Research article

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Studies on the antioxidant activity of some thiazolidinedione, imidazolidinedione and rhodanine derivatives having a flavone core

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ABSTRACT: A series of flavonyl-2,4-thiazolidinedione, imidazolidinedione and rhodanine derivatives were tested for their antioxidant activity as scavengers of oxygen free radicals. Free radical scavenging activities, including superoxide anion radical ($O_2^{\bullet}$), hydroxyl radical ($HO^{\bullet}$) and 2,2′-diphenyl-1-picrylhydrazyl free radical have been evaluated using chemiluminescence, electron paramagnetic resonance and spin trapping with 5,5-dimethyl-1-pyrroline-1-oxide as a spin trap. Potassium superoxide in dimethylsulfoxide and 18-crown-6 ether were used for the production of $O_2^{\bullet}$. Hydroxyl radical was generated using the Fenton reaction. Ten of the eleven examined compounds exhibited decrease in chemiluminescence, but there were large differences in the decrease, ranging from 16% to 89%; also, two of these compounds increased light emission by about 200%. On the contrary, all compounds tested exhibited 30–68% scavenging $HO^{\bullet}$ and 25–96% scavenging the $DPPH^{\bullet}$ radical respectively. Possible mechanisms are proposed to explain the results. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: flavone derivatives; radical scavenging activity; chemiluminescence; EPR study

Introduction

Flavonoids are a well-known class of natural compounds that possess radical scavenging properties due to their ability to form stable radicals (1). The reactive oxygen species (ROS)-scavenging abilities of flavonoids give them beneficial effects, such as anti-inflammatory, neuroprotective, cardioprotective and ultraviolet (UV)B-protective activities (2). All these activities have been associated with beneficial health effects (3,4). Therefore, flavonoids are important components of the human diet. However, most interest has been devoted to their antioxidant activity, which is due to their ability to reduce free radical formation and to scavenge free radicals (5). Among various ROS, superoxide anion radical ($O_2^{\bullet}$) is known as an initiator of several reactions leading to the formation of other oxygen species, such as hydrogen peroxide (H_2O_2) that can be converted in the presence of transition metal ions into a highly reactive short-lived hydroxyl radical ($HO^{\bullet}$) able to cause tissue injury. In recent years, flavonoids as potent free radical scavengers have attracted tremendous interest as possible therapeutics against free radical mediated pathophysiology of several conditions, such as neurodegenerative and cardiovascular diseases, cancer and ageing, caused by oxidative stress (6). The excellent free radical scavenging (chain-breaking antioxidation) property of flavonoids is due to their high reactivity as hydrogen or electron donors (7).

In this study, we report the *in vitro* antioxidant capacity of flavonylrhodanines (F1–F6), flavonyl-thiazolidine-2,4-diones (F7–F10), and flavonyl-imidazolidine-2,4-diones (F11, F12) (Fig. 1) as scavengers of $O_2^{\bullet-}$ and HO radicals using chemiluminescence (CL) and electron paramagnetic resonance spectrometry (EPR) techniques.

Experimental

Chemistry

The compounds F1–F6 (8), F7 (9), F8, F10, F11 (10) and F12 (11) were synthesized by the Knoevenagel reaction of flavone carboxaldehyde with thiazolidine-2,4-dione/imidazolidine-2,4-dione/2-thioxo-imidazolidine-4-one using acetic acid/sodium acetate mixture. Methylated compound (F9) (8) was obtained by reacting F7 with methyl iodide in dimethylformamide/anhydrous sodium carbonate mixture. All reagents were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA).

The chemical reagents used for evaluation of antioxidant activity were purchased from the following sources: tiron (4,5-dihydroxy-1,3-benzene-disulfonic acid), 5,5-dimethyl-1-pyrroline oxide (DMPO), 18-crown-6-ether (1,4,7,10,13,16)-hexaoxacyclooctane,

