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A concise synthesis of polyhydroxydihydrochalcones and homoisoflavonoids

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Abstract—A general and single step synthesis of polyhydroxydihydrochalcones from the readily available phenols and dihydrocinnamic acids using $BF_3 \cdot Et_2O$ is described. The method allows the synthesis of a wide range of compounds with multiple phenolic hydroxyls and other substituents. These dihydrochalcones are converted into homoisoflavonoids by DMF/PCl_5 and the methodology has been applied to the synthesis of naturally occurring phloretin and 5,7-dihydroxy-3-[(4-hydroxyphenyl)methyl]-4*H*-chromen-4-one. The antioxidant activity of dihydrochalcones and homoisoflavonoids was determined by superoxide free radical (NBT) and DPPH free radical scavenging methods. Polyhydroxydihydrochalcones **3c**, **3f**, **3g** and homoisoflavonoids **4c**, **4f**, **4g** displayed excellent antioxidant activity.

1. Introduction

Naturally occurring polyphenolic compounds display wide spectrum of biological activities. Polyphenolic flavonoids have gained increasing importance in view of their strong antioxidant activity and preventing role in free radical mediated disorders such as cancer, Alzheimer's, Parkinson's, and cardiovascular diseases.[1] Dihydrochalcones (DHCs), the reduced form of chalcones, have been known to occur widely in nature and are important intermediates for many natural products and pharmaceutical drugs.[1] DHCs have been reported to have various biological activities[2,3] and have received considerable attention as food sweeteners (neohesperidin).[4] Up to now, DHCs were mostly synthesized through a Claisen–Schmidt condensation[5,6] to obtain chalcone, followed by reduction to DHC or through the alkaline reduction of a corresponding flavanone.[7,8] Although widely used, the procedures are not suitable for polyhydroxyDHCs, as these methods require protection of all phenolic hydroxyls. Recently, palladium mediated coupling of iodobenzenes and the enol of acetophenone was reported[9] as an alternative route to DHCs. The homoisoflavonoids (3-benzylchromen-4-ones), are naturally occurring compounds and are structurally related to flavonoids,[10] and display a wide spectrum of biological activities.[11–15] A few methods of synthesis have been reported in the literature for homoisoflavonoids and these were based on (i) the condensation of 4-chromanones with arylaldehydes in methanol by passing HCl gas or by using piperidine as a base[16,17] followed by isomerisation of the double bond using Pd/C at 250 °C, (ii) hydrogenation of chalcones followed by one carbon extension using ethyl formate/sodium[18] or methanesulfonyl chloride/DMF.[19] Both the methods have disadvantages; while the first method has multiple steps, in the second method, the phenolic hydroxyls have to be protected to get chalcones in good yield. Our interest in the chemistry of the flavonoids[20] and an increasing demand for a short, and efficient method prompted us to develop a simple and general method for the synthesis of DHCs and homoisoflavonoids. The methodology has been applied to the synthesis of phloretin,[21] a naturally occurring DHC and 5,7-dihydroxy-3-[(4-hydroxyphenyl)-methyl]-4*H*-chromen-4-one, a homoisoflavonoid from *Ophiopogon jaburan*.[22] Moreover, to the best of our knowledge, there is no report in the literature on the antioxidant activity of homoisoflavonoids. So we report in this paper, the details of synthesis of DHCs, homoisoflavonoids and their antioxidative activity results.

Keywords: Dihydrochalcones; Homoisoflavonoids; Boron trifluoride etherate; Antioxidant activity.
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2. Results and discussion

