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Synthetic and Biological Activity Evaluation Studies on Novel 1,3-Diarylpropenones

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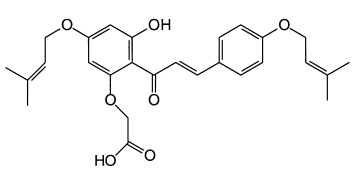
Abstract—Fourteen novel *C*-prenylated and *O*-allylated 1,3-diarylpropenones (chalcones) were synthesized by Claisen–Schmidt condensation reaction of *C*-prenylated/*O*-allylated acetophenones with appropriate aldehydes; twelve of these model chalcones were screened in an assay based on the confrontation of invasive human MCF-7/6 mammary carcinoma cells with fragments of normal embryonic chick heart in vitro. Out of the twelve chalcones tested, three were found to exhibit potent anti-invasive activity. Some of these chalcones and their precursor acetophenones were also tested for inhibition of initiation of lipid peroxidation in rat liver microsomes; a prenylated acetophenone carrying two methoxy groups and two free phenolic hydroxy functions was found to be a potential antioxidant.

Introduction

There is currently a great deal of interest in the health benefits of phytochemicals, in particular prenylated (3-methyl-2-butenylated) flavonoids, because of their interesting biological activities. 1,3-Diarylpropenones (chalcones) constitute an important group of natural products and some of them possess a wide range of biological activities, such as antibacterial,[1,2] antifungal,[3–6] anti-inflammatory,[7,8] antimicrobial,[9–12] antitumour,[13–15] insect antifeedant,[16] antimutagenic[17] and inhibitor of adenosine 3′,5′-cyclic monophosphate phosphodiesterase.[18] Certain prenylated chalcones isolated from *Sophora subprostrata* have been studied in polorus-ligated, stress-induced ulcers in rat and found to possess potent inhibitory action on ulcer formation.[19,20] A di-*O*-prenylated chalcone **1**, synthesized by Kyogoku et al.,[21] is a clinical candidate for the treatment of ulcers. Chalcones also serve as precursors for the synthesis of different classes of flavonoids, *viz*. dihydrochalcones, flavanones, flavones, flavonols, aurones and isoflavanones.[22–25] Flavone acetic acid, a compound of the same family, is an antitumour agent against murine adenocarcinoma cells in vivo and this compound has undergone phase I and II clinical trials.[26–28] Chalcones have found uses in the production of nematic liquid crystals,[29] photosensitive polymers[30] and as antioxidants.[31,32]



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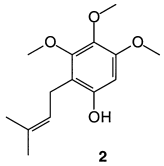

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In our laboratory, we are actively engaged in the synthesis of prenylated chalcones and their cyclic analogues and some of them were found to possess moderate to good antimicrobial, antiviral, anti-invasive and anti-insecticidal activities.[33,34] A prenylated chalcone, 1-[2,4-dimethoxy-5-(3-methyl-2-butenyl)phenyl]-3-phenylpropenone, synthesized by base-catalysed condensation of 2,4-dimethoxy-5-(3-methyl-2-butenyl)acetophenone with benzaldehyde, has been found to exhibit appreciable anti-invasive activity at concentrations ranging from 1

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to 100 μM and 3,7-dimethoxyflavone has been discovered to exhibit anti-invasive activity even at 1 μM concentration.[33] At these concentrations, no cytotoxic effects could be detected. The anti-invasive effect was reversible upon omission of the molecule. We are also engaged in the isolation of bioactive compounds from traditional medicinal plants; 2-(3-methyl-2-butenyl)-3,4,5-trimethoxyphenol (**2**), isolated from *Piper clarkii*, has exhibited anti-invasive activity against human breast carcinoma cells at 100 μM concentration.[35,36] Owing to aforementioned activities of chalcones and prenylated phenols, we undertook the synthesis of quite a few chalcones from the acetophenone derived from the phenol **2**, i.e., the *C*-prenylated acetophenone **8** and the *O*-allylated acetophenone **20**, and have accomplished the synthesis of fourteen novel chalcones, *viz*. **9**–**19** and **21**–**23**.

