

## SYNTHESIS AND HYDROXYL RADICAL SCAVENGING ACTIVITY OF 4-ARYL-3,4-DIHYDROCOUMARINS

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*Twelve 4-aryl-3,4-dihydrocoumarins were synthesized by the condensation of phenols with the corresponding substituted cinnamic acids in the presence of nitrobenzene and sulfated montmorillonite K-10. This method displayed the property of green chemistry with respect of reuse of the catalyst. These compounds were evaluated for hydroxyl radical scavenging activity in vitro. Results showed that compounds with 7,8-dihydroxy groups had relatively strong hydroxyl radical scavenging activity.*

**Keyword:** 4-aryl-3,4-dihydrocoumarins, montmorillonite K-10, hydroxyl radical scavenging activity.

Natural compounds with the 4-aryl coumarin structure have diverse biological activities and provide a platform for chemical modification [1]. Just as Ajit Kumar and co-workers have verified, 4-aryl-3,4-dihydrocoumarins have similar activity, with 4-aryl coumarins as its dihydro analogs [2]. Notably, several investigators have demonstrated that 4-aryl-3,4-dihydrocoumarins that possess two hydroxyl groups in ortho position exhibit potent antioxidant property [3, 4]. However, the free radicals they used for evaluating the antioxidant activity of 4-aryl-3,4-dihydrocoumarins were limited to DPPH, ABTS<sup>+</sup>, and NO. In this paper, we focus on its hydroxyl radical scavenging activity. In addition, this kind of compounds also have estrogen-like activity [5], immunomodulatory activity [6], and antiaging activity [7]. Hence, as part of our further study of 4-aryl-3,4-dihydrocoumarins, we report the synthesis and antioxidant activity of twelve 4-aryl-3,4-dihydrocoumarins.

The most common method for the synthesis of 4-aryl-3,4-dihydrocoumarins is the Ponnendorf reaction of phenols with substituted cinnamic acids promoted by acids including H<sub>2</sub>SO<sub>4</sub>, HCl, and PPA, Lewis acid, or clay [8]. For example, *p*-toluenesulfonic acid mediates hydroarylation of cinnamic acids with phenols under metal and solvent-free conditions [9], PPA mediates condensation of cinnamic acids with phenols [10], condensation of resorcinol and methylcinnamate in the presence of anhydrous AlCl<sub>3</sub> and HCl [11], condensation of phenols and cinnamic acids, in particular with hydroxy and methoxy substituents in the presence of CF<sub>3</sub>COOH or BF<sub>3</sub>–Et<sub>2</sub>O and POCl<sub>3</sub> [12, 13], with or without microwave-assisted synthesis from phenols and cinnamoyl chloride in the presence of montmorillonite K-10 catalyst [3, 14], and recently solvent-free synthesis of 4-aryl-3,4-dihydrobenzopyran-2-ones via [3+3] cyclocoupling of phenols with cinnamic acid catalyzed by molecular iodine [15]. However, most of these methods involve relatively harsh reaction conditions, low functional group tolerance, severe environmental pollution, or difficulty in separation and purification of products. Hence their applications have been somewhat constrained. In this article, we disclosed another methodology for the synthesis of 4-aryl-3,4-dihydrocoumarins based on sulfated montmorillonite K-10.

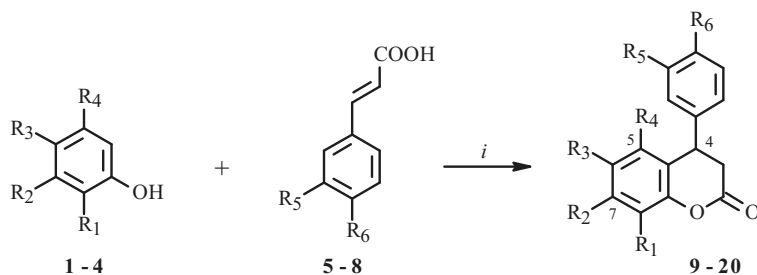
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TABLE 1. Repeated Use of Montmorillonite K-10 for Synthesis of 7-Hydroxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin<sup>a</sup>

Entry	Number of uses	Reaction time, h	Yield, % <sup>b</sup>
1	0	3	79.2
2	1	4.5	77.8
3	2	4.5	74.4
4	3	4.5	73.7
5	4	6	71.9
6	5	4	71.1

<sup>a</sup> Reagents and conditions: **1** and **2** (5 mmol·mL<sup>-1</sup>), nitrobenzene as solvent, recycled catalyst (2 g); <sup>b</sup> isolated yields.



