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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new phenylcarbamoylbenzoic acid derivatives and evaluation of their in vitro antioxidant activity

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Abstract In this study, new derivatives of phenylcarbamoylbenzoic acid were synthesized and evaluated for their in vitro antioxidant activity. The target compounds were prepared by bonding pharmacophoric moieties possessing antioxidant activity, including hydrazones, acid hydrazides, imino, Schiff's bases, and phthalimides with phenylcarbamoylbenzoic acid via simple and efficient synthetic strategies. The structures of the newly synthesized compounds were confirmed by physical and spectral data. The in vitro antioxidant activity was carried out using ABTS antioxidant assay. All the tested compounds showed low antioxidant activity except compound **7**, which showed moderate antioxidant activity compared with ascorbic acid.

Keywords Synthesis · Phenylcarbamoylbenzoic acid · Hydrazone · Acyl hydrazone · Phthaloyl derivatives · Antioxidant activity

Introduction

Monoamides of 1,2-dicarboxylic acid and their derivatives started receiving significant chemical and biological interest since the later part of the last century by many Russian workers (Koz'minykh, 2006). From a chemical point of view, monoamides of 1,2-dicarboxylic acids, especially monoamides of succinic, maleic, and phthalic acids (Fig. 1), are easily obtained under mild conditions (Filho *et al.*, 2003). They can be regarded as precursors for cyclic imides synthesis, in which facile dehydration conditions are used and high or almost quantitative yields are obtained (Argade and Balasubramaniyan, 2000; Feuer and Asunskis, 1962). Biologically, many of the amides and hydrazides of 1,2-dicarboxylic acids and their derivatives are characterized by low toxicity and wide variety of biological and pharmacological activity (Lange *et al.*, 1979; Dolzhenko *et al.*, 2002, 2003).

A collection of biological activity and acute toxicity data of several substituted amides and hydrazides of 1,2-dicarboxylic acids and their derivatives was reviewed (Koz'minykh, 2006).

Antioxidants are molecules, natural, or synthetic, capable to interact with free radicals and stop their chain reactions before essential vital molecules are damaged (Aruoma, 1998). Owing to the contribution and implication of oxidative stress in many human diseases, antioxidants are intensively studied in medicinal chemistry and pharmacology (Knight, 1998; Mates *et al.*, 1999). Their main use is for treatment, prevention, or protection against many disease conditions.

Diverse biological and pharmacological activities of this class of organic compounds were reported by many workers. The literature survey did not reveal any study about the antioxidant activity of phenylcarbamoylbenzoic acid derivatives. Recently, Phthalic acid was reported to alter the antioxidant system of specific type of plant, leading to the formation of reactive oxygen species (Bai *et al.*, 2009). In this study, phthalic acid as a precursor for phenylcarbamoylbenzoic acid, was chemically modified by conversion into amides and hydrazide derivatives.

The objective of this research is to investigate and discover the expected antioxidant activity of this class of compounds via design, synthesis, and in vitro antioxidant screening of selective representatives of phenylcarbamoylbenzoic acid derivatives bearing pharmacophoric moieties.

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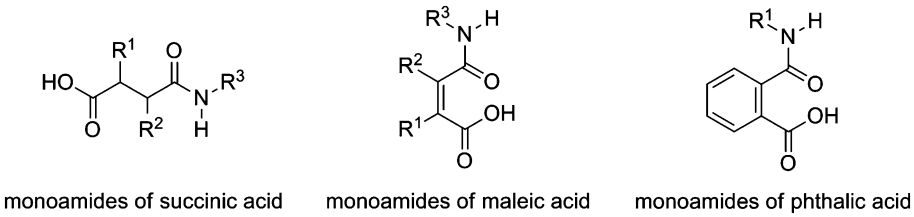


Fig. 1 General formulas of most famous 1,2-dicarboxylic acid amides (R^1, R^2, R^3 = different alkyl and aryl derivatives)

The synthesis was achieved through construction of different series of phenylcarbamoylbenzoic acid derivatives (mainly, amides, hydrazides of phthalic acid, and their derivatives) conjugated and bonded with a variety of pharmacophoric moieties possessing antioxidant activity. These, include hydrazones derivatives (Angelo *et al.*, 2003; Gürkök *et al.*, 2009), acid hydrazide (Gürkök *et al.*, 2009), imino derivatives (Vukovic *et al.*, 2010), Schiff's bases (Chen *et al.*, 2003; Guo *et al.*, 2005), and phthalimide derivatives (Shankar *et al.*, 2000).

