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Structure-activity relationship of *in vitro* radical-scavenging activity of 2-(hydroxyphenyl) benzothiazole derivatives

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The introduction of the lipophilic benzothiazole group increases the oxidant stability of the phenols and modifies their antioxidant activity. Finding a suitable method for detecting these modifications will afford studying of the structure-activity relationship. The aim of this study is to examine the antioxidant activity of 2-(hydroxyphenyl) benzothiazole derivatives obtained in high yields (56%–94%) by one-pot *α*-amidoalkylation reactions of benzothiazole, alkyl chloroformates with various phenols. Synthetic series of 2-(4-hydroxyphenyl), 2-(dihydroxyphenyl) and 2-(trihydroxyphenyl) benzothiazole derivatives were evaluated *in vitro* for their DPPH and ABTS free radical scavenging activities and compared to the radical scavenging activity of natural compounds – rutin, quercetin, gallic acid, catechol, resorcinol, hydroquinone and pyrogallol, defined under the same conditions. The radical scavenging activity of 2-(hydroxyphenyl) benzothiazole derivatives was analysed by taking rutin as positive standard and compared their IC_{50} values. Antioxidant activity mainly depends on the number and position of phenolic hydroxyl groups. The benzothiazole compounds **6ac**, **7bd** containing pyrogallol and resorcinol fragment demonstrated similar activity as the natural antioxidant - quercetin. The results obtained using the ABTS method showed possibility for studying the structure - activity relationship of the tested examples.

Keywords: Antioxidant activity, free radical scavenging potential, phenols, structure-activity relationship, benzothiazole derivatives, DPPH and ABTS assays.

INTRODUCTION

Antioxidant activity is one of the important characteristics for estimating the medicinal properties of polyphenols and various synthetic analogues. The definition of antioxidants as substances that can efficiently reduce pro-oxidants with concomitant formation of products without any or low toxicity is well known [1]. Conventional definition of antioxidant was suggested by Halliwell and Gutteridge [2] as "any substance that when present at low concentrations, compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate". The presence of free radical-scavenging activity is necessary but not sufficient condition for the manifested antioxidant capacity of a compound [3].

The activity of antioxidants depends not only on their structure, but also on the concentration in which they are present, the properties of the oxidized substrate, the conditions of oxidation, including the environment and temperature [4]. From the tested set of flavonoids in the literature, quercetin exhibits the highest free radical-scavenging and antioxidant activity explained by the presence of the structural fragments according to the criteria [5].

It is possible for one compound to play a role as a free radical scavenger and as lipid oxidation inhibitor, but quite often the strongest radical traps appear as weak inhibitors (antioxidants) and *vice versa* [6].

Phenols and their derivatives show various ranges of biological activity due to their ability to inhibit various enzymes. They are widely used in medicine as antioxidants and preservatives in the food industry [7]. Catechol and pyrogallol are allelochemicals which belong to the phenolic compounds synthesized in plants [8].

Benzothiazole and its derivatives are an important class of heterocyclic compounds which are a common feature of many natural products and pharmaceutical agents. 2-Aryl benzothiazoles show a wide variety of chemotherapeutic activities including their use as antitumor and antibacterial agents. In recent years, the discovery of new methods for synthesis of 2-substituted benzothiazoles plays an important role in organic synthesis [9].

In earlies studies we synthesized new benzothiazole derivatives *via* one-pot approach of *α*-amidoalkylation. This method was successfully applied for obtaining various 2-(hydroxyphenyl) benzothiazoles as well [10, 11].

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It is known that introduction of the lipophilic benzothiazole group increases the oxidant stability of the phenols and modifies their antioxidant activity. Finding a suitable method for detecting these modifications will enable the subsequent studying of the structure-activity relationship. In this regard, nowadays many of the antioxidants are synthetic modifications of naturally occurring compounds [12, 13].