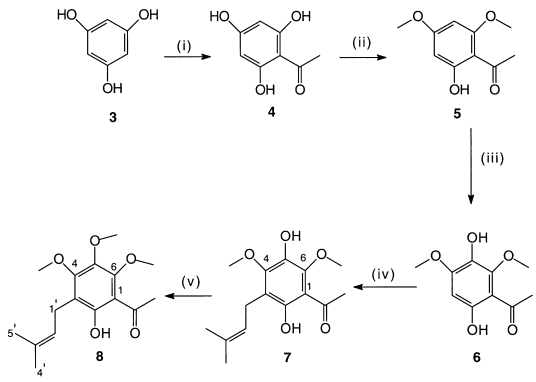
Invasion is the hallmark of malignant tumours and generally leads to metastasis, which is the major cause of death of cancer patients. Anti-invasive agents are being studied for both the development of new therapeutic rationales in cancer treatment and the analysis of tumour invasion mechanisms. In recent years, the role of oxidative stress in mechanism of tumour promotion has been established. Reactive oxygen species induce membrane damage, DNA base oxidation, DNA strand breaks, chromosomal aberrations and protein alterations leading to diverse pathologies, such as carcinogenesis and ageing. For instance, organic peroxides and free radical generators have tumour-promoting activities and, on the contrary, antioxidants and free radical scavengers inhibit the biochemical and biological effects of tumour promoters. Twelve model chalcones, *viz*. **9**, **11**, **12**, **14**–**19**, and **21**–**23** synthesized above, have been tested for their anti-invasive activity against solid tumours and three of them, i.e., compounds **11**, **16**, and **23**, were found to exhibit considerable activity. Further, three acetophenones, i.e., **7**, **8**, and **20**, and four chalcones, i.e., **9**, **12**, **14**, and **22**, were evaluated for their inhibitory activity of NADPH-catalyzed lipid peroxidation in rat liver microsomes.

Results and Discussion

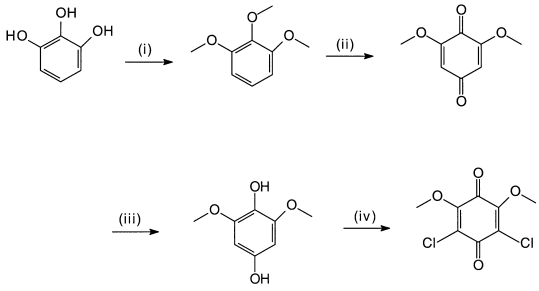
Synthesis

In all, eleven prenylated chalcones **9**–**19** were synthesized using $Ba(OH)_2$-catalyzed Claisen–Schmidt condensation of 2-hydroxy-3-prenyl-4,5,6-trimethoxyacetophenone (**8**) with appropriate aromatic aldehyde in 48 to 69% yields (Scheme 3). The prenylated acetophenone **8** was prepared in five steps starting from phloroglucinol (**3**) in an overall yield of 5% (Scheme 1). The Hoesch reaction product of **3**, i.e., phloracetophenone (**4**),[37] was partially methylated to yield 2 - hydroxy - 4,6 - dimethoxyacetophenone (**5**),[38] which was subjected to persulphate oxidation to yield 2,5-dihydroxy-4,6-dimethoxyacetophenone (**6**).[38] The compound **6** was prenylated using 2-methyl-3-buten-2-ol and $BF_3{\cdot}Et_2O$ as Lewis acid catalyst to afford compound **7**, which was further methylated to yield prenylated acetophenone **8**. An attempt to synthesize 2,5-dihydroxy-4,6-dimethoxyacetophenone **6**, a precursor of prenylated acetophenone **8**, was made by oxidation of 1,2,3-trimethoxybenzene to 2,6-dimethoxyquinone, followed by its reduction to 1,4-dihydroxy-2,6-dimethoxybenzene; the last step of nuclear acetylation involving the Hoesch reaction on 1,4-dihydroxy-2,6-dimethoxybenzene failed and resulted in the formation of 3,5-dichloro-2,6-dimethoxy-1,4-benzoquinone instead of the target acetophenone (Scheme 2).[39]