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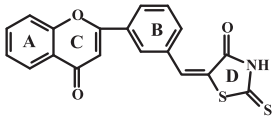
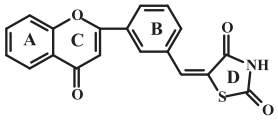
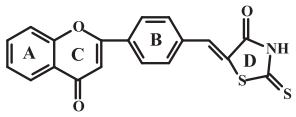
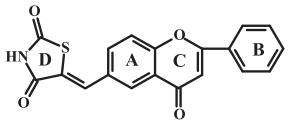
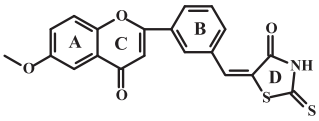
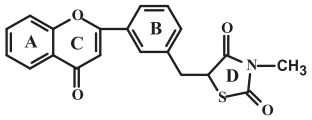
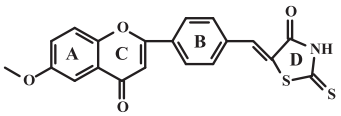
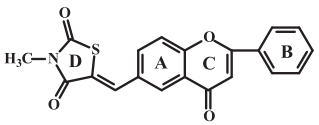
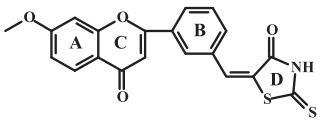
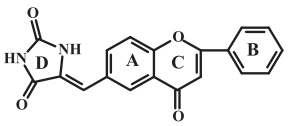
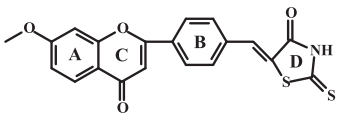
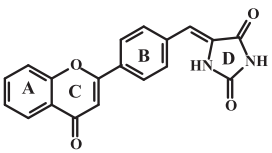
F1		F7	
F2		F8	
F3		F9	
F4		F10	
F5		F11	
F6		F12	

Figure 1. Chemical structures of the tested compounds (flavonyl-2,4-thiazolidinedione, imidazolidinedione and rhodanine derivatives).

Trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid from Merck (Darmstadt, Germany); ammonium ferrous sulfate hexahydrate and anhydrous dimethylsulfoxide (DMSO) from Aldrich (Milwaukee, WI, USA); 1,1-diphenyl-2-picryl hydrazyl (DPPH•), catalase from bovine liver and *p*-nitroblue-tetrazolium chloride (NBT) from Sigma-Aldrich GmbH (Sternheim, Germany); and potassium superoxide from Fluka (Buchs, Switzerland). Other reagents were obtained from POCH (Gliwice, Poland). They were of analytical grade and were prepared fresh daily. The flavonyl-2,4-thiazolidindione, imidazolidinedione and rhodanine derivatives (TZD compounds) were dissolved in DMSO, which was suitable for dissolving both water-soluble and water-insoluble reagents.

Superoxide anion radical ($O_2^{\bullet}$) was prepared from KO_2 according to the method described by Valentine *et al.* (12). 18-Crown-6 ether (60 mg) was dissolved in 10 mL dry DMSO, and then 7 mg KO_2 was added quickly to avoid contact with air humidity. The mixture of reagents was stirred for 1 h to give a pale yellow solution of 10 mmol/L superoxide anion radical. The $O_2^{\bullet}$ concentrated solution could be stored at 255 K for weeks without major decomposition. For CL experiments, $O_2^{\bullet}$ was used as a 1 mmol/L solution. The radical concentration was determined using the UV spectrum ($\lambda_{max} = 251$ *nm*, $\varepsilon = 2686 \pm 29$ mol/cm).

The reactivity of the tested compounds towards $O_2^{\bullet}$ was monitored using the CL technique (13).

CL kinetics and the effect of the examined compounds (TZD derivatives), tiron, salicylate and NBT on light intensity were measured using an EMI9553Q photomultiplier (Photek ,East Sussex, UK) with an S20 cathode sensitive in the range 200–800 nm, interfaced with a computer for data acquisition and handling. Reagents were introduced to a thermostated glass reactor cell placed just before the light detector in a light-tight camera. Reagents were injected through polyethylene pipes with the aid of semiautomatic syringes. The cuvette was exhausted using a B-169 vacuum system (Büchi, Flawill, Switzerland). All measurements were carried out at 295 ± 1 K. The CL signal from the $O_2^{\bullet}$/DMSO system or that influenced by the tested compounds was recorded as the kinetic curve of the CL decay. The CL intensity was used to determine the reactivity of the tested compounds and their reactivity was compared with those well known in the subject literature as inhibitors of O_2^{-} radicals. The quenching

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activity was calculated as ratio $Q(\%) = [(I_o - I)/I_o] \times 100\%$, where I_o is the light intensity measured in the absence of an inhibitor and I is the light intensity measured in the presence of the inhibitor. Because addition of DMSO to the $O_2^{\bullet}$/DMSO system caused a short-lasting small "flash" followed by a small increase in the CL, this reaction was considered as the control reaction.

