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One-pot synthesis of potential antioxidant agents, 3-carboxylate coumarin derivatives

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A simple and efficient route to synthesize 3-carboxylate coumarin derivatives through three-component one-pot reaction in a single step has been recommended. This method provides a direct and rapid access to get 3-carboxylate coumarin derivatives. The structures of these synthetic products were identified and the antioxidant activities were tested by inhibiting DPPH· and ·OH radicals capacities. The data suggested that 2H-1-penzopyran-3-carboxylicacid, 7,8-dihydroxy-2-oxo-, ethyl ester showed excellent activity in reducing both DPPH· and ·OH radicals in concentration-dependent manners with IC_{50} value of 83.1 µg/ml in DPPH· radicals and less than 0.12 mg/ml in the ·OH scavenging activity.

Key words: 3-Carboxylate coumarin, synthesis, antioxidant activity.

INTRODUCTION

Coumarins (2*H*-1-benzopyran-2-ones) are one family of naturally occurring compounds that are widely distributed in plants (Vazquez-Rodriguez et al., 2013). Coumarins could also be synthesized through chemical processes. In fact, more than 1,300 coumarins have been identified from natural sources, especially green plants (Hoult et al., 1996). Clinical and experimental studies have found that coumarin derivatives are known to possess a wide range of biological activities (Gabriele et al., 2008). These compounds possess anticancer (Gabriele et al., 2008; Paul et al., 2013), antioxidant, trypanocidal, anti-inflammatory (Vazquez-Rodriguez et al., 2013; Melagraki et al., 2009; Ćavar et al., 2012), antibacterial and cytotoxic properties (Canning et al., 2013). Coumarins are therefore used in treating metastatic malignant melanoma, renal cell carcinoma (Marshall et al., 1991; Thati et al., 2007; Thornes et al., 1994), and many other diseases. Moreover, coumarins have been extensively used in diverse sectors, such as pharmaceuticals, fragrances, agrochemicals, additives in food, cosmetics and insecticides (Kostova and Momekov, 2006).

Coumarin-3-carboxylate is one of the important coumarin derivatives, and an important intermediate that could be used in the synthesis of coumarins (Song et al., 2003). Coumarin-3-acyl derivatives were tested *in vitro* for their distinct human monoamine oxidase A and B (hMAO-A and hMAO-B) inhibitory activity, especially the 3-ethyl ester coumarin ring. They are considered as highly potent and selective hMAO-B inhibitors with IC_{50} values in the nanomolar range (Secci et al., 2011). 7,8-dihydroxy-coumarin and TGF-β1 have a synergistic effect on strongly induced, rat adipose-derived mesenchymal

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stem cells (ADMSCs) that are differentiated from the cartilage (Liu et al., 2013).

A one-pot reaction, one simple and rapid method, consists of a combination of three components that are used to generate new products in a single step. This resource-effective method is also used in the emerged multicomponent reactions (MCRs) (Karami et al., 2012) without isolating the intermediate. The economical use of material in this reaction along with post-processing is associated with a less tedious process of recycling and regenerating the catalyst. Compared with multistep reactions, it would be a convenient and greener way to get series coumarins.

In this paper, we gave the simple and rapid way, one-pot, to get hydroxy 3-carboxylate coumarin derivatives, and elaborated the process in detail (Scheme 1). The procedures and results were introduced and analyzed comprehensively. We found out that the compounds 2*H*-1-benzopyran-3-carboxylic acid, 7,8-dihydroxy-2-oxo-, ethyl ester showed definite activity in reducing both DPPH· and ·OH radicals. In addition, 2*H*-1- benzopyran-3-carboxylic acid, 7,8-dihydroxy-2-oxo-, ethyl ester, along with 2*H*-1-benzopyran-3-carboxylic acid, 8-hydroxy-2-oxo-, ethyl ester and 2*H*-1-benzopyran-3-carboxylic acid, 7-hydroxy-2-oxo-, ethyl ester showed more activity than ascorbic acid when there was low concentration in the ·OH scavenging. These experimental results showed that three of the synthesized 3-carboxylate coumarin derivatives were potential antioxidant agents.

EXPERIMENTAL

General

With the exception of piperidine (technically pure), all reagents used in this study were analytically pure. The products were synthesized using DF-101D solar collector heating thermostat magnetic stirrer. The melting points were measured using melting point apparatus. The synthesized compounds were purified by recrystallization and analyzed by thin-layer chromatography (TLC), melting point determination (X-6), and HPLC (5 μm, 250 × 4.60 mm Gemini, C_{18} preparation column, Phenomenex). Infrared spectra was recorded in KBr and determined on a Perkin Elmer fourier transform infrared (FT-IR) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz Nuclear Magnetic Resonance Spectrometer.