- 1:** R<sub>1</sub> = R<sub>3</sub> = R<sub>4</sub> = H, R<sub>2</sub> = OH; **2:** R<sub>1</sub> = R<sub>2</sub> = OH, R<sub>3</sub> = R<sub>4</sub> = H; **3:** R<sub>1</sub> = R<sub>3</sub> = H, R<sub>2</sub> = H, R<sub>4</sub> = OH  
**4:** R<sub>1</sub> = R<sub>2</sub> = R<sub>4</sub> = H, R<sub>3</sub> = OH; **5:** R<sub>5</sub> = H, R<sub>6</sub> = OCH<sub>3</sub>; **6:** R<sub>5</sub> = R<sub>6</sub> = OCH<sub>3</sub>; **7:** R<sub>5</sub> = OCH<sub>3</sub>, R<sub>6</sub> = OH  
**8:** R<sub>5</sub> = OH, R<sub>6</sub> = OCH<sub>3</sub>; **9:** R<sub>1</sub> = R<sub>3</sub> = R<sub>4</sub> = R<sub>5</sub> = H, R<sub>2</sub> = OH, R<sub>6</sub> = OCH<sub>3</sub>  
**10:** R<sub>1</sub> = R<sub>3</sub> = R<sub>4</sub> = H, R<sub>2</sub> = OH, R<sub>5</sub> = R<sub>6</sub> = OCH<sub>3</sub>; **11:** R<sub>1</sub> = R<sub>3</sub> = R<sub>4</sub> = H, R<sub>2</sub> = R<sub>6</sub> = OH, R<sub>5</sub> = OCH<sub>3</sub>  
**12:** R<sub>1</sub> = R<sub>2</sub> = OH, R<sub>3</sub> = R<sub>4</sub> = R<sub>5</sub> = H, R<sub>6</sub> = OCH<sub>3</sub>; **13:** R<sub>1</sub> = R<sub>2</sub> = OH, R<sub>3</sub> = R<sub>4</sub> = H, R<sub>5</sub> = R<sub>6</sub> = OCH<sub>3</sub>  
**14:** R<sub>1</sub> = R<sub>2</sub> = R<sub>6</sub> = OH, R<sub>3</sub> = R<sub>4</sub> = H, R<sub>5</sub> = OCH<sub>3</sub>; **15:** R<sub>1</sub> = R<sub>2</sub> = R<sub>5</sub> = OH, R<sub>3</sub> = R<sub>4</sub> = H, R<sub>6</sub> = OCH<sub>3</sub>  
**16:** R<sub>1</sub> = R<sub>3</sub> = R<sub>5</sub> = H, R<sub>2</sub> = R<sub>4</sub> = OH, R<sub>6</sub> = OCH<sub>3</sub>; **17:** R<sub>1</sub> = R<sub>3</sub> = H, R<sub>2</sub> = R<sub>4</sub> = OH, R<sub>5</sub> = R<sub>6</sub> = OCH<sub>3</sub>  
**18:** R<sub>1</sub> = R<sub>3</sub> = H, R<sub>2</sub> = R<sub>4</sub> = R<sub>6</sub> = OH, R<sub>5</sub> = OCH<sub>3</sub>; **19:** R<sub>1</sub> = R<sub>2</sub> = R<sub>4</sub> = R<sub>5</sub> = H, R<sub>3</sub> = OH, R<sub>6</sub> = OCH<sub>3</sub>  
**20:** R<sub>1</sub> = R<sub>2</sub> = R<sub>4</sub> = H, R<sub>3</sub> = OH, R<sub>5</sub> = R<sub>6</sub> = OCH<sub>3</sub>

