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Short communication

Synthesis of (±)Abyssinone I and related compounds: Their anti-oxidant and cytotoxic activities

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Abstract

An efficient and facile synthesis of naturally occurring prenylated flavonoids and their analogs have been described. Abyssinone I (**9a**) was prepared by condensing 2,2-dimethyl chrom-3-en-6-carboxaldehyde (**5a**) with protected resacetophenone under phase transfer conditions followed by deprotection and cyclization. The influence of prenyl group on anti-oxidant and cytotoxic activities was studied. The presence of 3′-prenyl group as in **8c** enhanced radical scavenging activity but decreased reducing power activity when compared to non-prenylated analog **8f**. In vitro testing in MCF-7 cell line revealed that prenylated chalcones and flavanones showed better inhibitory activity than their non-prenylated counterparts. Abyssinone I and its chalcone though exhibited negligible anti-oxidant activity their cytotoxic activities were comparable with other prenylated analogs.

Keywords: Abyssinone; Chalcone; Lipophilicity; Prenyl; Reducing power; Radical scavenging; MCF-7 cell line

1. Introduction

Flavonoids represent an important class of naturally occurring compounds and within the flavonoid class the prenylated derivatives are quite rich in structural variety and pharmacological activity. These compounds are known to exhibit a variety of biological activities such as anti-oxidant [1,2], anti-cancer [3], anti-viral [4,5], anti-inflammatory [6], anti-ulcer [7], anti-fungal [8] and also act as potential modulators in multidrug resistance [9–12]. Most of the above activities are influenced by the number and position of prenyl and hydroxyl groups present in the flavonoid ring [13]. Barron et al. [14] reported that substitution of the flavonoid ring system with prenyl groups increased the lipophilicity and conferred the molecule a strong affinity to biological membranes [15].

Abyssinones which are prenylated flavonoids/polyphenols were isolated from East African medicinal plant *Erythrina abyssinica* [16]. This plant has been widely used in folk medicine for treating malaria and syphilis [17] and methanolic extract of roots was found to possess anti-fungal and antibacterial properties [18]. Further Kinghorn et al. [19,20] reported the chemopreventive and aromatase inhibitory activity of prenylated flavanones. Because abyssinones exhibited the above biological activities many chemists turned their attention towards synthesis of these molecules. Recently, Moriarty et al. [21] have reported the aromatase inhibitory activity of Abyssinone II and its synthesis. Though Abyssinone I is isolated from natural source [16], there is no report of its synthesis or its biological activity and hence we have undertaken its synthesis and studied anti-oxidant and cytotoxic activities.

2. Result and discussion

Key intermediates used in the synthesis of Abyssinone I are 3,3-dimethyl allyl bromide (**2**), 4-hydroxy-3-(3-methylbut-2-enyl)

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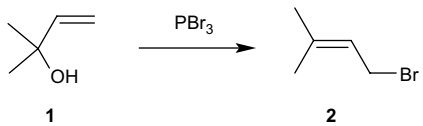
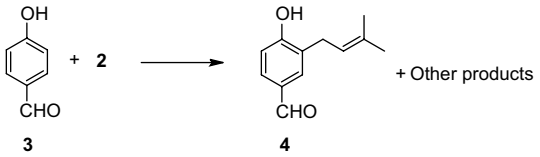
benzaldehyde (**4**), 2,2-dimethylchrom-3-enyl-6-carboxaldehyde (**5a**) and 2,4-bis (methoxymethoxy) acetophenone (**7**). Compound **2** was prepared (Scheme 1) in high purity using much cheaper 2-methyl-3-butene-2-ol instead of isoprene. Earlier reports of C-alkylation of phenols to make compound **4** suffer from set backs such as poor yields [22], scalability [23] and usage of alkyl lithium reagents [24]. Hence, we have modified the method reported by Moriarty et al. to improve the yield of compound **4** from 7.5 to 38% by simultaneous addition (portion wise) of alkali and 3,3-dimethyl allyl bromide to 4-hydroxybenzaldehyde (**3**) at room temperature (Scheme 2). Compound **4** on heating in toluene with 2,3-dichloro-5,6-dicyanobenzoquinone(DDQ) afforded **5a** (Scheme 3).

2,4-Dihydroxyacetophenone was efficiently protected with methoxymethyl (MOM) chloride using sodium hydride (NaH) in dimethylformamide (DMF) to get 2,4-bis (methoxymethoxy) acetophenone (**7**). Compound **8** was prepared (Scheme 4) by condensing compound **7** with **5a** in 50% aqueous potassium hydroxide (KOH) and methylene dichloride (MDC) using phase transfer catalyst at room temperature, which was then deprotected with 2 N HCl and cyclised using sodium acetate to give Abyssinone I (**9a**) (Scheme 5).