Hydroxyl radical scavenging activity was evaluated using the Haber Weiss/Fenton reactions as a source of the radical (14,15) as follows:

$$H_2O_2 + Fe(II) \rightarrow HO^{\bullet} + Fe(III) + HO^-$$

$$Fe(II) + HO^{\bullet} \rightarrow Fe(III) + HO^-$$

$$H_2O_2 + HO^{\bullet} \rightarrow HOO^{\bullet} + H_2O$$

and EPR conjugated with the spin-trapping technique using DMPO as a spin trap (16).

The Fenton reaction mixtures in the sample cell contained the following reagents at the indicated final concentrations (in the final volume of 2 mL): ammonium ferrous sulfate (0.0625 mmol/L), sodium trifluoroacetate buffer (0.02 mmol/L, pH 6.15), H_2O_2 (0.5 mmol/L), DMPO (25 mmol/L).The reaction was started by addition of the Fe(II) ion. The mixture was placed in the EPR cavity using a quartz flat cell within an optical path length of 0.25 mm. The spin-trapped $DMPO-O\dot{H}$ signal was analyzed 1 min after the addition ammonium ferrous sulfate on a standard EPR spectrometer operating at 9.3 GHz with 100 kHz modulation of the steady magnetic field. Tested TZD derivatives were dissolved in DMSO because of insolubility in water and were added before the Fe ion.

The ability to scavenge $HO^{\bullet}$ was calculated using the following formula:

$$R(\%) = [(H_o - H)/H_o] \times 100\%$$

where H_o and H represent the relative height of the second peak in the EPR spectrum of the spin-adduct from the blank (DMSO) and in the presence of a sample dissolved in an appropriate amount of DMSO, respectively.

$DPPH^{\bullet}$ scavenging activity was measured according to the method given by Nanjo *et al.* (17), except for the DMSO as a solvent instead of ethanol. The DMSO solution of each sample (100 μL) or DMSO itself as a control was added to 100 μL of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) dissolved in DMSO. After mixing for 10 s, the solution was introduced into flat cell, and DPPH signal was detected 2 min later. The scavenging ratio was calculated using the following equation $R(\%) = [(H_o - H)/H_o] \times 100\%$, where H_o and H are the relative heights of the third peak in the EPR spectrum of the DPPH radical in the absence of a tested compound and in the presence of the compound, respectively.

Results and discussion

Superoxide anion radical scavenging capacity

The shorter half-life of $HO^{\bullet}$ and $O_2^{\bullet}$ in aqueous solution makes them difficult to measure and only a few techniques can be utilized. In our laboratory, the $O_2^{\bullet}$ antiradical measurement of several pharmaceuticals and plant extracts showing antioxidant activity have been carried out based on CL accompanying the reaction of $O_2^{\bullet}$ produced in DMSO (13). The emission spectrum was found to have four bands with maxima at 480 nm, 580 nm, 640 nm and 700 nm. The CL bands at 640 nm and 700 nm were described to the vibrational component (0,0),(1,0) transition in O_2 ($^1\Delta_g$) dimole (18,19):

$$2\ O_2\left(^1\Delta_g\right) \rightarrow 2\ O_2\left(^3\Sigma_g^-\right) + h\nu$$

where $^1\Delta_g$ and $^3\Sigma_g^-$ are the first excited state and the ground state of the O_2 molecule, respectively. The band at 580 nm can be attributed to the same transition but with a vibrational quantum number of (1,0), whereas the band at 480 nm corresponds to the simultaneous transition in the O_2 dimole:

$$O_2\left(^1\Delta_g\right)\ O_2\left(^1\Sigma_g^+\right) \rightarrow 2\ O_2\left(^3\Sigma_g^-\right) + h\nu$$

with a quantum number of (0,0). The spectra were presented in a recent paper (13). Based on the spectroscopic and literature data the following reactions may be responsible for the $^1O_2$ generation (18,20).

$$O_2^{\bullet} \rightarrow {}^1O_2 + \text{electron} \quad (1)$$

$$O_2^{\bullet} + O_2 \rightarrow O_4^{\bullet} \quad (2)$$

$$O_4^{\bullet} + O_2^{\bullet} \rightarrow \left(^1O_2\right)_2 + O_2^{\bullet} \quad (3)$$

$$2O_2^{\bullet} + 2(CH_3)_2SO \rightarrow {}^1O_2 + (CH_3)_2SO_2 + CH_3SO(CH_2)^- + OH^- \quad (4)$$

$$2\ {}^1O_2 \rightarrow \left(^1O_2\right)_2 \rightarrow 2\ {}^3O_2 + \text{light} \quad (5)$$

Singlet oxygen dimoles $(^1O_2)_2$ emit ultraweak CL during their radiative deactivation to the ground state ($^3O_2$).