*i.* Nitrobenzene and montmorillonite K-10, 100–120°C, 2–10 h

Scheme 1

In spite of the synthesis of some 4-aryl-3,4-dihydrocoumarins successfully catalyzed by montmorillonite K-10, these reactions usually needed long reaction times, tedious isolation and purification processes, and used phenols and cinnamoyl chloride as reactants. To the best of our knowledge, the conversion of cinnamic acid to cinnamoyl chloride required absolutely anhydrous conditions that added difficulty to the operation in the experiment. Moreover, crude products are usually purified by chromatography on silica gel, leading to waste of eluent and pollution of the environment. In our experiment, we used phenols and substituted cinnamic acids as initial reactants and crystallization to purify the crude products. What is more, the montmorillonite K-10 that was used as catalyst for synthesizing certain 4-aryl-3,4-dihydrocoumarin can be applied to synthesize the same compound without extra treatment.

Twelve 4-aryl-3,4-dihydrocoumarins **9–20** were synthesized by the condensation of phenols **1–4** with the corresponding substituted cinnamic acids **5–8** in the presence of nitrobenzene and sulfated montmorillonite K-10, resulting in 52.2–83.3% yields (Scheme 1). Before its use as a high-efficient catalyst, commercially available montmorillonite K-10 was treated in 30% H<sub>2</sub>SO<sub>4</sub> solution for 4 h under 95°C in a three-neck round-bottom flask. This treatment method [16] was used with slight modification.

The structures of all the synthesized compounds were supported by FT-IR, MS, <sup>1</sup>H NMR, and <sup>13</sup>C NMR spectral data.

We have also examined the reuse of the catalyst on the tandem esterification alkylation of substituted cinnamic acids and resorcinol to get dihydrocoumarin **9**. The results shown in Table 1 indicate that another advantage of montmorillonite K-10 lies in the fact that it can be reused without extra treatment, together with the cheapness, nontoxicity, and commercial availability of this clay, rendering the synthetic process more environmentally friendly and economic.

TABLE 2. Quenching Effect of 4-Aryl-3,4-dihydrocoumarins on Hydroxyl Radical

Compound	IC <sub>50</sub> , mg/mL	Compound	IC <sub>50</sub> , mg/mL
<b>12</b>	1.00 ± 0.04 <sup>a</sup>	<b>15</b>	0.98 ± 0.03
<b>13</b>	1.06 ± 0.03	Vitamin C	0.84 ± 0.03
<b>14</b>	0.91 ± 0.03		

<sup>a</sup>Values represent the mean values from three identically treated cultures ± SD.

For compounds **9–11** and **16–20** IC<sub>50</sub> (mg/mL) > 2.00.

All the synthesized compounds were evaluated for their antioxidant activities by way of scavenging hydroxyl radicals. The hydroxyl radical scavenging activity of the twelve 4-aryl-3,4-dihydrocoumarins, as indicated by the half maximal inhibitory concentration (IC<sub>50</sub>) values, are listed in Table 2. The results showed that 4-aryl-3,4-dihydrocoumarins with 7,8-dihydroxy groups had relatively strong hydroxyl radical scavenging activity, which was a little weaker than vitamin C, while the other compounds presented little or no ability to scavenge hydroxyl radical, even at the highest test concentration of 2.00 mg·mL<sup>-1</sup>. Notably, compound **14** showed the highest activity, with an IC<sub>50</sub> value of 0.91 ± 0.03 mg·mL<sup>-1</sup> (Table 2).

In conclusion, twelve 4-aryl-3,4-dihydrocoumarins were synthesized using sulfated montmorillonite K-10 as catalyst. This method possesses such advantages as being more environmental friendly, and more sustainable and economic, requiring simple isolation and purification processes, producing few byproducts, the use of earth-abundant catalysts, and producing relatively high yields. With respect to their antioxidant activity, compounds **12**, **13**, **14**, and **15** have strong hydroxyl radical scavenging activity.

## EXPERIMENTAL

**General.** Melting points were determined using a Thiele tube and were uncorrected. The FT-IR spectra were recorded using a Thermo-Nicolet Nexus 670 spectrometer with KBr pellets. The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded with a Bruker AM-600 spectrometer with TMS as the internal standard. Chemical shifts are reported in ppm with DMSO-d<sub>6</sub> as solvent. Spin multiplets are given as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were obtained with an Agilent Trap VL LC/MS spectrometer. The absorbance were recorded by a Hitachi U-3000 UV spectrophotometer.