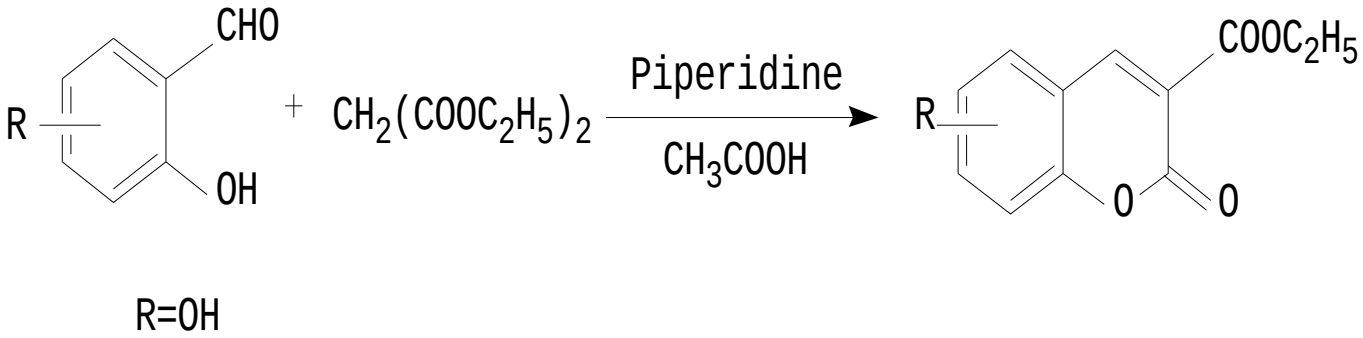
Synthesis procedure

2 *H-1-benzopyran-3-carboxylic acids*, 7-hydroxy-2-oxo-, ethyl ester. 2,4-Dihydroxybenzaldehyde (5.522 g, 0.040 mol), diethyl malonate (6.8 ml, 0.045 mol) with ethyl alcohol (25 ml), piperidine (0.5 ml), were added to a round-bottom flask(250 ml) that was equipped with a magnetic stirrer and spherical condenser. A small amount of glacial acetic acid was added to this flask to dissolve these chemicals. This mixture was heated to about 85°C for 3 h. The completion of this reaction was monitored by thin layer chromatography (TLC) using EtOAc: petroleum (3:1) as eluent. After completion of this reaction, the mixture was transferred to a beaker containing 35 ml of water. Then, the beaker was cooled and leached. The filter cake was washed thrice using 50% ethyl alcohol. Thereafter, the filter cake was washed using 95% ethyl alcohol. Finally, it was dried to obtain high purity product (faint yellow crystal, 60.5% yield), m.p. 171.4 to 173.5°C (otherwise the references 172 to 173°C (Valizadeh and Vaghefi, 2009). ^{1}H NMR (acetone-d_6, 500 MHz), *δ:* 8.58(s, 1H), 7.71 (d, *J*=7.0 Hz, 1H), 6.90(dd, *J*=2.0 Hz, *J*=5.5 Hz, 1H), 6.76(s, 1H), 4.29(d, *J*=7.0 Hz, 2H), 1.32(t, *J*=7.0 Hz, 3H). ^{13}C NMR (Acetone-d_6, 125 MHz), *δ*: 163.6, 163.1, 157.6, 156.1, 148.7, 131.8, 113.8, 113.6, 111.1, 102.1, 60.8, 13.6. IR(KBr), $\tilde{\nu}$ /cm^{-1}: 3550, 3471, 3056, 1739, 1680, 1606, 1466, 1447, 1245, 1145, 1087.

2H-1-Benzopyran-3-carboxylicacid, 8-hydroxy-2-oxo-, ethyl ester: 2,3-dihydroxybenzaldehyde (4.143 g, 0.030 mol), diethyl malonate (6.0 ml, 0.040 mol), ethyl alcohol (20 ml), and piperidine (0.5 ml) were added to a round-bottom flask (250 ml) that was equipped with a magnetic stirrer and spherical condenser. A small amount of glacial acetic acid was added to this flask to dissolve these chemicals. The mixture was heated to about 85°C for 4 h. Use the TLC monitor condition with EtOAc: petroleum (3:1) as an eluent to test the completion of this reaction. Then, the mixture was transferred to a beaker and 50 ml of water was added for cooling purposes. Finally, the cooled mixture was leached. The filter cake was washed twice using 50% ethyl alcohol. Then, it was dissolved using 25% ethyl alcohol and recrystallized. Finally, it was washed twice with 50% ethyl alcohol and dried to obtain high purity product (yellow crystal, 68.6% yield), mp 178.9 to 180.5°C (otherwise the references 174 to 175°C (Alvim et al., 2005)). ^{1}H NMR (acetone-d_6, 500 MHz), *δ:* 8.60(s, 1H), 7.26(m, 3H), 4.32(q, *J*=7.0 Hz, 2H), 1.33(t, *J*=7.0 Hz, 3H). ^{13}C NMR (Acetone-d_6, 125 MHz), *δ*: 162.8, 155.5, 148.4, 144.4, 143.5, 124.8, 120.5, 120.4, 118.8, 118.5, 61.1, 13.3. IR(KBr), $\tilde{\nu}$ /cm^{-1}: 3306, 3047, 1748, 1697, 1612, 1584, 1473,1265, 1231, 1032.

2*H*-1-benzopyran-3-carboxylicacid, 7,8-dihydroxy-2-oxo-, ethyl ester. 2,3,4-Trihydroxybenzaldehyde (3.084 g, 0.020 mol), diethyl malonate (4.0 ml, 0.026 mol), ethyl alcohol (20 ml), piperidine (0.5 ml) were added to a round-bottom flask (250 ml) that was equipped with a magnetic stirrer and spherical condenser. A small amount of glacial acetic acid was used as a solvent to dissolve these chemicals in the flask. The mixture was heated to about 85°C for 4 h. The same procedure was replicated and analyzed with TLC. After completion of this reaction, the mixture was transferred to a beaker and 50 ml water was added for cooling purposes. Finally, the cooled reaction mixture was leached. The filter cake was washed twice using 50% ethyl alcohol and then this filtered cake was dissolved using 95% ethyl alcohol. Thereafter, 50 ml water was added and the mixture was leached. The mixture was washed again with 95% ethyl alcohol and 50 ml water was added. Then, the mixture was leached and washed twice with 50% ethyl alcohol. Finally, the leached mixture was dried to obtain the product (faint yellow crystal, 39.9% yield), mp 238.4 to 240.0°C (otherwise the references 233 to 234°C (Alvim et al., 2005)). ^{1}H NMR (Acetone-d_6, 500 MHz), *δ:* 8.56 (s, 1H), 7.27 (d, *J*=8.5 Hz, 1H), 6.93(d, *J*=9.0 Hz, 1H), 4.29(q, *J*=7.0 Hz, 2H), 1.32(t, *J*=7.0 Hz, 3H). ^{13}C NMR (Acetone-d_6, 125 MHz), *δ*: 163.0, 155.6, 151.7, 149.3, 144.6, 131.5, 121.5, 113.4, 113.1, 111.7, 60.7, 13.6. IR(KBr), $\tilde{\nu}$ /cm^{-1}: 3482, 3218, 3055, 1694, 1611, 1588, 1514, 1263, 1193, 1083.

