Synthesis and investigation of antioxidant activities of 2-benzylidene-3-coumaranones

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ABSTRACT

A number of 6-hydroxy-2-benzylidene-3-coumaranones were synthesized from condensation of 6-hydroxy-3-coumaranone with appropriate aldehydes and were evaluated for their antioxidant activities. The antioxidant activity was assessed using two methods, including, 1,1-biphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and reducing power assays. Some of the benzylidene coumaranones showed antioxidant activity more than Trolox as reference antioxidant.

Keywords: Antioxidant activity; Coumaranones; Silica sulfuric acid; Ferric reducing antioxidant power assay; DPPH.

INTRODUCTION

Free radicals are produced in the normal or pathological cell metabolism. Oxidation is one of the essential processes to many living organisms to produce energy for biological processes [1]. On the other hand, generation and uncontrolled production of reactive oxygen species (ROS) and free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis, arteriosclerosis inflammation, ischemic heart disease, neurodegenerative such as Alzheimer and Parkinson diseases [2]. There are many kind of (ROS) in human body including superoxide anion, hydrogen peroxide, hydroxyl radical, and by-product of a variety of aerobic metabolism. The human body possesses innate defense mechanism in the form of enzymes such as glutathione peoxidase, superoxide dismulate and catalase [3-4]. In many latest studies has reported that antioxidant compounds in foods play an important role as a health-protecting factor. Scientific evidence suggested that antioxidants reduce the risk of chronic diseases including cancer, and heart diseases [5]. Primary source of naturally occurring antioxidants are grains, fruits, and vegetables. Plant sourced food antioxidant such as vitamin C, vitamin E, carotenes, and other carotenoids have been used as a supplementary antioxidant.

Apart from these, polyphenolic plant secondary metabolites such as flavonoids play an important role in the defense against free radicals. These compounds also show antibacterial, antiviral, anti-inflammatory, antiatherogenic properties, and preventing role in cancer, Alzheimer's, Parkinson's and cardiovascular diseases. Some of these activities are at least partially related to their antioxidant properties [5-6]. Aurones are very similar to flavonoids in structure and a series of aurone derivatives have been used for in vivo imagining of β-amyloid plaques in the brain of Alzheimer's disease [7].

Recently, silica sulfuric acid (SSA) has been introduced as a novel solid inorganic acidic proton source catalyst by the reaction of silica gel and chlorosulfonic acid for the various

functional group transformations [8-10]. In continuation of our studies in this regards[9], we report our efforts towards a new heterogeneous procedure for the synthesis of 6-hydroxy-2-benzylidene-3-coumaranones and evaluate their in vitro antioxidant activities (Figure1). In this project we used solid silica sulfuric acid (SSA) as a heterogeneous catalyst for the synthesis of 6-hydroxy-3-coumaranone from resorcinol and bromoacetyl bromide. Remarkable point about this reaction is that it is easy, clean and without any work-up procedure because hydrogen bromide gas is evolved from the reaction vessel immediately. This catalyst has no solubility in general solvents, so it is recovered by a simple filtration after ending the reaction.

MATERIALS AND METHODS

General

All chemicals were purchased from Merck and Fluka chemical companies. Column chromatography was performed using silica gel 60 (230-400 mesh). All yields refer to isolated yield. The structure of compounds was characterized by IR, ^{1}H NMR, and ^{13}C NMR spectra. The melting point was taken on a Kofler hot stage apparatus and is uncorrected. IR spectrum was recorded on a Shimadzu 470 spectrophotometer (KBr disk). ^{1}H NMR spectrum was recorded on a Bruker FT-300 NMR spectrophotometer using DMSO-d_6 as solvent and TMS as an internal standard. The purity of the compound was monitored by thin layer chromatography.

General procedure for synthesis of 6-hydroxy-3-coumaranone

The starting 6-hydroxy-3-coumaranone **4** (see Figure1) was prepared by dissolving resorcinol (1 mmol) and bromoacetyl bromide (1 mmol) in acetonitrile (3 ml) with 400 mg silica sulfuric acid added as catalyst. The reaction mixture was heated at 80 ^{o}C for 3 h. After cooling to room temperature and filtering the reaction mixture, the compound **3** was purified by column chromatography. Subsequently, the compound **3** was cyclized using NaOH 2 M to produce 6-hydroxy-3-coumaranone **4** in 75% yield after 1 h. m.p. = 243-246 ^{o}C; ^{1}H NMR (DMSO-d_6): δ 7.41 (d, J = 8.5 Hz, 1H), 6.54-6.56 (d, J = 8.5 Hz, 1H), 6.66 (s, 1H), 4.60 (s, 2H), 4.58 (s, 1H) ppm; IR (KBr, cm^{-1}): υ 1745 (C=O), 3452 (OH).