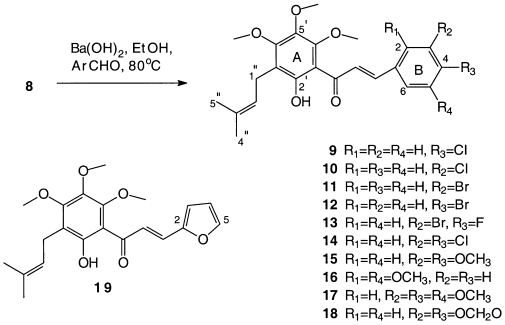
Further, three allylated chalcones 1 - [2 - hydroxy - 4,6 - dimethoxy-5-(prop-2-enyloxy)phenyl]-3-(3,4,5-trimethoxyphenyl)propenone (**21**), 1-[2-hydroxy-4,6-dimethoxy-5 -(prop-2-enyloxy)phenyl]-3-(3,4-methylenedioxyphenyl)-propenone (**22**) and 1-[2-hydroxy-4,6-dimethoxy-5-(prop-2 - enyloxy)phenyl] - 3 - (2 - furanyl)propenone (**23**) were synthesized using $Ba(OH)_2$-catalyzed Claisen–Schmidt



Scheme 1. Reagents and conditions: (i) $ZnCl_2$, HCl, CH_3CN, 0 °C; (ii) Me_2SO_4, K_2CO_3, acetone; (iii) $K_2S_2O_8$, 10% NaOH; (iv)  , $BF_3{\cdot}Et_2O$, dioxane; (v) Me_2SO_4, K_2CO_3, acetone.



Scheme 2. Reagents and conditions: (i) Me_2SO_4, 50% NaOH, EtOH; (ii) HNO_3, EtOH; (iii) $Na_2S_2O_4$, H_2O–$CHCl_3$; (iv) $ZnCl_2$, HCl, CH_3CN, 0°C.



9 R_1=R_2=R_4=H, R_3=Cl
10 R_1=R_3=R_4=H, R_2=Cl
11 R_1=R_3=R_4=H, R_2=Br
12 R_1=R_2=R_4=H, R_3=Br
13 R_1=R_4=H, R_2=Br, R_3=F
14 R_1=R_4=H, R_2=R_3=Cl
15 R_1=R_4=H, R_2=R_3=OCH_3
16 R_1=R_4=OCH_3, R_2=R_3=H
17 R_1=H, R_2=R_3=R_4=OCH_3
18 R_1=R_4=H, R_2=R_3=OCH_2O

Scheme 3.

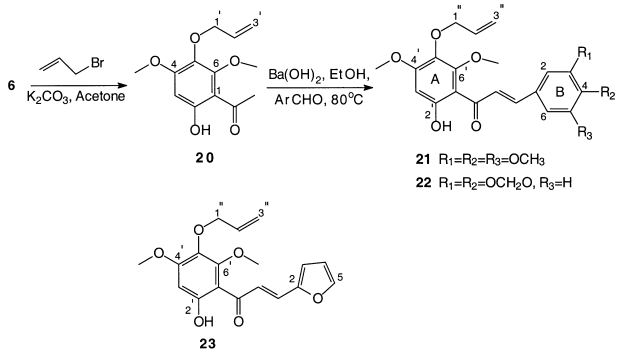
condensation of 5-allyloxy-2-hydroxy-4,6-dimethoxyacetophenone (**20**) with the corresponding aldehyde in 66 to 76% yields (Scheme 4). The allyloxyphenol **20** was synthesized by allylation of the acetophenone **6** with allyl bromide and anhydrous K_2CO_3 in acetone. The prenylated and allylated phenols **7**, **8**, and **20**, and all the fourteen 1,3-diarylpropenones **9**–**19** and **21**–**23** are new in the literature. The prenylated chalcones **9**–**19** with fully substituted A-ring are novel and belong to a rare category of flavonoids. The structures of these novel acetophenones and 1,3-diarylpropenones have been established unambiguously on the basis of their spectral studies (cf. Experimental).

Anti-invasive activity evaluation

Twelve chalcones, *viz*. **9**, **11**, **12**, **14**–**19**, and **21**–**23**, out of fourteen synthesized have been tested for their anti-invasive activity. The assay of anti-invasive activity was based on confrontation of invasive human MCF-7/6 mammary carcinoma cell lines on embryonic chick heart fragments. Briefly, 9-day-old embryonic chick heart fragments were precultured and confronted with aggregates of MCF-7/6 cells. After an overnight incubation on the top of semi-solid agar, the confronting pairs were cultured for another 8 days in a suitable medium. The interaction between MCF-7/6 carcinoma cells and embryonic chick heart fragments was evaluated histologically. In such confronting cultures, effect on the tumour cells can be discerned from effects on the normal host tissue. Human MCF-7/6 breast carcinoma cells were selected as test cells because they were invasive in this assay and are sensitive to the effect of a number of flavonoids.

