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Free radical scavenging activity of several caffeic acid derivatives

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Abstract

Several caffeic acid (CA) derivatives have been synthesized and estimated for their free radical scavenging activity by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. It was shown that the free radical scavenging activity of CA derivatives strongly depends on the nature of the substituents. The most active compounds among the synthesized compounds appeared to be dihydroxylated CA derivatives.

Keywords: Caffeic Acid Derivatives; Free Radical Scavenging Activity; DPPH assay; $ABTS^{\bullet+}$ assay

1. Introduction

Hydroxycinnamic acids (caffeic-, ferulic-, coumaric-, and sinapic acids) are naturally occurring compounds with a wide spectrum of biological activities [1-7]. Derivatives of these phenolic acids also characterized with remarkable biological activities and in some cases have superior antioxidant and antimicrobial activities than their precursor phenolic acids [8-15]. Among these derivatives are CA esters and amides [16-21]. Free radical scavenging and antibacterial activities are chemically and biologically linked through shared redox mechanisms and membrane interactions. Phenolic antioxidants like caffeic and gallic acid exemplify this overlap - they protect host tissues while simultaneously impairing bacterial survival [22, 23]. Previously we have reported about synthesis and activity of several CA derivatives [24-27]. It was shown that embedding two hydroxy groups in the side chain of CA increases antioxidant properties of obtained dihydroxylated *trans*-CA derivative, as in racemic -2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid and as well its enantiomeric forms - (+)-(2R,3S)-2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid and (–)-(2S,3R)-2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid, showing 3-fold higher antioxidant activity than CA against reactive oxygen species, such as hypochlorite or free radicals - DPPH [24]. Furthermore, racemic 2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid effectively suppressed the growth and induced death in prostate cancer cells, with comparatively lesser cytotoxicity towards non-neoplastic human prostate epithelial cells [25]. Antimicrobial assays of the tested CA derivatives revealed that some of them exceed the activity of the reference drugs ampicillin and streptomycin, and exhibit higher antifungal properties, than that of reference drugs ketoconazole and bifonazole [26,27]. The results provide a good background for further research, in particular, to determine the influence of different substituents on radical scavenging properties. In the current study several previously synthesized CA derivatives [24, 26] were examined for their free radical scavenging activity in DPPH and ABTS assays.

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2. Materials and Methods

Starting materials and reagents: *trans*-caffeic acid, methyl iodide, benzyl bromide (BnBr), dimethyl sulphate, citric acid, N-methylmorpholine-N-oxide (NMO), potassium osmate (K_2OsO_4 x $2H_2O$), palladium on carbon (10% Pd/C), DPPH, ABTS, were of analytical grade and used without further purification. All reagents obtained from Merck and Co., Inc.

2.1. Synthesis of derivatives of caffeic acid

trans-CA (1) was used as a starting material for synthesis of all obtained compounds (2-13) (Figure 1). Synthesis of compounds (2-5, 8-11) and (7, 13) is described in our recent papers respectively [26,24]. Spectral data (^1H NMR, ^{13}C NMR and IR) are in accordance with those of previously reported [24, 26].

