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Synthesis and antioxidant activities of novel 4-Schiff base-7-benzyloxy-coumarin derivatives

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ABSTRACT

4-Schiff base-7-benzyloxy-coumarins **5a$_1$**–**5h$_2$** and its derivative **6** were designed and synthesized based on the 7-benzyloxy-coumarin structure as novel antioxidants. The in vitro antioxidant activities screening revealed that 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of compounds **5b$_1$**, **5d$_1$**, **5f$_1$**, **5f$_2$**, **5g$_1$** and **5g$_2$**, and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) cation ($ABTS^+$) radical scavenging activities of compounds **5a$_1$**, **5b$_1$**, **5c$_1$**, **5c$_2$**, **5d$_1$**, **5e$_1$**, **5e$_2$**, **5f$_2$**, **5g$_1$**, **5g$_2$** and **5h$_1$** were better than that of the commercial antioxidant butylated hydroxytoluene (BHT), while the superoxide anion radical scavenging activities of **5a$_2$** and **5g$_2$** were stronger than that of the commercial antioxidant butylated hydroxyanisole (BHA), and the hydroxyl radical scavenging activity of **5e$_1$** was much better than that of the common antioxidant ascorbic acid.

High levels of free radicals and reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), extremely reactive hydroxyl, and several other free radicals produced by cells, would cause damage to lipids, proteins and DNA, and thus may lead to various diseases such as carcinogenesis, drug-associated toxicity, inflammation, atherogenesis and aging in aerobic organisms.[1–3] So the significance of free radicals and ROS in the pathogenesis of multifarious diseases has attracted considerable attention. Antioxidants are currently fabricated as the drug candidates to counter these diseases. Minor dietary compositions have been seriously considered to combat the ill effects of free radicals and ROS.

It is known that coumarins and their derivatives display different biological and pharmacological activities,[4] thus a great deal of effort have been devoted to design and synthesize the functional coumarin derivatives. Among the different existing active skeletons of coumarins, those compounds with a 7-benzyloxy-coumarin structure core (Fig. 1) have been studied and it have been found that they own good inhibitory activity and selectivity towards monoamine oxidase-B (MAO-B).[5] Previous studies have indicated that various diseases are characteristically associated with free radicals and ROS.[6] In addition, the potential therapeutic or preventive effects of antioxidative agents may be mentioned in the course of inhibition of MAO-B,[7–9] it is thus to expect that 7-benzyloxy-coumarin derivatives may contribute to good antioxidant activity. Therefore, 7-benzyloxy-coumarin structure is chosen in the present work as active structural core and some structural modifications are constructed to explore their antioxidant activities. Our previous studies have shown that good electronic fluidity may contribute to superior antioxidant activity,[10] so the multifunctional and conjugation-effective salen group is also designed to introduce at position 4 of 7-benzyloxy-coumarin structural core to enhance the donor–acceptor electronic effect and thus to increase the electronic fluidity. Our present work in this paper is to design and synthesize 4-Schiff base-7-benzyloxy-coumarin derivatives, and to evaluate their in vitro antioxidant activities.

4-Schiff base-7-benzyloxy-coumarins **5a$_1$**–**5h$_2$** and its derivative **6** were synthesized and the reaction route was given in Scheme 1. The synthesis of 4-methylcoumarin derivatives **2** were carried out according to Pechmann reaction,[11] which included the condensation of phenols with ethyl acetoacetate in the presence of the catalysis sodium bisulfate. Compounds **3** were then obtained in good yields by the coupling reactions of **2** with benzyl chloride in the presence of potassium carbonate. The oxidation reaction of compounds **3** were carried out to offer 4-formyl-coumarins **4** in dimethylbenzene, using selenium dioxide as oxidant. 4-Schiff base-7-benzyloxy-coumarins **5** were then obtained by the condensation of **4** with different primarily amines or hydrazinium in refluxing ethanol, respectively. It was important to note that 4-Schiff base-coumarin **5e$_1$** could be easily transformed to be benziminazole **6** in 93% yields when was treated with hot dimethyl

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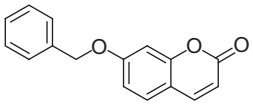


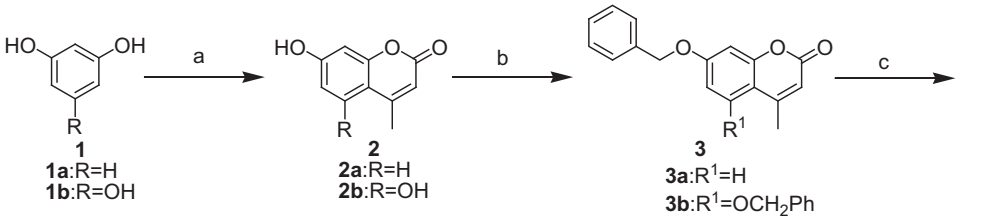
Figure 1. 7-Benzyloxy-coumarin.

