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6-Methyl 3-chromonyl 2,4-thiazolidinedione/ 2,4-imidazolidinedione/2-thioxo-imidazolidine-4-one compounds: novel scavengers of reactive oxygen species

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ABSTRACT: The benefits of antioxidants on human health are usually ascribed to their potential ability to remove reactive oxygen species providing protection against oxidative stress. In this paper the free radicals scavenging activities of nine 6-methyl 3-chromonyl derivatives (CMs) were evaluated for the first time by the chemiluminescence, electron paramagnetic resonance, spin trapping and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) methods. The total antioxidant capacity was also measured using a ferric-ferrozine reagent. Compounds having a hydrogen atom at the N3-position of the β-ring were effective in quenching CL resulted from the KO_2/18-crown-6-ether system (a source of superoxide anion radical, $O_2^{\bullet-}$) in a dose-dependent manner over the range of 0.05–1 mmol/L [IC_{50} ranged from 0.353 (0.04) to 0.668 (0.05) mmol/L]. The examined compounds exhibited a significant scavenging effect towards hydroxyl radicals ($HO^{\bullet}$ *$HO^{\bullet}$*), produced by the Fenton reaction, and this ranged from 24.0% to 61.0%, at the concentration of 2.5 mmol/L. Furthermore, the compounds examined were also found to inhibit $DPPH^{\bullet}$ and this ranged from 51.9% to 97.4% at the same concentration. In addition, the use of the total antioxidant capacity assay confirmed that CM compounds are able to act as reductants. According to the present study, CM compounds showed effective *in vitro* free radical scavenging activity and may be considered as potential therapeutics to control diseases of oxidative stress-related etiology. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: 2,4-Thiazolidinedione; 2,4-imidazolidinedione; 2-thioxo-imidazolidine-4-one; radical scavenging; chemiluminescence; electron paramagnetic resonance; spin trapping

Introduction

Much work has been carried out recently on antioxidant activity of naturally occurring pharmotherapeutic compounds such as flavonoids. These compounds exhibit important health protecting properties such as anti-inflammatory, antidiabetic, antimicrobial, antioxidant, antifungal and anti-HIV activities, and can be used to treat cardiovascular diseases and different types of cancer (1–10). The identification of the antioxidant effect of flavonoids has been demonstrated in many studies as important in many scientific fields and deals with a great number of flavonoid residues containing aromatic hydroxyl groups. This antioxidant function is due to both their chelating properties as well as to their interaction with membrane lipids and protein (11–13). Many *in vitro* studies have reported the potent reactive oxygen species (ROS) scavenging abilities of flavonoids.

ROS, generated in excess in the human body cause oxidative damage to various biomolecules, including lipids, proteins and DNA (13,14). Flavonoids, as the most widespread compounds, are present in plants, thereby in the human diet, and play an important role in antioxidant defenses against ROS-induced oxidative stress.

In the literature, studies of the redox behavior of flavonoids deal in general with those containing aromatic OH groups. In a previous study (15), we have presented a synthesis and antidiabetic activity of a novel group of compounds: 6-methyl-3-chromonyl-2,4-thiazolidinedione/2,4-imidazolidinedione/2-thioxo-imidazolidine-4-one (CMs) (Fig. 1) of which neither chromone nucleus nor substitutions at a C-2 are phenyl substituted chromones. In the present study, the antioxidant capacity of these chromone derivatives was investigated using

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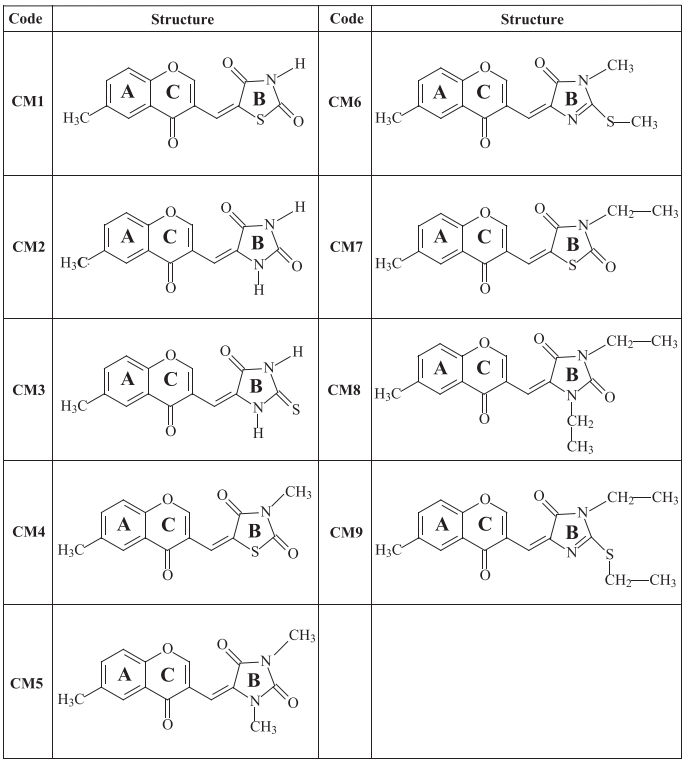