2.1. Synthesis

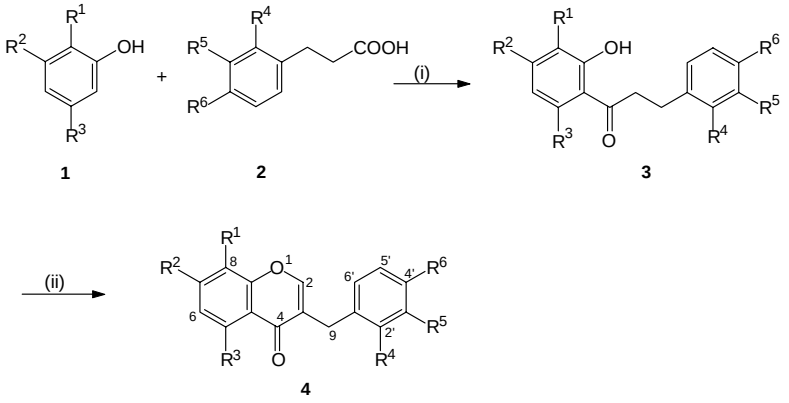
During the course of our investigations of new synthetic routes to flavonoids, we have found that a Friedel-Crafts reaction constitutes a novel and efficient approach to DHCs. The method involves the preparation of DHCs in a single step from phenols and dihydrocinnamic acids by Friedel-Crafts acylation using boron trifluoride etherate, which serves as the Lewis acid for the acylation and as solvent for the reaction (Scheme 1). In this method, protection of the phenolic hydroxyls is not necessary. In a typical experiment, substituted phenol (**1**, 3 mmol) and dihydrocinnamic acid (**2**, 3 mmol) was treated with boron trifluoride etherate at 80–90 °C for 90 min. After completion of the reaction (monitored by TLC), it was poured into aqueous sodium acetate and extracted with ethyl acetate to give DHCs (**3**). The generality of the reaction was established with various phenols and substituted dihydrocinnamic acids and in all cases (Table 1, 7 examples) the reaction was completed within 90 min. In the second step, the DHCs (**3a–3g**) were treated with *N*,*N*′-dimethyl(chloromethylene)ammonium chloride[23] generated in situ from DMF and PCl_5 for one carbon extension to get homoisoflavonoids (**4a–4g**) in 78–88% yield (Table 1). In all cases the reaction was complete in 2 h and the products were characterized by their spectral data (IR, NMR and Mass). The methodology has been applied to the synthesis of naturally occurring phloretin,[21] a DHC and 5,7-dihydroxy-3-[(4-hydroxyphenyl)methyl]-4*H*-chromen-4-one (**4h**), isolated from *Ophiopogon jaburan*.[22] Reaction of phloroglucinol and 4-hydroxydihydrocinnamic acid with $BF_3 \cdot Et_2O$ gave phloretin (**3h**), which was converted further into **4h** (85% yield) using *N*,*N*′-dimethyl(chloromethylene)ammonium chloride. The spectral data of synthetic **3h** and **4h** were found to be identical with those of the corresponding natural products.[19]

2.2. Antioxidant activity

We have determined the antioxidative activity of DHCs (**3a–3h**) and homoisoflavonoids (**4a–4h**) by nitro blue tetrazolium (NBT)[24,25] and 1,1-diphenyl-2-picrylhydrazyl (DPPH)[26] free radical scavenging methods. The IC_{50} values of these compounds are presented in Table 2. DHCs **3f** (IC_{50}: 12.9 μM), **3g** (IC_{50}: 19.6 μM) and **3c** (IC_{50}: 30.2 μM) and homoisoflavonoids **4f** (IC_{50}: 6.3 μM), **4g** (IC_{50}: 8.2 μM) and **4c** (IC_{50}: 24.8 μM) having catechol moieties were the most active compounds. Interestingly **3f**, **3g** and **3c** and **4f**, **4g** and **4c** showed several-fold more potent activity than vitamin C (IC_{50}: 852 μM), vitamin E (IC_{50}: 726 μM), BHA (IC_{50}: 966 μM) and BHT (IC_{50}: 381 μM). The same order of activity was followed by DHCs **3a–3h** and homoisoflavonoids **4a–4h** with the DPPH method. Again **3c**, **3f** and **3g** and **4c**, **4f** and **4g** showed good DPPH free radical scavenging activity. The superior antioxidative activity of these compounds lends further support to the fact that the catechol system enhances the antioxidative activity.[27]

3. Conclusions

In conclusion, we have described a general, single step method for the synthesis of polyhydroxydihydrochalcones using phenols and dihydrocinamic acids with $BF_3 \cdot Et_2O$. One carbon extension of these dihydrochalcones into homoisoflavonoids by DMF/PCl_5 was achieved in good yields. The DHCs and homoisoflavonoids were evaluated for their antioxidative potential by two commonly used



Scheme 1. Reagents and conditions: (i) $BF_3 \cdot Et_2O$, 80–90 °C, 90 min, 30–71% (ii) $BF_3 \cdot Et_2O$, DMF/PCl_5, rt, 2 h, 78–88%.