**Treatment of Montmorillonite K-10.** The montmorillonite K-10 used in this study was supplied by Shanghai Aladdin Bio-Chem. To a 250 mL oven-dried three-neck round-bottom flask, 13 g montmorillonite K-10 was mixed with 130 mL 30% H<sub>2</sub>SO<sub>4</sub> solution. The suspension was heated to 95°C and kept for 4 h under magnetic stirring. Then the acid-treated clays were washed thoroughly with distilled water until the pH of the washings was 7. The samples were dried in a vacuum oven at 40°C to get 13.95 g sulfated montmorillonite K-10.

**General Procedure for the Synthesis of 4-Aryl-3,4-dihydrocoumarins 9–20.** A mixture of a substituted cinnamic acid (5 mmol) and a substituted phenol (5 mmol) in nitrobenzene (10 mL) was heated to 100°C. Sulfated montmorillonite K-10 (2 g) was added to the mixture after all reagents were dissolved, and the mixture was stirred at 100°C for 3–12 h. The reaction process was monitored by TLC (CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>OH, 10:1). The suspension was directly filtered, and a suitable amount of petroleum ether was added to the filtrate. Then the mixture was cooled and stored or stirred under low temperature for a certain length of time to promote crystallization of the product. The crude product was recrystallized from EtOAc–PE to afford **9–20**.

**(±)-7-Hydroxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (9).** White solid, mp 177.2–178.3°C, yield 79.2%. IR (KBr, v, cm<sup>-1</sup>): 3332 (OH), 3040, 2800 (OCH<sub>3</sub>), 1736 (C=O), 1614, 1583, 1507, 1450, 1236, 1146 (C–O), 1105, 1028, 984, 832 (Ar). PMR spectrum (600 MHz, DMSO-d<sub>6</sub>, δ, ppm, J/Hz): 3.03 (2H, m, H-3), 3.73 (3H, s, 4'-OCH<sub>3</sub>), 4.32 (1H, t, J = 6.0, H-4), 6.53 (1H, dd, J = 2.4, 8.4, H-6), 6.55 (1H, d, J = 2.4, H-8), 6.84 (1H, d, J = 8.5, H-5), 6.90 (2H, d, J = 8.8, H-3', 5'), 7.06 (2H, d, J = 8.6, H-2', 6'), 9.74 (1H, s, 7-OH). <sup>13</sup>C NMR (150 MHz, DMSO-d<sub>6</sub>, δ, ppm): 36.76, 37.80, 54.95, 103.17, 111.51, 114.03, 116.47, 128.23, 128.81, 133.53, 151.81, 157.43, 158.10, 167.73. MS *m/z*: 270.7 [M + 1]<sup>+</sup>, 162.6.

**(±)-7-Hydroxy-4-(3,4-dimethoxyphenyl)-3,4-dihydrocoumarin (10).** White solid, mp 144.8–146.1°C, yield 53.3%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3433 (OH), 3030, 2790 ( $\text{OCH}_3$ ), 1767 (C=O), 1628, 1597, 1514, 1447, 1267, 1142 (C–O), 1101, 1025, 848, 810 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.05 (2H, m, H-3), 3.70 and 3.71 (each 3H, s, 3', 4'- $\text{OCH}_3$ ), 4.29 (1H, t, J = 6.0, H-4), 6.52 (1H, s, H-8), 6.53–6.56 (2H, m, H-6', 5'), 6.83–6.89 (3H, m, H-6, 2', 5), 9.74 (1H, s, 7-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 37.27, 38.81, 55.93, 55.95, 103.72, 111.76, 112.09, 112.34, 119.54, 129.40, 149.37, 158.00, 168.37. MS  $m/z$ : 300.8  $[\text{M} + 1]^+$ , 258.7, 190.6, 162.7.