formamide (DMF), and the treatment of **4a** with *o*-diaminobenzene in the same condition also offered **6** in good yield. The results suggested that the formation of imidazole ring should be easily achieved. So the similar work-up process was designed to deal with compounds **4b** and **5e$_2$**, respectively. However, it was probably due to the big steric hindrance of benzyloxy group at position 6 of coumarin skeleton, neither the treatment of **4b** with *o*-diaminobenzene in hot DMF, nor the heating of **5e$_2$**, offered the corresponding benziminazole. The experimental data of compounds **5**–**6** were cited in Ref. 12

In vitro antioxidant activities were measured against 2,2-diphenyl-1-picrylhydrazyl (DPPH),[13] 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) cation ($ABTS^+$),[14] hydroxyl[15] and superoxide anion[16] radicals, respectively, according to the literatures[13–16] with a little modification. The values of IC_{50}, the effective concentration at which 50% of the radicals were scavenged, were calculated to evaluate the antioxidant activities. A lower IC_{50} value indicated greater antioxidant activity. IC_{50} values of lower than 10 mg/mL usually implied effective activities in antioxidant properties.[17] The IC_{50} of butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ascorbic acid was also determined for comparison. The tested results were shown in Table 1.

DPPH radical scavenging activity evaluation is a rapid and convenient technique for screening the antioxidant activities of the antioxidants. It can be seen from Table 1 that, in DPPH assay, compounds **5b$_1$**, **5d$_1$**, **5f$_1$**, **5f$_2$**, **5g$_1$** and **5g$_2$** showed better radical scavenging activities than the synthetic commercial antioxidant **BHT**, with IC_{50} values of 57.72, 10.51, 36.63, 6.91, 33.29 and 16.21 μM, respectively. In addition, compounds **5d$_1$**, **5f$_2$** and **5g$_2$** showed better DPPH radical scavenging activities than both ascorbic acid and BHA. Obviously, of these compounds, **5f$_2$** showed the best radical scavenging activity in this assay. Compound **5b$_2$** displayed effective DPPH radical scavenging activity close to BHT, with IC_{50} of 76.37 μM. Compound **5c$_2$** exhibited very low DPPH radical scavenging activity and its IC_{50} was found to be 0.84 mg/mL and much lower than 10 mg/mL, demonstrating great DPPH radical scavenging activities of these compounds. On the basis of the above observation, the phenol and phenylhydrazine in the Schiff base group of compounds **5**, as well as the dinitro functionality at the position 2 and 4 of phenylhydrazine, appeared to be major contributors to the DPPH radical scavenging activities.

$ABTS^+$ radical assay is a conventional and excellent model for assessing the antioxidant activities of hydrogen-donating and chain breaking antioxidants.[18] In this assay, $ABTS^+$ radical was produced by reacting ABTS with potassium persulfate in sodium phosphate buffer solution.[13,14] It was found from Table 1 that most compounds showed good inhibition of $ABTS^+$ radical. Compounds **5a$_1$**, **5b$_1$**, **5c$_1$**, **5c$_2$**, **5d$_1$**, **5e$_1$**, **5e$_2$**, **5f$_2$**, **5g$_1$**, **5g$_2$** and **5h$_1$** exhibited better $ABTS^+$ radical scavenging activities than the synthetic commercial antioxidant BHT, with IC_{50} of 9.74, 13.23, 9.51, 15.52, 6.49, 9.25, 7.53, 3.57, 1.29, 7.76 and 22.81 μM, respectively. Besides, compounds **5a$_1$**, **5c$_1$**, **5d$_1$**, **5e$_1$**, **5e$_2$**, **5f$_2$**, **5g$_1$** and **5g$_2$** displayed better radical scavenging activities than ascorbic acid in this assay,



	R^1	R^2
5a$_1$	H	NH_2
5a$_2$	OCH_2Ph	NH_2
5b$_1$	H	C_6H_5NH
5b$_2$	OCH_2Ph	C_6H_5NH
5c$_1$	H	*p*-NO_2-C_6H_4NH
5c$_2$	OCH_2Ph	*p*-NO_2-C_6H_4NH
5d$_1$	H	*o*-NO_2-*p*-NO_2-C_6H_3NH
5d$_2$	OCH_2Ph	*o*-NO_2-*p*-NO_2-C_6H_3NH
5e$_1$	H	*o*-NH_2-C_6H_4
5e$_2$	OCH_2Ph	*o*-NH_2-C_6H_4
5f$_1$	H	*p*-OH-C_6H_4
5f$_2$	OCH_2Ph	*p*-OH-C_6H_4
5g$_1$	H	*o*-OH-C_6H_4
5g$_2$	OCH_2Ph	*o*-OH-C_6H_4
5h$_1$	H	OH
5h$_2$	OCH_2Ph	OH

Scheme 1. Synthetic route of 4-Schiff base-7-benzyloxy-coumarin derivatives. Reagents and conditions: (a) ethyl acetoacetate, $NaHSO_4.H_2O$, 110 °C; (b) benzylchloride, K_2CO_3, acetone, 56 °C; (c) SeO_2, dimethylbenzene, 150 °C; (d) R^2-NH_2, ethanol, 80 °C; (e) DMF, 100 °C.