Table 1. Dihydrochalcones **3** and homoisoflavonoids **4**

S.no.	Entry	R_1	R_2	R_3	R_4	R_5	R_6	**3** Yield (%)[a]	**4** Yield (%)[a]
1	a	H	OH	H	H	H	OH	62	85
2	b	H	OH	H	H	H	OCH_3	71	88
3	c	H	OH	H	H	OH	OH	55	82
4	d	H	OH	H	H	OCH_3	OCH_3	68	87
5	e	H	OH	H	OCH_3	H	OCH_3	61	78
6	f	OH	OH	H	H	H	OH	58	80
7	g	OH	OH	H	H	H	OCH_3	65	81
8	h	H	OH	OH	H	H	OH	30	85

[a] Unoptimized isolated yields.

Table 2. Antioxidant activity of homoisoflavonoids

Compound no.	NBT superoxide scavenging activity (IC_{50} in μM)	DPPH free radical scavenging activity (IC_{50} in μM)
3a	>100	>100
3b	>100	>100
3c	30.2	7.8
3d	>100	>100
3e	>100	>100
3f	12.9	11.3
3g	19.6	14.9
3h	16.6	95
4a	>100	>100
4b	>100	>100
4c	24.8	13.4
4d	>100	>100
4e	>100	>100
4f	6.3	19.0
4g	8.2	19.6
4h	17.6	>100
Vitamin C	852	25.1
Vitamin E	726	>100
BHA	966	34.0
BHT	381	22.5

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; NBT, nitro blue tetrazolium; DPPH, 1,1-diphenyl-2-picrylhydrazyl. The lower the IC_{50} values, the higher is the antioxidant activity.

methods, the superoxide and DPPH free radical scavenging methods. DHCs **3c**, **3f** and **3g** and homoisoflavonoids **4c**, **4f** and **4g** were potent antioxidants.

4. Experimental

4.1. General

Melting points were recorded on a Mel-Temp melting point apparatus, in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer BX1 FTIR Spectrophotometer and ^{1}H NMR (400 MHz), ^{13}C NMR-DEPT (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer using TMS as internal standard and the values for chemical shifts (δ) being given in ppm and coupling constants (J) in Hertz (Hz). Mass spectra were recorded on an Agilent 1100 LC/MSD. Acme silica gel G and silica gel (100–200 mesh) were used for analytical TLC and column chromatography, respectively.

4.2. General procedure for dihydrochalcones (3)

A mixture of phenol (**1**, 3 mmol), 3-phenylpropanoic acid (**2**, 3 mmol) and $BF_3\cdot Et_2O$ (1.94 mL, 15.3 mmol) was stirred at 80–90 °C for 90 min under N_2. The reaction mixture was poured into 10% aqueous NaOAc solution (100 mL) and allowed to stand for 4 h and the solution was extracted with EtOAc (3×100 mL). The combined EtOAc layer was washed with water (20 mL), brine (20 mL) and dried over Na_2SO_4. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using hexane–EtOAc mixtures as eluent to give **3a–h**.

4.2.1. 1-(2,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl)-propan-1-one (3a). Light brown powder (480 mg, 62%), mp 140–142 °C; IR (KBr): 3456, 3270, 1626, 1214, 1165, 1134, 987 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.63 (1H, s, Ar-OH), 11.69 (1H, s, Ar-OH), 10.60 (1H, s, Ar-OH), 7.79 (1H, d, J=8.8 Hz, H-6″), 7.04 (2H, d, J=8.3 Hz, H-2′,6′), 6.65 (2H, d, J=8.3 Hz, H-3′,5′), 6.35 (1H, dd, J=8.8, 2.4 Hz, H-5″), 6.24 (1H, d, J=2.4 Hz, H-3″), 3.20 (2H, t, J=7.6 Hz, H-3), 2.81 (2H, t, J=7.6 Hz, H-2); ^{13}C NMR (DMSO-d_6): δ 203.9, 164.7, 164.3, 155.5, 133.0, 131.0, 129.3, 115.1, 112.6, 108.2, 102.4, 39.1, 29.1; MS (ESI, negative ion mode): m/z 257 $(M-H)^-$. Analysis found: C, 69.68; H, 5.52%. Calcd for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46%.