Results and discussion

Chemistry

The synthetic methodologies for obtaining the newly synthesized compounds were illustrated in Schemes 1, 2, and 3. Starting compounds, carbamoyl hydrazone **2** (Scheme 1) and carbamoyl acid hydrazide **7** (Scheme 2), were prepared by condensing hydrazine hydrate in refluxing ethanol with ketone **1** and carboxylic acid ester **6**, respectively. Both **1** (Dolzhenko *et al.*, 2003) and **6** were prepared from the corresponding aromatic amines; 4-aminoacetophenome and ethyl 4-aminobenzoate through nucleophilic acylation with phthalic acid anhydride in the presence of ethyl acetate as a solvent, in a typical phthalic acid monoamide formation reaction (Koz'minykh, 2006).

Starting compounds **2** and **7** were converted to different derivatives through reaction with the appropriate aromatic aldehydes, aromatic ketone, and phthalic acid anhydride; to afford the target compounds **3–5** (Scheme 1) and **8–10** (Scheme 3).

The ketone hydrazone **2** afforded the benzylidene hydrazono derivatives **3a–c** by condensation with the appropriate aromatic aldehyde in refluxing ethanol and phenyl ethylidene hydrazono derivatives **4a**, **b** by condensation with the appropriate aromatic ketone in refluxing glacial acetic acid. 1,3-Dioxoisoindolin-2-yl-imino **5** was obtained by reacting the ketone hydrazone **2** with phthalic acid anhydride in refluxing glacial acetic acid.

On the other hand, the acid hydrazide **7** afforded the benzylidene acyl hydrazono derivatives **8a–c** by condensation with the appropriate aromatic aldehyde in refluxing ethanol and phenyl ethylidene acyl hydrazono derivatives **9a**, **b** by condensation with the appropriate aromatic ketone in refluxing glacial acetic acid. 1,3-Dioxoisoindolin-2-yl-imino **10** was obtained by condensing the acid hydrazide **7** with phthalic acid anhydride in refluxing glacial acetic acid.

Antioxidant screening

All the newly synthesized compounds **1–10** were evaluated for their potential in vitro antioxidant activity by preliminary qualitative screening using *L*-ascorbic acid as a positive control according to ABTS antioxidant assay method (Re *et al.*, 1999; Miller and Rice-Evans, 1997).

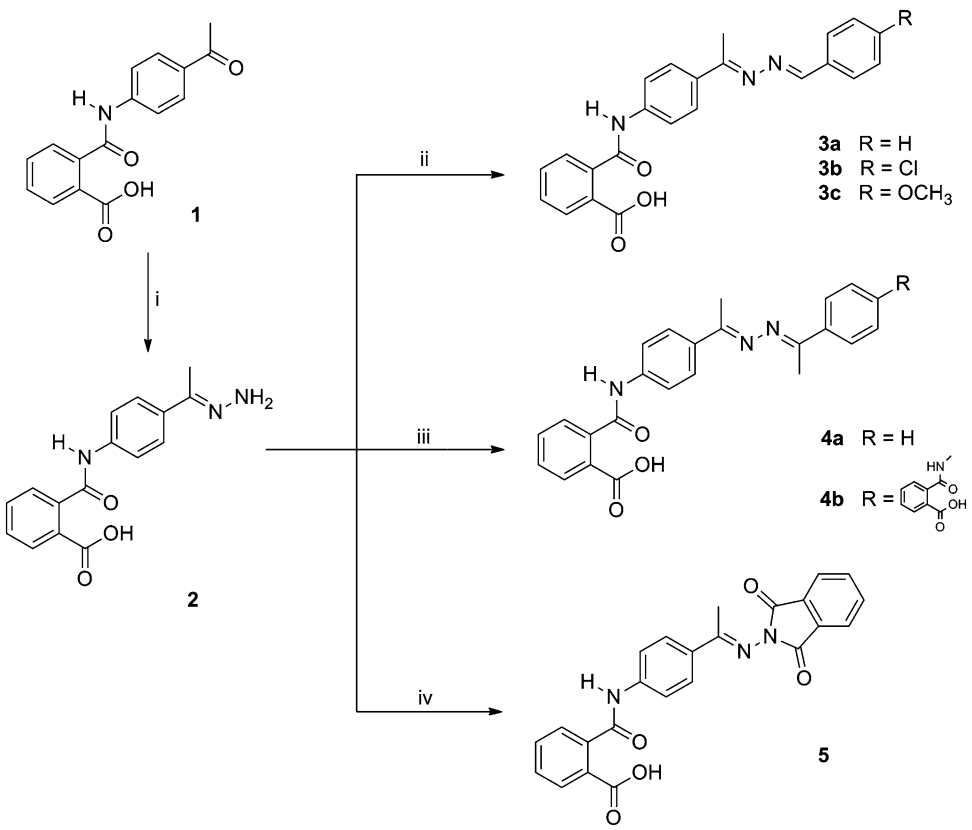
ABTS is an abbreviation to 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt, a radical cation decolonization test and is also a spectrophotometric method widely used for the assessment of antioxidant activity of various substances. It is applicable for both lipophilic and hydrophilic compounds. The radical cation ($ABTS^{\bullet+}$) was generated by oxidation of ABTS with potassium persulfate. The absorbance is bleached by antioxidants because of their capacity to reduce the preformed radical. The % inhibition of the absorbance was calculated to indicate the inhibition of superoxide production and to measure the antioxidant activity.