Fig. 2 presents the CL kinetics detected from the $O_2^{\bullet}$/DMPO system alone (blank) (curve 1), and in the presence of well-known inhibitors of $O_2^{\bullet}$ -Tiron (curve 6) and NBT (curve 5), and in the presence of representative compounds F7 and F10 (curves 4 and 3, respectively). Tiron and NBT showed high responses against $O_2^{\bullet}$ and $^1O_2$, which was in good agreement with the literature data (21). Compound F7, similarly as compounds F1–F6, F8, F11 and F12 (kinetics not shown) represented antiradical properties. Compounds F9 and F10 enhanced the maximal intensity of CL. The $O_2^{\bullet}$ scavenging ratios of the TZD derivatives were examined and compared to that of Tiron and NBT as shown in Fig. 3. The results indicated that the $O_2^{\bullet}$ -dependent light emission was quenched by flavones F1–F8, F11 and F12 varying from 16% to 89% at concentration of 1 mmol/L. The majority of the compounds exhibited the $O_2^{\bullet}$ scavenging activity at least equal with that monitored specifically for these radical scavengers Tiron and NBT (at the same concentration) or higher activity. It is noteworthy that the absolute second order rate constant interaction between $O_2^{\bullet}$ and Tiron was determined as 5×10^8 L/mol per s (22). This means that the tested TZD derivatives in the reaction with $O_2^{\bullet}$ could serve as efficient reducing agents or as a proton source (7):

$$O_2^{\bullet} + TZD(NH) \rightarrow TZD(N^{\bullet}) + HO_2^- \quad \text{H–atom transfer} \quad (6)$$

$$HO_2^- + (CH_3)_2SO \rightarrow OH^- + (CH_3)_2SO_2 \quad (7)$$

$$O_2^{\bullet} + TZD(N\text{-}CH_3) \rightarrow TZD\left(N\text{-}CH_2^-\right) + HO_2^{\bullet} \text{ proton transfer} \quad (8)$$

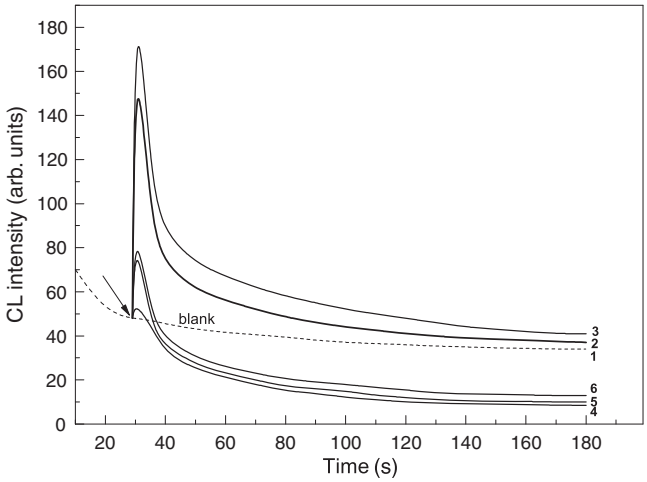

Figure 2. Scavenging effect of the representative compounds F7 (curve 4) and F10 (curve 3) and reference $O_2^{\bullet}$ inhibitors Tiron (curve 6) and *p*-nitroblue-tetrazolium chloride (curve 5) on the CL intensity recorded from 1 mmol/L $O_2^{\bullet}$ in DMSO after adding DMSO (0.5 mL) (curve 2) (control). Curve 1 represents blank ($O_2^{\bullet}$/DMSO). Reaction mixtures contained (1 mmol/L) of the flavonyl-2,4-thiazolidinedione, imidazolidinedione and rhodanine derivatives or reference inhibitors. An arrow indicates the moment of the compounds dissolved in 0.5 mL DMSO or 0.5 mL of DMSO addition. Denotations of the examined compounds are given in Fig. 1. CL, chemiluminescence; DMSO, dimethylsulfoxide.

