Screening for antiradical efficiency of 21 semi-synthetic derivatives of quercetin in a DPPH assay

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ABSTRACT
The group of 21 novel semi-synthetic derivatives of quercetin was screened for the antiradical efficiency in a DPPH assay. The initial fast absorbance decrease of DPPH, corresponding to the transfer of the most labile H atoms, was followed by a much slower absorbance decline representing the residual antiradical activity of the antioxidant degradation products. Initial velocity of DPPH decolorization determined for the first 75-s interval was used as a marker of the antiradical activity. Application of the kinetic parameter allowed good discrimination between the polyphenolic compounds studied. The most efficient chloronaphthoquinone derivative (compound Ia) was characterized by antiradical activity higher than that of quercetin and comparable with that of trolox. Under the experimental conditions used, one molecule of Ia was found to quench 2.6± 0.1 DPPH radicals.

KEY WORDS: antioxidant; quercetin derivatives; DPPH assay; kinetics; stoichiometry

Introduction
The antioxidant action of flavonoids, the best described biological activity of this group of natural polyphenolic substances, is covered by a number of excellent reviews (Bors et al., 1990; Cao et al., 1997; Pietta, 2000; Rice-Evans, 2001; Nijveldt et al., 2001; Bors & Michel, 2002; Heim et al., 2002; Williams et al., 2004; Amič et al., 2007; Bischoff, 2008; Boots et al., 2008). Flavonoids exert antioxidant effects by different mechanisms as e.g. free radical scavenging, hydrogen donating, singlet oxygen quenching, and metal iron chelating. Within the flavonoid family, quercetin (Qc) is the most potent scavenger of reactive oxygen species, including superoxide, peroxyl, alkoxyl and hydroxyl radicals, and reactive nitrogen species like NO· and ONOO· (Pietta, 2000; Butkovič et al., 2004; Amič et al., 2007; Boots et al., 2008). Flavonoids were found also to scavenge efficiently the model free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Butkovič et al., 2004). Quercetin O-glycosides, represent one of the most ubiquitous structures of all plant phenolics (Materska 2008). In addition, synthetic acyl derivatives of Qc, including aliphatic acids such as acetic, malonic and 2-hydroxypropionic acid, or aromatic acids, including benzoic, gallic, caffeic and ferulic acid, are frequently used as synthetic alternative to natural glycoside moieties (Harborne ed., 1994). Acylated Qc derivatives constitute useful active principles for cosmetic, dermatopharmaceutical, pharmaceutical or dietetic compositions (Perrier et al., 2001; Golding et al., 2001). The glycosidic structure has a large impact on quercetin bioavailability (Arts et al., 2004; Cao et al., 1997; Pietta, 2000; Rice-Evans, 2001; Nijveldt et al., 2001; Bors & Michel, 2002; Heim et al., 2002; Williams et al., 2004; Amič et al., 2007; Bischoff, 2008; Boots et al., 2008). Flavonoids exert antioxidant effects by different mechanisms as e.g. free radical scavenging, hydrogen donating, singlet oxygen quenching, and metal iron chelating. Within the flavonoid family, quercetin (Qc) is the most potent scavenger of reactive oxygen species, including superoxide, peroxyl, alkoxyl and hydroxyl radicals, and reactive nitrogen species like NO· and ONOO· (Pietta, 2000; Butkovič et al., 2004; Amič et al., 2007; Boots et al., 2008). Flavonoids were found also to scavenge efficiently the model free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Butkovič et al., 2004). Quercetin O-glycosides, represent one of the most ubiquitous structures of all plant phenolics (Materska 2008). In addition, synthetic acyl derivatives of Qc, including aliphatic acids such as acetic, malonic and 2-hydroxypropionic acid, or aromatic acids, including benzoic, gallic, caffeic and ferulic acid, are frequently used as synthetic alternative to natural glycoside moieties (Harborne ed., 1994). Acylated Qc derivatives constitute useful active principles for cosmetic, dermatopharmaceutical, pharmaceutical or dietetic compositions (Perrier et al., 2001; Golding et al., 2001). The glycosidic structure has a large impact on quercetin bioavailability (Arts et al., 2004; Cao et al., 1997; Pietta, 2000; Rice-Evans, 2001; Nijveldt et al., 2001; Bors & Michel, 2002; Heim et al., 2002; Williams et al., 2004; Amič et al., 2007; Bischoff, 2008; Boots et al., 2008). Flavonoids exert antioxidant effects by different mechanisms as e.g. free radical scavenging, hydrogen donating, singlet oxygen quenching, and metal iron chelating. Within the flavonoid family, quercetin (Qc) is the most potent scavenger of reactive oxygen species, including superoxide, peroxyl, alkoxyl and hydroxyl radicals, and reactive nitrogen species like NO· and ONOO· (Pietta, 2000; Butkovič et al., 2004; Amič et al., 2007; Boots et al., 2008). Flavonoids were found also to scavenge efficiently the model free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Butkovič et al., 2004).
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standard antioxidant trolox. Stoichiometry of the DPPH quenching reaction was determined for the most efficient derivative.