Chalcones **9**, **11**, **12**, **14**–**19**, and **21**–**23** were dissolved in dimethyl sulphoxide (DMSO) and diluted further with culture medium at a final concentration of 10 μM. Confronting cultures were treated with compounds at 10 μM concentration for 8 days. Control cultures contained same amount of DMSO as the treated ones but no test compound. According to the subjective scale, we could distinguish four grades of interaction between MCF-7/6 cells and precultured heart fragments (PHF) that had been cultured in confrontation during 8 days. Grade I, the confronting cells grew around the PHF without occupation of the peripheral PHF cells; grade II, the occupation by MCF-7/6 cells was limited to the outermost cell layers of the PHF; grade III, the confronting cells occupied and replaced less than half of the PHF; grade IV, the occupation and replacement exceeded half. Grades I and II are considered as non-invasive situations, while grades III and IV are typical for invasion. The negative and positive control experiments were done for quality control. The negative control is the solvent treatment at 0.1% DMSO. The positive control is treatment with 500 ng/mL insulin-like growth factor I. This standard anti-invasive treatment yielded grades I or II.[40] Control cultures, treated just with the solvent DMSO, showed invasion by MCF-7/6 cells. The results obtained with the assay are summarized in Table 1.

Among nine prenylated 1,3-diarylpropenones, *viz*. **9**, **11**, **12**, and **14**–**19**, tested for anti-invasive activity, compound **11** was found to exhibit highest activity as the carcinoma cells completely failed to invade the treated precultured heart fragment cells, followed by compound **16**, in which case the invasive cells either failed to invade or were restricted to outermost cell layer of the treated PHF cells (Table 1). The compound **11** has bromine atom at the C-3 position in the B-ring and showed high activity, whereas the chalcone with bromine atom at C-4 position in the same ring, i.e., compound **12**, does not show any appreciable anti-invasive activity. As mentioned above, the evaluation of invasion is done in accordance with a subjective scale, and is not quantitative. However, no quantitative techniques are currently available for this histological method; yet, the results of this assay are reproducible. The "large error limits", as for compound **12**, are usually encountered when anti-invasive compounds are tested at the lower limits of their potency. So, the variation for compound **12** presumably means that an anti-invasive activity can be achieved above 10 μM, but that this concentration is "on the edge". Prenylated chalcones **9** and **14** with chloro substituent(s) at C-4 and the C-3 and C-4 positions in the B-ring, respectively, and compound **18** with 3,4-methylenedioxy group in the B-ring exhibited appreciable anti-invasive activity against human MCF-7/6 carcinoma cells. Among the chalcones having two and three methoxy groups in the B-ring, i.e., **15**, **16**, and **17**, only compound **16** with 2,5-dimethoxyphenyl ring has exhibited anti-invasive activity, whereas the chalcones **15** and **17**, having respectively the 3,4-dimethoxyphenyl and 3,4,5-trimethoxyphenyl



Scheme 4.

Table 1. Effect of 1,3-diarylpropenones on invasion of MCF-7/6 cells in vitro

Compound	Invasion grade(s)[a] (10 μM conc.)		Compound	Invasion grade(s)[a] (10 μM conc.)	
	Exp. 1	Exp. 2		Exp. 1	Exp. 2
9	II	II	**17**	II	III
11	I	I	**18**	II	II
12	II	IV	**19**	III	III
14	II	II	**21**	II	
15	II	III	**22**	II	II
16	I	II	**23**	I	II

[a]Two data for each compound are the result of two different experiments involving confrontations treated in suspension with 10 μM concentration of each compound for 8 days (cf. Experimental).

moieties as B-rings were practically non-anti-invasive at 10 μM concentration. Thus, the methoxy groups at C-2 and C-5 positions and bromine atom at C-3 position in B-ring of prenylated chalcones were found to be most suitable for exhibiting anti-invasive activity among the compounds synthesized.