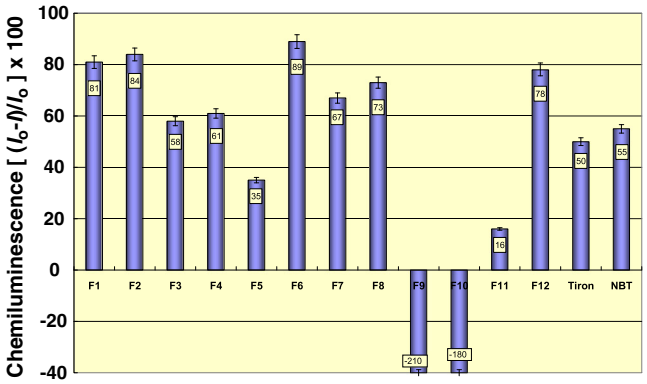

Figure 3. Superoxide radical scavenging activity of the flavonyl-2,4-thiazolidinedione, imidazolidinedione and rhodanine derivatives (F1–F12), Tiron and NBT (1 mmol/L) monitored using the chemiluminescence from 1 mmol/L $O_2^{\bullet}$ arising in DMSO. Temperature 295 K. The remaining conditions are reported in Experimental section. NBT, *p*-nitroblue-tetrazolium chloride.

Depending on the nature of products arising from $O_2^{\bullet}$ in reactions 6 and 8, consistent with the CL mechanism, it is likely that reaction 6 was responsible for the scavenging of $O_2^{\bullet}$ and reaction 8 might be responsible for an increase of the light emission observed for compounds F9 and F10 as follows (21,23):

$$2\ HO_2^{\bullet} \rightarrow {}^1O_2 + H_2O_2 \quad \left(k \approx 10^6 L/mol/s\right) \tag{9}$$

$$HO_2^{\bullet} + O_2^{\bullet} \rightarrow H_2O_2 + {}^1O_2 \quad \left(k \approx 8.5 \times 10^7 L/mol/s\right) \tag{10}$$

The structural feature of TZD derivatives provides the conjugation between the D-ring and B-ring or A-ring, and contributes to the stabilization of the products arising from TZD transformation.

Hydroxyl radical scavenging capacity

The hydroxyl radical as a short-lived ($t_{1/2} \approx 10^{-9} s$) species would be detected using the EPR spectroscopy in combination with the spin-trapping method, i.e., the radical addition to a spin trap. The method is based on the formation of a stable free radical by reacting covalently with a trap with an unstable free radical. Nitrones, e.g., DMPO are the spin traps most commonly used. Using this method and the Fenton reaction as a generator of $HO^{\bullet}$ (14,15), we examined reactivity of TZD derivatives towards the radical (Fig. 4). The hydroxyl radical trapped by DMPO gives a spin-adduct for which the EPR spectrum shows four-splitted lines with an intensity ratio 1: 2: 2: 1 and hyperfine splitting constant of $a_N = a_H^{\beta} = 14.9$ G. The parameters we measured are consistent with those values for the DMPO − OH spin adduct reported by other authors (16). All examined TZD derivatives were able to decrease the EPR signal amplitude of the DMPO − OH spin adduct at least by 30% (ranging from 25% to 68%) at concentration of 2.5 $mmolL^{-1}$ (Fig. 4). The scavenging effects exhibited by compounds F1, F2, F4, F7, F8 and F11 are significantly higher than an inhibition of the signal found for positive control salicylate. A salicylate concentration of 3.5 mmol/L exhibited the $HO^{\bullet}$ scavenging ratio equal to about 40%, although the rate constant for the salicylate reaction with $HO^{\bullet}$ was reported to be high 1.2×10^{10} L/mol per s (24). We checked the specificity of the Fenton reaction as a generator of $HO^{\bullet}$ by the addition of catalase (19,900 U/mg) from bovine liver, an enzyme decomposing H_2O_2. Catalase at a concentration of 180 μg/L prevented the appearance of any EPR signal.

The ERP spin-trapping method used for estimating the ability of the tested compounds to scavenge $HO^{\bullet}$ is the technique routinely applied for detection of short-lived oxygen species. The hydroxyl radical exhibits the high reactivity with ethanol (20); therefore, addition of this compound should have an influence on the DMPO-OH spin adduct formation. Indeed, the presence of 4.9 mol/L ethanol in the Fenton–DMPO reaction caused inhibition of the EPR signal amplitude by about 75%, and resulted in the appearance of a new six-line spectrum due to trapping of the α-hydroxyethyl radical (16) (data not shown). In turn, the addition of catalase (a key enzyme playing a part in the decomposition of H_2O_2) at the concentration of 150 μg/mL