The results of the antioxidant screening (scavenger activity) are listed (Table 1). The bar-chart of the % of inhibition of the absorbance is shown (Fig. 2). All compounds showed low antioxidant activity except compound **7** (carrying the carboxylic acid hydrazide moiety), which showed moderate antioxidant activity, in comparison to *L*-ascorbic acid. The results also revealed that the core nucleus "phenylcarbamoylbenzoic acid" even after chemical modification with pharmacophores, showed a low in vitro antioxidant activity.

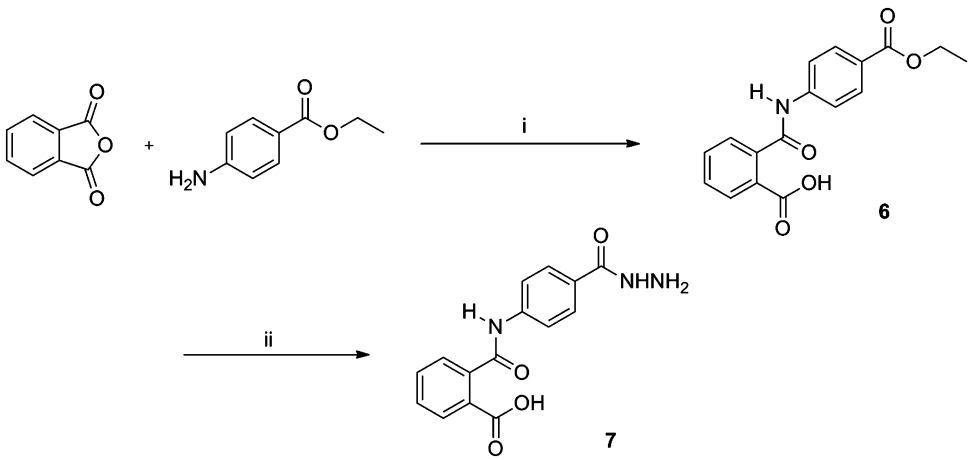
Experimental

Chemistry

Melting points were determined on Fisher-Johns melting point apparatus and were uncorrected. Elemental Microanalyses were within ±0.04% with theoretical calculated values. ^{1}H NMR spectra were recorded on Bruker Ac 250 FT NMR spectrometer (250 MH_Z) in DMSO-d_6 or $CDCl_3$

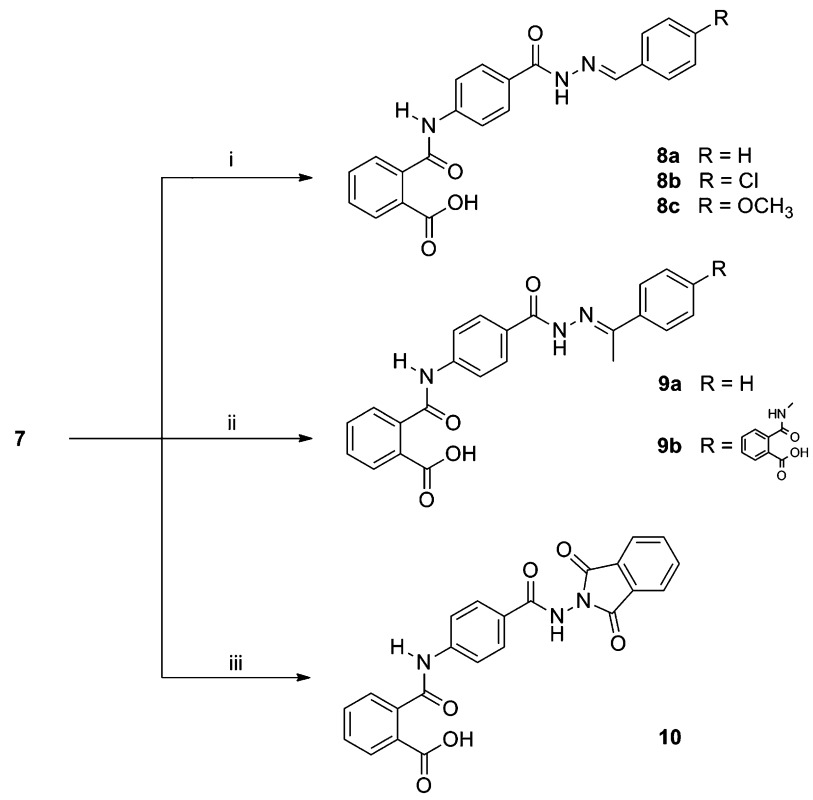


Scheme 1 Synthesis of 2-((4-(1-hydrazonoethyl)phenyl)carbamoyl) benzoic acid and its hydrazone and phthaloyl derivatives. Reagents and conditions: (*i*) hydrazine hydrate, ethanol, reflux; (*ii*) aromatic aldehyde, ethanol, reflux; (*iii*) acetophenone, glacial acetic acid, reflux; (*iv*) phthalic acid anhydride, glacial acetic acid, reflux