Table 1
Radicals scavenging activities of compounds **5**–**6**

Compound	DPPH. IC_{50} (μM)	$ABTS^{+}$. IC_{50} (μM)	O_2^{-}. IC_{50} (μM)	OH. IC_{50} (μM)
$5a_1$	1288.10	9.74	131.29	None
$5a_2$	None	363.13 (0.15 [a])	36.51	>2000
$5b_1$	57.72	13.23	415.84	455.47
$5b_2$	76.37	40.13	302.75	401.15
$5c_1$	1346.75	9.51	>2000	>2000
$5c_2$	1608.25 (0.84[a])	15.52	418.54(0.22 [a])	97.89
$5d_1$	10.51	6.49	181.47	1967.39 (1.03 [a])
$5d_2$	None	None	291.71	734.28
$5e_1$	857.30	9.25	217.16	34.61
$5e_2$	314.56	7.53	257.77	623.80
$5f_1$	36.63	64.53	524.56	232.83
$5f_2$	6.91	3.57	248.58	106.72
$5g_1$	33.29	1.29	468.73	None
$5g_2$	16.21	7.76	33.13	348.42
$5h_1$	2408.14	22.81	>2000	>2000
$5h_2$	None	38.65	>2000	None
6	None	None	145.92	None
BHT	65.85	36.98	253.66	>2000
Ascorbic acid	43.81	12.83	15.56	73.81
BHA	20.25	1.02	38.83	>2000

None: ineffective.
[a] IC_{50} of the very low radical scavenging activities compounds countered by mg/mL unit.

though all their radical scavenging activities were lower than that of BHA. Evidently, compound **$5g_1$** showed the best $ABTS^{+}$ radical scavenging activity, while compound **$5a_2$** showed the lowest except compounds **$5d_2$** and **6**. IC_{50} of **$5a_2$** was found to be 363.13 μM, which was equivalent to 0.15 mg/mL and was clearly much lower than 10 mg/mL, implying good $ABTS^{+}$ radical scavenging activities of these compounds. In addition, since these compounds contain NH or OH groups, it could be concluded that NH and OH groups were important contributors to their $ABTS^{+}$ radical scavenging activities.

Superoxide anion radical, an initial radical, plays a significant role in the formation of other reactive oxygen species such as hydroxyl radical, hydrogen peroxide and singlet oxygen in living systems.[19] Table 1 revealed that compounds **$5a_2$** and **$5g_2$** displayed stronger superoxide anion radical scavenging activities than the synthetic commercial antioxidant BHA, with IC_{50} of 36.51 and 33.13 μM, respectively. Compound **$5c_2$** exhibited very low radical scavenging activity countered by mg/mL unit in this assay, with IC_{50} of 0.22 mg/mL, which was equivalent to 418.54 μM. Since IC_{50} values of lower than 10 mg/ml usually implied effective activities in antioxidant properties,[17] the result suggested that these compounds display effective radical scavenging effect on superoxide anion radical generation that may help prevent or ameliorate oxidative damage. By the respective comparation **$5a_1$** with **$5a_2$** and **$5g_1$** with **$5g_2$**, it could be summarized that the benzyloxy group at position 6 of coumarin structure may have important effect on their superoxide anion radical scavenging activities.

The radical scavenging effects were also examined in the present study using hydroxyl radicals generated by Fenton reagent.[15] As shown in Table 1, IC_{50} values of compound **$5e_1$** and common antioxidant ascorbic acid were found to be 34.61 and 73.81 μM, respectively. The result demonstrated that compound **$5e_1$** showed better hydroxyl radical scavenging activity than common antioxidant ascorbic acid and its radical scavenging activity was more than twice of that of ascorbic acid. Compound **$5d_1$** exhibited very low hydroxyl radical scavenging activity countered by mg/mL unit, with IC_{50} of 1.03 mg/mL, which was much lower than 10 mg/mL. The result suggested effective hydroxyl radical scavenging activity of these compounds.