2 H-1-Benzopyran-3-carboxylic acids, 6-hydroxy-2-oxo-, ethyl ester. 2,5-Dihydroxybenzaldehyde (2.762 g, 0.020 mol), diethyl malonate (4.0 ml, 0.026 mol), ethyl alcohol (20 ml), piperidine (0.5 ml) and a small amount of glacial acetic acid were added to a round-bottom flask (250 ml) equipped with a magnetic stirrer and spherical condenser. This mixture was heated to about 85°C for 4 h. The same conditions were monitored by TLC. After completion of the reaction, the mixture was transferred to a beaker and 40 ml water was added for cooling purposes. Thereafter, this reaction mixture was leached. The filter cake was washed twice using a small amount of 50% ethyl alcohol. Subsequently, the filter cake



Scheme 1. The synthesis route for 3-carboxylate coumarin derivatives.

was dissolved in 95% ethyl alcohol and recrystallized. Thereafter, 30 ml water was used for washing the filter cake. A small amount of 50% ethyl alcohol was used for washing the filter cake again. The crude mixture was heated to dissolve with 25% $_{ethyl}$ alcohol (40 ml) and recrystallized. Finally, it was dried to obtain this purity product (primrose yellow crystal, 70.2% yield), mp 188.8 to 190.4°C (otherwise the references 182 to 184°C (Kraus and Pezzanite, 1979)). ^{1}H NMR (Acetone-d_6, 500 MHz), *δ:* 8.83(s, 1H), 8.53(s, 1H), 7.23 (m, 3H), 4.31 (q, *J*=7.5 Hz, *J*=7.0 Hz 2H), 1.33(t, *J*=7.5 Hz, *J*=7.0 Hz, 3H). ^{13}C NMR (Acetone-d_6, 125 MHz), *δ*: 163.0, 156.0, 154.0, 148.9, 147.5, 122.3, 119.0, 118.6, 117.2, 114.6, 61.1, 13.6. IR (KBr), $\tilde{\nu}$ /cm^{-1}: 3333, 3090, 1748, 1605, 1574, 1502, 1245, 1191, 1044.

The antioxidant activity test

Assay for the DPPH· radical-scavenging activity: a series of sample solutions was prepared in dimethyl sulfoxide (0.5 ml). The concentrations of these solutions ranged between 10 to 200 μg ml^{-1}. 3.0 ml of DPPH· solution in 95% alcohol was added to each of these solutions. The reaction mixtures were protected from light and incubated at room temperature for 30 min. The absorption was read at 517 nm and the mean value was measured for three duplicated readings. The ascorbic acid was used as a positive control. The scavenging activity was determined from the following equation (Lin et al., 2008; Tyagi et al., 2005; Tantry et al., 2012).

DPPH· scavenging activity (%) = 100 × [1 – (A_1 - A_2) / A_0]

A_0: absorbance of 3 ml DPPH· solution containing 0.5 ml DMSO;
A_1: absorbance of 3 ml DPPH· solution containing 0.5 ml sample;
A_2: absorbance of 3 ml 95% alcohol added 0.5 ml sample.

Assay for the scavenging effect on hydroxyl radicals: The scavenging effect was evaluated using the hydroxyl radical system that was generated by the Fenton reaction (Heo et al., 2005) with a minor modification. Briefly, samples were dissolved in dimethyl sulfoxide at 0 (control), 0.04, 0.08, 0.16, 0.24, 0.40 and 0.80 mg/ml. The reaction mixture consisted of the following reagents: 2 ml of salicylic acid and absolute ethanol solution (9 mM), 2 ml of $FeSO_4$ (9 mM), 2 ml of H_2O_2 (9 mM), and 2 ml samples of varying concentrations. The absorbance of this mixture was measured at 510 nm after incubating it at 37°C for 30 min. The hydroxyl radical-scavenging rate was calculated with the following equation (Sun et al., 2010).

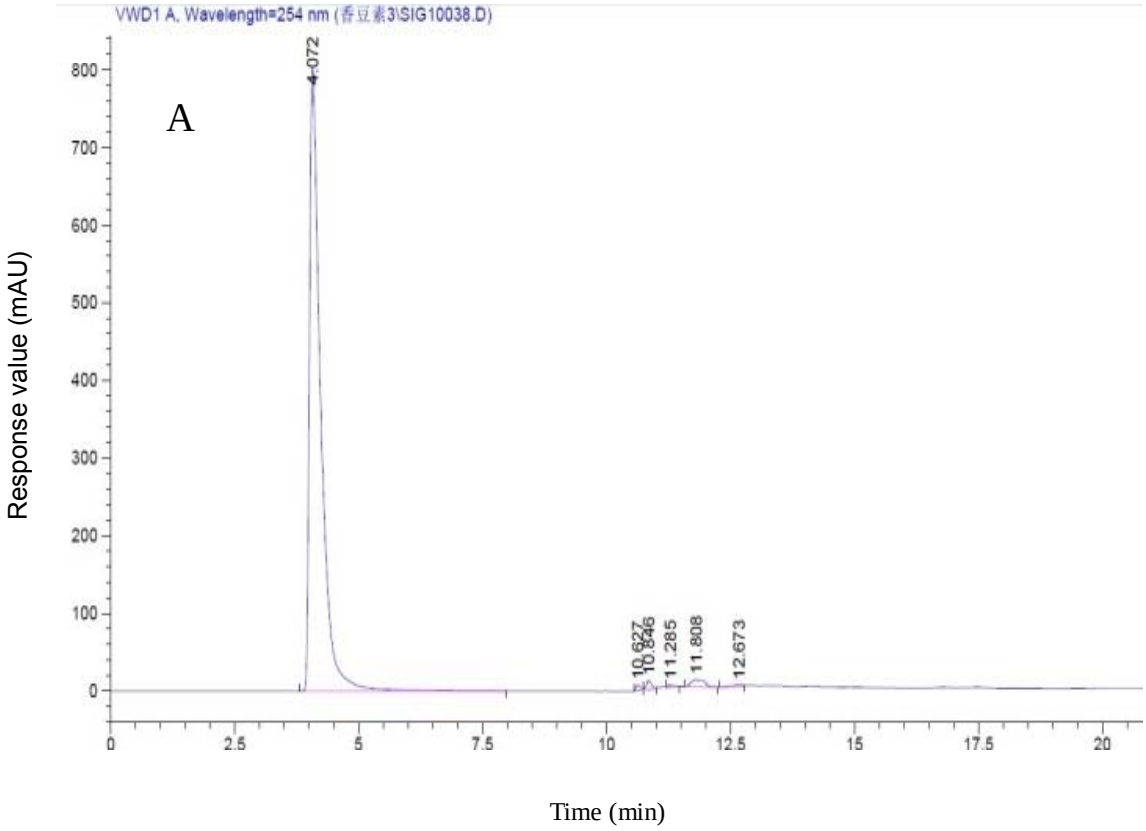
Hydroxyl radical-scavenging rate (%) = 100 × [1 - (A_s - A_w) / A_c]
A_s: absorbance of the mixture solution containing 2 ml sample;
A_w: absorbance of the mixture solution in which 2 ml water was replaced with 2 ml H_2O_2;
A_c: absorbance of the mixture solution in which 2 mL water was replaced with either 2 ml sample or vitamin C.

