inflammation, atherosclerosis, stroke, heart attack, diabetes, etc.) [1, 2].

Hydroxyl radical [HO$^{\bullet}$, $E°$(HO$^{\bullet}$, H^+/H_2O) = 2.73 V, $E°'$ = 2.31 V at pH = 7.25] is the most reactive and toxic of reactive oxygen species described to date [2]. The HO$^{\bullet}$ radicals initiate fast (10^9–10^{10} $M.^{-1}s^{-1}$) destruction of important biomolecules and are thus crucial for the development of pathologies [2, 3]. No *in vivo* enzymatic reaction of HO$^{\bullet}$ deactivation has been known. One of the paths of HO$^{\bullet}$ formation in an organism is decomposition of H_2O_2 catalyzed by transition metal ions (Fe^{2+}, Cu^+, etc.) (Scheme 1) [2, 3].

The increase in concentration of free (not bound to proteins or weakly bound to amino acids) copper and iron ions due to homeostasis is correlated to the development of the diseases, pathogenesis of which is related to the formation of reactive oxygen species (cardiovascular and neurogenerative ones, cancer, etc.) [4].

The role of amino acids in the control of the processes related to reactive oxygen species has been intensively studied [1, 2]. However, despite huge experimental material available, detailed mechanism of the regulation of the free-radical reactions, for instance under conditions of Fe^{2+}(Cu^{2+})-mediated generation of HO$^{\bullet}$, by amino acids and their derivatives has remained unclear. Amino acids (alone or in combination with other compounds) can either inhibit or promote the oxidation processes in the

Scheme 1.

$$Cu^{2+}(Fe^{3+}) + H_2O_2 \rightarrow Cu^{+}(Fe^{2+}) + O_2^{\bullet-} + 2H^+,\ k_v \sim 4.6\times10^2\ M^{-1}\ s^{-1}$$

$$O_2^{\bullet-} + Fe^{3+}(Cu^{2+}) \rightarrow Fe^{2+}(Cu^{+}) + O_2,\ k_v \sim 1\times10^8\ (1\times10^9)\ M^{-1}\ s^{-1}$$

$$Fe^{2+}(Cu^{+}) + H_2O_2 \rightarrow Fe^{3+}(Cu^{2+}) + HO^{\bullet} + OH^-,\ k_v \sim 76\ (4.7\times10^3)\ M^{-1}\ s^{-1}$$

Table 1. IC_{50} indexes and rate constants of the reaction with hydroxyl radicals for the studied compounds

Compound	IC_{50}, mmol/L			$k_{HO^{\cdot}}$, M^{-1} s^{-1} [3, 11]
	Cu^{2+}–H_2O_2 (0.05 : 5 mmol/L)	Fe^{2+}–H_2O_2 (0.1 : 1 mmol/L)	Fe^{2+}/EDTA–H_2O_2 (0.1 : 0.1 : 1 mmol/L)	
Trp	0.015 ± 0.001	0.072 ± 0.008	0.071 ± 0.007	1.3×10^{10} (pH = 6.0–8.5)
Phe	0.071 ± 0.006	–	–	6.5×10^{9} (pH = 5.8–8)
Met	0.075 ± 0.006	0.047 ± 0.005	–	8.3×10^{9} (pH = 6–7)
MetSO	0.046 ± 0.004	0.083 ± 0.008	–	1×10^{10}
His	0.078 ± 0.007	0.038 ± 0.004	0.280 ± 0.030	5×10^{9} (pH = 6.0–7.0)
Gly	0.158 ± 0.015	24.8 ± 1.70	> 30	1.7×10^{7} (pH = 5.8–6.0)
Glu	0.189 ± 0.021	7.88 ± 0.46	1.55 ± 0.14	2.3×10^{8} (pH = 6–7)
α-Ala	0.241 ± 0.026	9.26 ± 0.74	9.97 ± 0.80	7.7×10^{7} (pH = 5.5–6.8)
Cys	0.69 ± 0.08	0.082 ± 0.008	3.28 ± 0.29	3.5×10^{10}, 4.7×10^{10} (pH = 7)
NAC	1.16 ± 0.093	0.099 ± 0.01	1.99 ± 0.18	1.36×10^{10}
β-Ala	5.67 ± 0.39	3.42 ± 0.32	5.25 ± 0.47	–
Tau	>30	7.37 ± 0.59	–	2.4×10^{6}–1.4×10^{7}
GSH	0.111 ± 0.010	0.034 ± 0.003	0.739 ± 0.072	1.3×10^{10} (pH = 5.5)
GSSG	0.024 ± 0.002	0.222 ± 0.024	0.466 ± 0.050	9.6×10^{9} (pH = 7)
Cyst	0.344 ± 0.035	–	–	–
Car	0.073 ± 0.007	0.041 ± 0.004	0.289 ± 0.030	4.0×10^{9}
NaN_3	0.051 ± 0.005	0.044 ± 0.004	0.046 ± 0.005	1.2×10^{10} (pH = 7.9–13)
DMSO	0.035 ± 0.004	–	–	7.0×10^{9}