Apart from Abyssone I (**9a**), six different chalcones **8b–g** and corresponding flavanones **9b–g** including Abyssinone II (**9c**) were synthesized using different aldehydes **5b–g**. Compound **9e** was not taken up for further studies because of stability problem. Radical scavenging and reducing power assays for compounds **8a–g** and **9a–g** were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) by Blois [25] method and by Oyaizu [26] method, respectively. Chalcones **8a–g** were found to have better radical scavenging as well as reducing power activity than their respective flavanones **9a–g** (Fig. 1) which is in agreement with the general observation made by Miranda et al. [27]. Further, it was noticed that the 4′-hydroxy group of flavanone has exerted major influence on these activities. When the 4′-hydroxy group is free, the radical scavenging and reducing power activities are maximum and when it is blocked with methyl (**8d**, **9d**, **8g**, **9g**) or prenyl (**8e**) or any other alkyl group (**8a**, **9a**, **8b**, **9b**) these activities are minimal. Moreover, the presence of 3′-prenyl group as in **8c** enhances radical scavenging activity but decreases reducing power activity when compared to its non-prenylated analog **8f**. Anti-oxidant values of these compounds were measured at 200 μg concentrations and compared with trolox at concentrations of 200 μg for reducing power and 50 μg for DPPH assays. A study by Maiti et al. [24] revealed that flavones exhibited enhanced aromatase inhibitory activity compared to precursor chalcones, and also concluded that non-prenylated flavanones showed higher aromatase inhibitory activity compared to prenylated ones. Since it is known that chalcones



Scheme 1.



Scheme 2.

show better anti-oxidant properties over their flavanone counterparts, aromatase inhibitory activity and anti-oxidant activity are not complimentary to each other.

Compounds **8a–8g** and **9a–9d**, **9f** and **9g** were subjected to in vitro testing using breast carcinoma MCF-7 cell line for their cytotoxic activity (Fig. 2). It is observed that flavonoids containing prenyl groups in its ring system showed higher inhibitory activity than their non-prenylated analogs. This may be due to lipophilicity conferred to the molecule by the prenyl group and which may increase the affinity for the cell membrane.

In conclusion, we have synthesized Abyssinone I for the first time, also made related compounds including Abyssinone II, and studied their anti-oxidant and cytotoxic activities. Chalcones were found to have better radical scavenging as well as reducing power activity than their respective flavanones. Further prenylated flavonoids showed better cytotoxic activity than their non-prenylated analogs.

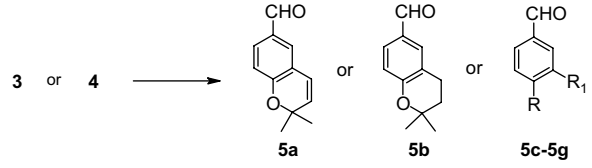
3. Experimental

3.1. General experimental procedures

Crystallizations were performed in chloroform and hexane mixtures. Melting points were determined on Acro melting point apparatus and are uncorrected. IR spectra were recorded on Thermo Nicolet FT-IR Instrument in KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 200 MHz instrument in $CDCl_3$ with tetramethylsilane as an internal standard. Mass spectra were recorded on Shimadzu-QP 2010 S GC–MS instrument. All chemicals and solvents used in the synthesis were of laboratory grade procured from local sources except for 2-methyl-3-butene-2-ol and DPPH which were obtained from Acros Organics (Belgium) and Sigma–Aldrich (USA), respectively.

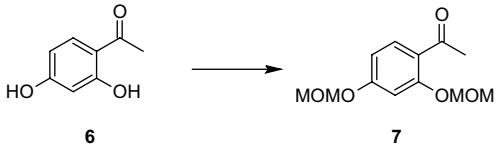
3.1.1. 3,3-Dimethyl allyl bromide (2)

To a stirred solution of phosphorous tribromide (780 g, 2.88 mol) in *n*-hexane (3.4 L) a mixture of 2-methyl-3-butane-2-ol (600 g, 6.98 mol) and pyridine (100 g, 1.27 mol) was



5c R = OMOM; R_1 = Prenyl 5e R = Oprenyl; R_1 = H 5g R = OMe; R_1 = H
5d R = OMe; R_1 = Prenyl 5f R = OMOM; R_1 = H

Scheme 3.



Scheme 4.

added drop wise over a period of 1–2 h by maintaining the temperature between 0 and 5 °C. Stirring was continued for a further period of 1 h at room temperature. Quenched the reaction mass in ice water (3.5 L), separated the hexane layer. Hexane layer was washed with 10% sodium bicarbonate solution, water and dried over anhydrous Na_2SO_4. Evaporated hexane under reduced pressure and distilled the residue under vacuum to get the product (450 g, 45%yield). GC–MS showed M^+ peak at *m/z* 148 with a base peak at *m/z* 69.

*3.1.2. 4-Hydroxy-3-(3-methylbut-2-enyl) benzaldehyde (**4**)*

4-Hydroxybenzaldehyde (200 g, 1.64 mol) was dissolved in aqueous potassium hydroxide (108 g in 1080 mL water, 1.64 mol) solution at room temperature. To this solution, 3,3-dimethyl allyl bromide (340 g, 2.28 mol) and aqueous potassium hydroxide (108 g in 1080 mL water, 1.64 mol) were added parallely, in portions at 1 h intervals over 48 h under vigorous stirring at around 30 °C. After the complete addition, the reaction mass was basified further with potassium hydroxide (1.64 mol, 108 g in 1080 mL water) extracted with toluene (2 × 750 mL) to remove *O*-prenylated products. The aqueous layer was then acidified with acetic acid at ice cold to pH 5.0 and extracted product into ethyl acetate (3 × 500 mL). Organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get the product (150 g). The crude product obtained was column chromatographed on silica gel eluting with 9% ethyl acetate in hexane to afford the pure product (120 g, 38% yield) mp 66–68 °C; GC–MS showed M^+ peak at *m/z* 190 with a base peak at *m/z* 135.