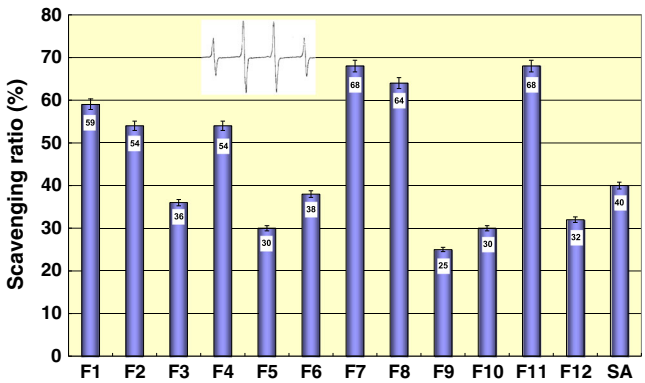

Figure 4. The inhibitory effect of the flavonyl-2,4-thiazolidinedione, imidazolidinedione and rhodanine derivatives (F1–F12) and SA (2.5 mmol/L) exerted on the 5,5-dimethyl-1-pyrroline oxide-OH spin adduct formation. Measurement conditions are reported in the Experimental section. The electron paramagnetic resonance spectrometry settings were microwave power 20 mW, modulation amplitude 0.5 G, time constant 0.3 s and receiver gain 4×10^4. Temperature 295 K. SA, salicylate.

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completely eliminated the spectrum. These findings further indicate that H_2O_2 was the necessary reagent for HO• generation by the Fenton reaction, and confirm that the observed EPR spectrum is attributed to the HO• – *DMPO* reaction.

As one of the modes of action in antioxidants is chelating metal ions, such as Fe(II)/Fe(III) and Cu(I)/Cu(II) involved in the conversion of $O_2^{\bullet-}$ and H_2O_2 into HO• in the Fenton reaction (14,15), we examined if TZD derivatives were able to form complexes with Fe ions under our experimental conditions. We found, using UV-visible spectrophotometry, that the compounds tested might not exert their antioxidant effects through binding Fe(II) and Fe(III) ions (data not shown). These findings are consistent with those of Linxiang and co-workers (25).

The hydroxyl radical is one of the strongest oxidizing agents able to react with the first encountered biomolecule *in vivo* (21,26). The species is involved in several reactions, such as adding to a double bond, e.g., in the benzene molecule, reaction with hydrogen-containing molecules resulting in H-abstraction, and the rapid electron transfer reaction. The mutagenic capacities of HO• and pathophysiological implications have been extensively studied (27,28). Therefore, a study on the inhibition of HO• and the search for compounds as good antioxidants, particularly of natural origin, have attracted attention as agents to prevent against oxidative stress.

2,2′-Diphenyl-1-picrylhydrazyl radical scavenging activity

The ability of the tested compounds to exhaust their hydrogen donating activity was detected using DPPH radical. This compound as a stable free radical is able to abstract the hydrogen atom directly or via an electron transfer process (29–31). A typical EPR spectrum of DPPH• monitored in DMSO is presented in Fig. 5.

The spectrum measured is very similar to that reported by other authors (30). All tested TZD derivatives were able to decrease the EPR signal from the DPPH• radical due to its reduction. We compared the influence of TZD derivatives on the EPR spectrum of DPPH• to the behavior of the well-known radical scavengers, ascorbic acid and vitamin E analog, Trolox.

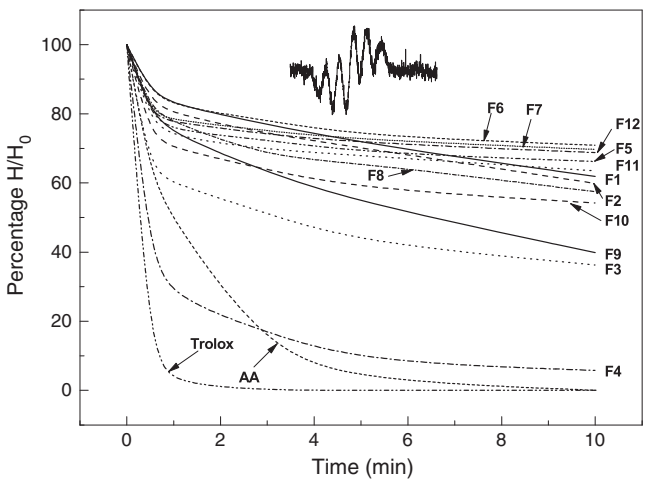

Figure 5. The disappearance of the 2,2′-diphenyl-1-picrylhydrazyl radical as a function of time in the presence of flavonyl-2,4-thiazolidinedione, imidazolidinedione and rhodanine derivatives, Trolox and AA (1.25 mmol/L). The reaction contained ethanol (75%, v/v) and dimethylsulfoxide (25%, v/v). AA, ascorbic acid.

