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Original article

In *Vitro* Antioxidant Properties of 2-Imino-benzimidazole and 1,3-Thiazolo[3,2-*a*]benzimidazolone Derivatives

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SUMMARY

Antioxidant properties of 2-[2-imino-5-nitro-3-(2-oxo-2-phenylethyl)-2,3-dihydro-1*H*-benzimidazol-1-yl]-1-phenylethanone (compound 1) and 2-(4-fluorobenzylidene)-6-(phenylcarbonyl)[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one (compound 2) were evaluated *in vitro*. Compounds 1 and 2 did not show significant radical scavenging activity. It has been suggested that antioxidant strategies should not be based on direct scavengers but rather on the potentiation of endogenous antioxidant defenses, or on the reduction of the sources of reactive species. Although a direct scavenging mechanism is missing, the assayed compounds (1 and 2) as evidenced inhibitors of xanthine oxidase and dipeptidyl peptidase-4 might act antioxidatively by employing other mechanisms.

Key words: **2-imino-benzimidazoles, 1,3-thiazolo[3,2-*a*]benzimidazolones, antioxidant activity, antioxidant mechanisms**

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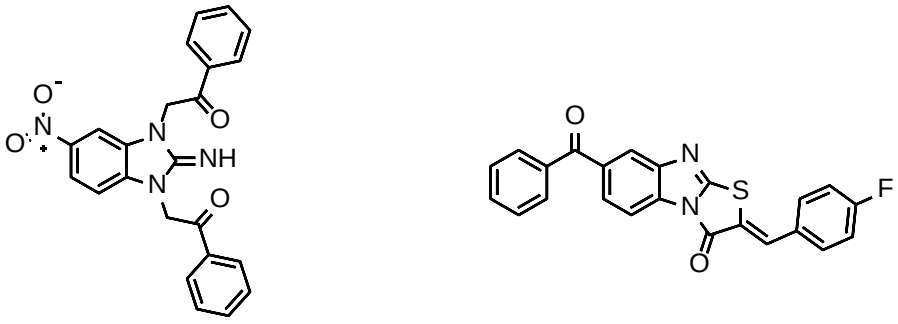
INTRODUCTION

Oxidative stress occurs when reactive oxygen/nitrogen species overwhelm antioxidative defenses, leading to oxidative modification of biomacromolecules and tissue injury (1). Aging and the pathogenesis of many diseases, including diabetes mellitus, atherosclerosis and neurodegenerative disorders, have been associated with oxidative stress (2). Reactive oxygen species are derived in many metabolic processes that include xanthine oxidase, uncoupled nitric oxide synthase, nicotinamide adenine dinucleotide phosphate oxidase and mitochondrial respiratory enzymes (3). Modulations of the activity/expression of the mentioned enzymes might decrease oxidative stress burden (4, 5).

An antioxidant is a molecule, ion, or a relatively stable radical that is capable of delaying or preventing the oxidation of substrates (proteins, lipids, carbohydrates and DNA). There are many possible mechanisms of antioxidant activity, by hindering a generation of reactive species, directly scavenging them, or indirectly by raising the levels of endogenous antioxidant defenses (2, 6, 7). The enzymatic/nonenzymatic, electron/hydrogen atom transfer-based assays enable the evaluation of the mechanism of antioxidant activity *in vitro* and *in vivo* (1, 6). Due to the complexity of the research topic, none of a multitude of tests has gained total acceptance (1). The target, the environment, and the source of reactive species are important in the characterization of an antioxidant (6). Assays may be used together to reveal more complete sight on the profile of antioxidative action (1).

Herein, *in vitro* antioxidant properties of 2-[2-imino-5-nitro-3-(2-oxo-2-phenylethyl)-2,3-dihydro-1*H*-benzimidazol-1-yl]-1-phenylethanone (compound **1**) and 2-(4-fluorobenzylidene)-6-(phenylcarbonyl)[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one (compound **2**) (Figure 1) were evaluated in order to assess their free radical scavenging capacity.



1 **2**

Figure 1. Structures of the assayed compounds

MATERIALS AND METHODS

Chemicals

The synthesis of the studied 2-imino-benzimidazole and 1,3-thiazolo[3,2-*a*]benzimidazolone derivative (compounds **1** and **2**) was performed, as described in our previous study (8).

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), iron(III) chloride hexahydrate and copper(II) chloride dyhidrate were purchased from Acros Organics (Morris Plains, New Jersey, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tris(2-pyridyl)-S-triazine and neocuproine were purchased from Sigma Aldrich (Steineheim, Germa-ny). Glacial acetic acid, ammonium acetate, po-tassium persulfate, iron(II) sulfate heptahydrate, ascorbic acid, hydrochloric acid, ethanol and methanol were purchased from Merck (Darmstadt, Ger-many).