Scheme 2 Synthesis of 2-((4-(hydrazinecarbonyl)phenyl)carbamoyl)benzoic acid. Reagents and conditions: (*i*) ethyl acetate, stirring, room temperature; (*ii*) hydrazine hydrate, ethanol, reflux

using TMS as internal standard; chemical shifts in ppm were expressed in δ units. MS analyses were performed on JEOL JMS-600H spectrometer. Reaction times and purity of compounds were decided and checked using TLC on Silica gel plates 60 F_{245} E. Merck, and the spots were visualized by UV (366, 245 nm). Compound **1** was prepared in a 65% yield, according to the published procedure (Dolzhenko *et al.*, 2003).



Scheme 3 Synthesis of acyl hydrazone and phthaloyl derivatives of phenylcarbamoylbenzoic acid. Reagents and conditions: (*i*) the appropriate aromatic aldehyde, ethanol, reflux; (*ii*) the appropriate acetophenone, glacial acetic acid, reflux; (*iii*) phthalic acid anhydride, glacial acetic, reflux

Table 1 Results of ABTS antioxidant assay

Tested compounds	Absorbance of samples (mean)	% Inhibition of superoxide production
Control	0.512	0
Ascorbic acid	0.051	90.04
1	0.474	7.42
2	0.419	18.16
3a	0.446	12.87
3b	0.385	24.80
3c	0.394	23.02
4a	0.462	9.85
4b	0.458	10.63
5	0.491	4.19
6	0.491	4.09
7	0.229	55.21
8a	0.467	8.78
8b	0.412	19.53
8c	0.490	4.29
9a	0.499	2.63
9b	0.461	9.95
10	0.469	8.39

*2-((4-(1-Hydrazonoethyl)phenyl)carbamoyl)benzoic acid (**2**)*

A mixture of **1** (2.83 g, 10 mmol) and hydrazine hydrate 99% (5 g, 0.1 mol) in absolute ethanol (30 ml) was stirred under reflux for 5 h. The product formed was collected by filtration, dried, and recrystallized from isopropyl alcohol to afford pure compound. Pale yellow crystals, mp 212–214°C, yield 81%. Analysis for $C_{16}H_{15}N_3O_3$ (297.31), Calcd.: C, 64.64; H, 5.09; N, 14.13; Found: C, 64.49; H, 5.00; N, 13.92. ^{1}H NMR (DMSO-d_6): δ 2.95 (s, 3H, CH_3), δ 6.98 (s, 2H, NH_2, D_2O exchangeable), δ 7.68–8.39 (m, 8H, Ar–H), δ 9.65 (s, 1H, NH, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 297 (15.56, M^+).

*(Un)substituted benzylidenehydrazono derivatives **3a–c***

A mixture of 2 (0.59 g, 2 mmol) and the appropriate aromatic aldehyde (2 mmol) in absolute ethanol (30 ml) was refluxed for 5 h, then allowed to cool to room temperature. The formed precipitate was filtered, dried, and recrystallized from dioxan to afford pure products.