All the three *O*-allylated chalcones **21**–**23** were tested for anti-invasive activity against MCF-7/6 carcinoma cells and exhibited appreciable activity (Table 1). Among the three *O*-allylated chalcones, compound **23** with furan-2-yl group as B-ring in place of the phenyl ring was found to be the most active as the MCF-7/6 carcinoma cells either failed to invade or were restricted to the outermost cell layer of the treated PHF cells. Antiinvasive activity of *O*-allylated chalcone **23** is comparable to the activity of prenylated chalcone **16**. Allylated chalcones **21** and **22** having 3,4,5-trimethoxyphenyl and 3,4-methylenedioxyphenyl as B-rings exhibited appreciable anti-invasive activity at 10 μM concentration as the invading MCF-7/6 cells were restricted to the outermost cell layer of the treated PHF cells.

Antioxidant activity

Peroxidation of lipids of biomembranes is a complicated process involving formation and propagation of lipid radicals, oxygen uptake and rearrangement of double bonds in unsaturated lipids. Uncontrolled lipid peroxidation ultimately leads to deterioration of membrane lipids resulting in various pathological conditions. Investigations from the laboratory of Svingen et al.[41] demonstrated that NADPH-dependent lipid peroxidation proceeds through the formation of lipid hydroperoxides, called 'initiation step'. This is followed by 'propagation', which involves the breakdown by hydroperoxides formed during initiation yielding reactive radicals and products unique to lipid peroxidation.

We have examined the effect of two prenylated acetophenones **7** and **8**, the *O*-allylated acetophenone **20** and four prenylated/allylated 1,3-diarylpropenones **9**, **12**, **14**, and **22** on the initiation of lipid peroxidation in rat liver microsomes. NADPH-dependent liver microsomal lipid peroxidation was assayed by the method of Ernster and Nordenbrand.[42] All activity testings have been carried out at 100 μM concentration. The results compiled in Table 2 illustrate the influence of these precursor acetophenones and 1,3-diarylpropenones on the initiation of enzymatic lipid peroxidation.

Out of the seven model compounds screened, 2,5-dihydroxy-4,6-dimethoxy-3-(3-methyl-2-butenyl)acetophenone (**7**) was found to be the most active and exhibited 90.2% inhibition of the NADPH-catalysed lipid peroxidation in rat liver microsomes at the initiation stage. The inhibitory activity of compound **7** is quite comparable with the activity of the well known natural antioxidant, α-tocopherol (Table 2). When the hydroxyl group at the C-5 position in compound **7** was methylated or allylated as in the case of compounds **8** and **20**, respectively, the activity went down significantly (Table 2). Conversion of prenylated acetophenone **8** to 1-[2-hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(3,4-dichlorophenyl)-propenone (**14**) and the *O*-allylated acetophenone **20** to 1-[2-hydroxy-4,6-dimethoxy-5-(prop-2-enyloxy)phenyl]-3-(3,4-methylenedioxyphenyl)propenone (**22**) increases the activity by almost 3- and 2-fold, respectively. The other two 1,3-diarylpropenones, i.e., **9** and **12**, did not exhibit any significant activity.

Table 2. Effect of acetophenones and 1,3-diarylpropenones on NADPH-dependent lipid peroxidation in rat liver microsomes

Compound	% of Inhibition[a] (100 μM conc.)		Compound	% of Inhibition[a] (100 μM conc.)	
7	90.2[b]	±2.8	**14**	60.3	± 3.7
8	18.7	±1.2	**20**	28.4	± 2.6
9	29.2	±0.7	**22**	51.0	±3.1
12	30.4	±2.4	α-tocopherol (reference)	91.7	±1.9

[a]The values are mean±SEM of three experiments.
[b]Compound **7** exhibited 91.1% inhibition of DPPH radical.

One of the mechanism of action of a compound as antioxidant in biological systems is to scavenge the reactive lipid hydroperoxide or other reactive radicals. We presume that 2,5-dihydroxyacetophenone **7** gives rise to aryloxy radical, which acts as a radical scavenger. The radical scavenging potency of compound **7** was found to be 91.1%, determined on the basis of drop in the UV-absorption of the stable radical 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH).