RESULTS AND DISCUSSION

Herein we gave detailed description of the synthesized compounds. In accordance with the references described in the procedure, the melting points of compounds differed by about 5 to 6°C. This could be attributed to the difference in the usage of the dissolved solvent and the measurement condition effects. The products were found to be highly purified as the melting point of every compound altered by only 1 to 2°C. The crystals of all compounds were yellow in appearance (Valizadeh and Azimi, 2011), except for the crystals of the compound 6-hydroxycoumarin-3-carboxylic acid ethyl ester. The crystals of this compound appeared yellowish green in color. The high performance liquid chromatography (HPLC) data is summarized and illustrated through Figure 1. The mean peak area of the samples was over 97%, and this indicated the high purity of products. While performing the experiments, the sample size was set in accordance with the concentration of every sample. In this case, a good, symmetrical, mean peak shape was obtained, which benefited from effective separation, high purity products, and medium sample size.

The reported data was identical with the standard spectra data (Lin et al., 2008; Horváth et al., 2005; Gong and Ding, 2006). Figure 2 displays the infrared (IR) spectra of 7,8-hydroxycoumarin-3-carboxylic acid ethyl ester. On the other hand, Table 1 illustrates those of other coumarins. They could also be recognized by ^{1}H NMR and ^{13}C NMR analysis (Table 2). Figure 3 displayed the partial nuclear magnetic resonance (NMR) spectra of compounds.

The abilities of inhibiting DPPH· and ·OH radicals of test compounds were assessed, and the findings were given as visualized in Figure 4. This examination of radical scavenging effect reflected the antioxidant activities of examined compounds to a certain extent. 2*H*-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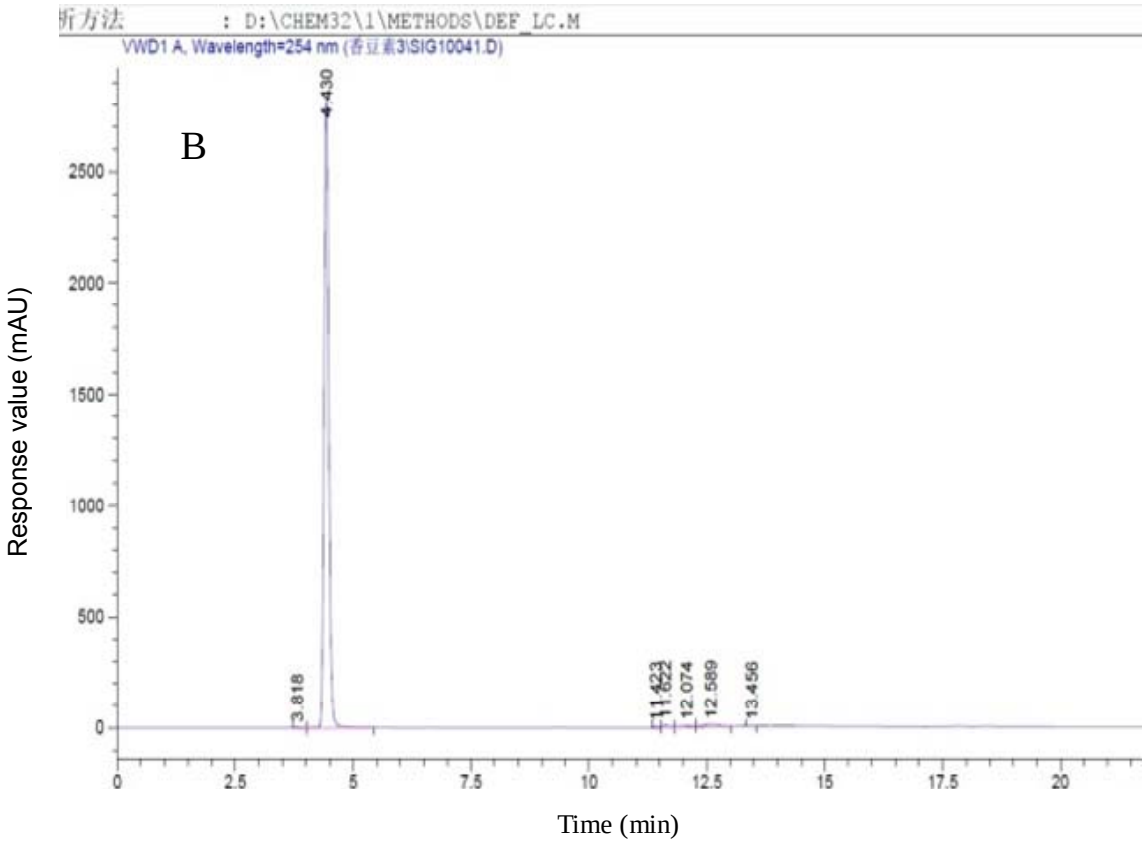
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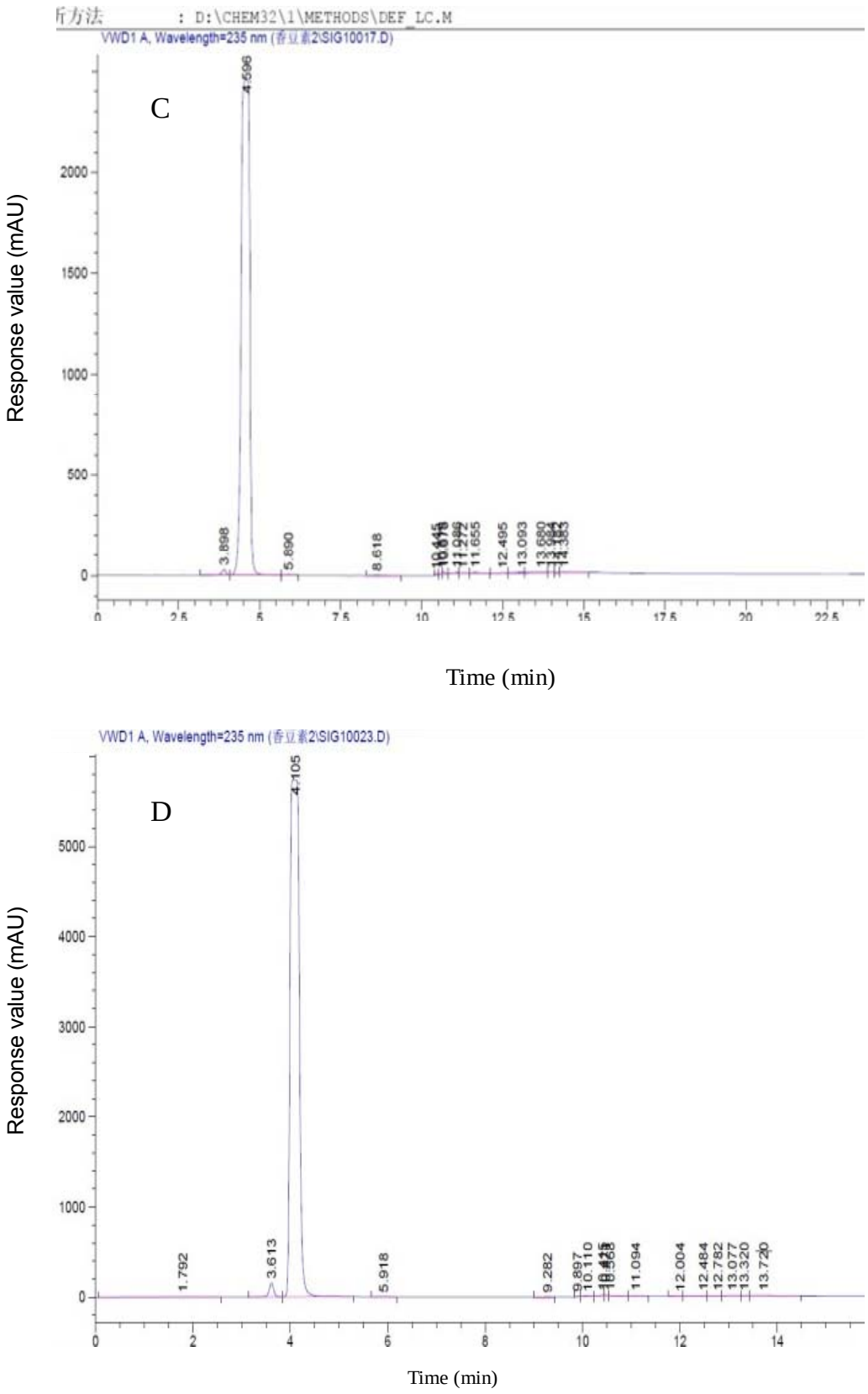