Figure 1. Structures of 2,4-thiazolidinedione, 2,4-imidazolidinedione and 2-thioxo-imidazolidine-4-one derivatives with 6-methyl chromonyl substituents (CMs).

chemiluminescence (CL), spectrophotometric and electron spin resonance techniques.

Materials and methods

Compounds CM1–CM3 were synthesized by the Knoevenagel reaction of 3-formyl chromone with thiazolidine-2,4-dione/imidazolidine-2,4-dione/2-thioxo-imidazolidine-4-one using an acetic acid/sodium acetate mixture. CM4–CM6 and CM7–CM9 were obtained by reacting CM1–CM3 with methyl iodide and ethyl iodide in dimethylformamide/anhydrous sodium carbonate mixture, respectively (14). All reagents for CM1–CM9 synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA).

5,5-Dimethyl-1-pyrroline-1-oxide (DMPO), tiron (4,5-dihydroxy-1,3-benzene-disulfonic acid), trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxyl acid), 18-crown-6-ether (1,4,7,10,13,16) hexaoxacyclooctadecane, β-carotene and ascorbic acid were purchased from Merck; 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\bullet}$) and 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine) from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany), anhydrous dimethylsulfoxide (DMSO) from Aldrich, potassium superoxide (KO_2) and ammonium ferrous sulfate hexahydrate from Fluka (Buchs, Switzerland).

All reagents were of analytical grade and those not specified were obtained from Merck. The CM compounds, trolox and tiron were dissolved in DMSO, which was suitable for dissolving both water-soluble and water-insoluble reagents.

Antioxidant activity determination

Chemiluminescence measurements. The superoxide anion scavenging property of the tested chromone derivatives was measured using a previously described methodology based on superoxide anion radical ($O_2^{\cdot-}$)-induced light emission in DMSO (16). The reaction between 18-crown-6-ether and KO_2 was used as a source of $O_2^{\cdot-}$ (17).

We successfully applied this reaction for the measurement of the antioxidant activity of several biologically important compounds. The reaction mixture contained a 60 mg aliquot of 18-crown-6-ether dissolved in 10 mL of dry DMSO and 7 mg of KO_2 was added quickly to avoid contact with air humidity. The mixture was stirred for 60 min to give a pale yellow solution of 10 mmol/L $O_2^{\cdot-}$ stable at room temperature. Solutions used in the experiments were prepared in DMSO, and the $O_2^{\cdot-}$ concentration was determined using the absorbance in the ultraviolet region at $\lambda_{max} = 251$ nm [$\epsilon = 2686 \pm 29$/M/cm, (17)]. For the measurement, $O_2^{\cdot-}$ was used as a 1 mmol/L solution. Light-emitting reactions were carried out in a thermostated glass cuvette placed in a light-tight chamber just before an EMI9553Q photomultiplier with an S20 cathode sensitive in the range 200–800 nm, interfaced with a computer for data acquisition and handling. The cuvette was exhausted using a B-169 vacuum system (Büchi, Flawill, Switzerland). All measurements were carried out at 22 ± 1°C. The resulting CL signal derived from the $O_2^{\cdot-}$ solution (control) or that influenced presence of the CM compound was recorded as the kinetic curve of light emission decay. The percentage of $O_2^{\cdot-}$ radical scavenging activity (Q) or enhancing was calculated using the following equation:

$$Q(\%) = [(I_o - I)/I_o] \times 100$$

where I_o is the light intensity from the control reaction and I is the light intensity in the presence of CM or other scavenger. Concentration of the CM compound providing 50% scavenging

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(IC_{50}) was calculated from the graph plotted I_o/I against the CM concentration (mmol/L).