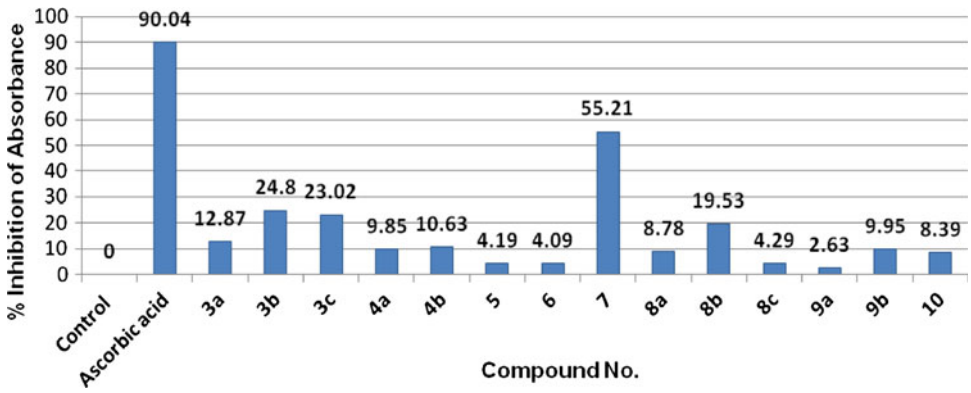


Fig. 2 Bar-chart of the % inhibition of absorbance of the tested compounds

*2-((4-(1-(Benzylidenehydrazono)ethyl)phenyl) carbamoyl)benzoic acid (**3a**)*

Yellow crystals, mp 241–43°C, yield 61%. Analysis for $C_{23}H_{19}N_3O_3$ (385.42), Calcd.: C, 71.67; H, 4.97; N, 10.90; Found: C, 71.52; H, 5.09; N, 10.78. ^{1}H NMR (DMSO-d_6): δ 2.14 (s, 3H, CH_3), δ 7.48–8.21 (m, 13H, Ar–H), δ 8.42 (s, 1H, N=CH–), δ 9.98 (s, 1H, CONH, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 385 (7.34, M^+).

*2-((4-(1-((4-Chlorobenzylidene)hydrazono)ethyl)phenyl) carbamoyl)benzoic acid (**3b**)*

Yellow crystals, mp 204–206°C, yield 73%. Analysis for $C_{23}H_{18}ClN_3O_3$ (419.86), Calcd.: C, 65.79; H, 4.32; N, 10.01; Found: C, 65.65; H, 4.27; N, 10.08. ^{1}H NMR (DMSO-d_6): δ 2.13 (s, 3H, CH_3), δ 7.37–8.21 (m, 12H, Ar–H), δ 8.65 (s, 1H, N=CH–), δ 10.74 (s, 1H, CONH, D_2O exchangeable), δ 11.25 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 419 (3.12, M^+).

*2-((4-(1-((4-Methoxybenzylidene)hydrazono)ethyl) phenyl)carbamoyl)-benzoic acid (**3c**)*

Yellow crystals, mp 241–43°C, yield 61%. Analysis for $C_{24}H_{21}N_3O_4$ (415.44), Calcd.: C, 69.39; H, 5.10; N, 10.11; Found: C, 69.43; H, 5.06; N, 10.23. ^{1}H NMR (DMSO-d_6): δ 2.12 (t, 3H, =C–CH_3), δ 3.79 (s, 3H, OCH_3), δ 7.18–8.22 (m, 12H, Ar–H), δ 8.48 (s, 1H, N=CH–), δ 9.84 (s, 1H, CONH, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 415 (10.89, M^+).

*Phenylethylidenehydrazono derivatives **4a, b***

A mixture of **2** (0.59 g, 2 mmol), acetophenone (0.24 g, 2 mmol) or **1** (0.57 g, 2 mmol), and catalytic amount of sodium acetate (0.1 g) in glacial acetic acid (20 ml) was refluxed for 5 h. The resultant solution was allowed to stand overnight. The precipitated solid was filtered, dried, and recrystallized from glacial acetic acid to afford pure products.

*2-((4-(1-((1-Phenylethylidene)hydrazono)ethyl)phenyl) carbamoyl)benzoic acid (**4a**)*

Yellow crystals, mp 241–43°C, yield 61%. Analysis for $C_{24}H_{21}N_3O_3$ (399.44), Calcd.: C, 72.16; H, 5.30; N, 10.52; Found: C, 72.10; H, 5.22; N, 10.61. ^{1}H NMR (DMSO-d_6): δ 2.13 (s, 6H, two CH_3), δ 7.51–8.20 (m, 13H, Ar–H), δ 9.99 (s, 1H, CONH, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 399 (14.63, M^+).

*2-((4-(1-((1-(4-(2-Carboxybenzamido)phenyl)ethylidene) hydrazono)ethyl)-phenyl)amino)benzoic acid (**4b**)*

Yellow crystals, mp 241–43°C, yield 61%. Analysis for $C_{32}H_{26}N_4O_6$ (562.57), Calcd.: C, 68.32; H, 4.66; N, 9.96; Found: C, 68.39; H, 4.71; N, 9.87. ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 6H, two CH_3), δ 6.99–8.20 (m, 16H, Ar–H), δ 9.99 (s, 2H, two CONH, D_2O exchangeable), δ 11.00 (s, 2H, two COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 562 (9.78, M^+).

*2-((4-(1-((1,3-Dioxoisoindolin-2-yl)imino)ethyl)phenyl) carbamoyl)benzoic acid (**5**)*

A mixture of **2** (0.59 g, 2 mmol), phthalic acid anhydride (0.30 g, 2 mmol), and catalytic amount of sodium acetate (0.1 g) in glacial acetic acid (20 ml) was refluxed for 5 h and allowed to cool to room temperature. The precipitated solid was filtered, dried, and recrystallized from glacial acetic acid to afford pure product. Orange crystals, mp 267–69°C, yield 78%. Analysis for $C_{24}H_{17}N_3O_5$ (427.41), Calcd.: C, 67.44; H, 4.01; N, 9.83; Found: C, 76.51; H, 4.20; N, 10.02. ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), δ 7.52–8.20 (m, 12H, Ar–H), δ 9.84 (s, 1H, CONH, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 427 (12.63, M^+).