The aim of this study was to investigate the radical scavenging activity of 2-(hydroxyphenyl) benzothiazole derivatives obtained in high yields (56%–94%) *via* one-pot *α*-amidoalkylation of various phenols with benzothiazole and alkyl chloroformates.

MATERIALS AND METHODS

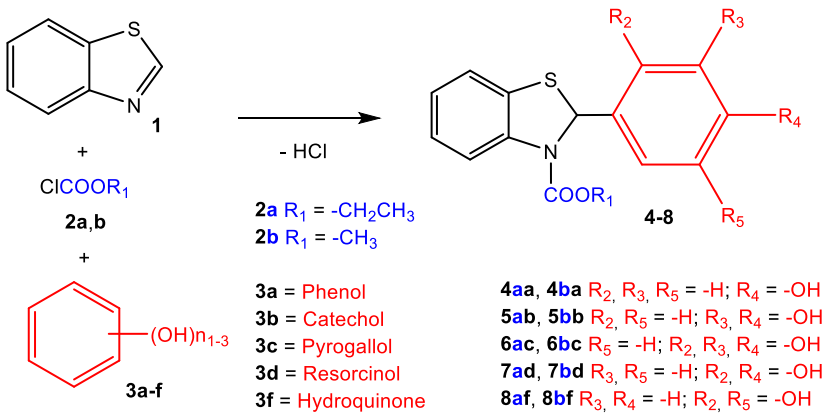
Reagents and equipment

All reagents and solvents as 2,2-diphenyl-1-picrylhydrazyl (CAS Number 1898-66-4), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (CAS number: 30931-67-0), methyl alcohol (Cas. No 67-56-1), potassium persulfate (CAS No 7727-21-1), quercetin (CAS No 117-39-5), rutin (CAS No 153-18-4), gallic acid (CAS No 149-91-7), pyrogallol (CAS No 87-66-1), catechol (CAS No 120-80-9), resorcinol (CAS No 108-46-3), hydroquinone (CAS No 123-31-9) were purchased from Sigma-Aldrich, USA.

The absorbance of free radical scavenging assay was measured by a Spectroquant Pharo 300, UV/Vis spectrophotometer.

Materials

2-(Hydroxyphenyl) benzothiazole derivatives were synthesized by one-pot *α*-amidoalkylation according to Stremski *et al*. [10, 11] (Scheme 1). The obtained products were purified by column chromatography and characterized by IR, ^{1}H- ^{13}C-NMR and ESI-MS analyses.



Scheme 1. Synthesis of 2-(hydroxyphenyl) benzothiazole derivatives by one-pot α-amidoalkylation reactions

Methods

DPPH free radical scavenging assay. The DPPH free radical (Figure 1) scavenging activities were measured as previously reported by Docheva *et al*. 2014 [14]: 0.12 mM DPPH was dissolved in methanol. The absorbance change was measured at 515 nm on a UV-Vis spectrophotometer within 30 min. The total DPPH radical scavenging activity within 30 min was measured in triplicate in the absence of light. The blank sample was prepared as above by replacing the test sample with equivalent methanol. The radical scavenging activity (RSA%) was calculated. IC_{50} value determined the effective concentration at which 50% of DPPH radicals were scavenged and it is obtained by interpolation from linear regression analysis. Lower IC_{50} value indicates a higher antioxidant activity.

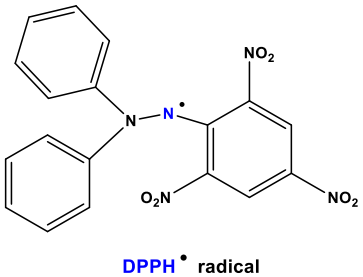


Figure 1. DPPH free radicals

ABTS free radical scavenging assay.