General procedure for synthesis of 6-hydroxy-2-benzylidene-3-coumaranone

To a solution of compound **4** (1 mmol), and appropriate aryl aldehyde (1 mmol) in ethanol (3 ml) was added piperazine (0.1 mmol) as the basic catalyst. The reaction mixture was heated in 80 ^{o}C for 2-3 h. The reaction mixture was allowed to stand at room temperature for 24 h. The precipitate was filtered, dried, and crystallized from acetic acid to afford pure 6-hydroxy-2-benzylidene-3-coumaranones **5a-e** (Table 1). Structural assignments of the products are based on their IR, ^{1}H NMR, ^{13}C NMR spectra, and melting point.

2-(4'-Hydroxybenzylidene)-6-hydroxy-3-coumaranone (5a)

Yield = 75%; Orange solid; m.p. 254-256 $^\circ$C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.68 (dd, J_1 = 1.8 Hz, J_2 = 10 Hz, H_5), 6.70 (s, overlap, =CH), 6.78 (d, J = 1.8 Hz, H_7), 6.88 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 8.4 Hz, H_4), 7.81 (d, J = 8.7 Hz, 2H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ 99.7, 113.5, 114.1, 114.9, 117.2, 124.9, 126.9, 134.7, 147.6, 160.7, 167.9, 169.5, 183.8 ppm; IR (KBr, cm^{-1}): υ 1740 (C=O), 3445 (OH).

2-(2',4'-dihydroxybenzylidene)-6-hydroxy-3-coumaranone (5b)

Yield = 65%; m.p. = 330-332 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.96-7.90 (m, 2H, $H_{6'}$, H_4), 7.35 (s, 1H, =CH), 6.70 (s, 1H, H_7), 6.55 (d, J = 8.7 Hz, 1H, H_5), 6.25 (d, J = 8.7 Hz, 1H, H_5), 6.15 (s, 1H, H_3), 3.49-3.42 (s, 3H, OH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ 182.1, 167.0, 165.2, 159.3, 157.3, 146.7, 131.3, 128.3, 115.2, 112.3, 108.3, 105.7, 103.5 ppm; IR (KBr, cm^{-1}): υ 1735 (C=O), 3455 (OH).

2-(2',5'-dihydroxybenzylidene)-6-hydroxy-3-coumaranone (5c)

Yield 60%; m.p. = 340-342 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.59 (d, J = 8.5 Hz, 1H, $H_{3'}$), 7.51 (d, J = 8.5 Hz, 1H, $H_{4'}$), 7.46 (s, 1H, $H_{6'}$), 7.11 (d, J = 8.7 Hz, 1H, H_4), 7.10 (s, 1H, H_7), 6.95 (d, J = 8.7 Hz, 1H, H_5), 6.75 (s, 1H, =CH), 3.38-3.42 (s, 3H, OH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ 182.2, 167.1, 165.0, 151.2, 149.5, 147.0, 128.4, 117.5, 116.5, 113.2, 103.4, 101.2 ppm; IR (KBr, cm^{-1}): υ 1750 (C=O), 3115 (OH).

2-(2'-hydroxy-3'-methoxybenzylidene)-6-hydroxy-3-coumaranone (5d)

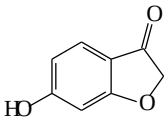
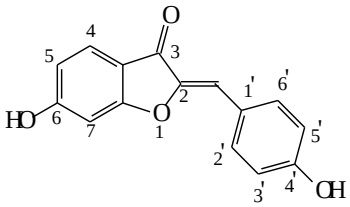
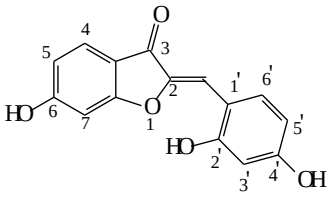
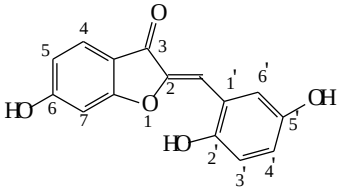
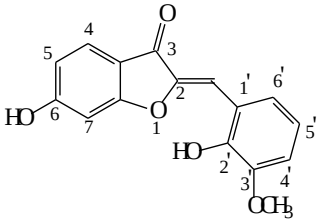
yield 71%; m.p. = 305-306 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.71 (d, J = 8.7 Hz, 1H, H_4), 7.62 (s, 1H, =CH), 7.11-7.17 (m, 2H, $H_{5'}$, $H_{6'}$), 7.04 (s, 1H, H_7), 6.91 (s, 1H, H_7), 6.73 (d, J = 8.8 Hz, 1H, H_4), 6.50 (d, J = 8.8 Hz, 1H, H_5), 5.22 (s, 2H, OH), 3.84 (s, 3H, OCH_3)