Electron spin resonance measurements

Spin-trapping measurements for hydroxyl radical. The second test for the radical scavenging capacity of the CM compounds is based on the electron paramagnetic resonance (EPR) spin trapping technique with DMPO as a spin trap. The Fenton reaction in a sodium trifluoroacetate, well known in the subject literature as a source of hydroxyl radical ($HO^{\bullet}$), was used to examine whether CMs could scavenge $HO^{\bullet}$ (18,19). The final concentrations of reagents (in volume of 2 mL) were ammonium ferrous sulfate (62.5 μmol/L), sodium trifluoroacetate buffer (10 mmol/L, pH 6.15), H_2O_2 (0.5 mmol/L) and DMPO (25 mmol/L, water solution). The reaction was initiated by adding ammonium ferrous sulfate, and the reaction mixture was introduced in the EPR cavity using a quartz flat cell.

The EPR signal was analyzed approximately 1 min after the start of the reaction at room temperature. The percentage of inhibition of the $DMPO-O^{\bullet}H$ spin adduct formation was calculated as follows:

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

where H is the relative height of the second peak in the spectrum of the spin adduct in the presence of a tested compound, and H_o is that of the control reaction. DMSO is known to react with DMSO producing the EPR signal that can affect observations of the $DMPO-O^{\bullet}H$ adduct. Because the CM samples were made in DMSO, due to their insolubility in water, the control samples also contained an equivalent concentration of DMSO (2%).

Measurements for 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity. $DPPH^{\bullet}$ scavenging activities of CM compounds were determined by the method described by Nanjo *et al.* (20) with slight modification, i.e., using DMSO as a solvent instead of ethanol. The DMSO solution of each examined compound (100 μL) or DMSO itself as a control was mixed with 300 μL of DPPH solution in ethanol. After mixing for 10 s, the mixture was introduced into a flat cell, and a DPPH signal was detected 2 min later. The decrease in the EPR spectrum amplitude of the third peak (H_{DPPH}) was measured every 5 min until the reaction reached a plateau. For each antioxidant tested, the reaction kinetics were plotted, and the percentage of inhibition of the $DPPH^{\bullet}$ signal was calculated based on an area under the kinetic curve of the signal decrease, within 30 min of reaction time. The percentage of inhibition was measured using the equation:

$$Q_{DPPH}(\%) = [(H_o - H)/H_o] \times 100$$

where H_o and H are the relative EPR signal integrals of the control reaction ($DPPH^{\bullet}$ solution) in the absence of the tested antioxidant, and in the presence of the tested antioxidant, respectively.

All EPR spectra were recorded on a standard X-band spectrometer operating at 9.3 GHz with a 100 kHz modulation of a steady magnetic field. Samples were placed in the EPR cavity using an EPR quartz flat cell with an optical path length of 0.25 mm.

Ferric reducing capacity measurements. Ferric reducing activity of the CM compounds was measured using a novel antioxidant assay elaborated by Berker and co-workers (21). The method is based on the reduction of a Fe(III)-ferrozine reagent by a compound with the antioxidant power to the stable Fe(II)-ferrozine complex that exhibits a single sharp absorbance peak with maximum at 562 nm.

The ferric ferrozine complex was prepared as follows. An aqueous solution containing 0.024 g of $NH_4Fe(SO_4)_2.12\ H_2O$ and 1 mL of 1 mol/L HCl was mixed with a solution of 0.123 g ferrozine dissolved in water. After mixing, the mixture was diluted to 25 mL with distilled water to obtain the final Fe(III) concentration of 2 mmol/L and ferrozine concentration 0.1 mmol/L. This Fe(III)-ferrozine complex was kept in a stoppered dark colored bottle; 1.5 mL of the complex was added to 1 mL of antioxidant solution at 1 mmol/L concentration and 2 mL of buffer solution (0.2 mol/L acetic acid/sodium acetate pH 5.5). The increase in absorbance at 562 nm was monitored after 1.5 h standing at room temperature.

Absorption spectra were recorded using a ultraviolet-visible spectrophotometer from Carl Zeiss Technology M-40 with Win-Aspect software (Jena, Germany).