*2-((4-(Ethoxycarbonyl)phenyl)carbamoyl)benzoic acid (**6**)*

A solution of ethyl 4-aminobenzoate (8.26 g, 50 mmol) in ethyl acetate (80 ml) was gradually added under vigorous stirring to a suspension of phthalic acid anhydride (7.41 g, 50 mmol) in ethyl acetate (100 ml). Stirring was continued for 3–4 h (TLC). The formed product was filtered, washed with ethyl acetate (2 × 25 ml), air-dried, and recrystallized from dioxan. White crystals, mp 219–221°C, yield 75%. Analysis for $C_{17}H_{15}NO_5$ (313.30), Calcd.: C, 65.17; H, 4.83; N, 4.47; Found: C, 65.31; H, 4.76; N, 4.99. ^{1}H NMR (DMSO-d_6): δ 1.32 (t, 3H, CH_3), δ 4.30 (q, 2H, CH_2), δ 7.55–7.97 (m, 8H, Ar–H), δ 9.99 (s, 1H, CONH, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 313 (6.39, M^+).

*2-((4-(Hydrazinecarbonyl)phenyl)carbamoyl)benzoic acid (**7**)*

A mixture of **6** (3.13 g, 10 mmol) and hydrazine hydrate (5 g, 99%) in ethyl alcohol (50 ml, 95%) was heated under reflux for 8 h. The formed precipitate was collected by filtration, washed with ethanol, dried, and crystallized from isopropyl alcohol. White crystals, mp 246–248°C, yield 68%. Analysis for $C_{15}H_{13}N_3O_4$ (299.28), Calcd.: C, 60.20; H, 4.38; N, 14.04; Found: C, 60.03; H, 4.51; N, 13.78. ^{1}H NMR (DMSO-d_6): δ 7.65–8.20 (m, 8H, Ar–H), δ 8.96 (s, 1H, CONHNH$_2$, D_2O exchangeable), δ 9.92 (s, 1H, CONHC$_6$H$_4$–, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). NH_2 protons seemed to be exchanged by the solvent. MS *m/z* (% Rel. Int.): 299 (13.40, M^+).

*(Un)substituted benzylidenehydrazinecarbonyl derivatives **8a–c***

A mixture of **7** (0.60 g, 2 mmol) and the appropriate aromatic aldehyde (2 mmol) in absolute ethanol (30 ml) was refluxed for 5 h and allowed to cool to room temperature. The formed crystalline product was separated by filtration, dried, and recrystallized from ethanol:acetone (1:1) to afford pure products.

*2-((4-(2-Benzylidenehydrazinecarbonyl)phenyl) carbamoyl)benzoic acid (**8a**)*

Yellow crystals, mp 223–25°C, yield 73%. Analysis for $C_{22}H_{17}N_3O_4$ (387.39), Calcd.: C, 68.21; H, 4.42; N, 10.85; Found: C, 68.34; H, 4.56; N, 10.77. ^{1}H NMR (DMSO-d_6): δ 7.32–8.20 (m, 13H, Ar–H), δ 8.44 (s, 1H, N=CH), δ 9.95 (s, 1H, CONH, D_2O exchangeable), δ 10.55 (s, 1H, CONH–N, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 387 (18.23, M^+).

*2-((4-(2-(4-Chlorobenzylidene)hydrazinecarbonyl)phenyl) carbamoyl)benzoic acid (**8b**)*

Yellow crystals, mp 254–56°C, yield 68%. Analysis for $C_{22}H_{16}ClN_3O_4$ (421.83), Calcd.: C, 62.64; H, 3.82; N, 9.96; Found: C, 62.56; H, 3.79; N, 10.04. ^{1}H NMR (DMSO-d_6): δ 7.34–8.20 (m, 12H, Ar–H), δ 8.44 (s, 1H, N=CH), δ 9.95 (s, 1H, CONH, D_2O exchangeable), δ 10.55 (s, 1H, CONH–N, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 421 (6.63, M^+).

*2-((4-(2-(4-Methoxybenzylidene)hydrazinecarbonyl) phenyl)carbamoyl)-benzoic acid (**8c**)*

Yellow crystals, mp 237–39°C, yield 79%. Analysis for $C_{23}H_{19}N_3O_5$ (417.41), Calcd.: C, 66.18; H, 4.59; N, 10.07; Found: C, 66.25; H, 4.47; N, 9.96. ^{1}H NMR (DMSO-d_6): δ 3.79 (s, 3H, OCH_3) δ 6.95–8.20 (m, 12H, Ar–H), δ 8.43 (s, 1H, N=CH), δ 9.99 (s, 1H, CONH, D_2O exchangeable), δ 10.57 (s, 1H, CONH–N, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 417 (8.78, M^+).