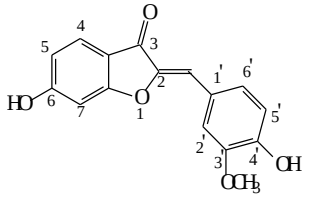
ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ 182.1, 167.5, 165.2, 151.2, 148.4, 146.7, 128.0, 122.6, 121.4, 117.4, 115.2, 111.8, 105.3, 101.3, 56.4 ppm; IR (KBr, cm^{-1}): υ 1730 (C=O), 3250 (OH).

2-(4'-hydroxy-3'-methoxybenzylidene)-6-hydroxy-3-coumaranone (5e)

Yield 80%; m.p. = 280-282 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.61 (d, J = 8.7 Hz, 1H, $H_{5'}$), 7.49 (d, J = 8.7 Hz, 1H, $H_{6'}$), 7.53 (s, 1H, $H_{2'}$), 6.91 (d, J = 8.8 Hz, 1H, H_4), 6.80 (s, 1H, H_7), 6.71 (d, J = 8.7 Hz, 1H, H_5), 6.80 (s, 1H, =CH), 3.85 (s, 3H, OCH_3), 3.80 (s, 2H, OH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ 182.3, 167.5, 165.1, 149.2, 147.4, 146.7, 128.1, 125.6, 121.5, 116.4, 115.3, 111.6, 101.2, 56.2 ppm; IR (KBr, cm^{-1}): υ 1743 (C=O), 3250 (OH).

Table 1. Antioxidant activities of 2-benzylidene-3-coumaranones in comparison to Trolox

Entry	Compound	DPPH radical scavenging activity (IC_{50}, µM)	FRAP value (Fe^{2+} µM)
1	 **4**	>100	42
2	 **5a**	50.22	49
3	 **5b**	23.99	65
4	 **5c**	27.53	63
5	 **5d**	24.71	52

6	 **5e**	26.47	68
7	**Trolox**	36.27	42

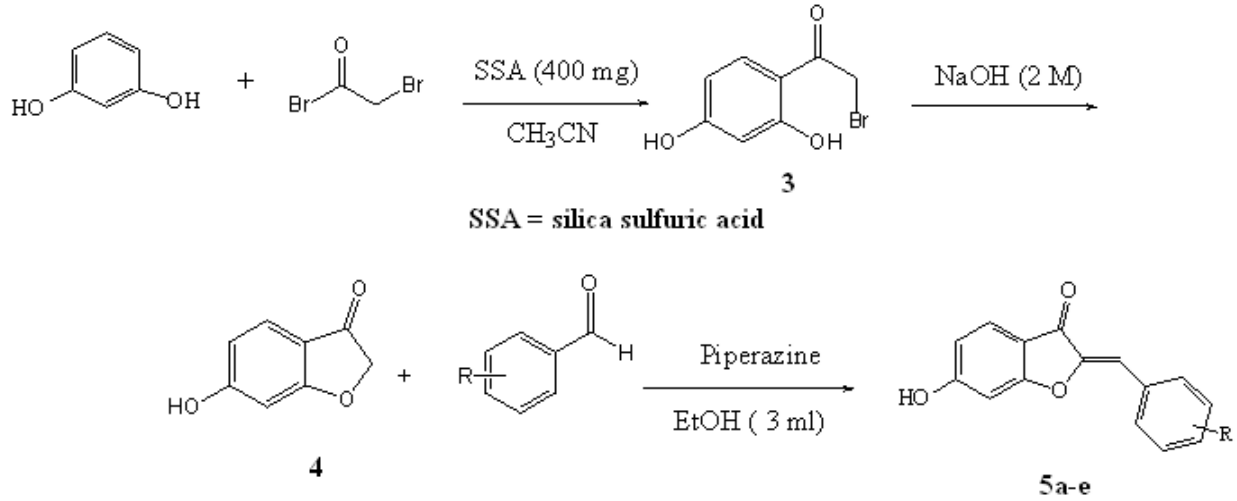


Figure 1. Synthetic route for 6-hydroxy-2-benzylidene-3-coumaranones.