In conclusion, we have designed and synthesized some 7-benzyloxy-coumarins derivatives with different salen groups which are constructed at position 4, leading to a general structural core compounds **5**–**6**. Of all the compounds, compounds **$5b_1$**, **$5d_1$**, **$5f_1$**, **$5f_2$**, **$5g_1$** and **$5g_2$** showed better radical scavenging activities than BHT in DPPH assay; compounds **$5a_1$**, **$5b_1$**, **$5c_1$**, **$5c_2$**, **$5d_1$**, **$5e_1$**, **$5e_2$**, **$5f_2$**, **$5g_1$**, **$5g_2$** and **$5h_1$** demonstrated better $ABTS^{+}$ radical scavenging activities than BHT; compounds **$5a_2$** and **$5g_2$** exhibited stronger superoxide anion radical scavenging activities than BHA; compound **$5e_1$** displayed more potent inhibition of hydroxyl radical than ascorbic acid. The above results demonstrate that the rational design of 4-Schiff base-7-benzyloxy-coumarin derivatives as novel antioxidant is feasible. Further studies on the relevant action mechanisms and problematic of the potential toxicity of these compounds are in progress, and will be published in the future.

Acknowledgments

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12. (a) General procedure for the preparation of compounds **3**–**6**: The mixture of compound **2** (5 mmol), potassium carbonate (5 mmol), potassium iodide (0.1 mmol), benzyl chloride (7 mmol) and acetone (50 mL) was refluxed at 56 °C for 18 h and then filtered to give a clear solution. Powder of derivative **3** was obtained through vacuum distillation. The mixture of compound **3**