Results and discussion

The extent of O_{2^-} radical scavenging activity of the CM compounds was estimated using the CL technique, i.e., by measurements of the change in light emission from decomposition of KO_2 in DMSO in the presence of 18-crown-6-ether. Typical CL kinetics measured from this reaction mixture are shown in Fig. 2. The light intensity from the O_{2^-}/DMSO system (curve 1) was decreased in the presence of compounds CM1, CM2 and CM3 (curve 2) or the positive controls β-carotene and methionine (curves 4 and 5, respectively). Compounds CM1, CM2 and CM3 were able efficiently scavenge O_{2^-}, in a concentration-dependent manner (Fig. 2B). The IC_{50} values of these compounds were measured in units of mmol and were compared with that of the powerful inhibitor of O_{2^-} such as tiron under the same experimental conditions (Table 1). Compound CM3 was considerably more efficient than the rest of the examined compounds and the positive control, tiron presenting an IC_{50} of 0.353 ± 0.04 mmol/L. Tiron has been reported to react with O_{2^-} with the high rate constant of 5×10^8 L/mol per s (22). The scavenging effect of the CM1–CM3 compounds on the superoxide anion radical decreased in the order of CM3 > CM1 > CM2 reaching 74.5%, 62.3% and 58.7% at the concentration of 1 mol/L, respectively. The other compounds CM4–CM9 revealed a strong increase in the CL within the examined concentration range (see Fig. 2A curve 3 as an example).

The strong quenching effect of the $^1O_2$ scavengers such as β-carotene (a compound that is frequently used as a diagnostic tool for involvement of $^1O_2$, or methionine) observed in our CL assay (Fig. 2A, curves 4 and 5, respectively) confirms generation of $^1O_2$ in the O_{2^-}/DMSO reaction. The rate constant k_q for quenching of $^1O_2$ by carotene was found to be 1.0×10^{10} L/mol per s, and by methionine 3.0×10^7 L/mol per s (23).

Results from the O_{2^-} scavenging assay show that the tested CM compounds could act as the reducing agents or as a proton source (24). The hydrogen atom transfer from compound CM to $O_2^{\cdot-}$ occurs with the decomposition of $O_2^{\cdot-}$ to HO_2^- species, followed by reaction 2:

$$O_2^{\cdot-} + H \rightarrow HO_2^- \quad (1)$$

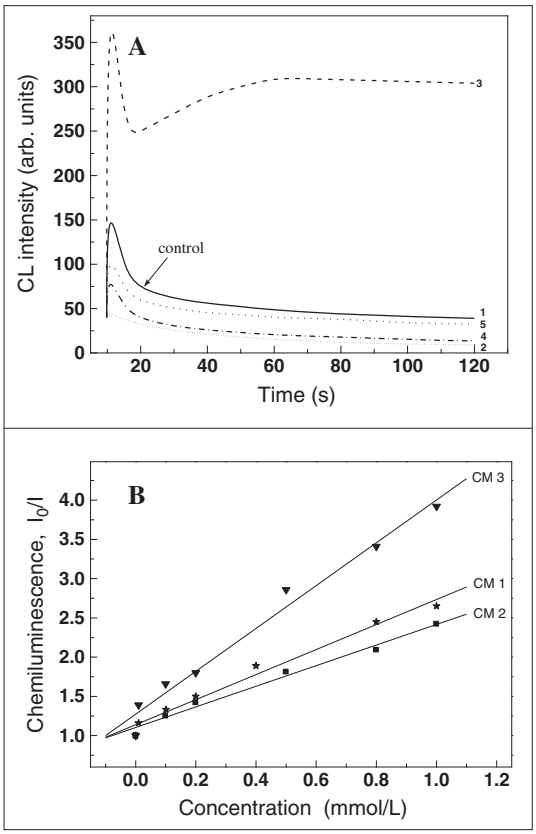

Figure 2. (A) The effect of the 6-methyl chromonyl (CMs), DMSO, β-carotene, and methionine on the chemiluminescence (CL) intensity recorded from 1 mmol/L of $O_2^{\cdot-}$ produced in DMSO (curve 1, control). Curve 2: the CL recorded under the same conditions but after an addition of 1 mmol/L of compound CM3 as a representation. Curve 3: after addition of 1 mmol/L of CM5. Curves 4 and 5: after addition of 1 mmol/L of β-carotene and methionine, respectively. (B) A dose-dependent inhibition of the CL intensity (I_o/I) from the $O_2^{\cdot-}$ system detected in the presence of CM1, CM2 and CM3, under the same conditions as in part (A). Temperature, 295 K. Denotations of the CMs are given in Fig. 1.