Conclusions

The present synthesis and screening of prenylated and allylated chalcones against invasive human MCF-7/6 mammary carcinoma cells have led to the identification of a potent anti-invasive chalcone, 1-[2-hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(3-bromophenyl)propenone (**11**) which is effective in inhibiting the invasion of cancer cells onto normal cells at 10 μM concentration. The chalcone **11** exhibits comparable anti-invasive activity at one tenth of concentration of the prenylated phenol **2** isolated from *Piper clarkii*.[35] Thus, a 10-fold increase in the activity with respect to active concentration of test compound has been achieved by derivatization of the natural phenol **2**. Though the breakthrough for a suitable and safe drug for the treatment of different types of cancer and to stop the inherent tendency of cancer cells to migrate beyond their natural tissue boundaries is still to come, these compounds could prove to be a lead for further studies. Furthermore, prenylated acetophenone **7** was found to be effective in inhibiting the initiation of lipid peroxidation.

Experimental

Reactions were monitored by TLC on precoated Merck silica gel $60F_{254}$ aluminium plates. Flash column chromatography was carried out using silica gel (Speckpure,

100–200 mesh). The spots on TLC were visualized either under UV light (254 nm) or by developing with alcoholic $FeCl_3$ solution (3%). Melting points were determined either in a sulphuric acid bath or on a Mettler FP62 instrument and are uncorrected. The IR spectra were recorded with a Perkin–Elmer RX1FT-IR spectrometer. The ^1H NMR spectra (300 MHz or 250 MHz) and ^{13}C NMR spectra (75.5 MHz or 62.9 MHz) were recorded on Bruker AC 300 or Bruker AC 250 spectrometer. TMS has been used as an internal standard for both ^1H and ^{13}C NMR spectral recordings. The chemical shift values are on δ scale and the coupling constants (J) are in Hz. The EI mass spectra were recorded on a Jeol JMA-DA 5000 mass spectrometer at 70 eV. MCF-7/6 cells, a variant of the MCF-7 cell family, were obtained from Dr Henri Rochefort, Unite d'Endocrinologie Cellulaire et Moleculaire, Montpellier, France.

2,5-Dihydroxy-4,6-dimethoxy-3-(3-methyl-2-butenyl)acetophenone (7). To a stirred solution of 2,5-dihydroxy-4,6-dimethoxyacetophenone (**6**,[38] 40 g, 188 mmol) in dry dioxane (200 mL) was added gradually $BF_3{\cdot}Et_2O$ (8.40 mL) at room temperature, when the solution acquired a pinkish-red colour. To this was added a solution of 2-methyl-3-buten-2-ol (27.51 mL, 263 mmol) in anhydrous dioxane (30.0 mL) and the whole solution stirred for 4 h at room temperature. The reaction mixture was diluted with Et_2O (250 mL) and washed with water (2×50 mL). The ethereal layer was dried over Na_2SO_4, concentrated under reduced pressure and the oily residue obtained was subjected to column chromatography using ethyl acetate: petroleum ether (1:19) as eluent to afford the desired compound **7** as yellow solid (10.80 g) in 20.5% yield; mp 70–71 °C. R_f: 0.33 (ethyl acetate:petroleum ether, 1:4); EIMS, m/z (% rel. int.): 280 $[M]^+$(90), 265 (33), 237 (20), 225 (100), 209 (40), 181 (12), 163 (8) and 43 (87); ^1H NMR (300 MHz, $CDCl_3$): δ 1.68 and 1.77 (6H, 2s, 3H each, C-4′H and C-5′H), 2.68 (3H, s, $COCH_3$), 3.33 (2H, d, J = 6.7 Hz, C-1′H), 3.89 (6H, s, 2×OCH_3), 5.20–5.28 (2H, m, C-2′H and C-5OH) and 12.89 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.73 (C-5′), 22.52 (C-1′), 25.61 (C-4′), 31.40 ($CO\mathit{C}H_3$), 61.16 and 61.30 (2×OCH_3), 110.81 (C-3), 118.73 (C-1), 122.16 (C-2′), 131.85 (C-3′), 134.61 (C-5), 146.42 (C-2), 152.71 (C-6), 155.77 (C-4) and 203.74 (C=O); IR (KBr) ν_{max}: 3362, 2924, 2361, 1607, 1474, 1428, 1282, 1221, 1073, 1014, and 832 cm^{-1}.