cells in the presence of transition metal ions (Fe^{2+}, Cu^{2+}, Co^{2+}) [1, 2, 5–9]. The studies of the antioxidant properties of amino acids and their derivatives have been performed under inconsistent *in vitro*/*vivo* conditions, and many of the obtained data are often discrepant; they can hardly be compared to predict the action of individual amino acids.

This study aimed to estimate the ability of various amino acids and their derivatives to accept $HO^{\cdot}$ radicals and/or promote their formation in the presence of Fe^{2+} and Cu^{2+}, other conditions being the same. The experiments were performed by means of the fluorescent probe method based on the use of terephthalic acid; this is an easy and highly sensitive method. The $HO^{\cdot}$ radicals were generated using the Cu^{2+}(Fe^{2+})–H_2O_2 and Fe^{2+}/EDTA–H_2O_2 redox systems. The concentration range of the studied compounds generally was of 5×10^{-6}–2×10^{-2} mol/L, being extended in certain cases.

Terephthalic acid is a sensitive and specific detector of $HO^{\cdot}$ (~10^{-9} M), it does not react with other reactive oxygen species ($O_2^{\cdot-}$, $^1O_2$, H_2O_2) [10]. Due to its symmetrical structure, terephthalic acid (pK_{a1} = 3.51, pK_{a2} = 4.82) interacts with $HO^{\cdot}$ with the formation of a single monohydroxylated isomer, 2-hydroxyterephthalate **2**. The product is stable and, in contrast to the parent terephthalic acid, exhibits fluorescence (λ_{ex} = 315 nm, λ_{em} = 418 nm). According to the reaction stoichiometry, the concentration of $HO^{\cdot}$ radicals is proportional to the intensity of 2-hydroxyterephthalate fluorescence.

Amino acids and their derivatives can bring different effects on the concentration of $HO^{\cdot}$ in the system containing Fe^{2+}(Cu^{2+}) ions and terephthalic acid. First, the interaction of amino acids with Fe^{2+}(Cu^{2+}) can alter the efficiency of the redox system to generate of $HO^{\cdot}$. Second, they can accept the formed $HO^{\cdot}$, thus competing with terephthalic acid **1** (Scheme 2). Overall, these processes change the concentration of 2-hydroxyterephthalate **2**.

Pro/antioxidant properties of the compounds were estimated from their ability to affect the kinetics of

Scheme 2.

$$\mathbf{1} + HO^{\bullet} \xrightarrow{k_1,\ HO} \mathbf{2}$$

$$S + HO^{\bullet} \xrightarrow{k_S,\ HO} \text{Reaction product}$$

hydroxylation of terephthalic acid and the IC_{50} index (concentration of half inhibition).

Effect of amino acids and their derivatives in the presence of copper(II) ions. The most prominent difference between the amino acids and their derivatives in their effect on the oxidation of terephthalic acid under conditions of Cu^{2+}-mediated generation of $HO^{\bullet}$ were revealed at the lower concentration range. The strongest ability to accept $HO^{\bullet}$ was observed for aromatic amino acids Trp and Phe: the yield of 2-hydroxyterephthalate was significantly reduced in their presence, and they revealed low IC_{50} values (Table 1).

The highest antiradical activity among the sulfur-containing amino acids was observed for Met and its sulfoxide. Hydroxylation of terephthalic acid was significantly enhanced in the presence of Cys ($c = 5\times10^{-6}$–1×10^{-4} mol/L) and its N-acetylated analog (NAC). That fact evidenced the increase in the $HO^{\bullet}$ content in the system. Glu and proteinogenic α-Ala also exhibited the promoting effect over the considered concentration range. The increase in the yield of 2-hydroxyterephthalate was also observed in the presence of His, yet its effect was revealed in a narrower concentration range (5×10^{-6}–1×10^{-5} mol/L) and its IC_{50} value was lower in comparison with Cys, Glu, and α-Ala. The amino acids exhibiting the prooxidant effect acted as antioxidants at the amino acid : Cu^{2+} molar ratio >2 : 1 (≥1 : 1 for His). $HO^{\bullet}$-Accepting properties of Gly were observed at the amino acid : Cu^{2+} ratio > 2 : 1, and those of the non-proteinogenic β-Ala were found at the ratio of >10 : 1. The IC_{50} value for β-Ala was more than 20 times higher than that for α-Ala. The sulfonic acid Tau (not found in proteins) did not affect the hydroxylation of terephthalic acid.