*3.2. General procedure for the preparation of compounds **5c–f***

To a stirred solution of respective aldehyde (**3** or **4**) in DMF (50 mL) sodium hydride (2.0 M equiv.) was added in portions

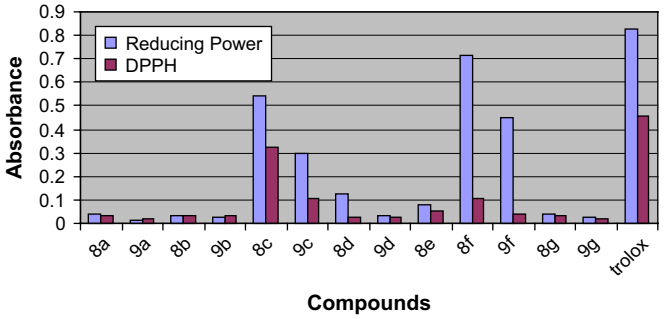


Fig. 1. Absorbance values of reducing power and radical scavenging activity.

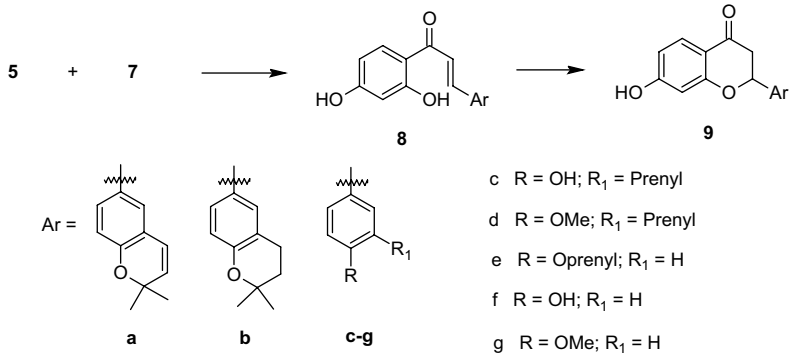
by maintaining temperature at 10 °C. Stirred the reaction mass for further 10 min and the respective alkyl halide (2.0 M equiv.) was added drop wise over 10 min at ice cold and then continued stirring at room temperature for further 1 h. Solvent was evaporated under reduced pressure, quenched reaction mass in cold water and extracted product into toluene. Organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get the product. The crude product obtained was column chromatographed on silica gel eluting with 6% ethyl acetate in hexane to afford the pure product.

*3.2.1. 2,2-Dimethylchrom-3-ene-6-carboxaldehyde (**5a**)*

Compound **4** (5 g, 26.3 mmol) and DDQ (8 g, 35.2 mmol) were taken in toluene heated at reflux for 2 h. Reaction mass was cooled to room temperature, filtered insoluble solids and washed solids thoroughly with toluene. The filtrate obtained was washed with 2% NaOH solution (10 mL) and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get the product (3.5 g, 71%yield): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.46 (6H, s), 5.68 (1H, d, $J = 10$ Hz), 6.36 (1H, d, $J = 10$ Hz), 6.85 (1H, d, $J = 8.4$ Hz), 7.50 (1H, d, $J = 2.0$ Hz), 7.63 (1H, dd, $J = 8.4$, 2.0 Hz), 9.82 (1H, s).

*3.2.2. 2,2-Dimethylchroman-6-carboxaldehyde (**5b**)*

Compound **4** (5 g, 26.3 mmol) and *p*-toluene sulphonic acid (0.2 g) were taken in toluene heated at reflux for 2 h. The reaction mass was cooled to room temperature, washed with 2%



Scheme 5.

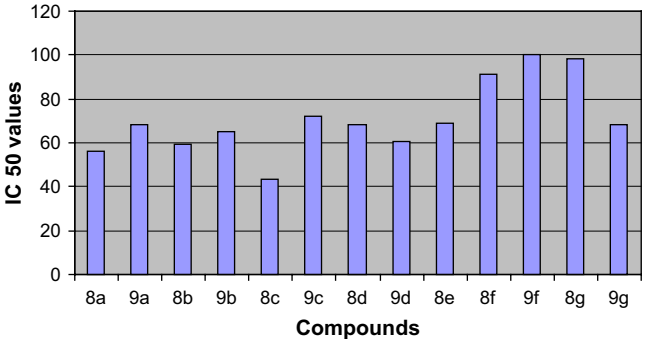


Fig. 2. Cytotoxic activity of compounds (**8a–8g** and **9a–9g**) on MCF-7 (breast adeno carcinoma) cell line. IC 50 (μM solutions); determined by MTT assay; (values are average of at least two independent experiments ±10% (SD) conducted in triplicate for each concentration).

NaOH solution (10 mL) and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get the product (4.5 g, 90%yield): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.36 (6H, s), 1.83 (2H, t, $J = 6.8$ Hz), 2.81 (2H, t, $J = 6.8$ Hz), 6.86 (1H, d, $J = 8.8$ Hz), 7.62 (2H, m), 9.81 (1H, s).