Materials and methods
Chemicals
Samples of new semi-synthetic derivatives of Qc Ia–Ir (Figure 1) were synthesized by reaction of appropriate acyl chloride with Qc or the corresponding protected derivative and then purified by repeated column chromatography of the rich reaction mixture. Qc was oxidized to generate heterodimer IIa (Figure 2). Diquercetin was treated with an anhydride to yield corresponding acyl derivatives IIb–IIc (Figure 2; Veverka et al. 2013).

1,1’-Diphenyl-2-picrylhydrazyl (DPPH) radical was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were purchased from local commercial sources and were of analytical grade quality.

DPPH test
To investigate the antiradical activity of the compounds studied, the ethanolic solution of DPPH (50 μM) was incubated in the presence of the given compound tested (50 μM) at laboratory temperature. The absorbance decrease, recorded at λmax = 518 nm, during the first 75-s interval was taken as a marker of the antiradical activity. During the 75-s

| No. | Position 1 | Position 2 | Position 3 | Position 4 |
|-----|------------|------------|------------|------------|
| la  | H          | H          | H          | 2-chloro-1,4-naphthoquinone-3-yl |
| lb  | H          | H          | 3-chloro-2,2-di methylpropanoyl |
| lc  | H          | H          | tetracetyl quinoyl tetracetyl quinoyl |
| ld  | H          | H          | 3,4-O-diacetyl caffeoyl |
| le  | H          | H          | 4-O-prenyl-feruoyl |
| lf  | H          | 4-(3,4,5-O-trimethyl galloyloxy)feruoyl |
| lg  | 3,4-diacyl-O-caffeoyl |
| lh  | H          | 3-chloro-2,2-di methylpropanoyl |
| lch | H          | H          | 3,4-O-diprenyl-caffeoyl |
| li  | 4-O-acetyl feruloyl |
| lj  | H          | H          | tetra-O-acetyl chlorogenoyl |
| lk  | H          | 3,4-O-diacetyl caffeoyl |
| ll  | H          | H          | 4-O-acetyl feruoyl |
| lm  | H          | H          | 1-N-(2,6-dimethyl morfolino)prop-3-yl |
| ln  | H          | 1-N-(2,6-dimethyl morfolino)H |
| lo  | O-acetyl salicyloyl |
| lp  | acetyl |
| lr  | 3,4,5-O-trimethyl galloyl |

Figure 1. Compounds la–lr.
interval used, an approximately linear decrease of DPPH absorbance was observed, which was considered as a good assessment of the initial velocity of the radical reaction.

The stoichiometry of the radical reaction was determined by spectrophotometric titration of the ethanolic solution of DPPH (50 μM) by increasing concentrations of an antioxidant with the reaction time long enough for completion of the reaction as indicated.

The radical studies were performed at the laboratory temperature.