Derivatives of amino acids such as carnosine (Car) and glutathione (GSH) play important part in the control of the processes mediated by reactive oxygen species [1, 2]. We found that Car (β-alanyl-*L*-histidine) efficiently reduced the $HO^{\bullet}$ level at the same concentration range (>1×10^{-5} mol/L) corresponding to the analogous properties of His. The results obtained for Car, His, and β-Ala evidenced the importance of the presence of peptide bond for the antiradical properties of dipeptide.

GSH (L-γ-glutamyl-L-cysteinyl-glycine) promoted the oxidation of terephthalic acid in 2-hydroxyterephthalate at $c = 5\times10^{-6}$–5×10^{-5} mol/L. Its oxidized form (GSSG) did not exhibit such effect. The prooxidant action was not observed either in the case of cystamine disulfide (Cyst), a well-known radioprotector. The obtained data showed that the effect of GSH (and other free amino acids) on the hydroxylation of terephthalic acid depended on the peptide : Cu^{2+} ratio. The strongest prooxidant effect of GSH was observed at the 1 : 1 ratio, the antioxidant effect being noticeable at >2 : 1. Its IC_{50} was found of 0.056, 0.111, 0.160, and 0.210 mmol/L at $c_{Cu^{2+}} = 0.025$, 0.05, 0.075, and 0.1 mmol/L, respectively, $[H_2O_2]$ = const. Prooxidant properties of GSH were correlated with those of the constituents (Cys and Glu). Despite the promoting action at low dose, the IC_{50} value of GSH was low, comparable to Car.

The amino acids were arranged in the following series according to the increasing IC_{50} values in the Cu^{2+}-containing system (sodium azide was added as reference): Trp < NaN_3 ≤ Phe ≤ Met ≤ His < Gly ≤ Glu < α-Ala < Cys << β-Ala << Tau.

The series corresponding to the decrease in the rate constant of the reaction with hydroxyl radical ($k_{HO\cdot}$) was as follows: Cys ≥ Trp ≥ NaN_3 > Met > His ≈ Phe >> Glu > α-Ala > Gly > Tau (Table 1) [3, 11]. Comparison of the $k_{HO\cdot}$ and IC_{50} values for the considered amino acids showed that they were involved in the complex processes under conditions of the Cu^{2+}-mediated $HO^{\bullet}$ formation. The obtained data suggested that the amino acids effect on the Cu^{2+} : Cu^{+} ratio and their coordination with copper ions thus changing the metal catalytic activity in the reaction with H_2O_2 should be considered in the analysis, on top of the rate of the amino acids interaction with $HO^{\bullet}$. For example, the absence of the $HO^{\bullet}$-accepting activity of Tau was in line with the low $k_{HO\cdot}$ value and the data in [9, 12] revealing weak inhibition of the $HO^{\bullet}$-induced free-radical destruction of biomolecules by Tau. However, that correlation was not observed in the case of Cys, Gly, and α-Ala, even though Cys revealed strong $HO^{\bullet}$-accepting properties under conditions of γ-radiolysis, in contrast to the simplest amino acids [2]. Biothiols have been recognized as efficient radioprotectors [2].

Prooxidant effect of amino acids and their derivatives could be explained by nucleophilic properties of their functional groups capable of Cu(II) reduction into Cu(I), hence accelerating the decomposition of H_2O_2 and increasing the concentration of $HO^{\bullet}$. The strongest prooxidant action was observed for Cys and its derivatives bearing a SH group. That fact coincided with the dual role of biothiols in the control of the free-radical processes [5, 6, 9]. His could also act as a nucleophile due to the presence of the imidazole group and favor the reduction of Cu^{2+} [13], thus activating the decomposition process,