The ABTS free radical (Figure 2) was prepared by the method of Re *et al*., 1999 [15] with some modification. ABTS radical cation ($ABTS^{\bullet+}$) was produced by 7 mM ABTS and 2.45 mM $K_2S_2O_8$ (the mixture stayed in the dark at room temperature for 12–16 h before use) dissolved in deionized H_2O. Mixture of the reagents as 1:1 (v/v) $ABTS^{\bullet+}$ solution was diluted with methanol to an absorbance of

0.70±0.02 at 734 nm. The ABTS radical-scavenging activity within 10 min was measured in triplicate in the absence of light at room temperature. The percentage inhibition (%) of radical scavenging activity was calculated according to the corresponding equation of method.

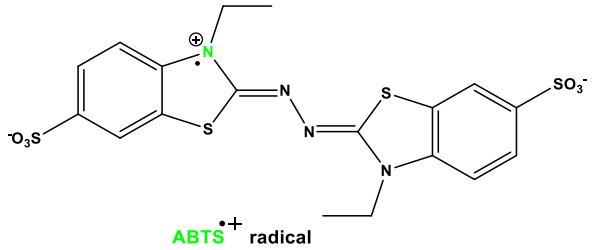


Figure 2. ABTS free radical

The stock solution of all compounds was prepared in concentration of 1 mg/ml. The working solutions were prepared by dissolving aliquot parts of the stock solution with methanol.

Statistical analysis

Experimental results were presented as the mean ± standard deviation (SD) of three parallel measurements.

RESULTS AND DISCUSSIONS

A series of synthetic 2-(4-hydroxyphenyl), 2-(dihydroxyphenyl) and 2-(trihydroxyphenyl) benzothiazole derivatives were evaluated for their DPPH and ABTS free radical-scavenging activities and compared to the radical-scavenging activity of natural compounds - rutin, quercetin, gallic acid, catechol, resorcinol, hydroquinone and pyrogallol, defined under the same conditions.

The DPPH method was related with a colour change from violet to yellow (Figure 3). The results were presented as IC_{50} for every compound in μM. The antioxidant activity of phenols – pyrogallol (IC_{50} = 5.71±0.45 μM) and catechol (IC_{50} = 5.72±0.45 μM) using DPPH method was similar to the antioxidant activity of natural antioxidant rutin (IC_{50} = 5.02±0.35 μM) and quercetin (IC_{50} = 4.60±0.30 μM) (Table 1).

DPPH •

$$2\ ABTS + S_2O_8^{2-} \longrightarrow 2\ ABTS^{\bullet+} + 2\ SO_4^{2-}$$

Figure 3. DPPH and ABTS assays

The final results for IC_{50} of rutin and quercetin confirmed those established by Docheva *et al.* (2014) [13]. The free radical scavenging activity of the benzothiazole derivatives varied between of IC_{50} = 3.66±0.21 μM – 38.22±2.19 μM, lower than the values defined for natural compounds - rutin, quercetin, pyrogallol and catechol (Table 1).

The results obtained for IC_{50} using ABTS method were associated with colour change – from blue-green to colourless (Figure 3). As a result, high activity of the natural compounds gallic acid (IC_{50} = 37.7±1.25 μM) and quercetin (IC_{50} = 48.01±4.36 μM) was observed (Table 1). The highest free radical-scavenging activity of phenols determined by ABTS the method possessed pyrogalol (IC_{50} = 22.7±1.45 μM), lower activity for resorcinol (IC_{50} = 48.2±4.93 μM), hydroquinone (IC_{50} = 88.9±5.59 μM) and the lowest - catechol (IC_{50} = 111.2±0.5 μM).

The free radical scavenging activity of the benzothiazole derivatives containing catechol **5ab**, **5bb**, resorcinol **7ad**, **7bd**, hydroquinone **8af**, **8bf** and pyrogallol **6ac**, **6bc** fragment, varied between of IC_{50} = 45.9±2.09 μM – 129.0±3.8 μM (Figure 4). The compound **4aa** with one phenolic hydroxyl group showed low antioxidant activity in concentration > 1000 μM. We suggest that the difference of activity is associated with the number and position of phenolic groups in their structures.