*3.2.3. 4-Methoxymethoxy-3-(3-methylbut-2-enyl) benzaldehyde (**5c**)*

To a stirred solution of compound **4** (90 g, 0.47 mol) in DMF (500 mL), sodium hydride (45 g, 0.93 mol) was added in portions at around 10 °C. Stirred the reaction mass for further 10 min and chloromethyl methyl ether (80 g, 1.0 mol) was added slowly over a period of 1 h at ice cold and then continued stirring for further 1 h at room temperature. Distilled off DMF under reduced pressure quenched the reaction mass in cold water and extracted with toluene (4 × 250 mL). Organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get the product. The crude product obtained was subjected to silica gel column chromatography and on elution with 6% ethyl acetate in hexane afforded pure product (98 g, 89% yield). ^{1}H NMR ($CDCl_3$, 200 MHz), δ 1.72 (3H, s), 1.75 (3H, s), 3.37 (2H, d, $J = 7.5$ Hz), 3.49 (3H, s), 5.29 (2H, s), 5.30 (1H, t, $J = 7.5$ Hz), 7.16 (1H, d, $J = 9.0$ Hz), 7.68 (1H, dd, $J = 2.5$, 9.0 Hz), 7.70 (1H, d, $J = 2.5$ Hz), 9.87 (1H, s).

*3.2.4. 4-Methoxy-3-(3-methylbut-2-enyl) benzaldehyde (**5d**)*

Compound **4** (5 g, 26.3 mmol), sodium hydride (2.5 g, 52 mmol), iodomethane (3.7 g, 54 mmol) and product (5 g, 94% yield); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.70 (3H, s), 1.75 (3H, s), 3.34 (2H, d, $J = 7.4$ Hz), 3.92 (3H, s), 5.30 (1H, t, $J = 7.4$), 6.94 (1H, d, $J = 8.4$ Hz), 7.68 (1H, d, $J = 2.4$ Hz), 7.71 (1H, dd, $J = 2.4$, 8.4 Hz), 9.85 (1H, s).

*3.2.5. 4-(3-Methylbut-2-enyl)oxy phenyl benzaldehyde (**5e**)*

Compound **3** (5 g, 40 mmol), sodium hydride (3.9 g, 84.7 mmol), prenyl bromide (12.2 g, 81.8 mmol) and product (6.5 g, 84% yield).

*3.2.6. 4-Methoxymethoxybenzaldehyde (**5f**)*

Compound **3** (5 g, 40.9 mmol), sodium hydride (3.9 g, 84.7 mmol), chloromethyl methyl ether (6.6 g, 80 mmol) and product (6 g, 88% yield).

*3.2.7. 2,4-bis (Methoxymethoxy) acetophenone (**7**)*

To a stirred solution of 2,6-dihydroxy acetophenone (150 g, 0.99 mol) in DMF (1.2 L), sodium hydride (94.7 g, 2.05 mol) was added in portions at around 10 °C. Stirred the reaction mass for further 10 min and chloromethyl methyl ether (169 g, 2.1 mol) was added slowly over a period of 1 h at ice cold and then continued stirring at room temperature for further 1 h. Distilled off DMF under reduced pressure, quenched the reaction mass in cold water and extracted with toluene (4 × 250 mL). Organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get the product. The crude product obtained was subjected to silica gel column chromatography and on elution with 6% ethyl acetate in hexane afforded pure product (201 g, 85% yield) ^{1}H NMR ($CDCl_3$, 200 MHz), δ 2.60 (3H, s), 3.48 (3H, s), 3.52 (3H, s), 5.20 (2H, s), 5.27 (2H, s), 6.72 (1H, dd, $J = 2.0$, 8.6 Hz), 6.82 (1H, d, $J = 2.0$ Hz), 7.78 (1H, d, $J = 8.6$ Hz).

*3.3. General procedure for the preparation of compounds **8a–g***

To hydroxy protected aldehydes (**5a–5g**) in MDC and 50% aqueous potassium hydroxide solution (1:1) were added compound **7** and tetra butyl ammonium sulphate (5 mol%). The reaction mass was stirred at room temperature till starting materials disappeared (24–72 h). MDC layer was separated, washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get thick mass. The material obtained was dissolved in a mixture of methanol and MDC (40 mL:10 mL) and 2 N HCl (5 mL) was added under stirring at room temperature. After 24 h stirring, evaporated solvent under reduced pressure and quenched reaction mass in water. Extracted the mixture with MDC and organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent to get the product. The crude product obtained was subjected to silica gel column chromatography and on elution with 12–15% ethyl acetate in hexane afforded the pure product.