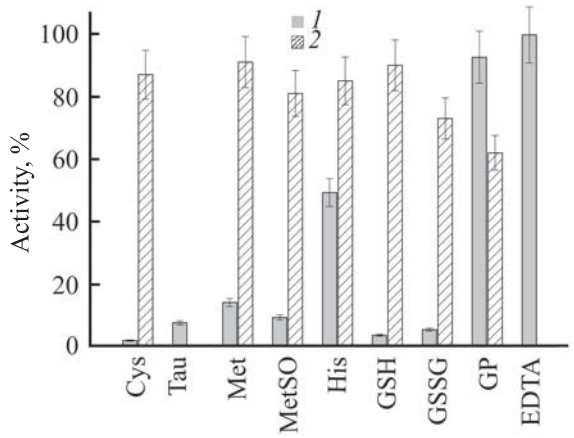

Fig. 1. Ability of the tested compounds (S) to chelate Fe^{2+} (*1*, [S] = 10 mmol/L) and to inhibit the formation of 2-hydroxyteraphthalate **2** in the terephthalic acid–Fe^{2+}–H_2O_2 system (0.06 : 0.1 : 1 mmol/L) (*2*, [S] = 0.5 mmol/L).

in line with the data of [14]. It should be noted that the disulfide GSSG exhibited stronger antiradical activity in comparison with GSH. It has been shown that cystine and GSSG are more efficient inhibitors of DNA damage than Cys and GSH under conditions of Cu^+-mediated generation of $HO^{\bullet}$ [15].

The obtained data pointed at the contribution of the amino acids to form complexes with Cu^{2+}/Cu^+ in the protecting action [1, 16–19]. Cys and GSH form complexes with both Cu^{2+} and Cu^+, those with the reduced metal ion being more stable [16]. The antioxidant effect of Gly, α-Ala, and Glu observed despite low $k_{HO\cdot}$ values was likely due to their affinity to Cu^{2+}/Cu^+ [17–19]. Gly, α-Ala, and His in low concentration have exhibited ~3–4 times stronger chelation to Cu^{2+} in comparison with Tau and β-Ala [20]. His and Car (0.5–5 mmol/L) have efficiently inhibited the H_2O_2–$CuSO_4$–ascorbate-induced DNA oxidation, whereas β-Ala has not been efficient [7]. Antioxidant properties of MetSO have been reduced by 25% for the system with the Cu^+ chelated to 2,2'-bipyridyl in comparison to the free metal ions [21].

The action of the amino acids and peptides was dependent on their molar ratio to Cu^{2+}, which could be related to the complexes formation. The increase in Cys or GSH content to twofold excess with respect to Cu(I) led to the formation of stable complexes, preventing the Fenton reaction of Cu(I) (Scheme 1) [16, 22, 23].

Effect of amino acids and their derivatives in the presence of iron(II) ions. The effect of the studied compounds under the conditions of Fe^{2+}–H_2O_2-mediated oxidation of terephthalic acid was different from that in the presence of Cu^{2+} (Table 1). An important difference was the absence of the prooxidant effect in the considered cases. The lowest IC_{50} value was observed for His exhibiting strong antioxidant activity. However, His accelerated the Fe^{2+}–H_2O_2-induced damage of biomolecules in the presence of ascorbate. Cys and its derivatives were efficient $HO^{\bullet}$-acceptors, whereas Glu, α-Ala, and Gly did not show any activity, which was reflected in the high values of IC_{50}. Among the peptides, the behavior of GSH was different: it was more efficient than its oxidized form at 5×10^{-6}–5×10^{-4} mol/L.

The following series reflects the increase in the IC_{50} value in the systems containing free Fe^{2+} ions: His ≤ NaN_3 ≤ Met < Trp ≤ Cys << β-Ala < Tau ≤ Glu < α-Ala < Gly.

$HO^{\bullet}$-Accepting properties of the amino acids were studied also in the Fe^{2+}–H_2O_2 system in the presence of ethylenediaminetetraacetic acid (EDTA) forming the Fe^{2+}/EDTA complex (log K = 14.3). The addition of EDTA, on one hand, increased the system efficiency via enhancement of iron(II) catalytic effect in the Fenton reaction (Scheme 1) {$E°(Fe^{3+}/Fe^{2+})$ = 0.77 V, $E°(Fe^{3+}$–EDTA/Fe^{2+}–EDTA) = 0.13 V [2]}, but on the other hand prevented the binding of iron ions with the tested compounds.