4.2.2. 1-(2,4-Dihydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (3b). Light brown powder (580 mg, 71%), mp 58–60 °C; IR (KBr): 3456, 3105, 1629, 1225, 1131, 1029, 990 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.60 (1H, s, Ar-OH), 10.61 (1H, s, Ar-OH), 7.79 (1H, d, J=8.8 Hz, H-6″), 7.16 (2H, d, J=8.3 Hz, H-2′,6′), 6.82 (2H, d, J=8.3 Hz, H-3′,5′), 6.34 (1H, dd, J=8.8, 2.0 Hz, H-5″), 6.23 (1H, d, J=2.0 Hz, H-3″), 3.69 (3H, s, Ar-OCH_3), 3.22 (2H, t, J=7.5 Hz, H-3), 2.84 (2H, t, J=7.5 Hz, H-2); ^{13}C NMR (DMSO-d_6): δ 203.7, 164.8, 164.3, 157.6, 132.9, 129.4, 114.2, 113.7, 112.6, 108.2, 102.5, 55.0, 40.1, 29.0; MS (ESI, negative ion mode): m/z 271 $(M-H)^-$. Analysis found: C, 70.52; H, 5.97%. Calcd for $C_{16}H_{16}O_4$: C, 70.58; H, 5.92%.

4.2.3. 1-(2,4-Dihydroxyphenyl)-3-(3,4-dihydroxyphenyl)-propan-1-one (3c). Colorless powder (450 mg, 55%), mp 108–110 °C; IR (KBr): 3339, 1638, 1605, 1286, 1222, 1200, 1174, 1141, 968 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.63 (1H, s, Ar-OH), 8.72 (1H, s, Ar-OH), 8.56 (1H, s, Ar-OH), 8.29 (1H, s, Ar-OH), 7.77 (1H, d, J=8.8 Hz, H-6″), 7.10–7.20 (2H, m, H-2′,5′), 6.47 (1H, dd, J=7.8, 1.5 Hz, H-6′), 6.34 (1H, dd, J=8.8, 2.0 Hz, H-5″), 6.23 (1H, d, J=2.0 Hz, H-3″), 3.16 (2H, t, J=7.6 Hz, H-3), 2.74 (2H, t, J=7.6 Hz, H-2); ^{13}C NMR (DMSO-d_6): δ 205.0, 165.9, 165.4, 146.1, 144.5, 134.1, 133.0, 120.1, 117.0, 116.6, 113.7, 109.3, 103.6, 40.4, 30.4; MS (ESI, negative ion mode): m/z 273 $(M-H)^-$. Analysis found: C, 65.64; H, 5.18%. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15%.

4.2.4. 1-(2,4-Dihydroxyphenyl)-3-(3,4-dimethoxyphenyl)-propan-1-one (3d). Light brown powder (615 mg, 68%), mp 128–130 °C; IR (KBr): 3371, 1631, 1257, 1235, 1209, 1137, 1026, 991 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.64 (1H, s, Ar-OH), 10.63 (1H, s, Ar-OH), 7.82 (1H, d, J=8.8 Hz, H-6″), 6.88 (1H, d, J=1.8 Hz, H-2′), 6.83 (1H, d, J=8.0 Hz, H-5′), 6.76 (1H, dd, J=8.0, 1.8 Hz, H-6′), 6.37 (1H, dd, J=8.8, 2.1 Hz, H-5″), 6.26 (1H, d, J=2.1 Hz, H-3″), 3.73 (3H, s, Ar-OCH_3), 3.70 (3H, s, Ar-OCH_3), 3.25 (2H, t, J=7.6 Hz, H-3), 2.86 (2H, t, J=7.6 Hz, H-2); ^{13}C NMR (DMSO-d_6): δ 203.8, 164.8, 164.3, 148.7, 147.2, 133.5, 133.0, 120.2, 112.6, 112.4, 111.9, 108.2, 102.5, 55.5, 55.4, 39.2, 29.5; MS (ESI, negative ion mode): m/z 301 $(M-H)^-$. Analysis found: C, 67.52; H, 6.04%. Calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00%.