$$HO_2^- + (CH_3)_2SO \rightarrow OH^- + (CH_3)_2SO_2 \quad (2)$$

Gampp and Lippard suggested that $(CH_3)_2SO_2$ in the reaction generating $O_2^{\cdot-}$ serves both as a reducing agent and as a proton donor (25). It is a highly probable that the hydrogen transfer from the CM compound to $O_2^{\cdot-}$

$$O_2^{\cdot-} + CM \rightarrow HO_2^- + CM^{\cdot} \quad (3)$$

was responsible for the CL decrease in the presence of compounds CM1–CM3. In turn, an increase in the light emission, seen in the presence of the rest compounds, might result from proton transfer with the HO_2 radical formation:

$$O_2^{\cdot-} + CM \rightarrow HO_2^{\cdot} + CM^- \quad (4)$$

The dismutation of two hydroperoxy radicals generates $^1O_2$ (26,27).

$$2\ HO_2^{\cdot} \rightarrow {}^1O_2 + H_2O_2 \quad (5)$$

The enhancing effect of the CM compounds increased in the following order: CM8 > CM5 > CM9 > CM7 > CM6 > CM4 (data not shown). It is noteworthy that these compounds differ from compounds CM1, CM2 and CM3 the presence of a methyl group linked with the B-ring at its N3 position or in the side chain.

The next reactive radical derived from the reduction of molecular oxygen that can be generated in living systems is the hydroxyl radical, $HO^{\cdot}$.

The hydroxyl radical scavenging ability of the CM compounds is shown in Fig. 3 using the Fenton reaction as the $HO^{\cdot}$ generator, and EPR spectroscopy with spin trapping. $HO^{\cdot}$ radicals can form a nitroxide adduct with DMPO spin trap, and the spin adduct exhibits a characteristic EPR response. Spin trapping connected with EPR spectroscopy is a valuable method to characterize the short-lived oxygen radicals, as the formed adducts have relatively long lifetime. The rate constant for $HO^{\cdot}$ trapping by DMPO is high ($k = 3.4 \times 10^9$ L/mol per s) (28). Figure 3(A) shows the typical $DMPO-O^{\cdot}H$ spin adduct formed in the Fenton reaction alone (spectrum a), in the presence of DMSO (spectrum b) and in the presence of CM1 (spectrum c). The EPR spectra of the $DMPO-O^{\cdot}H$ spin adducts exhibit four split lines with an intensity ratio 1:2:2:1 and hyperfine splitting constants of $a_N = 14.86$ G and $a_H^{\beta} = 14.72$ G. These parameters are consistent with those found for the $DMPO-O^{\cdot}H$ spin adduct by other authors (29). The scavenging effect of DMSO, of the compound well known as the good $HO^{\cdot}$ scavenger, shown in Fig. 3(A) (spectrum b) is due to its high reactivity with $HO^{\cdot}$ ($k = 7.0 \times 10^9$ L/mol per s) (30). This finding agrees with the data reported, for example, Zhu and co-workers (31). As shown in Fig. 3(A) (spectrum c), compound CM1 significantly decreased formation of the $DMPO-O^{\cdot}H$ spin adduct ($P < 0.008$). In addition, direct addition of 1 mmol/L H_2O_2 efficiently increased the EPR signal intensity, whereas an addition of 180μg/mL catalase almost completely suppressed the $DMPO-O^{\cdot}H$ spin adduct

Table 1. Superoxide anion radical scavenging activity (IC_{50} mean ± SE) of the studied compounds exerting inhibition of chemiluminescence and tiron

	IC_{50} mmol/L	*a*	*b*	*R*
CM1 Q = 62.3%	0.517 (0.033)	1.183 ± 0.036	1.581 ± 0.068	0.9905
CM2 Q = 58.7%	0.668 (0.036)	1.103 ± 0.076	1.313 ± 0.122	0.9873
CM3 Q = 74.5%	0.353 (0.071)	1.378 ± 0.088	1.762 ± 0.127	0.9808
Tiron Q = 66.2%	0.451 (0.065)	1.222 ± 0.085	1.723 ± 0.698	0.9920

CM, 6-methyl chromonyl.
Results are mean of $n = 3$ measurements ± SE, $P < 0.05$.
$y = a + b$ [*c*], where $y = I_o/I$, Q(%) calculated with 1 mmol/L of the CM compound. Details are reported under Materials and methods.