**(±)-7-Hydroxy-4-(3-methoxy-4-hydroxyphenyl)-3,4-dihydrocoumarin (11).** White solid, mp 194.3–196.3°C, yield 54.9%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3415 (OH), 2994, 2929 ( $\text{OCH}_3$ ), 1724 (C=O), 1625, 1602, 1518, 1454, 1259, 1152 (C–O), 1112, 1028, 841, 809 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.03 (2H, m, H-3), 3.71 (3H, s, 3'- $\text{OCH}_3$ ), 4.24 (1H, t, J = 6.0, H-4), 6.44 (1H, d, J = 7.8, H-6), 6.51 (1H, s, H-8), 6.54 (1H, d, J = 8.4, H-6'), 6.70 (1H, d, J = 8.1, H-5'), 6.78 (1H, s, H-2'), 6.84 (1H, d, J = 7.8, H-5), 8.93 and 9.72 (each 1H, s, 4', 7-OH).  $^{13}\text{C}$  NMR (151 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 37.35, 38.81, 56.05, 103.69, 112.05, 115.93, 117.23, 119.84, 129.42, 132.94, 146.01, 148.20, 152.37, 157.95, 168.45. MS  $m/z$ : 287.1  $[\text{M} + 1]^+$ , 162.8.

**(±)-7,8-Dihydroxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (12).** White solid, mp 168.9–171.1°C, yield 79.4%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3330 (OH), 3050, 2805 ( $\text{OCH}_3$ ), 1738 (C=O), 1634, 1612, 1512, 1458, 1236, 1154 (C–O), 1109, 1030, 1002, 830, 802 (Ar). PMR spectrum (400 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.01 (2H, m, H-3), 3.72 (3H, s, 4'- $\text{OCH}_3$ ), 4.28 (1H, t, J = 6.8, H-4), 6.28 (1H, d, J = 8.0, H-6), 6.53 (1H, d, J = 8.0, H-5), 6.88 and 7.06 (each 2H, d, J = 8.0, H-2', 6', 3', 5'), 8.91 and 9.29 (each 1H, s, 7, 8-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 37.35, 38.93, 55.53, 111.7, 114.9, 118.5, 119.1, 128.83, 133.74, 134.11, 140.99, 146.31, 158.66, 168.25. MS  $m/z$ : 286.8  $[\text{M} + 1]^+$ , 244.6, 178.6, 160.6.

**(±)-7,8-Dihydroxy-4-(3,4-dimethoxyphenyl)-3,4-dihydrocoumarin (13).** White solid, mp 173.7–174.6°C, yield 83.3%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3423 (OH), 2935, 2829 ( $\text{OCH}_3$ ), 1754 (C=O), 1610, 1515, 1470, 1413, 1240, 1137 (C–O), 1025, 807 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.04 (2H, m, H-3), 3.70 and 3.71 (each 3H, s, 3', 4'- $\text{OCH}_3$ ), 4.27 (1H, t, J = 6.3, H-4), 6.28, 6.53, 6.56, and 6.87 (each 1H, d, J = 8.2, H-6', 5', 6, 5), 6.84 (1H, s, H-2'), 8.90 and 9.28 (each 1H, s, 7, 8-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 37.25, 39.98, 55.96, 111.48, 111.87, 112.32, 117.61, 118.26, 119.62, 133.68, 134.56, 140.99, 146.28, 148.26, 149.32, 168.34. MS  $m/z$ : 316.8  $[\text{M} + 1]^+$ , 178.5.

**(±)-7,8-Dihydroxy-4-(3-methoxy-4-hydroxyphenyl)-3,4-dihydrocoumarin (14).** White solid, mp 171.7–173.1°C, yield 53.1%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3373 (OH), 3018, 2810 ( $\text{OCH}_3$ ), 1743 (C=O), 1612, 1517, 1464, 1274, 1067 (C–O), 1033, 812, 693 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.01 (2H, m, H-3), 3.71 (3H, s, 3'- $\text{OCH}_3$ ), 4.22 (1H, t, J = 6.2, H-4), 6.29 (1H, d, J = 8.3, H-6), 6.45 (1H, dd, J = 8.1, 1.5, H-6'), 6.52 (1H, d, J = 8.1, H-5), 6.69 (1H, d, J = 8.1, H-5'), 6.78 (1H, d, J = 1.5, H-2'), 8.88, 8.90, and 9.26 (each 1H, s, 7, 8, 4'-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 37.34, 39.98, 56.08, 111.45, 112.19, 115.88, 117.63, 118.45, 119.90, 132.95, 133.65, 140.96, 145.97, 146.21, 148.14, 168.41. MS  $m/z$ : 303.1  $[\text{M} + 1]^+$ , 283.7, 260.7, 178.7, 150.6.