Figure 1. The HPLC spectra of 3-carboxylate coumarins. A, B, C and D (A) 7, 8-dihydroxycoumarin-3-carboxylicacid ethyl ester; (B) 8-hydroxycoumarin-3-carboxylicacid ethyl ester (C) 7-hydroxycoumarin-3-carboxylicacid ethyl ester (D) 6-hydroxycoumarin-3-carboxylicacid ethyl ester.

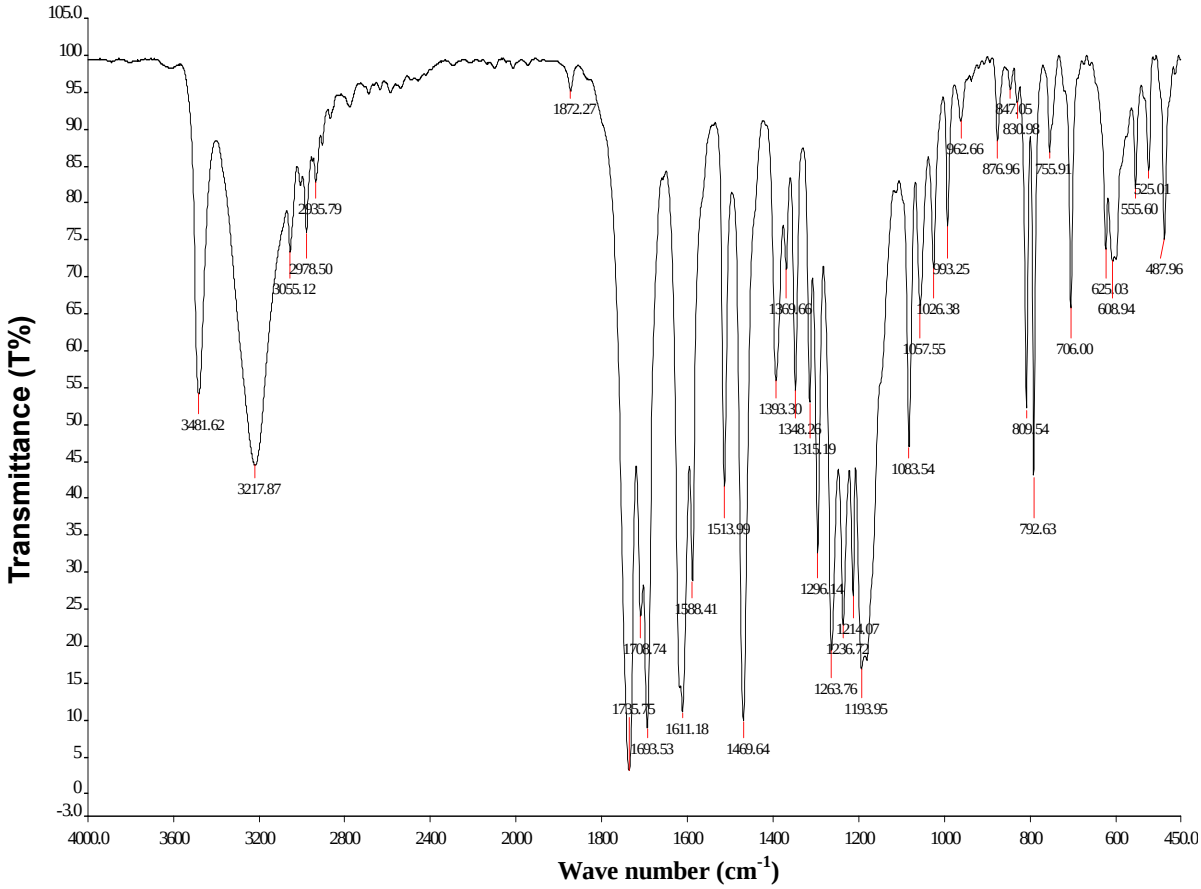


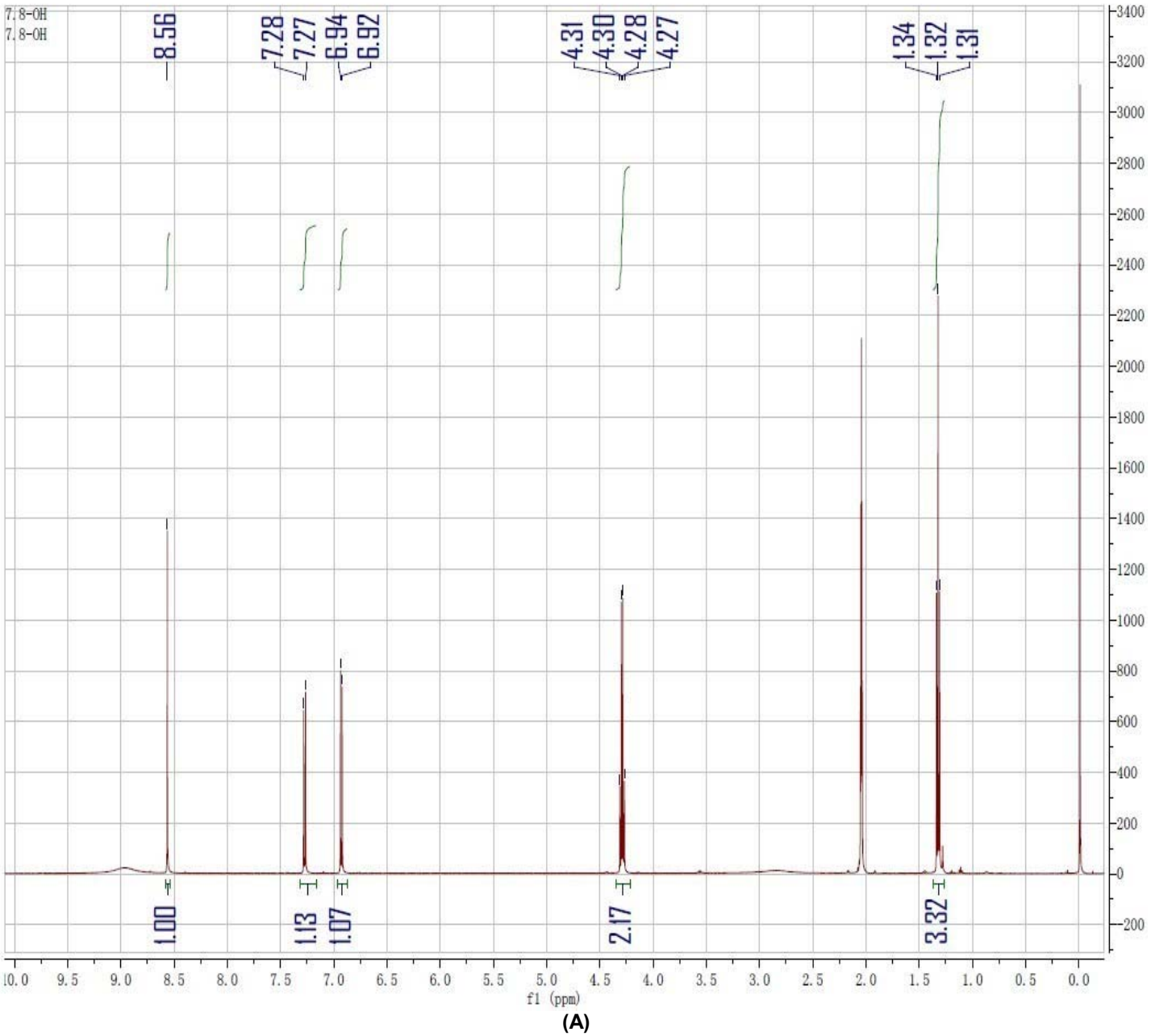
Figure 2. IR spectrum of 2H-1-Benzopyran-3-carboxylicacid, 7,8-dihydroxy-2-oxo-, ethyl ester

Table 1. IR spectra data of the objective products (KBr, cm^{-1}).

Product	ν_{O-H}	ν_{Ar-H}	$\nu_{C=O}$	$\nu_{C=C}$	$\nu_{Ar-C=C}$	ν_{C-O}
2H-1-Benzopyran-3-carboxylic acid, 7,8-dihydroxy-2-oxo-, ethyl ester	3482, 3218	3055	1694	1611	1611, 1588, 1514	1263, 1193, 1083
2H-1-Benzopyran-3-carboxylicacid, 8-hydroxy-2-oxo-, ethyl ester	3306	3047	1748, 1697	1612	1612, 1584, 1473	1265, 1231, 1032
2H-1-Benzopyran-3-carboxylicacid, 7-hydroxy-2-oxo-, ethyl ester	3550, 3471	3056	1739, 1680	1606	1606, 1466, 1447	1245, 1145, 1087
2H-1-Benzopyran-3-carboxylicacid, 6-hydroxy-2-oxo-, ethyl ester	3333	3090	1748	1605	1605, 1574, 1502	1245, 1191, 1044

Table 2. The objective products chemical shifts $\delta^{(ppm)}$.