Results and discussion

As a weak hydrogen atom abstractor, DPPH is considered a good kinetic model for peroxyl ROO· radicals (Blois, 1958; Ratty et al., 1988). DPPH assay is routinely used as a primary screening test of antiradical efficacy. Figure 3 shows UV-VIS spectra of DPPH and compound 1a with characteristic absorbance maxima and their time-dependent changes during the first 75 sec after mixing the reactants. The time-dependent decrease of the characteristic absorbance of the ethanolic solution of DPPH at 518 nm in the presence of Qc and one of its derivatives, 1a, is illustrated by Figure 4. As shown in Figure 4, the initial fast absorbance decrease, corresponding to the transfer of the most labile H atoms, is followed by a much slower absorbance decline representing the residual antiradical activity of the antioxidant degradation products. The initial velocity of DPPH decolorization determined for the first 75-s interval was used as a marker of antiradical activity. Based on the kinetic parameter the compounds studied were arranged according to their decreasing activity in comparison with the parent Qc and standard trolox, as shown in Table 1. It is apparent that a group of six new derivatives (Ia–Ie, IIa) exert antioxidant activity comparable with that of Qc and even slightly higher. The antiradical efficacy of the most efficient chloronaphthoquinone derivative 1a was found comparable with that of the standard trolox. The results indicate that application of the initial velocity of DPPH decolorization allows good discrimination between the polyphenolic compounds studied. In addition, the kinetic parameter is considered to be of primary importance in antioxidant evaluation since fast reaction with low concentrations of short-living damaging radicals is of utmost importance for antioxidant protection. Other authors applied the kinetic approach to rank flavonoids according to their antioxidant efficacy (Goupy et al. 2003; Butkovic et al. 2004; Villano et al. 2007).

In general, the antioxidant efficacy is characterized not only by kinetics of free radical quenching but also by stoichiometry of the scavenging reaction. So for the most efficient chloronaphthoquinone derivative 1a, the total stoichiometry of DPPH scavenging was determined in comparison with the parent Qc. The technique of spectrophotometric titration of fixed concentration of DPPH (50 μmol/l) with increasing concentrations of the antioxidant was used to determine the point of equivalence. In this approach the reaction time was set long enough to let the reaction run to completion. Fig. 5 shows the absorbance decrease of the ethanolic solution of DPPH radical in the presence of increasing concentrations of the compounds tested. By analyzing the titration curves, points of equivalence were determined and
corresponding stoichiometric factors were calculated. Under the experimental conditions used, one molecule of Ia was found to quench 2.6±0.1 DPPH radicals, while one molecule of Qc scavenged 5.5±0.2 DPPH radicals. The high stoichiometric ratio found for Qc is in agreement with findings of other authors (Goupy et al. 2003; Villano et al. 2007; Markovic et al. 2012) and indicates high antiradical activity of its decomposition products which is in contrast to compound Ia. To conclude, by using a DPPH assay, 21 novel derivatives of Qc were ranked according to their antiradical efficacy in comparison with the parent Qc and the standard trolox. For the most efficient derivative, stoichiometry of DPPH scavenging was determined.

Table 1. Antiradical activities of novel quercetin derivatives, in comparison with parent quercetin and trolox standard, in a DPPH test.

| Compound | MW     | Absorbance decrease (ΔA/75 s) |
|----------|--------|-------------------------------|
| Ia       | 492.82 | 0.462 ± 0.015                 |
| Ib       | 420.80 | 0.446 ± 0.024                 |
| Ic       | 986.85 | 0.414 ± 0.021                 |
| Id       | 836.72 | 0.391 ± 0.028                 |
| Quercetin| 302.24 | 0.386 ± 0.025                 |
| Il       | 602.46 | 0.374 ± 0.030                 |
| Ie       | 790.82 | 0.316 ± 0.017                 |
| If       | 1783.68| 0.195 ± 0.011                 |
| Ig       | 1040.9 | 0.121 ± 0.012                 |
| Ih       | 657.92 | 0.119 ± 0.030                 |
| Ic       | 899.00 | 0.115 ± 0.031                 |
| Ii       | 956.85 | 0.091 ± 0.012                 |
| Ij       | 1437.25| 0.063 ± 0.005                 |
| Ik       | 998.86 | 0.043 ± 0.007                 |
| Il       | 520.45 | 0.040 ± 0.026                 |
| Im       | 743.84 | 0.035 ± 0.021                 |
| Ib       | 1093.08| 0.033 ± 0.012                 |
| In       | 473.47 | 0.014 ± 0.009                 |
| Io       | 950.8  | 0.013 ± 0.003                 |
| Ip       | 512.42 | 0.010 ± 0.009                 |
| Ir       | 884.79 | 0.008 ± 0.004                 |
| Ilc      | 896.71/854.68 | 0.007 ± 0.006 |
| Trolox   | -      | 0.520 ± 0.025                 |

* The ethanolic solution of DPPH radical (50 μmol/l) was incubated in the presence of the compound tested (50 μM). Absorbance decrease at 518 nm during the first 75-s interval was determined. Results are mean values ± SD from at least three measurements.
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