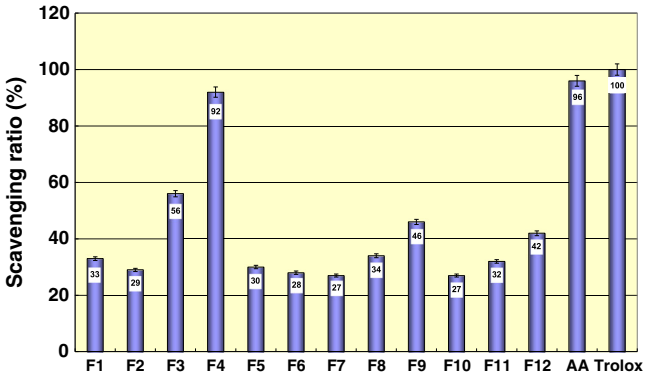

Figure 6. Scavenging effect of the tested flavonyl-2,4-thiazolidinedione, imidazolidinedione and rhodanine derivatives, AA and Trolox (1.25 mmol/L) on free radical 2,2′-diphenyl-1-picrylhydrazyl in ethanol (75%, v/v)/dimethylsulfoxide (25%, v/v) solution. Denotations of the examined compounds are given in Fig. 1. Measurement conditions are reported in the Experimental section. The electron paramagnetic resonance spectrometry settings were microwave power 20 mW, modulation amplitude 0.2 G, time constant 0.2 s and receiver gain 3.2×10^4. Temperature 295 K. AA, ascorbic acid.

As seen from Fig. 5 only ascorbic acid, Trolox and compound F4 reacted rapidly with the DPPH•, reaching a steady state in less than 2 min. The remaining compounds reacted more slowly with the radical.

As indicated in the Experimental section, the antiradical activity was evaluated from the plot of the percentage DPPH• remaining when the kinetics reached a steady state. Fig. 6 presents these data. Among the compounds examined, F4 was the most potent in the DPPH• scavenging, reaching about 90% effect. The remaining TZD derivatives presented the antiradical efficiency ranging from 27% to 56% being lower than that of ascorbic acid and Trolox.

It is commonly accepted that the ability of antioxidants to scavenge the DPPH• belongs to their hydrogen-donating ability or an electron process (28,31). The data reported in Fig. 5 showed that reactions of the tested derivatives are biphasic, similar to the reference antioxidants used in this study namely ascorbic acid and Trolox. The reduction of the DPPH radical was successfully used for the primary characterization of total antioxidant activity of several biologically important compounds (17,29,30,32).

Conclusions

The findings in the present study revealed promising flavone derivatives with outstanding oxygen free radicals scavenging property. The preliminary TZD derivatives evaluation indicated that 10 of the 12 examined compounds exhibited significant direct scavenging activities towards $O_2^{\bullet-}$, and all compounds were effective as HO• scavengers. This property is important with regard to the participation of ROS in cell damage under oxidative stress. In addition, TZD derivatives were found to be effective as reducing agents in the reaction with DPPH•. The design of flavone-based drugs against ROS and the primary screening of their antioxidant capacity constitutes a promising direction for novel anticancer pharmaceutical drugs, long-term prevention against neurodegenerative pathologies or natural product, such as antioxidants for use in, for example, food preservatives.