4.2.5. 1-(2,4-Dihydroxyphenyl)-3-(2,4-dimethoxyphenyl)-propan-1-one (3e). Colorless powder (555 mg, 61%), mp 110–112 °C; IR (Neat): 3347, 1626, 1290, 1208, 1153, 1037, 990 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.72 (1H, s, Ar-OH), 9.99 (1H, s, Ar-OH), 7.60 (1H, d, J=8.5 Hz, H-6″), 7.00 (1H, d, J=8.5 Hz, H-5″), 6.20–6.40 (4H, m, H-3′,5′,6′,3″), 3.81 (3H, s, Ar-OCH_3), 3.78 (3H, s, Ar-OCH_3), 3.10 (2H, t, J=7.5 Hz, H-3), 2.90 (2H, t, J=7.5 Hz, H-2); ^{13}C NMR (DMSO-d_6): δ 204.2, 164.8,

164.4, 159.2, 158.0, 132.9, 130.0, 120.8, 112.5, 108.2, 104.3, 102.5, 98.3, 55.5, 55.3, 38.0, 24.8; MS (ESI, negative ion mode): *m/z* 301 $(M-H)^-$. Analysis found: C, 67.51; H, 6.05%. Calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00%.

4.2.6. 1-(2,3,4-Trihydroxyphenyl)-3-(4-hydroxyphenyl)-propan-1-one (3f). Pale green powder (475 mg, 58%), mp 158–160 °C; IR (KBr): 3440, 3246, 1633, 1243, 1213, 1118, 1044, 1004, 899 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.63 (1H, s, Ar-OH), 10.06 (1H, s, Ar-OH), 9.15 (1H, s, Ar-OH), 8.59 (1H, s, Ar-OH), 7.34 (1H, d, $J=8.8$ Hz, H-6″), 7.04 (2H, d, $J=8.3$ Hz, H-2′,6′), 6.66 (2H, d, $J=8.3$ Hz, H-3′,5′), 6.38 (1H, d, $J=8.8$ Hz, H-5″), 3.20 (2H, t, $J=7.5$ Hz, H-3), 2.82 (2H, t, $J=7.5$ Hz, H-2); ^{13}C NMR (DMSO-d_6): δ 204.9, 155.7, 152.6, 152.4, 132.5, 131.2, 129.4, 122.6, 115.2, 113.0, 107.9, 39.0, 29.4; MS (ESI, negative ion mode): *m/z* 273 $(M-H)^-$. Analysis found: C, 65.66; H, 5.20%. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15%.

4.2.7. 1-(2,3,4-Trihydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (3g). Light brown oil (560 mg, 65%), IR (Neat): 3396, 2928, 1632, 1244, 1178, 1114, 1033, 999 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.60 (1H, s, Ar-OH), 10.08 (1H, s, Ar-OH), 8.61 (1H, s, Ar-OH), 7.33 (1H, d, $J=8.8$ Hz, H-6″), 7.16 (2H, d, $J=7.8$ Hz, H-2′,6′), 6.81 (2H, d, $J=7.8$ Hz, H-3′,5′), 6.38 (1H, d, $J=8.8$ Hz, H-5″), 3.69 (3H, s, Ar-OCH_3), 3.20 (2H, t, $J=7.5$ Hz, H-3), 2.85 (2H, t, $J=7.5$ Hz, H-2); ^{13}C NMR (DMSO-d_6): δ 204.8, 157.8, 152.7, 152.6, 133.1, 132.6, 129.6, 122.6, 114.0, 113.1, 108.0, 55.2, 39.4, 29.3; MS (ESI, negative ion mode): *m/z* 287 $(M-H)^-$. Analysis found: C, 66.62; H, 5.62%. Calcd for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59%.