*3.3.1. 1-(2,4-Dihydroxyphenyl)-3-(2,2-dimethylchrom-3-ene-6yl)-prop-2-en-1-one (**8a**)*

To compound **5a** (4 g, 21.2 mmol) in MDC and 50%KOH (20 mL:20 mL) were added compound **7** (5 g, 20.8 mmol) and 0.2 g of phase transfer catalyst. The reaction mass was stirred for 48 h and separated layers. Organic layer was washed with water (3 × 50 mL), dried under anhydrous Na_2SO_4 and evaporated under reduced pressure to get thick mass. The material obtained was dissolved in a mixture of methanol and MDC (40 mL:10 mL) and 5 mL of 2 N HCl was added at room temperature. The reaction mass was stirred at room temperature for 24 h, evaporated solvent under reduced pressure and quenched in water (20 L). Extracted the

product with MDC (2 × 50 mL) and organic layer was dried over anhydrous Na_2SO_4 and on evaporation of solvent obtained the product. The crude product obtained was subjected to silica gel column chromatography to get pure product (5 g, 75% yield); mp 180–183 °C; IR (KBr) υ_{max} 3193, 2931, 1631, 1600, 1550, 1488, 1361, 1307, 1226, 1141, 1103, 1026 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.47 (6H, s), 5.68 (1H, d, $J = 8.8$ Hz), 5.92 (1H, s), 6.36 (1H, d, $J = 8.8$ Hz), 6.46 (m, 2H), 6.81 (1H, d, $J = 8.4$ Hz), 7.28 (1H, d, $J = 2.0$ Hz), 7.42 (1H, d, $J = 15.4$ Hz), 7.44 (1H, dd, $J = 2.0$, 8.4 Hz), 7.82 (1H, d, $J = 15.4$ Hz), 7.83 (1H, d, $J = 8.4$ Hz), 13.53 (1H, s); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 28.20, 77.20, 103.65, 107.55, 115.22, 116.85, 117.38, 121.32, 121.52, 126.61, 127.39, 130.04, 131.36, 131.78, 144.58, 156.52, 162.41, 166.24, 191.84.

3.3.2. 1-(2,4-Dihydroxyphenyl)-3-(2,2-dimethylchroman-6yl)-prop-2-en-1-one (**8b**)

Compound **5b** (4 g, 21 mmol), compound **7** (5 g, 20.8 mmol), PTC (0.2 g) and product (5 g, 75% Yield); mp 188–189 °C; IR (KBr) υ_{max} 3209, 2931, 1631, 1600, 1546, 1488, 1372, 1307, 1230, 1145, 1118, 1026 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.36 (3H, s), 1.84 (2H, t, $J = 6.6$ Hz), 2.81 (2H, t, $J = 6.6$ Hz), 5.99 (1H, br s), 6.43 (2H, m), 6.81 (1H, d, $J = 8.4$ Hz), 7.41 (1H, d, $J = 15.0$ Hz), 7.43 (2H, d, $J = 9.6$ Hz), 7.82 (1H, d, $J = 15.0$ Hz), 7.83 (1H, d, $J = 9.6$ Hz); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 22.24, 26.82, 32.45, 75.28, 103.65, 107.55, 114.49, 116.91, 117.97, 121.37, 126.39, 127.88, 130.75, 131.79, 145.03, 156.83, 162.39, 166.21,191.99.

3.3.3. 1-(2,4-Dihydroxyphenyl)-3-[4-hydroxy-3-(3-methylbut-2-enyl) phenyl]-prop-2-en-1-one (**8c**)

Compound **5c** (5 g, 21.3 mmol), compound **7** (5 g, 20.8 mmol), PTC (0.2 g) and product (4.5 g, 67% yield): mp 153–155 °C; IR (KBr) υ_{max} 3413, 3313, 2927, 2854, 1627, 1600, 1550, 1504, 1431, 1373, 1242, 1137, 1103, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.57 (3H, s), 1.81 (3H, s), 3.40 (2H, d, $J = 7.0$ Hz), 5.33 (1H, m), 6.41 (1H, s), 6.42 (1H, m), 6.85 (1H, d, $J = 8.2$ Hz), 7.42 (3H, m), 7.84 (2H, m), 13.48 (1H, s); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 20.09, 27.88, 30.60, 104.95, 110.43, 115.36, 117.68, 119.44, 125.02, 128.06, 130.76, 131.20, 133.30, 133.71, 135.18, 147.00, 160.46, 167.27, 168.16, 193.85.

3.3.4. 1-(2,4-Dihydroxyphenyl)-3-[4-methoxy-3-(3-methylbut-2-enyl) phenyl]-prop-2-en-1-one (**8d**)

Compound **5d** (5 g, 24.5 mmol), compound **7** (5 g, 20.8 mmol), PTC (0.2 g) and product (5 g, 71%yield): mp 150–154 °C; IR (KBr) υ_{max} 3163, 2916, 1624, 1593, 1542, 1500, 1373, 1261, 1226, 1141, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.74 (3H, s), 1.77 (1H, s), 3.34 (2H, d, $J = 7.2$ Hz), 3.89 (3H, s), 5.30 (1H, t, $J = 7.2$ Hz), 6.37 (1H, s), 6.44 (1H, dd, $J = 8.6$, 2.0 Hz), 6.88 (1H, d, $J = 8.6$ Hz), 7.45 (3H, m), 7.84 (1H, d, $J = 9.4$ Hz), 7.86 (1H, d, $J = 15.4$ Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 17.87, 25.86, 28.49, 55.60, 103.79, 107.58, 110.46, 114.74, 117.44, 121.84, 127.20, 128.44, 129.78, 130.97, 131.92, 133.09, 145.15, 159.81, 162.39, 166.41,192.14.