**(±)-7,8-Dihydroxy-4-(3-hydroxy-4-methoxyphenyl)-3,4-dihydrocoumarin (15).** White solid, mp 185.0–187.1°C, yield 66.4%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3499, 3420 (OH), 3010, 2810 ( $\text{OCH}_3$ ), 1752 (C=O), 1610, 1591, 1518, 1469, 1277, 1068 (C–O), 1030, 786, 698 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 2.97 (2H, m, H-3), 3.72 (3H, s, 4'- $\text{OCH}_3$ ), 4.19 (1H, t, J = 5.5, H-4), 6.32 (1H, d, J = 8.2, H-6), 6.53 (3H, m, H-5, 6', 2'), 6.84 (1H, d, J = 8.2, H-5'), 8.90, 8.95, and 9.29 (each 1H, s, 7, 8, 3'-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 37.44, 39.11, 56.10, 111.47, 112.85, 114.92, 117.73, 118.25, 133.68, 134.92, 140.96, 146.23, 147.03, 168.26. MS  $m/z$ : 302.9  $[\text{M} + 1]^+$ , 283.7, 260.7, 178.6, 150.6.

**(±)-5,7-Dihydroxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (16).** White solid, mp 148.4–149.9°C, yield 71.6%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3510 (OH), 3050, 2805 ( $\text{OCH}_3$ ), 1759 (C=O), 1635, 1611, 1513, 1457, 1231, 1179 (C–O), 1134, 1061, 1032, 826 (Ar). PMR spectrum (400 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 2.97 (2H, m, H-3), 3.69 (3H, s, 4'- $\text{OCH}_3$ ), 4.38 (1H, d, J = 6.5, H-4), 6.02 (1H, d, J = 2.1, H-6), 6.17 (1H, d, J = 2.2, H-8), 6.83 and 6.98 (each 2H, d, J = 8.6, H-3', 5', and 2', 6'), 9.55 and 9.73 (each 1H, s, 5, 7-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 33.40, 37.75, 61.07, 94.51, 95.15, 99.18, 103.88, 114.44, 128.13, 134.74, 153.38, 155.79, 158.28, 168.41. MS  $m/z$ : 286.9  $[\text{M} + 1]^+$ , 178.7, 110.8.

**(±)-5,7-Dihydroxy-4-(3,4-dimethoxyphenyl)-3,4-dihydrocoumarin (17).** White solid, mp 185.8–187.7°C, yield 61.8%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3402 (OH), 1778 (C=O), 1632, 1610, 1500, 1460, 1237, 1148 (C–O), 1012, 825 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.02 (2H, m, H-3), 3.68 and 3.70 (each 3H, s, 3', 4'- $\text{OCH}_3$ ), 4.37 (1H, d, J = 6.7, H-4), 6.01 (1H, s, H-6), 6.17 (1H, s, H-8), 6.40 (1H, dd, J = 8.3, H-6'), 6.81 (1H, d, J = 8.3, H-5'), 6.81 (1H, d, J = 1.5, H-2'), 9.56 and 9.74 (each 1H, s, 5, 7-OH).  $^{13}\text{C}$  NMR (151 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 33.76, 37.74, 55.87, 55.95, 95.11, 99.17, 103.74, 111.49, 112.22, 118.39, 135.29, 148.08, 153.45, 155.81, 158.27, 168.47. MS  $m/z$ : 316.9  $[\text{M} + 1]^+$ , 178.7, 164.7, 110.8.