(3 mmol), xylene (15 mL) and selenium dioxide (6 mmol) was refluxed at 150 °C for 20 h and then the solvent was removed by vacuum distillation. The gum obtained was purified by silica column chromatography with petroleum ether–ethyl acetate (v/v = 5:1) as the eluent. Upon recrystallization, yellow crystals of compound **4** were obtained. The mixture of compound **4** (1 mmol) and different primarily amines (1.1 mmol) or hydrazinium (1.1 mmol) was refluxed in ethanol for 5 h. After cooling to room temperature, crystals of compound **5** were obtained. The mixture of compound **4** (1 mmol) and *o*-diaminobenzene (1 mmol) was refluxed in DMF for 6 h, and crystals of compound **6** were obtained when the reaction solution cooling to room temperature. In addition, the treatment of compound **5e$_1$** and hot DMF also offer compound **6**; (b) Experimental: Melting points were determined on a WRS-IA apparatus without correction. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 500 spectrometer in DMSO-*d* or $CDCl_3$. Mass spectra were recorded on BRUKER ESQUIRE HCT spectrometer. Compound **5a$_1$**: Yields 69%; mp: 150.2–150.8 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.39 (s, 1H, N=CH), 8.13 (s, 2H, NH_2), 7.82 (s, 1H, Ar-H), 7.48 (d, J = 7.4 Hz, 2H, Ar-H), 7.41 (t, J = 7.4 Hz, 2H, Ar-H), 7.35 (t, J = 7.2 Hz, 1H, Ar-H), 7.08–6.99 (m, 2H, Ar-H), 6.22 (s, 1H, C=CH), 5.22 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.15, 160.73, 155.56, 146.95, 136.43, 131.09, 128.59, 128.16, 127.95, 127.62, 112.68, 110.71, 106.18, 101.96, 69.94, 54.95; MS (m/z) 295 (M+1)$^+$; Compound **5a$_2$**: Yields 67%; mp: 214–215.7 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.46 (s, 1H, N=CH), 7.63 (s, 2H, Ar-H), 7.47 (d, J = 7.3 Hz, 2H, Ar-H), 7.43–7.30 (m, 2H, Ar-H), 7.30–7.07 (m, 5H, Ar-H), 6.87 (s, 1H, Ar-H), 6.31 (s, 1H, C=CH), 5.35 (s, 2H, CH_2), 5.24 (s, 2H, CH_2), 3.99 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 160.22, 159.34, 156.36, 154.11, 149.35, 140.69, 136.76, 136.61, 134.17, 129.11, 128.97, 128.65, 128.62, 128.50, 128.25, 128.00, 126.26, 109.88, 103.27, 95.92, 71.16, 70.54; MS (m/z) 401 (M+1)$^+$; Compound **5b$_1$**: Yields 83%; mp: 205.3–206.3 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ11.21 (s, 1H, NH), 8.52 (d, J = 8.9 Hz, 1H, Ar-H), 8.02 (s, 1H, N=CH), 7.49 (d, J = 7.3 Hz, 2H, Ar-H), 7.42 (t, J = 7.4 Hz, 2H, Ar-H), 7.38–7.29 (m, 3H, Ar-H), 7.18 (d, J = 7.7 Hz, 2H, Ar-H), 7.14 (dd, J = 9.0, 2.5 Hz, 1H, Ar-H), 7.10 (d, J = 2.5 Hz, 1H, Ar-H), 6.91 (t, J = 7.3 Hz, 1H, Ar-H), 6.46 (s, 1H, C=CH), 5.25 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.25, 160.54, 155.59, 145.98, 143.87, 136.42, 131.70, 129.49, 128.59, 128.14, 127.89, 127.50, 120.94, 113.12, 112.87, 110.49, 108.06, 102.24, 69.96; MS (m/z) 371 (M+1)$^+$; Compound **5b$_2$**: Yields 83%; mp:242.0–243.5 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.99 (s, 1H, NH), 8.75 (s, 1H, N=CH), 7.47 (s, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 7.45 (d, J = 7.6 Hz, 1H, Ar-H), 7.43 (s, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.38 (d, J = 3.1 Hz, 1H, Ar-H), 7.36 (s, 1H, Ar-H), 7.35–7.35 (m, 1H, Ar-H), 7.35 (d, J = 7.1 Hz, 1H, Ar-H), 7.33–7.26 (m, 3H, Ar-H), 7.14 (d, J = 7.7 Hz, 1H, Ar-H), 6.86 (t, J = 7.3 Hz, 1H, Ar-H), 6.72 (d, J = 7.3 Hz, 1H, Ar-H), 6.66 (d, J = 8.3 Hz, 1H, Ar-H), 6.53 (s, 1H, C=CH), 5.41 (s, 2H, CH_2), 5.17 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.68, 159.90, 157.20, 156.96, 147.77, 144.34, 136.32, 136.23, 133.47, 129.41, 128.69, 128.61, 128.24, 128.07, 128.03, 127.32, 120.42, 112.91, 104.08, 102.79, 98.26, 95.34, 70.42, 70.11; MS (m/z) 477 (M+1)$^+$; Compound **5c$_1$**: Yields 80%; mp: 255.2–257.0 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.84 (s, 1H, NH), 8.43 (d, J = 8.7 Hz, 1H, Ar-H), 8.21 (s, 1H, N=CH), 8.19 (d, J = 5.9 Hz, 2H, Ar-H), 7.49 (d, J = 7.5 Hz, 2H, Ar-H), 7.42 (t, J = 7.3 Hz, 2H, Ar-H), 7.37 (d, J = 7.3 Hz, 1H, Ar-H), 7.29 (d, J = 8.8 Hz, 2H, Ar-H), 7.14 (d, J = 10.0 Hz, 2H, Ar-H), 6.61 (s, 1H, C=CH), 5.26 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.80, 160.72, 155.99, 149.89, 145.57, 140.56, 137.34, 136.76, 129.00, 128.56, 128.30, 127.79, 126.55, 113.48, 113.05, 110.83, 110.62, 102.67, 70.38; MS (m/z) 414 (M−1)$^+$; Compound **5c$_2$**: Yields 82%; mp:226.7–227.9 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.62 (s, 1H, NH), 8.81 (s, 1H, N=CH), 8.18 (d, J = 9.1 Hz, 2H, Ar-H), 7.46 (s, 2H, Ar-H), 7.45–7.37 (m, 5H, Ar-H), 7.38–7.28 (m, 5H, Ar-H), 6.75 (d, J = 1.9 Hz, 1H, Ar-H), 6.69 (d, J = 1.7 Hz, 1H, Ar-H), 6.53 (s, 1H, C=CH), 5.39 (s, 2H, CH_2), 5.19 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.33, 160.17, 157.38, 157.16, 150.33, 147.28, 139.98, 139.68, 136.53, 129.04, 129.00, 128.65, 128.62, 128.46, 128.02, 127.71, 126.57, 112.60, 107.05, 102.96, 98.61, 95.67, 70.95, 70.52; MS (m/z) 522 (M+1)$^+$; Compound **5d$_1$**: Yields 88%; mp:295.2–297.0 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.90 (s, 1H, NH), 9.02 (s, 1H, Ar-H), 8.87 (s, 1H, N=CH), 8.42 (d, J = 9.5 Hz, 1H, Ar-H), 8.21 (d, J = 8.5 Hz, 1H, Ar-H), 8.12 (d, J = 9.8 Hz, 1H, Ar-H), 7.49 (d, J = 7.7 Hz, 2H, Ar-H), 7.42 (t, J = 7.4 Hz, 2H, Ar-H), 7.37 (d, J = 7.6 Hz, 1H, Ar-H), 7.15 (d, J = 9.6 Hz, 2H, Ar-H), 6.73 (s, 1H, C=CH), 5.26 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.09, 160.55, 156.04, 145.16, 143.98, 138.93, 136.70, 130.42, 129.01, 128.60, 128.33, 127.11, 123.19, 117.85, 113.69, 110.74, 102.77, 70.46; MS (m/z) 461 (M+1)$^+$; Compound **5d$_2$**: Yields 87%; mp: 236.8–238.8 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.62 (s, 1H, NH), 9.16 (s, 1H, N=CH), 8.36 (d, J = 9.3 Hz, 1H, Ar-H), 8.03 (d, J = 9.5 Hz, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 7.49–7.44 (m, 4H, Ar-H), 7.42 (s, 1H, Ar-H), 7.37 (d, J = 7.0 Hz, 1H, Ar-H), 7.30 (s, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.26 (d, J = 1.0 Hz, 1H, Ar-H), 7.20 (d, J = 7.4 Hz, 1H, Ar-H), 6.79 (d, J = 2.2 Hz, 1H, Ar-H), 6.77 (d, J = 2.0 Hz, 1H, Ar-H), 6.50 (s, 1H, C=CH), 5.30 (s, 2H, CH_2), 5.22 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.83, 162.70, 160.07, 157.37, 156.98, 147.89, 147.33, 146.77, 139.22, 138.73, 136.53, 136.31, 130.13, 129.01, 128.98, 128.67, 128.46, 128.25, 128.02, 123.29, 119.99, 117.59, 109.00, 102.73, 98.38, 95.64, 71.51, 70.59; MS (m/z) 565 (M−1)$^+$; Compound **5e$_1$**: Yields 85%; mp: 152.6–153.8 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.96 (s, 1H, N=CH), 8.66 (d, J = 8.9 Hz, 1H, Ar-H), 7.49 (d, J = 7.6 Hz, 2H, Ar-H), 7.41 (t, J = 7.3 Hz, 2H, Ar-H), 7.35 (t, J = 7.2 Hz, 2H, Ar-H), 7.14–7.04 (m, 3H, Ar-H), 6.97 (s, 1H, C=CH), 6.78 (d, J = 8.1 Hz, 1H, Ar-H), 6.59 (t, J = 7.5 Hz, 1H, Ar-H), 5.42 (s, 2H, NH_2), 5.25 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.81, 160.97, 156.09, 151.95, 146.48, 145.72, 136.74, 134.40, 130.15, 128.99, 128.56, 128.33, 128.04, 118.05, 116.54, 115.84, 113.55, 113.40, 111.21, 102.46, 70.36; MS (m/z) 371 (M+1)$^+$; Compound **5e$_2$**: Yields 85%; mp: 194.0–194.7 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.04 (s, 1H, N=CH), 7.51 (d, J = 5.4 Hz, 4H, Ar-H), 7.43 (dd, J = 14.0, 6.6 Hz, 3H, Ar-H), 7.37 (t, J = 7.3 Hz, 3H, Ar-H), 6.91 (d, J = 7.8 Hz, 1H, Ar-H), 6.89 (dd, J = 5.3, 1.5 Hz, 1H, Ar-H), 6.86 (s, 1H, C=CH), 6.82 (d, J = 2.1 Hz, 1H, Ar-H), 6.65 (d, J = 7.9 Hz, 1H, Ar-H), 6.17 (t, J = 7.9 Hz, 1H, Ar-H), 6.09 (d, J = 7.4 Hz, 1H, Ar-H), 5.32 (s, 2H, NH_2), 5.27 (s, 2H, CH_2), 5.23 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.58, 160.42, 157.87, 157.14, 153.79, 149.04, 145.17, 136.67, 135.81, 134.31, 129.44, 129.19, 129.01, 128.64, 128.45, 117.57, 116.55, 115.35, 109.89, 102.85, 98.03, 95.65, 72.07, 70.60; MS (m/z) 477 (M+1)$^+$; Compound **5f$_1$**: Yields 70%; mp: 210.2–211.3 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.82 (s, 1H, OH), 8.89 (s, 1H, N=CH), 8.85 (d, J = 9.0 Hz, 1H, Ar-H), 7.49 (d, J = 7.3 Hz, 2H, Ar-H), 7.45–7.39 (m, 4H, Ar-H), 7.36 (t, J = 7.2 Hz, 1H, Ar-H), 7.14 (s, 1H, Ar-H), 7.09 (d, J = 9.0 Hz, 1H, Ar-H), 6.87 (d, J = 8.6 Hz, 2H, Ar-H), 6.77 (s, 1H, C=CH), 5.26 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.47, 160.16, 158.12, 154.79, 153.81, 153.42, 136.42, 136.38, 128.59, 128.17, 128.14, 127.95, 127.89, 126.57, 123.71, 116.01, 114.63, 113.42, 113.00, 112.78, 111.34, 110.58, 102.07, 101.84, 69.98, 18.17; MS (m/z) 372 (M+1)$^+$; Compound **5f$_2$**: Yields 68%; mp: 231.5–233.1 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.58 (s, 1H, OH), 8.98 (s, 1H, N=CH), 7.57–7.26 (m, 10H, Ar-H), 6.85 (s, 1H Ar-H), 6.80 (s, 1H Ar-H), 6.69 (d, J = 8.5 Hz, 2H Ar-H), 6.61 (d, J = 8.5 Hz, 2H Ar-H), 6.50 (s, 1H, C=CH), 5.26 (s, 2H, CH_2), 5.20 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.79, 160.38, 157.91, 157.69, 157.21, 154.81, 149.27, 141.80, 136.73, 135.94, 129.53, 129.25, 129.12, 128.75, 128.57, 123.58, 116.31, 109.28, 102.86, 98.08, 95.71, 72.12, 70.71; MS (m/z) 478 (M+1)$^+$; Compound **5g$_1$**: Yields 83%; mp: 194.8–196.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.89 (s, 1H, OH), 8.36 (d, J = 8.9 Hz, 1H, Ar-H), 7.50–7.41 (m, 4H, Ar-H), 7.36 (m, 3H, Ar-H, N=CH), 7.10 (d, J = 8.8 Hz, 1H, Ar-H), 7.06–6.93 (m, 4H, Ar-H), 6.77 (s, 1H, C=CH), 5.20 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.51, 160.54, 155.76, 151.87, 145.95, 137.24, 136.38, 129.25, 128.60, 128.17, 127.99, 127.95, 120.04, 119.63, 116.76, 114.42, 112.99, 110.69, 102.11, 70.01; MS (m/z) 370 (M−1)$^+$; Compound **5g$_2$**: Yields 83%; mp: 249.7–250.9 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.11 (s, 1H, OH), 7.50 (dd, J = 12.5, 7.4 Hz, 4H, Ar-H), 7.44 (t, J = 7.5 Hz, 2H, Ar-H), 7.41–7.37 (m, 2H, Ar-H), 7.34 (t, J = 7.3 Hz, 2H, Ar-H), 6.99 (s, 1H, N=CH), 6.90–6.78 (m, 3H, Ar-H), 6.67–6.21 (m, 4H, Ar-H and C=CH), 5.27 (s, 2H, CH_2), 5.24 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.24, 160.08, 157.33, 156.65, 151.97, 148.31, 144.08, 136.73, 136.27, 135.44, 128.96, 128.77, 128.68, 128.63, 128.26, 128.08, 119.69, 118.72, 116.44, 114.51, 110.32, 102.38, 97.65, 95.27, 71.62, 70.23; MS (m/z) 478 (M+1)$^+$; Compound **5h$_1$**: Yields 73%; mp: 208.3–209.3 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.42 (s, 1H, N=CH), 8.36 (d, J = 9.0 Hz, 1H, Ar-H), 7.49 (s, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 7.41 (t, J = 7.4 Hz, 2H, Ar-H), 7.35 (t, J = 7.2 Hz, 1H, Ar-H), 7.10 (d, J = 2.2 Hz, 1H, Ar-H), 7.05 (dd, J = 9.0, 2.2 Hz, 1H, Ar-H), 6.52 (s, 1H, C=CH), 5.23 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.86, 160.59, 155.88, 146.84, 144.72, 136.68, 128.99, 128.58, 128.50, 128.35, 113.37, 112.57, 110.46, 102.44, 70.37; MS (m/z) 296 (M+1)$^+$; Compound **5h$_2$**: Yields 75%; mp: 210.4–211.3 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.72 (s, 1H, OH), 8.66 (s, 1H, N=CH), 7.47 (s, 4H, Ar-H), 7.39 (dd, J = 15.5, 7.3 Hz, 6H, Ar-H), 6.78 (d, J = 9.8 Hz, 2H, Ar-H), 6.26 (s, 1H, C=CH), 5.26 (s, 2H, CH_2), 5.23 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.25, 159.57, 157.17, 156.67, 147.33, 145.48, 136.23, 135.87, 128.66, 128.29, 128.24, 128.11, 127.88, 108.06, 102.28, 97.92, 95.27, 71.12, 70.21; MS (m/z) 402 (M+1)$^+$; Compound **6**: Yields 93%; mp: 229.4–231.3 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ13.32 (s, 1H, NH), 9.14 (d, J = 8.8 Hz, 1H, Ar-H), 7.85 (d, J = 7.8 Hz, 1H, Ar-H), 7.62 (d, J = 7.8 Hz, 1H, Ar-H), 7.51 (d, J = 7.1 Hz, 2H, Ar-H), 7.42 (t, J = 7.3 Hz, 2H, Ar-H), 7.37 (d, J = 7.2 Hz, 2H, Ar-H), 7.31 (t, J = 7.4 Hz, 1H, Ar-H), 7.16 (d, J = 12.9 Hz, 2H, Ar-H), 6.92 (s, 1H, C=CH), 5.27 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.68, 160.07, 155.89, 146.86, 143.80, 141.49, 136.34, 134.20, 129.82, 128.63, 128.22, 128.02, 124.62, 122.68, 120.10, 113.22, 112.06, 111.94, 110.24, 102.11, 70.07; MS (m/z) 369 (M+1)$^+$.