4.2.8. 1-(2,4,6-Trihydroxyphenyl)-3-(4-hydroxyphenyl)-propan-1-one (3h). Pale yellow solid (245 mg, 30%), mp 258–260 °C (lit.[19] mp 257–258 °C); IR (KBr): 3268, 1630, 1606, 1296, 1209, 1163, 1076 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.23 (2H, s, Ar-OH), 10.36 (1H, s, Ar-OH), 9.13 (1H, s, Ar-OH), 7.00 (2H, d, $J=8.3$ Hz, H-2′,6′), 6.65 (2H, d, $J=8.3$ Hz, H-3′,5′), 5.79 (2H, s, H-3″, 5″), 3.20 (2H, t, $J=7.8$ Hz, H-3), 2.74 (2H, t, $J=7.8$ Hz, H-2); ^{13}C NMR (DMSO-d_6): δ 204.4, 164.8, 164.4, 155.6, 131.9, 129.4, 115.3, 103.9, 94.9, 45.7, 29.6; MS (ESI, negative ion mode): *m/z* 273 $(M-H)^-$.

4.3. General procedure for homoisoflavonoids (4)

A mixture of **3** (3 mmol) and $BF_3\cdot Et_2O$ (1.2 mL, 9 mmol) was cooled to 10 °C and DMF (4.6 mL) was added drop wise for 5 min. In another flask, DMF (8 mL) was cooled to 10 °C and PCl_5 (0.939 g, 4.5 mmol) was added in small portions. The mixture was then allowed to stand to 55 °C for 20 min. The light yellow colored solution containing *N*,*N*′-dimethyl(chloromethylene)ammonium chloride was then added to the above reaction mixture slowly at 20–25 °C. The mixture was stirred at rt for 2 h and poured into boiling dil HCl slowly and cooled. The solution was extracted with EtOAc (3×50 mL) and the combined EtOAc layer was washed with water (20 mL), brine (20 mL) and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using chloroform–methanol mixtures as eluent to give **4a–h**.

4.3.1. 7-Hydroxy-3-[(4-hydroxyphenyl)methyl]-4*H*-chromen-4-one (4a). Colorless powder (680 mg, 85%), mp 212–214 °C; IR (KBr): 3430, 1628, 1600, 1267, 1244, 1176, 1140, 1099, 960 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.73 (2H, br s, Ar-OH), 8.04 (1H, s, H-2), 7.86 (1H, d, $J=8.8$ Hz, H-5), 7.06 (2H, d, $J=8.3$ Hz, H-2′,6′), 6.88 (1H, dd, $J=8.8$, 2.2 Hz, H-6), 6.79 (1H, d, $J=2.2$ Hz, H-8), 6.65 (1H, d, $J=8.3$ Hz, H-3′,5′), 3.53 (2H, s, H-9); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 176.3, 162.2, 157.8, 155.4, 152.2, 129.4, 128.9, 126.6, 123.8, 116.3, 115.1, 114.6, 102.0, 30.2; MS (ESI, negative ion mode): *m/z* 267 $(M-H)^-$. Analysis found: C, 71.59; H, 4.55%. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51%.

4.3.2. 7-Hydroxy-3-[(4-methoxyphenyl)methyl]-4*H*-chromen-4-one (4b). Light brown powder (740 mg, 88%), mp 162–164 °C; IR (KBr): 3433, 1631, 1248, 1161, 1132, 1096, 1034 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.55 (1H, s, Ar-OH), 7.96 (1H, s, H-2), 7.87 (1H, d, $J=8.8$ Hz, H-5), 7.19 (2H, d, $J=8.3$ Hz, H-2′,6′), 6.86 (1H, dd, $J=8.8$, 2.0 Hz, H-6), 6.80 (2H, d, $J=8.3$ Hz, H-3′,5′), 6.71 (1H, d, $J=2.0$ Hz, H-8), 3.72 (3H, s, Ar-OCH_3), 3.61 (2H, s, H-9); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 176.2, 162.2, 157.8, 157.6, 152.1, 130.6, 129.4, 126.6, 123.5, 116.3, 114.6, 113.4, 102.0, 54.7, 30.2; MS (ESI, negative ion mode): *m/z* 281 $(M-H)^-$. Analysis found: C, 72.29; H, 5.04%. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00%.