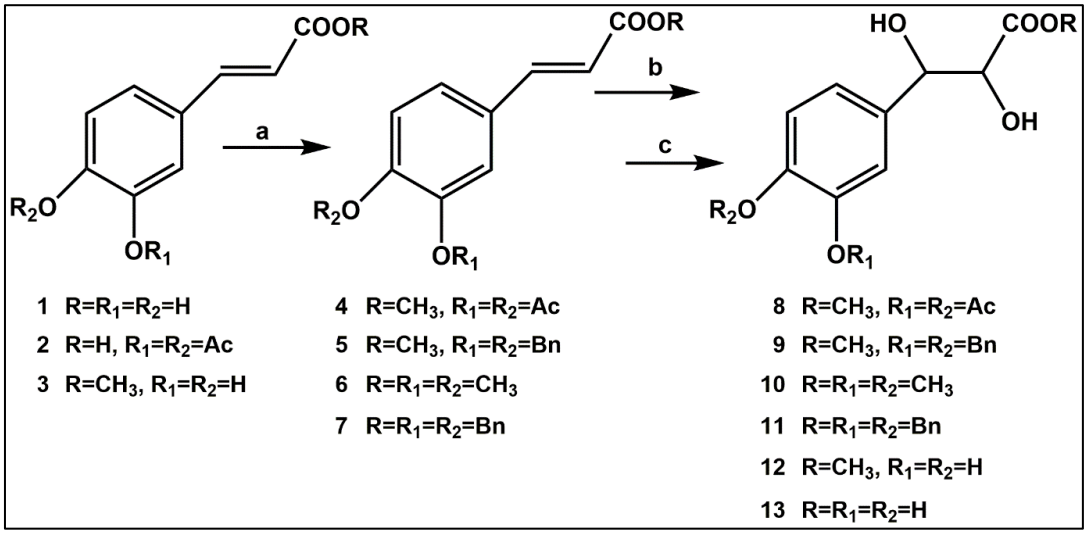

Figure 1 Synthetic route to CA derivatives. Reagents and conditions: (a) CH_3I, DMF, $NaHCO_3$, r.t., 24h (b) K_2OsO_4 x $2H_2O$, NMO, acetone: CH_3CN: H_2O (3:1:1), 4h; (c) H_2, Pd/C, THF-EtOH, 12 h

2.2. Radical Scavenging Assays

Radical scavenging-based assays such as $DPPH^{\cdot}$ and ABTS are common spectrophotometric assays due of their sensitivity, simplicity, speed, and reproducibility [28]. Prior to the assays, in silico experiment was carried out using the Prediction of Activity Spectra for Substances (PASS) platform [29] in order to estimate the probability of radical scavenging efficacy of synthesized derivatives. Gallic acid (GA) was chosen as reference compound in both predictive and in vitro assays.

2.2.1. In silico experiment

The probability of radical scavenging efficacy was assessed in an *in-silico* experiment using the PASS platform, which forecasts more than 3500 types of biological activity, covering over 300 pharmacological effects, mechanisms of action, toxic and adverse effects, interactions with enzymes and transporters. PASS analyses structure-activity relationships (SAR) for over 250,000 biologically active substances by comparing the structure of compound under investigation with one of a well-known bioactive compound (drug, drug candidate, lead, etc.) and forecasts a probability of either biological activity (Pa) or inactivity (Pi). Compounds with Pa > 0.7 are considered to be effective in animal tests, ones that fall in 0.5 < Pa < 0.7 range, are expected to show lower likelihood activity, whereas compounds with Pa<0.5 are not expected to exhibit any activity.

2.2.2. DPPH Radical Scavenging Assay

DPPH radical scavenging activity was determined using a modified method of Brand-Williams et al. [30]. A DPPH stock solution (10.4 mg in 40 mL methanol, sonicated) was diluted 10-fold to achieve an absorbance of 0.7–0.8. The assay mixture consisted of 1.98 mL of the DPPH solution and 0.02 mL of either the test sample (0.39–200 µg/mL in DMSO), GA standard (0.3125–10 µg/mL), or pure DMSO (blank control). Absorbance was monitored in triplicate at 517 nm using an HBS-1096 Microplate Reader every 30 minutes at room temperature until stable. The percentage of DPPH radical inhibition was calculated using the following formula:

$$In\ \% = A_0 - A_1/A_0 * 100$$

Where A_0 represents the absorbance of the DPPH methanolic solution with DMSO (control), and A_1 represents the absorbance of the test samples. The IC_{50} (µg/mL) values were subsequently calculated based on the obtained results.

2.2.3. ABTS Radical Scavenging Assay

ABTS radical scavenging activity was determined according to the method of Re et al. [31]. The $ABTS^{\bullet+}$ cation was generated by reacting 0.05 M ABTS with 0.02 M sodium persulfate overnight at room temperature in the dark, then diluted 300-fold with methanol. The assay mixture consisted of 1.98 mL of the diluted $ABTS^{\bullet+}$ solution and 0.02 mL of either the test sample (0.19–200 µg/mL in DMSO), GA standard (0.3125–10 µg/mL), or pure DMSO (blank control). Absorbance was monitored in triplicate at 734 nm using an HBS-1096 Microplate Reader every 30 minutes at room temperature until stable. The absorbance of the test samples was monitored every 30 minutes at room temperature until a constant value was reached. All experiments were performed in triplicate. Spectrophotometric measurements were carried out using an HBS-1096 Microplate Reader. The percentage of $ABTS^{\bullet+}$ inhibition was calculated using the following formula:

$$In\ \% = A_0 - A_1/A_0 * 100$$

Where A_0 represents the absorbance of the ABTS methanolic solution with DMSO (control), and A_1 represents the absorbance of the test samples. The IC_{50} (µg/mL) values were subsequently calculated based on the obtained results.