3.3.5. 1-(2,4-Dihydroxyphenyl)-3-[(4-(3-methylbut-2-enyl)oxyphenyl)]-prop-2-en-1-one (**8e**)

Compound **5e** (4.5 g, 23.7 mmol), compound **7** (5 g, 20.8 mmol), PTC (0.2 g) and product (3 g, 44% yield): mp 155–158 °C; IR (KBr) υ_{max} 3163, 2920, 1627, 1604, 1546, 1508, 1373, 1292, 1226, 1172, 1145, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.76 (3H, s), 1.81 (3H, s), 4.57 (2H, d, $J = 6.6$ Hz), 5.49 (1H, t, $J = 6.6$ Hz), 6.44 (2H, m), 6.95 (2H, d, $J = 8.8$ Hz), 7.44 (1H, d, $J = 15.44$ Hz), 7.60 (2H, d, $J = 8.6$ Hz), 7.83 (1H, d, $J = 9.2$ Hz), 7.86 (1H, d, $J = 15.4$ Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.13, 25.70, 64.90, 103.66, 107.46, 114.53, 115.08, 117.55, 119.02, 127.28, 130.27, 131.75, 138.70, 144.48, 161.12, 162.30, 166.29, 191.91.

3.3.6. 1-(2,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl)-prop-2-en-1-one (**8f**)

Compound **5f** (4 g, 24 mmol), compound **7** (5 g, 20.8 mmol), PTC (0.2 g) and product (4 g, 75% yield): mp 190–195 °C; IR (KBr) υ_{max} 3379, 3282, 1627, 1542, 1512, 1288, 1207, 1141, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.38 (2H, m), 6.83 (2H, d, $J = 8.4$ Hz), 7.35 (1H, d, $J = 15.4$ Hz), 7.46 (2H, d, $J = 8.4$ Hz), 7.72 (1H, d, $J = 9.4$ Hz), 7.75 (1H, d, $J = 15.4$ Hz), 9.21 (1H, br s), 9.74 (1H, br s), 13.48 (1H, s); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 103.06, 108.56, 113.48, 116.32, 117.89, 126.24, 131.69, 133.32, 144.74, 160.74, 165.42, 166.26, 192.01.

3.3.7. 1-(2,4-Dihydroxyphenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one (**8g**)

Compound **5g** (3.5 g, 25.7 mmol), compound **7** (5 g, 20.8 mmol), PTC (0.2 g) and product (4.5 g, 80% yield): mp 180–183 °C; IR (KBr) υ_{max} 3301, 2923, 1635, 1600, 1562, 1512, 1365, 1292, 1215, 1172, 1126, 1033 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.81 (3H, s), 6.38 (2H, m), 6.88 (2H, d, $J = 8.8$ Hz), 7.40 (1H, d, $J = 15.4$ Hz), 7.55 (2H, d, $J = 8.8$ Hz), 7.73 (1H, d, $J = 9.4$ Hz), 7.77 (1H, d, $J = 15.4$ Hz), 9.78 (1H, s), 13.42 (1H, s); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 55.88, 103.06, 108.62, 113.49, 114.92, 119.04, 127.75, 131.46, 133.45, 144.24, 161.95, 165.52, 166.28, 192.00.

3.4. General procedure for the preparation of compounds **9a–g**

Chalcones **8a–g** (10 mmol) and sodium acetate (20 mmol) were taken in 10% aqueous ethanol and heated to reflux for 24–48 h. Evaporated ethanol under reduced pressure diluted the reaction mass with cold water and extracted with MDC (3 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get the product. The product obtained mainly contains equal amount of products and starting materials which were separated by column chromatography on silica gel, eluting with 15–25% ethyl acetate in hexane.

*3.4.1. 2-(2′,2′-Dimethyl chrom-3′-en-6′-yl)-7-hydroxy chroman-4-one (**9a**)*

Chalcone **8a** (3 g, 9.2 mmol) and sodium acetate (1.5 g, 20 mmol) were taken in 10% aqueous ethanol and heated to reflux for 24 h. Evaporated ethanol under reduced pressure diluted the reaction mass with cold water and extracted with MDC (3 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated solvent under reduced pressure to get the product. The product obtained mainly contains equal amount of products and starting material which was separated by column chromatography on silica gel, eluting with 15% ethyl acetate in hexane (1.3 g, 43% yield): mp 161–165 °C; IR (KBr) υ_{max} 3039, 2927, 2715, 1651, 1569, 1492, 1353, 1245, 1164, 1118, 1060 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.44 (6H, s), 2.79 (1H, dd, $J = 3.0$, 17.0 Hz), 3.06 (1H, dd, $J = 17.0$, 13.2 Hz), 5.35 (1H, dd, $J = 3.0$, 13.2 Hz), 5.65 (1H, d, $J = 9.8$ Hz), 6.07 (1H, br s), 6.33 (1H, d, $J = 9.8$ Hz), 6.45 (1H, d, $J = 2.0$ Hz), 6.54 (1H, dd, $J = 8.6$, 2.0 Hz), 6.81 (1H, d, $J = 8.2$ Hz), 7.08 (1H, d, $J = 2.0$ Hz), 7.19 (1H, dd, $J = 2.0$, 8.2 Hz), 7.85 (1H, d, $J = 8.6$ Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.15, 44.10, 77.82, 79.79, 103.48, 110.61, 115.09, 116.60, 121.49, 121.98, 124.48, 127.26, 129.46, 130.81, 131.41, 153.48, 162.89, 163.74, 191.24.