References

1. Greeff J, Joubert J, Malan SF, van Dyk S. Antioxidant properties of 4-quinolones and structurally related flavones. Bioorg Med Chem 2012;20:809–18.
2. Yau-Hung C, Zhi-Shiang Y, Chi-Chung W, Yeong-Sheng C, Bo-Cheng W, Chih-Ang H, Tzenge-Lien S. Evaluation of the structure–activity relationship of flavonoids as antioxidants and toxicants of zebrafish larvae. Food Chem 2012;134(2):717–24.
3. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther 2002;96:67–202.
4. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: Mechanism and action. Mutat Res 2005;579:200–13.
5. Quideau S, Deffieux D, Douat-Casassus C, Pouysegu L. Plant polyphenols: chemical properties, biological activities, and synthesis. Angew Chem Int Ed 2011;50:586–621.
6. Middleton E Jr., Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52:673–751.
7. Seyoum S, Asres K, El-Fiky FK. Structure-radical scavenging activity relationships of flavonoids. Phytochemistry 2006;67:2058–70.
8. Research Organization of Ankara University (09B3336003) Project, Studies on the synthesis and antidiabetic activity of some new thiazolidinedione derivatives, 2009–2012, Oya Bozdağ-Dündar (Project coordinator).
9. Bozdağ-Dündar O, Verspohl EJ, Ertan R. Synthesis and hypoglycemic activity of some new flavone derivatives, III. Communication: 3′-flavonyl-2,4-thiazolidinediones. Arzneim-Forsch/Drug Res 2000;50(II):626–30.
10. Bozdağ-Dündar O, Waheed A, Verspohl EJ, Ertan R. Synthesis and hypoglycemic activity of some new flavone derivatives. 4th Communication: 6-flavonyl-2,4-thiazolidinediones. Arzneim-Forsch/Drug Res 2001;51(II):623–7.
11. Bozdağ O, Verspohl EJ, Ertan R. Synthesis and hypoglycemic activity of some new flavone derivatives II. Communication: 4′-flavonyl-2,4-thiazolidinediones. Arzneim-Forsch/Drug Res 2000;50(II):539–43.
12. Valentine JS, Miksztal AR, Sawyer DT. Methods for the study of superoxide chemistry in non-aqueous solution. Methods Enzymol 1984;105:71–80.
13. Kruk I, Michalska T, Kładna A, Berczyński P, Aboul-Enein HY. Chemiluminescence investigations of antioxidative activities of some antibiotics against superoxide anion radical. Luminescence 2011;26:598–603.
14. Goldstein S, Meyerstein D, Czapski G. The Fenton reagents. Free Radic Biol Med 1993;15:435–445.
15. Walling C. Fento's reagents revisted. Acc Chem Res 1975;8:125–31.
16. Finkenstein E, Rosek GM, Rauckamn EJ. Spin trapping of superoxide and hydroxyl radical: practical aspects. Arch Biochem Biophys 1980;200:1–16.
17. Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radic Biol Med 1966;21:895–952.
18. Khan AU, Kasha M. Chemiluminescence arising from simultaneous transition in pairs of singlet oxygen molecules. J Am Chem Soc 1970;92:3293–300.
19. Khan AU. Activated oxygen: singlet molecular oxygen and superoxide anion. Photochem Photobiol 1978;28:615–27.
20. Gampp H, Lippard SJ. Reinvestigation of 18-Crown-6 ether/potassium superoxide solutions in Me_2SO. Inorg Chem 1983;22:357–8.
21. Kruk I. Environmental Toxicology and Chemistry of Oxygen Species. In: Hutzinger O, Kruk I, editors. *The Handbook of Environmental Chemistry Reactions and Processes, Part 1*, Vol. 2. New York: Springer, 1998.
22. Greenstock CL, Miller RW. The oxidation of tiron by superoxide anion. Kinetics of the reaction in aqueous solution in chloroplast. Biochim Biophys Acta 1975;396:11–16.
23. Sawyer DT, Gibian MJ, Morrison MM, Seo ET. On the chemical reactivity of superoxide ion. J Am Chem Soc 1978;100:627–8.
24. Bartosz G, The second face of oxygen. Free radicals in nature Oxford University Press (second revised edition) Warsaw 2003, p.50.
25. Linxiang L, Abe Y, Kanagawa K, Shoje T, Mashino T, Mochizuki M, et al. Iron chelating agent never suppress Fenton reaction but participates in quenching spin-trapped radicals. Anal Chim Acta 2007;599:315–19.
26. Valko M, Izakovic M, Mazur M, Thodes CI, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Moll Cell Biochem 2004;266:37–56.
27. Halliwell B, Gutteridge IMC. *Free Radicals in Biology and Medicine*. New York: Oxford University Press, 1999.
28. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44–84.
29. Brand-Willams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 1995;28:25–30.
30. Tang Y-Z, Liu Z-Q. Free-radical scavenging effect of carbazole derivatives on DPPH and ABTS radicals. J Am Oil Chem Soc 2007;84:1095–100.
31. Foti MC, Daquino C, Geraci C. Electron-transfer reaction of cinnamic acids and their methyl esters with the $DPPH^{\bullet}$ radical in alcoholic solutions. J Org Chem 2004;69:2309–14.
32. Chen CW, Ho CT. Antioxidant properties of polyphenols extracted from green and black tea. Food Lipids 1995;2:35–46.