**(±)-5,7-Dihydroxy-4-(3-methoxy-4-hydroxyphenyl)-3,4-dihydrocoumarin (18).** White solid, mp 173.0–174.0°C, yield 53.1%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3404 (OH), 3030, 2790 ( $\text{OCH}_3$ ), 1767 (C=O), 1628, 1597, 1514, 1447, 1267, 1142 (C–O), 1101, 1025, 848, 810 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.01 (2H, m, H-3), 3.70 (3H, s, 3'- $\text{OCH}_3$ ), 4.33 (1H, d,  $J = 6.7$ , H-4), 6.00 (1H, s, H-6), 6.16 (1H, s, H-8), 6.30 (1H, d,  $J = 8.1$ , H-6'), 6.62 (1H, d,  $J = 8.1$ , H-5'), 6.75 (1H, s, H-2'), 8.91, 9.54, and 9.72 (each 1H, s, 5, 7, 4'-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 33.73, 37.86, 56.00, 95.09, 99.16, 103.95, 111.78, 115.72, 118.78, 133.68, 145.78, 148.05, 153.42, 155.78, 158.19, 168.55. MS  $m/z$ : 303.1  $[\text{M} + 1]^+$ , 261.1, 179.1, 151.1, 111.2.

**(±)-6-Hydroxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (19).** White solid, mp 167.7–169.7°C, yield 52.2%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3332 (OH), 3000, 2829 ( $\text{OCH}_3$ ), 1728 (C=O), 1613, 1600, 1502, 1450, 1236, 1146 (C–O), 1105, 1028, 831, 807 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.01 (2H, m, H-3), 3.73 (3H, s, 4'- $\text{OCH}_3$ ), 4.34 (1H, t,  $J = 6.3$ , H-4), 6.37 (1H, s, H-5), 6.68 (1H, d,  $J = 8.6$ , H-7), 6.92 (2H, d,  $J = 8.0$ , H-3', 5'), 6.96 (1H, d,  $J = 8.6$ , H-8), 7.10 (2H, d,  $J = 8.0$ , H-2', 6'), 9.34 (1H, s, 6-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 36.8, 39.99, 55.55, 114.68, 115.23, 117.76, 128.05, 129.05, 133.34, 144.43, 154.37, 158.81, 168.53. MS  $m/z$ : 207.7  $[\text{M} + 1]^+$ , 162.6.

**(±)-6-Hydroxy-4-(3,4-dimethoxyphenyl)-3,4-dihydrocoumarin (20).** White solid, mp 166.7–168.5°C, yield 53.3%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3259 (OH), 3071, 2790 ( $\text{OCH}_3$ ), 1709 (C=O), 1593, 1522, 1487, 1451, 1267, 1142 (C–O), 1106, 1022, 856, 817 (Ar). PMR spectrum (600 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, J/Hz): 3.04 (2H, m, H-3), 3.72 and 3.73 (each 3H, s, 3', 4'- $\text{OCH}_3$ ), 4.33 (1H, t,  $J = 6.3$ , H-4), 6.37 (1H, d,  $J = 2.6$ , H-5), 6.62 (1H, dd,  $J = 1.6, 8.2$ , H-6'), 6.68 (1H, dd,  $J = 2.7, 8.7$ , H-7), 6.88 (1H, d,  $J = 1.5$ , H-2'), 6.91 (1H, d,  $J = 8.3$ , H-5'), 6.96 (1H, d,  $J = 8.7$ , H-8), 9.32 (1H, s, 6-OH).  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 36.70, 39.99, 55.90, 111.99, 112.43, 114.67, 115.22, 117.70, 119.88, 128.06, 133.72, 144.42, 148.42, 149.41, 154.35, 168.59. MS  $m/z$ : 301.0  $[\text{M} + 1]^+$ , 259.0, 162.9.

**Hydroxyl Radical Scavenging Activity Assay.** The ability of **9–20** and vitamin C to scavenge the hydroxyl radical was evaluated with method as described by Cai et al. [17] with slight modifications. In a test tube 1 mL  $\text{H}_2\text{O}_2$  solution (0.03%), 1 mL  $\text{FeSO}_4$  solution (2  $\text{mmol}\cdot\text{L}^{-1}$ ), 1 mL salicylic acid solution (10  $\text{mmol}\cdot\text{L}^{-1}$ ), and 1 mL of 4-aryl-3,4-dihydrocoumarins and vitamin C with different concentrations (0.4–2.0 mg/mL) were mixed. Then 1 mL  $\text{H}_2\text{O}_2$  solution (0.03%) was added to this mixture to start the reaction. The solutions were kept at 37°C. After 30 min, absorbance was measured at 510 nm against a reagent blank which use distilled water to replace the  $\text{H}_2\text{O}_2$  solution. The percentage of OH scavenging by **9–20** and vitamin C was calculated using the following equation:

$$\text{OH scavenging effect (\%)} = [A_0 - (A_1 - A_2)]/A_0 \times 100\%$$

where  $A_0$  is the absorbance of 1.0 mL distilled water + 1.0 mL  $\text{H}_2\text{O}_2$  + 1 mL  $\text{FeSO}_4$  + 1 mL salicylic acid + 1.0 mL  $\text{H}_2\text{O}_2$ ;  $A_1$  is the absorbance of 1.0 mL 4-aryl-3,4-dihydrocoumarins or standard + 1.0 mL  $\text{H}_2\text{O}_2$  + 1.0 mL  $\text{FeSO}_4$  + 1.0 mL salicylic acid + 1.0 mL  $\text{H}_2\text{O}_2$ ;  $A_2$  is the absorbance of 1.0 mL 4-aryl-3,4-dihydrocoumarins or standard + 1.0 mL  $\text{H}_2\text{O}_2$  + 1.0 mL  $\text{FeSO}_4$  + 1.0 mL absolute ethyl alcohol + 1.0 mL  $\text{H}_2\text{O}_2$ .

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## REFERENCES

1. M. M. Garazd, Y. L. Garazd, and V. P. Khilya, *Chem. Nat. Compd.*, **39**, 54 (2003).
2. A. Kumar, B. K. Singh, R. Tyagi, S. K. Jain, S. K. Sharma, A. K. Prasad, H. G. Raj, R. C. Rastogi, A. C. Wattersonc, and V. S. Parmar, *Bioorg. Med. Chem.*, **13**, 4300 (2005).
3. J. M. Lee, T. H. Tseng, and Y. J. Lee, *Synthesis*, **15**, 2247 (2001).
4. K. Y. Zhang, W. X. Ding, J. Sun, B. Zhang, F. J. Lu, R. Lai, Y. Zou, and G. Yedid, *Biochimie*, **107**, 203 (2014).
5. F. Roelens, K. Huvaere, W. Dhooge, M. V. Cleemput, F. Comhaire, and D. D. Keukeleire, *Eur. J. Med. Chem.*, **40**, 1042 (2005).

6. X. F. Zhang, H. M. Wang, Y. L. Song, L. H. Nie, L. F. Wang, B. Liu, P. P. Shen, and Y. Liu, *Bioorg. Med. Chem. Lett.*, **16**, 949 (2006).
7. J. Posakony, M. Hirao, S. Stevens, J. A. Simon, and A. Bedalov, *J. Med. Chem.*, **47**, 2635 (2004).
8. M. M. Garazd, Y. L. Garazd, and V. P. Khilya, *Chem. Nat. Compd.*, **41**, 245 (2005).
9. A. R. Jagdale and A. Sudalai, *Tetrahedron Lett.*, **48**, 4895 (2007).
10. P. L. Majumder, *J. Ind. Chem. Soc.*, **78**, 743 (2001).
11. A. K. D. Gupta, K. R. Das, and A. D. Gupta, *Ind. J. Chem.*, **10**, 32 (1972).
12. E. Rizzi, S. Dallavalle, and L. Merlini, *Synth. Commun.*, **36**, 1117 (2006).
13. J. Sun, W. X. Ding, X. P. Hong, K. Y. Zhang, and Y. Zou, *Chem. Nat. Compd.*, **48**, 16 (2012).
14. Z. Zhang, Y. Ma, and Y. F. Zhao, *Synlett*, **7**, 1091 (2008).
15. D. P. Kamat, S. G. Tilve, and V. P. Kamat, *Tetrahedron Lett.*, **53**, 4469 (2012).
16. C. N. Rhodes and D. R. Brown, *Catal. Lett.*, **24**, 285 (1994).
17. B. Q. Cai, Z. Y. Cai, F. D. Zhang, and M. J. Hang, *Acta Agric. Univ. Jingxiensis*, **32**, 813 (2010).