*3.4.2. 2-(2′,2′-Dimethyl chroman-6′-yl)-7-hydroxy chroman-4-one (**9b**)*

Compound **8b** (3 g, 9.2 mmol), sodium acetate (1.5 g, 18.2 mmol), product (1.35 g, 45% yield): mp 155 °C; IR (KBr) υ_{max} 2927, 2854, 1651, 1573, 1500, 1353, 1330, 1242, 1153, 1114, 1060 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.35 (6H, s), 1.83 (2H, t, $J = 6.6$ Hz), 2.76 (3H, m), 3.06 (1H, dd, $J = 17.0$, 13.4 Hz), 5.35 (1H, dd, $J = 2.6$, 13.4 Hz), 6.48 (1H, d, $J = 2.0$ Hz), 6.57 (1H, dd, $J = 8.6$, 2.0 Hz), 6.82 (1H, d, $J = 9.0$ Hz), 7.17 (1H, s), 7.30 (1H, d, $J = 8.0$ Hz), 7.86 (1H, d, $J = 8.6$ Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 22.42, 26.77, 32.56, 43.86, 74.55, 79.82, 103.36, 110.68, 114.59, 114.45, 117.51, 121.16, 125.57, 127.69, 129.32, 154.50, 163.46, 163.89, 191.92.

*3.4.3. 7-Hydroxy-2-[4′-hydroxy-3′-(3-methylbut-2-enyl)phenyl]chroman-4-one (**9c**)*

Mixture of chalcone **8c** (25 g, 77 mmol) and sodium acetate (10 g, 120 mmol) was taken in aqueous ethanol (90:10 ethanol:water, 100 mL) and heated to reflux for 24 h. After that ethanol was removed under reduced pressure, quenched the reaction mass with ice-cold water (100 mL) and extracted with ethyl acetate (4 × 100 mL). Ethyl acetate extract was washed with water and dried over anhydrous sodium sulphate. On evaporation of ethyl acetate a solid residue was obtained which was subjected to silica gel column chromatography. The pure product (14 g, 56% yield) thus obtained was crystallized as pale yellow crystals from chloroform/*n*-hexane. mp 120–122 °C; IR (KBr) υ_{max} 3282, 2896, 2854, 1670, 1612, 1581, 1469, 1434, 1276, 1157, 1126 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.71 (3H, s), 1.74 (3H, s), 2.67 (1H, dd, $J = 2.6$, 16.8 Hz), 3.01 (1H, dd, $J = 16.8$, 8.6 Hz), 3.32 (2H, d, $J = 7.0$ Hz), 5.31 (2H, d, $J = 9.8$ Hz), 6.41 (1H, s), 6.51 (1H, d, $J = 8.6$ Hz), 6.86 (1H, d, $J = 8.0$ Hz), 7.12 (1H, d, $J = 8.0$ Hz), 7.60 (1H, s), 7.73 (1H, d, $J = 8.6$ Hz), 8.92 (1H, s), 9.99 (1H, s); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 20.03, 27.91, 30.49, 45.53, 81.49, 104.93, 112.81, 115.94, 117.03, 125.07, 127.81, 129.81, 130.53, 130.76, 131.63, 133.68, 157.56, 165.52, 166.96, 192.46.

*3.4.4. 7-Hydroxy-2-[4′-methoxy-3′-(3-methylbut-2-enyl) phenyl]chroman-4-one (**9d**)*

Compound **9d** (2.5 g, 7.4 mmol) and sodium acetate (1.2 g, 14.6 mmol), product (1.25 g, 50% yield): mp 135–139 °C; IR (KBr) υ_{max} 3325, 2958, 2850, 1662, 1593, 1500, 1461, 1323, 1215, 1122, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.70 (3H, s), 1.74 (3H, s), 2.78 (1H, dd, $J = 3.0$, 17.0 Hz), 3.07 (1H, dd, $J = 17.0$, 13.2 Hz), 3.33 (2H, d, $J = 7.4$ Hz), 3.85 (3H, s), 5.38 (1H, dd, $J = 3.0$, 13.2 Hz), 5.79 (1H, br s), 6.44 (1H, d, $J = 2.2$ Hz), 6.53 (1H, dd, $J = 2.2$, 8.6 Hz), 6.88 (1H, d, $J = 8.2$ Hz), 7.22 (1H, s), 7.27 (1H, d, $J = 8.2$ Hz), 7.85 (1H, d, $J = 8.6$ Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 17.82, 25.85, 28.50, 44.12, 55.56, 79.98, 103.49, 110.33, 110.44, 115.24, 122.02, 125.13, 127.63, 129.44, 130.39, 130.49, 130.76, 157.55, 162.61, 163.78, 191.22.