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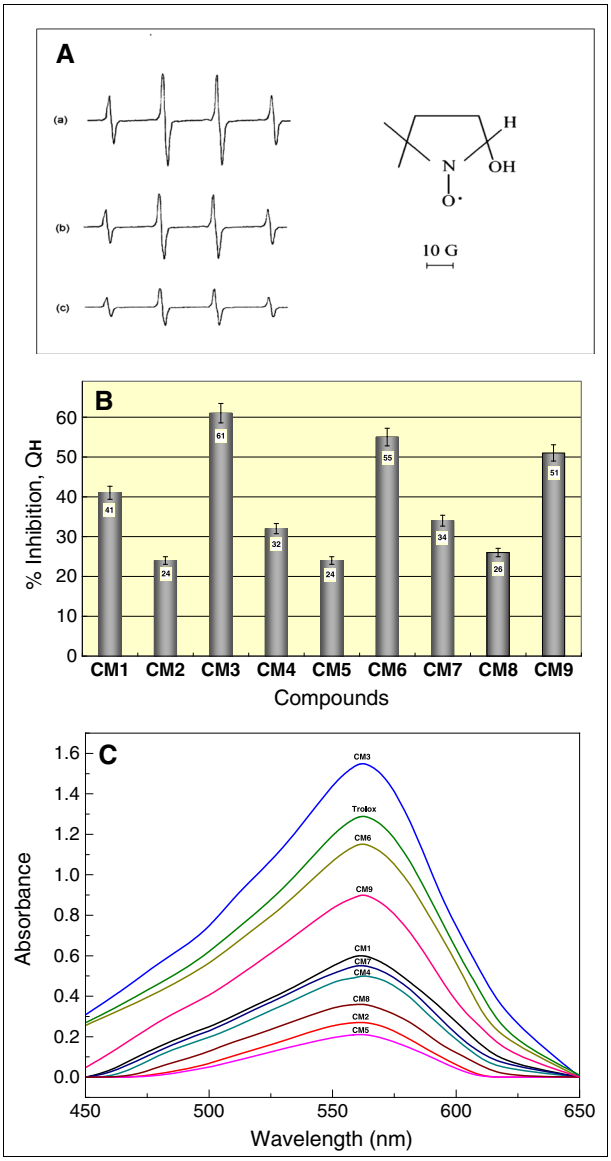

Figure 3. 6-Methyl chromonyl (CMs) are hydroxyl radical scavengers and act as reductants. (A) The electron paramagnetic resonance spectra of the DMPO — O· H spin adduct arising in the Fenton reaction. Spectrum (a): the reaction mixture contained 25 mmol/L DMPO, 0.5 mmol/L H_2O_2, 10 mmol/L sodium trifluoroacetate, pH 6.15, and 62.5 μmol/L $FeSO_4(NH_4)_2SO_4$. The reaction was initiated by an addition of the Fe(II) ion: (b) DMPO — O· H spin adduct formed after an addition of 0.1 mL DMSO (final concentration 3.5 mol/L, the control reaction); (c) DMPO — O· H spin adduct in the presence of 2.5 mmol/L CM1 dissolved in 0.1 mL of DMSO. (B) The inhibitory effect of CM compounds exerted on the DMPO spin adduct formation. The composition of the reaction mixtures was the same as in (A, spectrum c). Values are mean ± SD of at least free different experiments. The electron paramagnetic resonance setting were: microwave power 20 mW; modulation amplitude, 0.5 mT; time constant, 0.1 s; receiver gain 2.5×10^4. Temperature, 295 K. (C) Absorption spectra of iron(II)-ferrozine complex resulting from iron (III)-ferrozine complex in the presence of CM compounds (220 μg/mL) or of trolox (22 μg/mL). The data are averages of three measurements. Temperature, 294 K. The remaining conditions are reported under the Materials and methods section.