*Phenylethylidenehydrazinecarbonyl derivatives **9a, b***

A mixture of **7** (0.60 g, 2 mmol) and acetophenone (0.24 g, 2 mmol) or **1** (0.57 g, 2 mmol) in acetic acid (20 ml, 96%) was refluxed for 5 h and allowed to cool to room temperature. The precipitated solid was filtered, dried, and recrystallized from glacial acetic acid to afford pure products.

*2-((4-(2-(1-Phenylethylidene)hydrazinecarbonyl)phenyl) carbamoyl)benzoic acid (**9a**)*

Orange crystals, mp 272–74°C, yield 67%. Analysis for $C_{23}H_{19}N_3O_4$ (401.41), Calcd.: C, 68.82; H, 4.77; N, 10.47; Found: C, 68.86; H, 4.70; N, 10.54. ^{1}H NMR (DMSO-d_6): δ 2.54 (s, 3H, CH_3), δ 7.34–8.21 (m, 13H, Ar–H), δ 9.86 (s, 1H, CONH, D_2O exchangeable), δ 10.16 (s, 1H, CONH–N, D_2O exchangeable), δ 11.00 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 401 (11.98, M^+).

*2-((4-(1-(2-(4-(2-Carboxybenzamido)benzoyl)hydrazono) ethyl)phenyl)-carbamoyl)benzoic acid (**9b**)*

Orange crystals, mp >300°C, yield 45%. Analysis for $C_{31}H_{24}N_4O_7$ (564.54), Calcd.: C, 65.95; H, 4.28; N, 9.92; Found: C, 65.91; H, 4.33; N, 9.98. ^{1}H NMR (DMSO-d_6): δ 2.59 (s, 3H, CH_3), δ 7.57–8.24 (m, 16H, Ar–H), δ 9.86 and δ 10.10 (s, 2H, two CONH, D_2O exchangeable), δ 10.84 (s, 1H, CONH–N, D_2O exchangeable), δ 11.00 (s, 2H, two

COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 564 (2.56, M^+).

*2-((4-((1,3-Dioxoisoindolin-2-yl)carbamoyl)phenyl) carbamoyl)benzoic acid (**10**)*

A mixture of **7** (0.60 g, 2 mmol), phthalic acid anhydride (0.30 g, 2 mmol), and catalytic amount of sodium acetate (0.1 g) in glacial acetic acid (20 ml) was refluxed for 5 h and allowed to cool to room temperature. The precipitated solid was filtered, dried, and recrystallized from glacial acetic acid to afford pure product. Orange crystals, mp 228–230°C, yield 72%. Analysis for $C_{23}H_{15}N_3O_6$ (429.38), Calcd.: C, 64.34; H, 3.52; N, 9.79; Found: C, 64.53; H, 3.46; N, 9.83. ^{1}H NMR (DMSO-d_6): δ 7.62–8.17 (m, 12H, Ar–H), δ 8.82 (s, 1H, CONH–N, D_2O exchangeable), δ 10.02 (s, 1H, CONH, D_2O exchangeable), δ 11.96 (s, 1H, COOH, D_2O exchangeable). MS *m/z* (% Rel. Int.): 429 (8.45, M^+).

ABTS antioxidant assay

Preparation of reagents

ABTS solution was prepared as 0.1 g/100 ml. MnO_2 solution 25 mg/ml was used instead of potassium persulfate. All reagents were prepared in phosphate buffer (pH 7, 0.1 M). The two reagents ABTS/MnO_2 (2:3) were mixed and centrifuged. The supernatant was obtained as green–blue solution ($ABTS^{\bullet+}$ radical solution). This color remained stable more than 1 h. The absorbance was adjusted at ca., 0.2 at 734 nm. L-Ascorbic acid solution was prepared as 2% solution: 1 g/50 ml distilled water. Each test sample was used in concentration of 0.01 mg/ml in methanol/phosphate buffer (1:1).

Starting assay

900 µl of (ABTS/MnO_2) was added to cuvette of spectrophotometer (SPEKOL 11), and the absorbance was measured at 734 nm, against a blank, made of methanol/phosphate buffer (1:1).

900 µl of (ABTS/MnO_2) mixture was added to 100 µl standard L-ascorbic acid, and the absorbance was measured against blank: methanol/phosphate buffer (1:1) + 100 µl of L-Ascorbic acid.

900 µl of (ABTS/MnO_2) mixture was added to 100 µl of sample, and the absorbance was measured against blank: methanol/phosphate buffer (1:1) + 100 µl of sample).