According to the obtained data, His and Car were more efficient in the system containing free Fe^{2+} ions, which could point at the Fe^{2+}/Fe^{3+} binding contribution to the antioxidant action [24, 25]. In our experiments, the Fe^{2+}-chelating activity of His was the best correlated with the antiradical activity, in contrast to other amino acids (Fig. 1). The data in [24] shows that His is efficient in Fe(III) biding, whereas Glu is not. Gly and α-Ala can form the complexes with iron ions, yet less stable than in the case of copper [26]. However, those amino acids did not reveal any significant activity in the presence of Fe^{2+}. It should be noted that the formation of an amino acid complex with a metal ion can either promote (prooxidant effect) or weaken (antioxidant effect) the catalytic activity of the metal.

The most prominent difference in the action of the amino acids in comparison with the system containing free Fe^{2+} ions was observed for Cys and its N-acetylated analog. Those thiols favored hydroxylation of terephthalic acid at concentration of 5×10^{-6}–1×10^{-3} mol/L, noticeable inhibiting effect being observed at $c = 5\times10^{-3}$ mol/L. GSH did not exhibit prooxidant activity, but the protecting effect comparable in strength to that in the Fe^{2+}–H_2O_2

system was observed at more than tenfold higher concentration.

Prooxidant action of biothiols (RSH) could be explained by nucleophilic properties of the sulfhydryl group. The electron transfer from Cys and GSH to iron(III) ions in an aqueous medium under physiological conditions is very slow [2]. The thiols activity is correlated to the pK_a values of their SH groups (GSH 9.2, Cys 8.5, pH = 7.4). Oxidative modification of low-molecular thiols is favorable upon the increase in their nucleophilic properties due to the transformation in the thiolate form (RS^-). Under the experiment conditions, direct interaction of Cys with the Fe^{3+}/EDTA complex is likely possible, leading to the reduction of Fe^{3+} ions and thus returning them in the catalytic cycle of H_2O_2 decomposition. The activating action of GSH and Cys on the Fe^{2+}/EDTA-mediated free-radical process has been observed in [9], even though the tripeptide and N-acetylated cysteine accelerate lipid peroxidation induced by free Fe^{2+} ions [6].

In summary, the study by means of the fluorescent probe method revealed the difference in the antioxidant action of the amino acids and their derivatives depending on the ions mediating the formation of the HO$^\bullet$ radicals (Fe^{2+} or Cu^{2+}). Certain amino acids and their derivatives acted as efficient promotors or protectors only in the presence of Cu^{2+}. The obtained robust data suggested that the pro(anti)oxidant action of amino acids and peptides in the presence of Fe^{2+}(Cu^{2+}) was due to a balance of their HO$^\bullet$-scavenging, reducing, and complex forming properties. The obtained data are useful in the development of efficient antioxidants, nutriceutics, and drugs based on amino acids as well as the understanding of detailed mechaisms of their action.

EXPERIMENTAL

L-Cysteine (Cys), *N*-acetyl-*L*-cysteine (NAC), taurine (2-aminoethanesulfonic acid) (Tau), *L*-methionine (Met), *L*-methionine sulfoxide (MetSO), *L*-histidine (His), *L*-tryptophane (Trp), glycine (Gly), *L*-phenylalanine (Phe), *L*-glutamic acid (Glu), α-alanine (α-Ala), β-alanine (β-Ala), carnosine (Car), glutathione reduced (GSH) and oxidized (GSSG), glycerol-2-phosphate disodium salt (GP), terephthalic acid, and ferrozine (Fz) (all from Sigma-Aldrich, Germany), sodium azide, hydrogen peroxide, dimethyl sulfoxide, cystamine, $CuSO_4 \cdot 5H_2O$, $FeSO_4 \cdot 7H_2O$, and EDTA sodium salt (Vekton, Russia) were used. The chemicals and solvents were of "analytical pure" grade. The aqueous solutions were prepared using deionized water.

Fluorescence spectra over 350–550 nm range were obtained using a Solar CM2203 spectrofluorimeter. The absorption spectra were recorded using a Specord S600 spectrophotometer.

Determination of antiradical activity of the compounds. The following testing system was used for determination of the HO$^\bullet$-scavenging activity of the studied compounds: terephthalic acid–Cu^{2+} (Fe^{2+}, Fe^{2+}/EDTA)–H_2O_2, phosphate buffered saline (PBS, 10 mmol/L, pH = 7.4). Total volume of the reaction mixture was 2 mL. The initially prepared stock solutions were added to the mixture to ensure the demanded concentration of the components. Stock solution of terephthalic acid (c = 1×10^{-2} mol/L) was prepared via dissolution of 0.0118 g of the acid in 25 mmol/L solution of NaOH. The final concentration of terephthalic acid in the tested mixture was 6×10^{-5} mol/L.