*3.4.5. 7-Hydroxy-2-(4′-hydroxyphenyl) chroman-4-one (**9f**)*

Compound **8f** (4 g, 15.6 mmol) and sodium acetate (2.5 g, 30.5 mmol), product (1.6 g, 40% yield): mp 169–170 °C; IR (KBr) υ_{max} 3031, 2920, 2827, 1654, 1600, 1515, 1469, 1330, 1234, 1164, 1118 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.52–2.95 (3H, m), 5.18 (1H, d, $J = 13$ Hz), 6.27 (1H, s), 6.38 (1H, d, $J = 8.4$ Hz), 6.73 (2H, dd, $J = 2.0$, 7.2 Hz), 7.13 (2H, dd, $J = 2.0$, 7.2 Hz), 7.60 (1H, dd, $J = 2.6$, 8.4 Hz), 8.90 (1H, br s), 9.9 (1H, br s); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 43.64, 79.44, 103.03, 110.96, 114.01, 115.59, 128.73, 128.87, 129.79, 158.08, 163.65, 165.08, 190.58.

*3.4.6. 7-Hydroxy-2-(4′-methoxyphenyl) chroman-4-one (**9g**)*

Compound **8g** (4 g, 14.8 mmol) and sodium acetate (2.4 g, 29.2 mmol), product (1.8 g, 45% yield): mp 155–163 °C; IR (KBr) υ_{max} 3305, 2908, 1662, 1589, 1515, 1323, 1215, 1176, 1110, 1033 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.8 (1H, dd, $J = 3.0$, 16.8 Hz), 3.07 (1H, dd, $J = 16.8$, 13.0 Hz), 3.85 (3H, s), 5.41 (1H, dd, $J = 13.0$, 3.0 Hz), 6.01 (1H, br s), 6.45 (2H, m), 6.41 (1H, dd, $J = 2.2$, 8.6 Hz), 6.95 (2H, d, $J = 8.6$ Hz), 7.39 (2H, d, $J = 8.6$ Hz), 7.86 (1H, d, $J = 8.8$ Hz); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 43.09, 55.06, 78.63, 102.49, 110.44, 113.47, 113.75, 128.07, 128.31, 130.98, 159.26, 162.99, 164.53, 189.85.

4. Anti-oxidant assay

4.1. Reducing power assay

The reducing power assays of flavonoid compounds were determined by the method of Oyaizu. Samples of respective compounds (200 μg, 0.6–0.78 μM) in 1 mL of milli Q water were mixed with phosphate buffer (2.5 mL, 0.2 mM, pH 6.6) and potassium ferricyanide solution (2.5 mL, 1% w/v). The

mixture was incubated at 50 °C for 20 min, followed by the addition of aqueous trichloroacetic solution (2.5 mL, 10% w/v). The mixture was then centrifuged at 5000 rpm for 10 min using refrigerated bench top centrifuge (Rotina-38R). The upper layer of the solution (2.5 mL) was mixed with milli Q water (2.5 mL) and ferric chloride solution (0.5 mL, 0.1%w/v), and the absorbance was measured at 700 nm using UV–vis spectrophotometer (CARY 50 Bio). Increased absorbance of the reaction mixture indicates the increased reducing power. All experiments were performed in triplicate.

4.2. Radical scavenging assay

DPPH-scavenging activity was measured according to the procedure described by Blois. A total of 200 μg (40 μL of 5 mg of compound in 1 mL of methanol) of sample was added to 960 μL of freshly prepared DPPH solution (0.004% in methanol), and the mixture vortexed for 15 s. The decrease in absorbance at room temperature was determined at 515 nm using UV–vis spectrophotometer (CARY 50 Bio) after 30 min of incubation. All experiments were performed in triplicate. The difference in absorbance (Δ abs) was measured using formula $A_s - A_0$ where A_0 an A_s are the absorbances of the control and sample, respectively.

5. In vitro cell growth inhibition assay

The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) [Sigma–Aldrich Inc., USA] supplemented with 10% fetal bovine serum [Sigma–Chemical Co., USA] in a CO_2 incubator. The cytotoxicity of the compounds was measured by MTT assay [28]. The cells were plated in a 96-well plate at the density of 8000 cells/well for MCF-7. After 24 h, cell culture media were replaced with DMEM containing 0.1% FBS and the cells were treated with different concentrations of the compounds (25–150 μM). The cells were later incubated for 72 h. The cytotoxicity was measured by adding 5 mg/mL of MTT [Sigma–Aldrich Inc., USA] to each well and incubated for another 3 h. The purple formazan crystals were dissolved by adding 100 μl of DMSO to each well. The absorbance was read at 570 nm in a spectrophotometer [Spectra Max 340]. The cell death was calculated as follows.

Cell death = 100 − [(test absorbance/control absorbance) × 100].

The test result is expressed as the concentration of a test compound which inhibits the cell growth by 50% (IC_{50}).

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Appendix. Supporting information

The ^{1}HNMR, ^{13}C NMR and Mass spectroscopic data and data supporting anti-oxidant and cytotoxic activities of compounds **8a–8g**, **9a–9d**, **9f** and **9g** are available free of charge via the Internet at http://www.sciencedirect.org. Supplementary data associated with this article can also be found in the online version, at doi: 10.1016/j.ejmech.2008.05.032.

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