Antioxidant assays

DPPH radical scavenging assay

The compounds were dissolved in appropriate solvent mixed with 1 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in ethanol, and final volume was adjusted to 2 mL. Mixtures were variously shaken and left for 30 min in the dark. Absorbance was measured at 517 nm using a UV–VIS spectrophotometer. 1 mL of 0.2 mM DPPH diluted in 1 mL of ethanol was used as control. Neutralization of DPPH radical was calculated using the equation: S (%) = 100 $(A_o-A_s)/A_o$, where Ao is the absorbance of the control (containing all reagents except the test compound) and A_s is the absorbance of the test sample. Results were compared to activity of Trolox. The IC_{50} values represented the concentration of the test compounds that caused 50% inhibition are shown in Table 1.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay reagent was prepared by adding 10 vol of 30 mM acetate buffer, pH 3.6 (1 g sodium acetate and 16 mL glacial acetic acid), 1 vol of 10 mM 2,4,6-tripyridyl-triazine prepared in 40 mM HCl and 1 vol of 20 mM $FeCl_3$. The mixture was diluted to 1/3 with methanol and pre-warmed at 37 ^{o} C. This reagent (3 mL) was mixed with 0.1 mL diluted the test compounds. The mixture was shaken and incubated at 37 ^{o} C for 8 min and the absorbance was read at 593 nm. A blank with only 0.1 mL methanol was used for calibration. The difference in absorbance between the tested sample and the blank reading was calculated and the data were expressed as mM of ferric reduced to ferrous form (Table 1).

RESULTS AND DISCUSSION

Chemistry

The synthesis of final compounds begins by reaction of resorcinol with bromoacetyl bromide using SSA as the heterogeneous catalyst. The first intermediate product was then cyclized using NaOH 2 M to give 6-hydroxy-3-coumaranone **3** in 75% yield. In the next stage, condensation of 6-hydroxy-3-coumaranone **3** with different aryl aldehydes in basic condition and EtOH as the solvent afforded 6-hydroxy-3-croumanone derivatives **5a-e** in 60-80% yields.

Antioxidant activity

The antioxidant activity was assessed using two methods, including, 1,1-biphenyl-2-picrylhydrazyl (DPPH) radical scavenging [11], and ferric reducing antioxidant power [12] assays according to the methods described in

the literature (Table 1). The DPPH is a stable free radical with maximal absorption at 517 nm. It loses this absorption when reduced by an antioxidant or a free radical species. The decrease in absorbance at a reaction time is used in determining the antioxidant activity of the tested substances with Trolox (2,5,7,8-tetramethychroman-2-carboxylic acid) as the standard. In FRAP assay, Fe^{3+}, can be reduced by the antioxidant to a catalytically ion Fe^{2+} that provokes the antioxidant to behave as prooxidant. The FRAP assay measure the ability of a compound to reduce the 2,4,6-tripyridyl-s-triazine complex. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. FRAP values of coumaranones is shown in Table 1

The results of antioxidant activity in both methods are approximately similar to each other. All the synthesized compounds except **4, 5a,** exhibited very good antioxidant properties according to DPPH method. They were also more potent than Trolox as reference compound. It should be noted that when electron-donating groups such as hydroxyl and methoxy are added to the phenyl ring derived from aldehyde, the antioxidant activity is increased. This is due to the stabilization of the generated radical during oxidation. The compounds **5b, 5c, 5d, 5e** have well antioxidative activity with a major activity for **5b** (IC_{50} 23.99 µM).

The results of antioxidant activity indicated that in addition to phenyl moiety, benzofuranone ring was also effective and necessary in the assays. However, it is seems that benzofuranone moiety delocalized free electron during oxidation. For example, compound showed antioxidative activity having IC_{50} values higher than 100 µM respectively via DPPH method, whereas benzylidene coumaranone similarities (**5a**, **5b**, **5c**, **5d**, **5d**, **5e**) have IC_{50} values 50.22, 23.99, 27.53, 24.71, 26.47 µM respectively via the same assay. Overall, the result of DPPH assay was relatively consistent with that of reducing power assay. The potencies for the antioxidative activity of the test compounds compared to the reference drug are in the following order: **5b** > **5d** > **5e** > **5c** > Trolox > **5a** > 4.

CONCLUSION

In summary, we have reported biological evaluation of benzylidene coumaranones, which represent antioxidant activity. Also, we developed a new methodology for the synthesis of 6-hydroxy-3-coumaranone under heterogeneous condition by silica sulfuric acid. Some of the synthesized benzylidene coumaranones showed antioxidant activity more potent that the reference drug.

ACKNOWLEDGMENT

We gratefully acknowledge Vice Chancellor for Research and Technology, Kermanshah University of Medical Sciences for financial support. This work was performed in partial fulfillment of the requirements for a Pharm. D of Karim Najafi in Faculty of Pharmacy, Kermanshah University of Medical Sciences Kermanshah-Iran.

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