13. (a) Pan, Y. M.; Zhu, J. C.; Wang, H. S.; Zhang, X. P.; Zhang, Y.; He, C. H.; Ji, X. W.; Li H. Y. *Food Chem.* **2007**, *103*, 913; (b) General procedure for evaluation of DPPH radical activity: Each sample solution (0.1 mL) in DMF at different concentrations was added to the solution [3.9 mL, 0.004% (w/v)] of DPPH**.** in ethanol. The reaction mixture was incubated at 37 °C. The scavenging activity on DPPH**.** was determined by measuring the absorbance at 517 nm after 30 min. All tests were performed in triplicate and mean were centred. The scavenging activity was expressed as a percentage of scavenging activity on DPPH**.**: SC% = $[(A_{control} - A_{test})/A_{control})] \times 100\%$, where $A_{control}$ is the absorbance of the control (DPPH**.** solution without test sample) and A_{test} is the absorbance of the test sample (DPPH**.** solution plus scavenger). The control contains all reagents except the scavenger.
14. (a) Pan,Y. M.; He, C. H.; Wang, H. S.; Ji, X. W.; Wang, K.; Liu, P. Z.; *Food Chem.* **2010**, *121*, 497; (b) General procedure for evaluation of ABTS radical activity: Stock solution of ABTS (2 mM) was prepared by dissolving in phosphate buffered saline (PBS, 50 ml) and the pH of the solution should be 7.4. ABTS**.**$^+$ was produced by reacting of stock solution (50 ml) with $K_2S_2O_8$ water solution (200 ml, 70 mM). The mixture was left to stand in the dark at room temperature for 15–16 h before use. For the evaluation of antioxidant activity, the ABTS**.**$^+$ solution was diluted with PBS to obtain the absorbency of 0.700 ± 0.030 at 734 nm. Compounds **5**–**6** solution (0.1 ml) at different concentration were mixed with ABTS**.**$^+$ solution (1.9 ml), then absorbance was read at ambient temperature after 3 min. PBS solution was used as a blank sample. All tests were performed in triplicate and mean were centred. The radical scavenging activity of the sample was expressed as SC% = $[(A_{control} - A_{test})/A_{control}] \times 100\%$, where $A_{control}$ is the absorbance of the control (ABTS**.**$^+$ solution without test sample) and A_{test} is the absorbance of the test sample (ABTS**.**$^+$ solution plus extracts).
15. (a) Guo T.; Wei, L.; Sun, J.; Hou, C. L.; Fan, L.; *Food Chem.* **2011**, *127*, 1634; (b) General procedure for evaluation of hydroxyl radical activity: The following