(data not shown). No significant effect on DMPO — O· H formation was observed with superoxide dismutase, a major hydrophilic $O_2^{\cdot-}$-radical scavenger. These data confirm that detection of the DMPO — O· H spin adduct is a good indicator for HO· formation in the Fenton reaction under our experimental conditions, and further a good assay for HO· scavenging activity. The inhibitory effects for these CM compounds are summarized in Fig. 3(B). Compounds CM1, CM9, CM6 and CM3 were the most effective, presenting at least 40% scavenging activity at the highest tested concentration (2.5 mmol/L) (41%, 51%, 55% and 61%, respectively).

The scavenging effect of the CM compounds on the hydroxyl radical decreased in the order of CM3 > CM6 > CM9 > CM1 > CM7 > CM4 > CM8 ≥ CM2 ≥ CM5. Our observations showed that some CMs are good HO· scavengers. It is known that HO· is the most reactive oxidizing species among ROS, and reacts with most organic compounds at near diffusion controlled rates (usually exceeded 10^9 L/mol per s) (32). The high reactivity and low selectivity of HO· result from three basic types of HO· reactions: addition to double bonds, hydrogen abstraction and electron transfer from the negative ion to the radical. All the tested CMs have the double bond between position 2 and 3 in the C ring in conjugation with the 4-oxo group. The compounds showing the highest reactivity towards HO· (51–61%) are 2-thioxo-imidazolidine-4-one derivatives bearing 6-methyl chromonyl (CM3, CM6 and CM9). This property is less pronounced for chromones having the 2,4-thiazolidindione (32–41%) followed by the compounds comprising those having 2,4-imidazolidinedione (24–26%) being the least effective. The order of effectiveness in bringing about scavenging of HO radical correlates with their antioxidant activity. In the ferric-ferrozine antioxidant assay (21), Fe(III) ion in the presence of ferrozine oxidizes an antioxidant and is itself reduced to the Fe(II)-ferrozine complex exhibiting an absorbance maximum at 562 nm, and the absorbance depends on the reducing power of the antioxidant samples. Higher absorbance indicates higher Fe(III) reducing activity (electron transfer). Figure 3(C) shows the ferric reducing capacity of the examined CMs at the same concentration of 220 μmol and trolox at 10 times the lower concentration. The reducing power of the CM compounds and trolox exhibited the following order: trolox CM3 > CM6 > CM9 > CM1 > CM7 > CM4 > CM8 > CM2 > CM5. These data demonstrate that the reducing property of the tested CMs were at least 10-fold lower than that of trolox. The order of CMs antioxidant activity is also in a good accordance with their capacity to scavenge the DPPH radical (Fig. 4).

Figure 4(A) shows the kinetic behavior of the EPR amplitudes of the DPPH· obtained for the nine examined CMs and for the three standards, trolox, ascorbic acid and tiron. Figure 4(B) presents the antiradical activity evaluated from the plot of percentage DPPH· remaining when the kinetics reached a steady state that represents the percentage of inhibition. In this method the EPR signal magnitude decreased when the DPPH· was scavenged by an antioxidant through donation of hydrogen atom or an electron followed by formation of a stable diamagnetic molecule DPPH-H (33).

The DPPH· signal magnitude was inversely related to the examined antioxidant concentration and to the reaction time. As seen in Fig. 4(B), the percentage of the remaining area in the presence of an antioxidant under the relative EPR signal integrals of the DPPH· solution may be a useful parameter to compare the antioxidant capacity of the studied CMs at the same concentration of reference compounds (trolox, ascorbic acid and tiron).

The scavenging effect of CMs and standards on the DPPH radical decreased in the order of CM3 ≥ trolox > ascorbic acid CM6 ≥ tiron ≥ CM9 > CM1 > CM4 > CM7 > CM2 > CM8 > CM5, at the same concentration.