The difference in IC_{50} values between rutin (IC_{50} = 95.3±4.45 μM) and quercetin (IC_{50} = 48.01±4.36 μM), obtained by the ABTS method was significant, while that was not observed in the DPPH method.

Compounds **6ac**, **7ad** and **7bd** showed activity similar to quercetin and higher than the other 2-aryl benzothiazoles. This is explained by the fact that various substituents on "*o*-" and "*p*-" position in the phenolic fragment, form a resonance-stable radical (Kancheva *et al.* 2009) [12]. The results are presented in Table 1. The reported results (Table 1) are also illustrated by diagrams (Figures 4 and 5).

Table 1. DPPH and ABTS assay – free radical scavenging activities.

Compound	MW	DPPH Method	ABTS Method
		IC_{50}(μM)	IC_{50}(μM)
Quercetin	302.24	4.60±0.30	48.01±4.36
Rutin	610.52	5.02±0.35	95.3±4.45
Gallic acid	170.12	-	37.7±1.25
Pyrogallol	126.11	5.71±0.45	22.7±1.45
Catechol	110.11	5.72±0.45	111.2±0.5
Resorcinol	110.11	-	48.2±4.93
Hydroquinone	110.11	-	88.9±5.59
4aa	301.36	38.22±2.19	>1000
5ab	317.36	13.58±0.80	102±4.75
5bb	303.33	13.58±0.81	116.2±1.0
6ac	333.36	3.66±0.21	45.9±2.09
6bc	319.33	18.10±0.85	72.9±6.82
7ad	317.36	-	55.6±0.51
7bd	303.33	-	46.3±6.21
8af	317.36	14.00±0.82	111±11.2
8bf	303.33	-	129±3.8

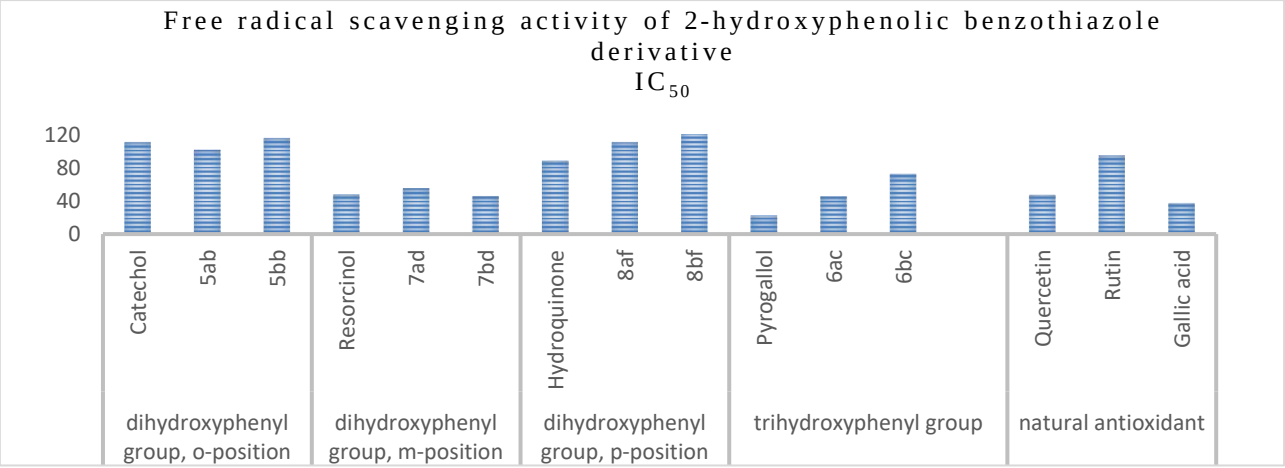


Figure 4. Free radical scavenging activity using ABTS-method (μM)