Carbon spectra	2H-1-Benzopyran-3-carboxylicacid, 7,8-dihydroxy-2-oxo-, ethyl ester	2H-1-Benzopyran-3-carboxylicacid, 8-hydroxy-2-oxo-, ethyl ester	2H-1-Benzopyran-3-carboxylicacid, 7-hydroxy-2-oxo-, ethyl ester	2H-1-Benzopyran-3-carboxylicacid, 6-hydroxy-2-oxo-, ethyl ester
C=O	155.6	155.5	163.1	156.0
C-2	163.0	162.8	163.6	163.0
C-3	121.5	124.8	131.8	122.3
C-4	149.3	144.4	148.7	147.5
C-5	113.4	118.5	113.8	114.6
C-6	113.1	120.5	113.6	154.0
C-7	151.7	118.8	157.6	119.0
C-8	131.5	148.4	102.1	117.2
C-9	144.6	143.5	156.1	148.9
C-10	111.7	120.4	111.1	118.6
$-CH_3$	60.7	61.1	60.8	61.1
$-OCH_2-$	13.6	13.3	13.6	13.6

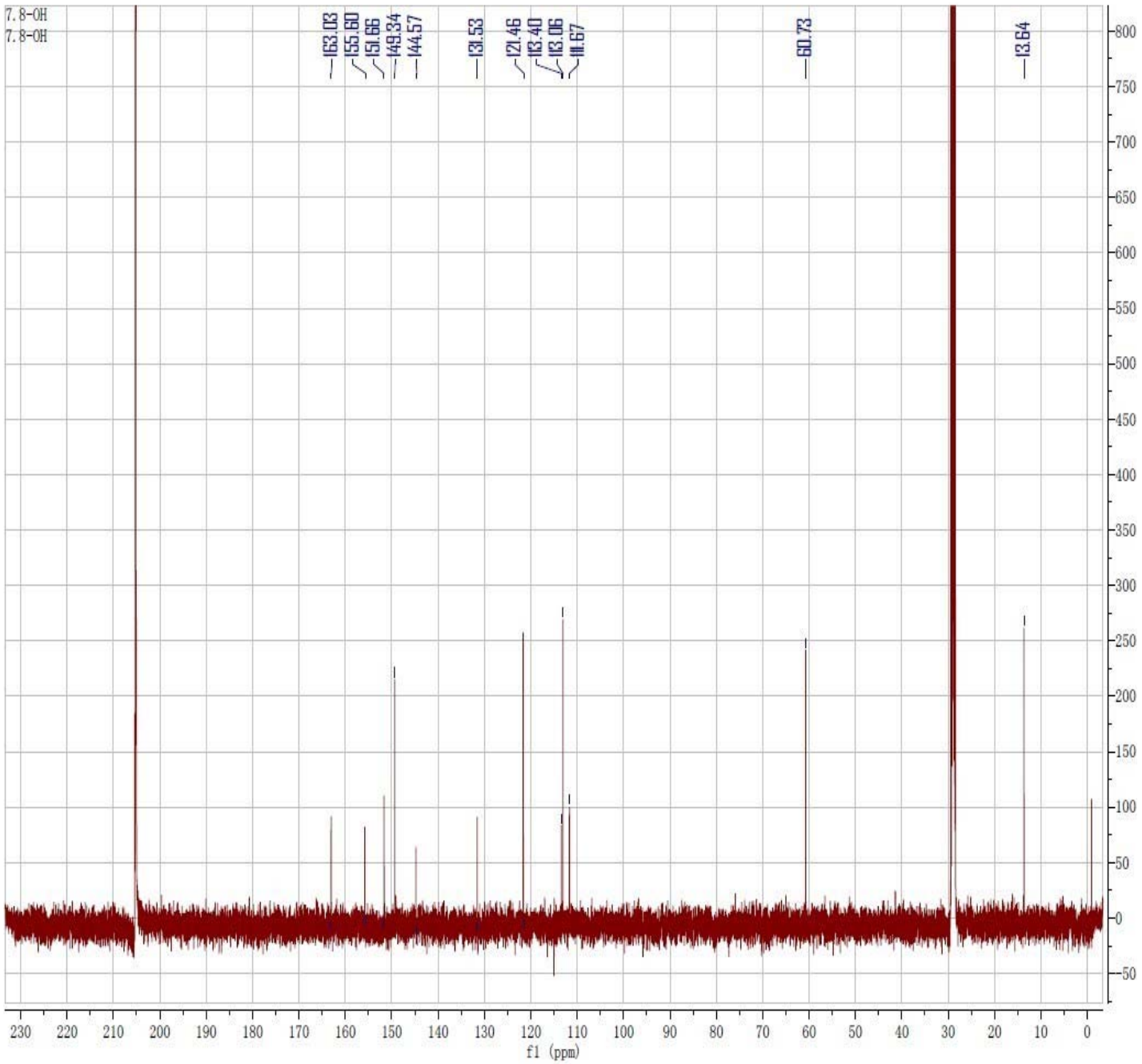


(A)

Figure 3A. 1H-NMR spectrum of 2H-1-Benzopyran-3- carboxylicacid, 7, 8-dihydroxy -2-oxo-, ethyl ester.

1-benzopyran-3-carboxylic acid, 7,8-dihydroxy-2-oxo-, ethyl ester was found to show excellent activity in reducing both DPPH• and •OH radicals in concentration-dependent manners. Figure 4a illustrates that 7,8-dihydroxyl coumarin inhibited DPPH· radicals with IC_{50} value of 83.1 µg ml^{-1}, compared to ascorbic acid with an IC_{50} value of 45.3 µg/ml. 7-hydroxyl coumarin displayed a less-potent effect with an IC_{50} value of 0.89 mg/ml. The other two selected coumarins had definite effect in scavenging DPPH· radicals. Figure 4b illustrates that similar activity was detected in the scavenging of ·OH radicals, whereas 7,8-dihydroxyl coumarin exhibited higher activity than ascorbic acid when the concentration was less than 0.12 mg/ml.