reagents were put into a reaction tube in the following order: 0.3 ml of 20 mM sodium salicylate, 1.0 ml of 1.5 mM ferrous sulfate, 1.0 ml of various concentrations of sample solution, 0.7 mL of 6 mM H_2O_2. They were mixed immediately, and then the reaction tubes were put in the 37 °C water bath for 1 h, the absorbance of the mixture was recorded at 510 nm against a blank. Ascorbic acid was used as the positive control. All tests were performed in triplicate and mean were centred. The hydroxyl radical-scavenging ability was calculated as follows: Hydroxyl radical-scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100\%$, where A_0 is the absorbance without samples and A_1 the absorbance in the presence of the samples.

16. (a) Marklund, S.; Marklund, G. *Eur. J. Biochem*. **1974**, *47*, 469; (b) Zhao, F.; Liang, H.; Cheng, H.; Wang, J. *Acta Chim. Sinica*, **2011**, *69*, 925; (b) General procedure for evaluation of superoxide anion radical activity: Under room temperature, to 4.5 mL of 0.05 M Tris–HCl buffered solution, 1.0 mL of sample in DMF solution (in different concentration) and 0.4 mL of 30 mM 1,2,3-trihydroxybenzene solution were added and reacted for 5 min. Then 0.5 mL of 8.0 M hydrochloric acid solution was added and the absorbance of the mixture was recorded at 320 nm against a blank. All tests were performed in triplicate and mean were centred. The superoxide anion radical-scavenging ability was calculated as follows: Superoxide anion radical-scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100\%$, where A_0 is the absorbance without samples and A_1 the absorbance in the presence of the samples.
17. Lee, Y. L.; Yen, M. T.; Mau, J. L. *Food Chem.* **2007**, *104*, 1.
18. Leong, L. P.; Shui, G. *Food Chem.* **2002**, *76*, 69.
19. Stief, T. W. *Med. Hypotheses* **2003**, *60*, 567.