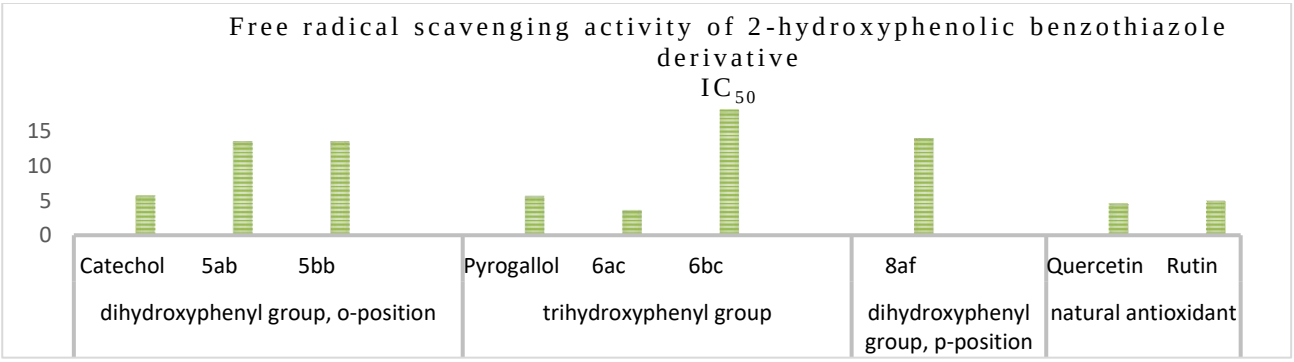


Figure 5. Free radical scavenging activity using DPPH-method (μM)

Antioxidant activity mainly depends on the number and position of phenolic hydroxyl groups. The benzothiazole compounds containing pyrogallol **6ac** (IC_{50} = 45.9±6.21 μM) and resorcinol - **7ad** (IC_{50} = 55.6±0.51 μM), **7bd** (IC_{50} = 46.3±6.21 μM) fragment demonstrated similar activity as quercetin, gallic acid and resorcinol examined *via* ABTS method (Table 1). The results obtained using ABTS method showed a possibility for studying of the structure-activity relationship of the tested examples.

CONCLUSION

A series of synthetic 2-(4-hydroxyphenyl), 2-(dihydroxyphenyl) and 2-(trihydroxyphenyl) benzothiazole derivatives were evaluated for their DPPH and ABTS free radical scavenging activity and compared to natural compounds - rutin, quercetin, gallic acid, catechol, resorcinol, hydroquinone and pyrogallol, defined under the same conditions. The results obtained for IC_{50} values showed a high radical scavenging activity for flavonoids - rutin (IC_{50} = 5.02±0.35 μM) and quercetin (IC_{50} = 4.60±0.30 μM), followed by phenols - pyrogallol and catechol (IC_{50} = 5.7±0.45 μM) using DPPH method. The free radical scavenging activity of the benzothiazole derivatives varied in the range of IC_{50} = 3.66±0.21 μM – 38.22±2.19 μM. The obtained IC_{50} values for ABTS method showed high activity for pyrogallol (IC_{50} = 22.7±1.45 μM), gallic acid (IC_{50} = 37.7±1.25 μM) and quercetin (IC_{50} = 48.01 ± 4.36 μM). The determined free radical scavenging activity of the benzothiazole derivatives was IC_{50} = 45.9±2.09 μM – 129±3.8 μM. The compound **4aa** with one phenolic hydroxyl group showed low antioxidant activity in concentration > 1000 μM. It was proved that antioxidant activity mainly depends on the number and position of phenolic hydroxyl groups. The benzothiazole compounds containing pyrogallol **6ac** (IC_{50} = 45.9±6.21 μM) and resorcinol **7ad** (IC_{50} = 55.6±0.51 μM), **7bd** (IC_{50} = 46.3±6.21 μM) fragment demonstrated similar activity as the natural antioxidants quercetin, gallic acid and resorcinol examined via ABTS method. The results obtained using ABTS method showed possibility for studying of the structure-activity relationship of the tested examples.

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