In both assays data were expressed as mean ± standard deviation (SD) relative to the control, which was set as 100%. IC_{50} values were determined by nonlinear regression using a four-parameter logistic model, with 95% confidence intervals and coefficient of determination (R2) reported. Statistical analysis was performed using GraphPad Prism version 11.0.1 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

3. Results and discussion

Free radical scavenging activity for compounds 2-13 predicted in *in silico* assay using PASS revealed 3 derivatives with Pa values > 0.5. The predicted values were further compared with in vitro results obtained in both DPPH and ABTS assays (Table 1)

Table 1 Free radical scavenging activity of caffeic acid derivatives 2-13

Compounds	PASS predicted probability		IC_{50} (µg/mL) in in vitro assay*	
	Pa	Pi	ABTS	DPPH
2	0.688	0.004	15.3 ± 0.51	94.5 ± 0.55
3	0.674	0.004	1.61 ± 0.89	2.5 ± 0.94
4	0.402	0.017	26.8 ± 0.38	64.6 ± 0.69
5	0.444	0.014	26.5 ± 0.46	>200
6	0.593	0.006	>200	>200
7	0.425	0.015	>200	>200
8	0.328	0.025	1.31 ± 0. 71	2.7 ± 0.58
9	0.198	0.078	96.4 ± 0.42	.>200
10	0.286	0.034	>200	>200
11	0.190	0.085	>200	>200
12	0.425	0.015	1.76 ± 0.94	3.8 ± 0.76
13	0.359	0.021	0.89± 0.35	1.61 ± 0.41
Galic Acid	0.570	0.007	1.21 ± 0.78	1.35 ± 0.5

* Data represented as mean of triplicates ± standard deviation (SD)

According to Table 1 dihydroxylated caffeic acid (13) was the most active compound in both ABTS and DPPH assays with IC_{50} = (0.89± 0.35) and (1.61 ± 0.41), respectively. The second one with IC_{50} = 1.61 ± 0.89 and 2.5 ± 0.94 was methyl caffeate (3). The high antioxidant activity of this caffeic acid methyl ester towards DPPH radical scavenging was reported previously in [32], and, in our study, it appeared even more pronounced in the ABTS test. Dihydroxylation of methyl caffeate side chain (compound 12) decreases activity in both assays - 1.76 ± 0.94 and 3.8 ± 0.76 respectively. It should be mentioned that diacetoxy caffeic acid (2) showed moderate activity towards ABTS radical 15.3 ± 0.51 and less against DPPH (94.5 ± 0.55). Methylation of carboxylic group of this acid (4), increases activity towards DPPH radical scavenging (64.6 ± 0.69) when compared to compound 2, whereas, dihydroxylation of 4 remarkably increases antiradical scavenging in both assays 1.31 ± 0. 71 and 2.7 ± 0.58. The results indicate that compounds with substituents in catechol moiety (6,7 and 9-11) show less activity. The free radical scavenging activity of the compounds towards ABTS radical decreases in the following order: (13) > (3) > (8) > (12) > (2) > (5) > (4) > (9) > (6) = (7) = (10) = (11). The same tendency persists for the first four active compounds towards DPPH radical: (13) > (3) > (8)> (12) > (4) > (2) > (6) = (7) = (10) = (11) = (9).

The SAR analysis revealed that in case of caffeic acid derivatives the presence of the catechol moiety (13,12) is beneficial for radical scavenging activity. As it was shown earlier, two hydroxy groups embedded in the side chain of CA increases its antioxidant activity [24]. The same tendency is observed in case of compounds (8 and 12). Conversely, the antiradical scavenging activity of derivatives with methoxy and benzyloxy substituents is reduced (6,7) and their activity is not enhanced by further dihydroxylation (10,11).

4. Conclusion

Thirteen compounds were synthesized and evaluated for their free radical scavenging activity by ABTS and DPPH assays based on in silico computer-aided prediction using PASS software. It was found that the activity of compounds depends on the nature of substituents in positions 3 and 4 of the catechol moiety. The presence of methoxy and benzyloxy groups decreases their activity, whereas embedding of two hydroxy groups in the side chain of caffeic acid increases their free radical scavenging activity. The obtained data show strong dependence of the free radical scavenging activity of caffeic acid derivatives on their structure modifications.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflicts of interest.

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