4.3.3. 7-Hydroxy-3-[(3,4-dihydroxyphenyl)methyl]-4*H*-chromen-4-one (4c). Colorless powder (695 mg, 82%), mp 192–194 °C; IR (KBr): 3393, 1627, 1239, 1180, 1113, 967 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.73 (1H, s, Ar-OH), 8.72 (1H, s, Ar-OH), 8.61 (1H, s, Ar-OH), 8.07 (1H, s, H-2), 7.86 (1H, d, $J=8.8$ Hz, H-5), 6.88 (1H, dd, $J=8.8$, 2.2 Hz, H-6), 6.79 (1H, d, $J=2.2$ Hz, H-8), 6.63 (1H, d, $J=2.1$ Hz, H-2′), 6.59 (1H, d, $J=8.3$ Hz, H-5′), 6.51 (1H, dd, $J=8.3$, 2.1 Hz, H-6′), 3.47 (2H, s, H-9); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 176.4, 162.1, 157.8, 152.3, 144.4, 142.9, 129.9, 126.6, 123.7, 119.8, 116.3, 115.7, 115.1, 114.6, 102.0, 30.3; MS (ESI, negative ion mode): *m/z* 283 $(M-H)^-$. Analysis found: C, 67.58; H, 4.29%. Calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26%.

4.3.4. 7-Hydroxy-3-[(3,4-dimethoxyphenyl)methyl]-4*H*-chromen-4-one (4d). Colorless powder (815 mg, 87%), mp 180–182 °C; IR (KBr): 3245, 1632, 1262, 1244, 1175, 1140, 1094, 1026, 963 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.62 (1H, s, Ar-OH), 8.01 (1H, s, H-2), 7.88 (1H, d, $J=8.8$ Hz, H-5), 6.78–6.90 (5H, m, Ar-H), 3.75 (3H, s, Ar-OCH_3), 3.72 (3H, s, Ar-OCH_3), 3.61 (2H, s, H-9); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 176.0, 162.3, 157.8, 152.4, 148.4, 147.1, 131.4, 126.6, 123.3, 120.5, 116.3, 114.7, 112.2, 111.2, 102.0, 55.4, 55.3, 30.6; MS (ESI, negative ion mode): *m/z* 311 $(M-H)^-$. Analysis found: C, 69.19; H, 5.21%. Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16%.

4.3.5. 7-Hydroxy-3-[(2,4-dimethoxyphenyl)methyl]-4*H*-chromen-4-one (4e). Colorless powder (730 mg, 78%), mp 182–184 °C; IR (KBr): 3224, 1631, 1241, 1207, 1158, 1124, 1040 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.72 (1H, s, Ar-OH), 7.86 (1H, d, $J=8.8$ Hz, H-5), 7.85 (1H, s, H-2), 7.02 (1H, d, $J=8.3$ Hz, H-6′), 6.88 (1H, dd, $J=8.8$, 2.0 Hz, H-6), 6.79 (1H, d, $J=2.0$ Hz, H-8), 6.51 (1H, d, $J=2.1$ Hz,

H-3′), 6.42 (1H, dd, $J=8.3$, 2.1 Hz, H-5′), 3.77 (3H, s, Ar-OCH_3), 3.70 (3H, s, Ar-OCH_3), 3.51 (2H, s, H-9); MS (ESI, negative ion mode): m/z 311 $(M-H)^-$. Analysis found: C, 69.18; H, 5.20%. Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16%.

4.3.6. 7,8-Dihydroxy-3-[(4-hydroxyphenyl)methyl]-4*H*-chromen-4-one (4f). Colorless powder (680 mg, 80%), mp 251–253 °C; IR (KBr): 3458, 1628, 1201, 1175, 1156, 1047, 985 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.17 (1H, s, Ar-OH), 9.15 (2H, br s, 2×Ar-OH), 8.14 (1H, s, H-2), 7.37 (1H, d, $J=8.6$ Hz, H-5), 7.07 (2H, d, $J=8.3$ Hz, H-2′,6′), 6.91 (1H, d, $J=8.6$ Hz, H-6), 6.65 (2H, d, $J=8.3$ Hz, H-3′,5′), 3.56 (2H, s, H-9); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 176.1, 155.6, 152.9, 149.9, 147.1, 132.9, 129.9, 129.5, 123.0, 117.1, 115.2, 115.0, 114.1, 30.0; MS (ESI, negative ion mode): m/z 283 $(M-H)^-$. Analysis found: C, 67.57; H, 4.30%. Calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26%.