In addition, 7-hydroxyl coumarin and 8-hydroxyl coumarin were found to be more productive than ascorbic acid at a dose of below 0.04 mg/ml. In contrast, 6-hydroxyl coumarin showed little activity. Overall, under the experimental conditions, 7,8-dihydroxyl coumarin exhibited the strongest activity followed by 7-hydroxyl coumarin and 8-hydroxyl coumarin, with 6-hydroxyl coumarin displaying the weakest activity.



(B)

Figure 3B. ^{13}C-NMR spectrum of 2H-1-Benzopyran-3- carboxylicacid, 7, 8-dihydroxy -2-oxo-, ethyl ester.

Conclusion

In summary, we applied a simple way to get a series of ethyl coumarin-3-carboxylate containing hydroxyl group using the one-pot and multicomponent synthesis methods. These synthesized compounds have been elaborately elucidated, with the confirmation of their organic structures. This illustrates that workup reaction condition could be used in the synthesis of these products. Among the synthesized coumarins,7,8-dihydroxyl coumarin displayed excellent activities in reducing both DPPH· and ·OH radicals. 7,8-dihydroxyl coumarin was found to be more active than ascorbic acid under the concentration of 0.12 mg/ml. 7-hydroxyl coumarin and 8-hydroxyl coumarin were found to be more active than ascorbic acid within some limits

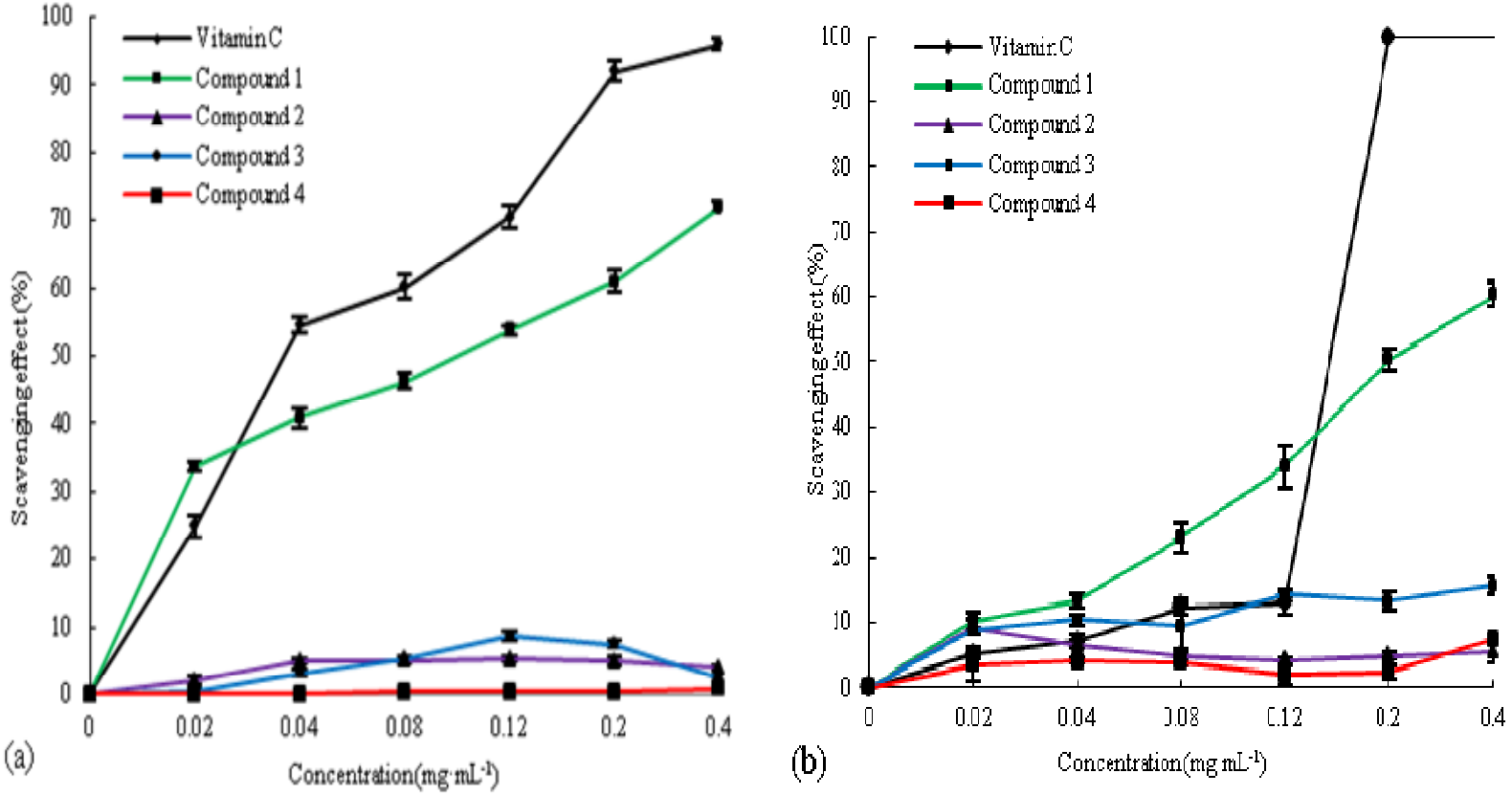


Figure 4. DPPH· scavenging effect (a) and ·OH scavenging effect (b) of the obtained coumarins. Compounds 1-4 are separated as follows: 2H-1-Benzopyran-3-carboxylicacid, 7,8-dihydroxy-2-oxo-, ethyl ester; 2H-1-Benzopyran-3-carboxylicacid, 8-hydroxy-2-oxo-, ethyl ester; 2H-1-Benzopyran-3-carboxylicacid, 7-hydroxy-2-oxo-, ethyl ester; 2H-1-Benzopyran-3-carboxylicacid, 6-hydroxy-2-oxo-, ethyl ester

concentration in the •OH scavenging. 6-hydroxylcoumarin exhibited little activity. The results of these experiments showed that three of the synthesized 3-carboxylate coumarin derivatives were potential antioxidant agents. New testing methods need to be devised to determine the other pharmacological activities of these compounds.

Conflict of Interest

Authors have declared no conflict of interest.

ACKNOWLEDGEMENTS

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