% Inhibition of absorbance for each of the tested compound was calculated from the following equation: % Inhibiton = [Abs(control) − Abs(test)]/Abs(control).

Conclusion

It was observed that compound **7**, carrying the acid hydrazide moiety, showed moderate antioxidant activity (55.21% inhibition), in comparison to L-ascorbic acid. The rest of the tested compounds showed low antioxidant activity.

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References

Angelo QM, George KB, Cezar SG, Ricardo GO, Antonio A, Marcelo H-L (2003) Pyridoxal isonicotinoyl hydrazone inhibits iron-induced ascorbate oxidation and ascorbyl radical formation. Biochim Biophys Acta 1620(1–3):15–24

Argade NP, Balasubramaniyan V (2000) Ring closure reactions of suitably *ortho*-substituted maleanilic acids: an avenue for heterocycles. Heterocycles 53(2):475–488. doi:10.3987/REV-99-525

Aruoma OI (1998) Free radicals, oxidative stress, and antioxidants in human health and disease. JAOCS 75(2):199–212. doi:10.1007/s11746-998-0032-9

Bai R, Ma F, Liang D, Zhao X (2009) Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of *Malus prunifolia*. J Chem Ecol 35(4):488–494. doi:10.1007/s10886-009-9615-7

Chen A, Taguchi T, Aoyama S, Sugiura M, Haruna M, Wang M, Miwa I (2003) Antioxidant activity of a Sciff base of pyridoxal and aminoguanidine. Free Radic Biol Med 35(11):1392–1403. doi:10.1016/j.freeradbiomed.2003.08.014

Dolzhenko AV, Kolotova NV, Kozminykh VO, Syropyatov BY, Kotegov VP, Godina AT (2002) Hypoglycemic effect of several substituted amides and acylhydrazides of succinic acid. Pharmazie 57(11):776–778

Dolzhenko AV, Koz'minykh VO, Kolotova NV, Burdi NZ, Kotegov VP, Novoselova GN, Syropyatov BY (2003) Substituted amides and hydrazides of dicarboxylic acids. Part 17. Synthesis and antibacterial and anticonvulsant activity of some amides and acylhydrazides of phthalic acid. Pharm Chem J 37(7):342–344. doi:10.1023/A:1026307221989

Feuer H, Asunskis JP (1962) The chemistry of cyclic hydrazides. VIII. The preparation of substituted N-aminomaleimides and their conversion to N-substituted maleic hydrazides. J Org Chem 27(12):4684–4686. doi:10.1021/jo01059a530

Filho VC, Campos F, Corrêa R (2003) Chemical aspects and therapeutic potential of cyclic imides: a review. Quim Nova 26(2):230–241

Guo Z, Xing R, Liu S, Yu H, Wang P, Li C, Li P (2005) The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl chitosan. Bioorg Med Chem Lett 15(20):4600–4603. doi:10.1016/j.bmcl.2005.06.095

Gürkök G, Coban T, Suzen S (2009) Melatonin analogue new indole hydrazide/hydrazone derivatives with antioxidant behavior: synthesis and structure-activity relationships. J Inhib Med Chem 24(2):506–515. doi:10.1080/14756360802218516

Knight JA (1998) Free radicals: their history and current status in aging and disease. Ann Clin Lab Sci 28(6):331–346

Koz'minykh VO (2006) Synthesis and biological activity of substituted amides and hydrazides of 1,4-dicarboxylic acids. Pharm Chem J 40(1):8–17. doi:10.1007/s11094-006-0048-0

Lange J, Rump S, Ilczuk I, Lapszewicz J, Rabsztyn T, Walczyna K (1979) Synthesis and properties of cyclic derivatives of succinic acid with anticonvulsant activity Part 3. Pharmazie 34(12):794–795

Mates JM, Perez-Gomez C, Nunez de Castro I (1999) Antioxidant enzymes and human diseases. Clin Biochem 32(8):595–603

Miller NJ, Rice-Evans C (1997) Factors influencing the antioxidant activity determined by the $ABTS^{+}$ radical cation assay. Free Rradic Res 26:195–199. doi:10.3109/10715769709097799

Re N, Pellegrini A, Proteggente A, Pannala MY, Evans CR (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 29:1231–1237. doi:10.1016/S0891-5849(98)00315-3

Shankar RA, Hideg K, Zweier JL, Kuppusamy P (2000) Targeted antioxidant properties of N-[(tetramethyl-3-pyrroline-3-carboxamido) propyl]phthalimide and its nitroxide metabolite in preventing postischemic myocardial injury. J Pharmacol Exp Ther 292(3): 838–845

Vukovic N, Sukdolak S, Solujic S, Niciforovic N (2010) An efficient synthesis and antioxidant properties of novel imino and amino derivatives of 4-hydroxy coumarins. Arch Pharm Res 33(1):5–15. doi:10.1007/s12272-010-2220-z