Evaluation of free radical scavenging activity

Antioxidant activity of compounds **1** and **2** was evaluated *in vitro* using DPPH radical scavenging assay (according to the method of Brand-Williams et al. (9), slightly modified by Miliauskas et al. (10)), ABTS radical cation decolourisation assay (using the method of Re et al. (11) and Arts et al. (12)), cupric reducing antioxidant capacity (CUPRAC) assay (according to the method of Apak et al. (13)) and ferric reducing antioxidant power (FRAP) assay (performed as previously described by Benzie and Strain (14) and modified by Vijayalakshmi and Ruckmani (15)). Ascorbic acid was used as a reference antioxidant. All measurements were done in triplicate. The results were expressed as micromoles of Trolox equivalents (TE) per gram of the sample (μmol TE/g) in DPPH and ABTS assays, as milligrams of TE per gram of the sample (mg TE/g) in CUPRAC assay, and as micromoles of Fe^{2+} equivalents (FE) per gram of the sample (μmol FE/g) in FRAP assay.

RESULTS AND DISCUSSION

In all performed *in vitro* assays, compounds **1** and **2** did not show significant antioxidant activity, in terms of direct scavenging of free radicals, when compared to the ascorbic acid as a reference antioxidant. The highest antioxidant activity of both tested compounds and ascorbic acid was measured in FRAP assay (Table 1).

Ascorbic acid is an antioxidant available in natural sources, with well-established biological functions (16). Among others, data from *in vitro* and *in vivo* studies suggest that ascorbic acid might inhibit xanthine oxidase and reduce serum uric acid levels (17, 18).

Table 1. *In vitro evaluated antioxidant activity of compounds **1** and **2***

	DPPH (μmol TE/g)	**ABTS** (μmol TE/g)	**CUPRAC** (mg TE/g)	**FRAP** (μmol FE/g)
Compound				
1	0.24 ± 0.01	13.79 ± 0.36	22.21 ± 0.16	66.75 ± 2.26
2	6.55 ± 0.08	5.63 ± 0.18	29.06 ± 0.16	60.37 ± 0.75
Ascorbic acid	4760 ± 68	6695 ± 108	3173 ± 52	14227 ± 132

Although the tested compounds did not show direct radical scavenging effects to a greater extent in the used assays, it has been evidenced that they act as inhibitors of xanthine oxidase and dipeptidyl peptidase-4 (19), which allows them to act antioxidatively by other mechanisms, suppressing the production of free radicals as well as enhancing endogenous antioxidant defense systems. Besides the inhibition of xanthine oxidase as a producer of reactive species, inhibition of dipeptidyl peptidase-4 has also been shown to reduce oxidative stress in various disease models in many ways (20).

The impairment of redox homeostasis, increased production of reactive oxygen species and/or weakened antioxidant defense system, associated with reduced NO bioavailability, contribute to vascular dysfunction (21). The levels of reactive oxygen species are determined by the rate of their production and clearance. Direct scavengers of free radicals and antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) participate in the removal of reactive species (22). It has been highlighted that the antioxidant strategies should not be based on the classical antioxidants as direct scavengers, but rather on the elevation of the levels of endogenous antioxidant enzymes, or on the suppression of the sources of reactive species (20). Missing enough effective radical scavenging, but showing other mechanisms of the antioxidant activity, compounds **1** and **2** assayed here fit into the abovementioned strategy.

CONCLUSION

Although compounds **1** and **2** are not sufficiently effective direct scavengers of free radicals, they act antioxidatively as inhibitors of xanthine oxidase, by which the generation of free radicals is

downregulated, and as inhibitors of dipeptidyl peptidase-4, which allows them to act antioxidatively by different mechanisms. The absence of substantial body of good results in the *in vitro* evaluation of direct reactive species scavenging means that this mechanism of antioxidant activity is missing. Direct scavenging is not more important when compared to other mechanisms, and it is not enough as the only way of action.

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Antioksidativna aktivnost 2-imino-benzimidazol i 1,3-tiazolo[3,2-*a*]benzimidazolon derivata: *in vitro* studija

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SAŽETAK

Antioksidativna aktivnost 2-[2-imino-5-nitro-3-(2-okso-2-feniletil)-2,3-dihidro-1*H*-benzimidazol-1-il]-1-feniletanona (jedinjenje 1) i 2-(4-fluorobenziliden)-6-fenilkarbonil)[1,3]tiazolo[3,2-*a*]benzimidazol-3(2*H*)-ona (jedinjenje 2) ispitivana je *in vitro*. Jedinjenja 1 i 2 nisu pokazala značajnu efikasnost u direktnom uklanjanju slobodnih radikala. Pretpostavka je da antioksidativne strategije ne bi trebalo da budu zasnovane na antioksidansima, koji uklanjaju radikale direktno, već na jačanju endogene antioksidativne odbrane ili na inhibiranju izvora reaktivnih vrsta. Iako izostaje efikasnost u direktnom uklanjanju radikala, ispitivana jedinjenja (1 i 2), kao dokazani inhibitori ksantin oksidaze i dipeptidil peptidaze-4 mogu da pokazuju antioksidativnu aktivnost drugim mehanizmima.

Ključne reči: **2-imino-benzimidazoli, 1,3-tiazolo[3,2-*a*]benzimidazoloni, antioksidativna aktivnost, antioksidativni mehanizmi**