The components were added to the mixture in the following order: PBS, terephthalic acid, tested compound, metal salt, H_2O_2. Upon the addition of each component, the mixture was vigorously stirred using a Vortex Mixer instrument. After the mixing, the sample was kept at room temperature during 5 or 9 min. The measurements were performed upon the same keeping duration for each sample. The keeping duration was determined from the kinetic data on the formation of 2-hydroxyterephthalate. To do so, the intensity of fluorescence at 418 nm was recorded as a function of time upon addition of H_2O_2 in the terephthalic acid–M^{2+} reaction mixture.

The data were processed using the competing reaction method. Terephthalic acid **1** and the tested compound (S) competed for the interaction with hydroxyl radical (Scheme 2). The fluorescence response (F) was measured as a function of the tested compound concentration ([**1**] = const) and plotted in the F–[S] coordinates.

Since the fluorescence signal of the sample was proportional to the concentration of 2-hydroxyterephthalate **2**, the data were processed using the modified Stern–Volmer equation (1).

$$\frac{F_0}{F} = 1 + \frac{k_{S,HO}[S]}{k_{1,HO}[1]}. \quad (1)$$

Here [S] and [**1**] being concentrations of the tested compound and terephthalic acid, respectively; F_0 and F being the intensity of fluorescence at λ_{em} = 418 nm in the absence and in the presence of the tested compound, respectively.

To calculate the IC_{50} value, Eq. (1) was reshaped to obtain the logarithmic function with a linear plot in the log [$F_0/F - 1$]–log[S] coordinates. Concentration of the tested compound leading to the twofold decrease in the fluorescence intensity with respect to the reference (the IC_{50} index), was obtained at log ($F_0/F - 1$) = 0 [if $F = 1/2F_0$, then log ($F_0/F - 1$) = 0]. NaN_3 was used as reference due to its stable antiradical properties under conditions of Fe^{2+}(Cu^{2+})-mediated generation of $HO^{\bullet}$ [2].

To calculate the relative rate constant ($k_{S,HO\cdot}$) of the reaction between the tested compound (S) and $HO^{\bullet}$, the plots in the ($F_0/F - 1$)–([S]/[**1**]) coordinates were used. The slope of that plot was equal to $k_{S,OH}/(k_{\mathbf{1},OH\cdot}[\mathbf{1}])$ was used to determine the $k_{S,HO\cdot}$ value, since $k_{\mathbf{1},HO\cdot} = 4.4\times10^9\ M^{-1}\ s^{-1}$ [10]. The $k_{S,HO\cdot}$ values for NaN_3, DMSO, Trp, and Tau determined in this study as 8.6×10^9, 5.2×10^9, 1.06×10^{10}, and $1.3\times10^7\ M^{-1}\ s^{-1}$, respectively, were in good agreement with the reference data (Table 1). That further confirmed the validity of the applied express method for estimation of the antiradical activity of the compounds.

Inhibiting activity of the compounds (%) was calculated using Eq. (2).

$$A = \frac{F_0 - F}{F_0}\times100. \quad (2)$$

Determination of the Fe^{2+}-chelating ability of the compounds. Estimation of the ability of the tested compounds to chelate the Fe^{2+} ions was based on the formation of stable water-soluble complex between ferrozine (Fz) and Fe^{2+} (pH = 4–9) [27]. The tested compounds completed with Fz for the binding with Fe^{2+}, and the concentration of the colored $Fe(II)Fz_3$ complex was decreased.

0.2 mL of $FeSO_4$ solution (5×10^{-5} mol/L) was added to 0.2 mL of the sample. The reaction was initiated by the addition of 0.2 mL of ferrozine solution (2.5×10^{-4} mol/L). The mixture was vigorously shaken and left in dark at room temperature for 15 min; after that, the absorbance at λ = 562 nm was measured. Glycero-2-phosphate and EDTA were used as references.

Chelating ability (%) of the compounds was calculated using Eq. (3).

$$A = \frac{A_c - A_s}{A_c}\times100. \quad (3)$$

Here A_c and A_s being the absorbance of the $Fe(II)Fz_3$ in the absence and in the presence of the studied compound, respectively.

Statistical analysis. The experimental data were processed using Excel and Origin 8 software. Validity of the results was confirmed using the Student t-test. Each experiment was performed in 3–5 independent runs. The linear relationship between the quantitative variables was estimated using the Pearson coefficient. The hypothesis of the significant positive correlation was considered confirmed at $R^2 = 0.97–0.99$ ($p < 0.05$).

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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