4.3.7. 7,8-Dihydroxy-3-[(4-methoxyphenyl)methyl]-4*H*-chromen-4-one (4g). Colorless powder (725 mg, 81%), mp 252–254 °C; IR (KBr): 3320, 3150, 1631, 1241, 1172, 1155, 1047, 980 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.25 (1H, s, Ar-OH), 9.39 (1H, s, Ar-OH), 8.21 (1H, s, H-2), 7.38 (1H, d, $J=8.8$ Hz, H-5), 7.21 (2H, d, $J=8.3$ Hz, H-2′,6′), 6.92 (1H, d, $J=8.8$ Hz, H-6), 6.82 (1H, d, $J=8.3$ Hz, H-3′,5′), 3.70 (3H, s, Ar-OCH_3), 3.61 (2H, s, H-9); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 176.2, 157.4, 152.1, 149.4, 146.8, 132.5, 130.9, 129.3, 122.7, 117.0, 115.1, 113.7, 113.3, 54.6, 30.0; MS (ESI, negative ion mode): m/z 297 $(M-H)^-$. Analysis found: C, 68.41; H, 4.79%. Calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73%.

4.3.8. 5,7-Dihydroxy-3-[(4-hydroxyphenyl)methyl]-4*H*-chromen-4-one (4h). Pale yellow powder (725 mg, 85%), mp 215–217 °C (lit.[19] mp 218–219 °C); IR (KBr): 3283, 1667, 1620, 1314, 1282, 1233, 1174, 1053 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.73 (1H, s, Ar-OH), 10.83 (1H, s, Ar-OH), 9.19 (1H, s, Ar-OH), 8.13 (1H, s, H-2), 7.06 (1H, d, $J=8.3$ Hz, H-2′,6′), 6.65 (2H, d, $J=8.3$ Hz, H-3′,5′), 6.32 (1H, d, $J=2.0$ Hz, H-6), 6.17 (1H, d, $J=2.0$ Hz, H-8), 3.53 (2H, s, H-9); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 180.8, 164.0, 161.6, 157.8, 155.6, 153.3, 129.3, 128.5, 122.3, 115.1, 104.4, 98.7, 93.5, 29.3; MS (ESI, negative ion mode): m/z 283 $(M-H)^-$.

4.4. Antioxidant activity

4.4.1. Superoxide free radical scavenging activity. The superoxide free radical scavenging activity was determined by the NBT method.[24,25] The reaction mixture contained EDTA (6.6 mM), NaCN (3 μg), riboflavin (2 μM), NBT (50 μM), various concentrations of the test drug in ethanol and a phosphate buffer (58 mM, pH 7.8) in a final volume of 3 mL. Optical density was measured at 560 nm. The test tubes were uniformly illuminated with an incandescent lamp for 15 min, after which the optical density was measured again at 560 nm. The percent inhibition of superoxide radical generation was measured by comparing mean absorbance values of the control and those of the test substances. IC_{50} values were obtained from the plot drawn of concentration in μg versus percentage inhibition and were converted into μM. All the tests were run in triplicate and averaged.

4.4.2. DPPH free radical scavenging activity. DPPH radical scavenging activity was measured based on the reduction of methanolic solution of the colored DPPH.[26] Free radical scavenging ability of the test drug in ethanol added to the methanolic solution of DPPH is inversely proportional to the difference in initial and final absorption of DPPH solution at 516 nm. Drug activity is expressed as the 50% inhibitory concentration (IC_{50}). The reaction mixture contained 1×10^{-4} mM methanolic solution of DPPH and various concentrations of test drugs. The percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the plot, drawn for concentration in microgram versus percentage inhibition.

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