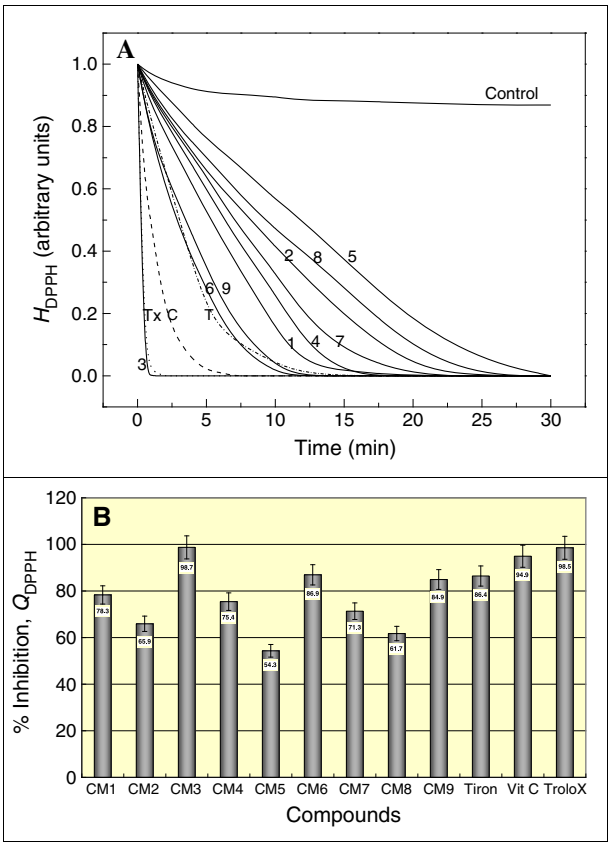

Figure 4. 6-Methyl chromonyl (CMs) are powerful DPPH radical scavengers. (A) Changes in the electron paramagnetic resonance signal amplitude of the DPPH radical with time in the presence of 2.5 mmol/L CMs (curves 1–9), trolox (Tx), ascorbic acid (C) and tiron (T) in DMSO (25% v/v/C_2H_5OH, 75% v/v). Denotation of the CMs are given in Fig. 1. The reaction mixture contained 0.125 mmol/L $DPPH^{\bullet}$. (B) The inhibitory effects of CMs (2.5 mmol/L), trolox, tiron and ascorbic acid on $DPPH^{\bullet}$ (0.125 mmol/L), detected 30 min after an addition of scavengers. The electron paramagnetic resonance setting were: microwave power 20 mW; modulation amplitude 0.2 mT; time constant 0.3 s; receiver gain 3.2×10^4. Temperature, 295 K. Values are mean ± SD of three individual experiments.

The antioxidant power involves some structural associations (reviewed in reference 34). Different studies have indicated that three structural requirements for antioxidant capacity of a flavonoid are required: (a) the catechol structure in the B-ring; (b) the presence of the 2,3-double bond in conjugation with the 4-oxo function; and (c) the presence of both 3- and 5-OH groups (35).

In addition, for the catechol in the B-ring of flavonoids, the number of hydroxyl substituents, their position, the presence of glycosides or aglycones, and the degree of conjunction play an important role in the antioxidant power (11). The examined CM compounds meet only the second (b) criterion. They have the unique structural characteristic featuring the 2, 4-thiazolidindione, imidazolidinedione or 2-thioxo-imidazolidine-4-one ring instead of the catechol in the B-ring. Our measurements show that compounds containing the 2-thioxo-imidazolidine-4-one ring showed the strongest scavenging activities with regard to O_{2^-}, $HO^{\bullet}$ and $DPPH^{\bullet}$. In addition, compounds CM3, CM6 and CM9 were the strongest reductants in the ferric-ferrozine assay. In contrast, CM compounds containing the imidazolidinedione ring exhibit the weakest antioxidant power. The differences were statistically significant ($P < 0.05$).

In conclusion, the scavenging activities for free radicals of nine 6-methyl 3-chromonyl derivatives previously synthesized and tested for their antidiabetic activity were evaluated for their antioxidant activity in the present paper. Some of the examined compounds were remarkable scavengers of ROS, such as hydroxyl and superoxide anion radicals. There is considerable knowledge concerning the role of ROS in carcinogenesis, for example, melanoma or other skin cancers. Moreover, ROS interfere with the expression of a number of genes in the signaling pathway and have a direct effect on cell proliferation, apoptosis and inflammation (reviewed recently in reference 36). Therefore, the studied compounds may have a potential therapeutic value, for controlling diseases of oxidative stress-related etiology. However, the therapeutic use of the tested 6-methyl 3-chromonyl derivatives requires further studies to confirm their efficacy as inhibitors of free radicals and assurance of their safety.

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