2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyacetophenone (8). To a solution of 2,5-dihydroxy-4,6-dimethoxy-3-(3-methyl-2-butenyl)acetophenone (**7**, 7.6 g, 27.1 mmol) in anhydrous acetone (120 mL) was added anhydrous potassium carbonate (4.0 g), followed by dimethyl sulphate (2.84 mL, 29.81 mmol) and the reaction mixture refluxed for 4 h. The reaction was worked up by removing the solvent under reduced pressure and by addition of ice-cold water (75 mL) into it and extraction with ethyl acetate (3×100 mL). The combined organic phase was dried over Na_2SO_4; the residue obtained after evaporation was purified by column chromatography with ethyl acetate:petroleum ether (1:19) as eluent to yield the title compound **8** as an oil (5.40 g) in 67.6% yield. R_f: 0.40 (ethyl acetate:petroleum ether, 1:19); EIMS, m/z (% rel. int.): 294 $[M]^+$ (100), 279 (60), 251 (38), 239 (74), 223 (25), 195 (14), 163 (9), and 91 (11); ^1H NMR (300 MHz, $CDCl_3$): δ 1.67 and 1.77 (6H, 2s, 3H each, C-4′H and C-5′H), 2.65 (3H, s, $COCH_3$), 3.31 (2H, d, J = 6.8 Hz, C-1′H), 3.82, 3.89, and 3.94 (9H, 3s, 3H each, 3×OCH_3), 5.20 (1H, t, J = 6.8 Hz, C-2′H) and 13.15 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.73 (C-5′), 22.32 (C-1′), 25.71 (C-4′), 32.13 ($CO\mathit{C}H_3$), 60.76, and 60.99 (3×OCH_3), 110.00 (C-3), 119.00 (C-1), 122.60 (C-2′), 131.64 (C-3′), 139.00 (C-5), 154.00 (C-2), 158.60 (C-6), 158.62 (C-4), and 204.00 (C=O); IR (KBr) ν_{max}: 2934, 1620, 1590, 1458, 1417, 1405, 1281, 1091, 1047, and 983 cm^{-1}.

2-Hydroxy-4,6-dimethoxy-5-(prop-2-enyloxy)acetophenone (20). To a solution of 2,5-dihydroxy-4,6-dimethoxyacetophenone (**6**, 7.58 g, 35.7 mmol) in acetone (100 mL) was added freshly ignited K_2CO_3 (3.0 g), followed by allyl bromide (3.09 mL, 35.7 mmol) and the reaction mixture refluxed for 5 h. The progress of the reaction was monitored by TLC. On completion of the reaction, solvent was removed under reduced pressure and ice-cold water (75 mL) was added into it. The aqueous reaction mixture was extracted with ethyl acetate (3×100 mL), combined organic layer dried over Na_2SO_4, solvent evaporated under reduced pressure and the residue was purified by column chromatography (ethyl acetate:petroleum ether, 3:17) to yield the title compound **20** as an oil (6.23 g) in 69.2% yield. R_f: 0.47 (ethyl acetate:petroleum ether, 1:4); EIMS, m/z (% rel. int.): 252 $[M]^+$ (35), 211 (100), 193 (21), 183 (81), 165 (54), 151 (46), 109 (23), 69 (28), and 43 (66); ^1H NMR (300 MHz, $CDCl_3$): δ 2.65 (3H, s, $COCH_3$), 3.96 and 3.99 (6H, 2s, 3H each, 2×OCH_3), 4.43 (2H, d, J = 5.8 Hz, C-1′H), 5.20–5.38 (2H, m, C-3′H), 6.04–6.22 (1H, m, C-2′H), 6.49 (1H, s, C-3H) and 13.44 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 31.85 ($CO\mathit{C}H_3$), 56.03, and 61.10 (2×OCH_3), 74.35 (C-1′), 96.11 (C-2′), 108.50 (C-1), 117.83 (C-3′), 133.32 (C-5), 134.06 (C-3), 155.52 (C-2), 160.28 (C-6), 161.97 (C-4), and 203.32 (C=O); IR (Nujol) ν_{max}: 2922, 2360, 1624, 1461, 1377, 1311, 1282, 1252, 1208, 1164, 1110, and 1082 cm^{-1}.

General method of synthesis of 1,3-diarylpropenones 9–19 and 21–23. Acetophenone **8** or **20** (3.4 mmol) was dissolved in ethanol (50 mL) and fused $Ba(OH)_2$ (500 mg) was added, followed by appropriate aldehyde (3.7 mmol). The reaction mixture was refluxed for 3–4 h, during which it acquired a dark red colour. On completion, the reaction mixture was concentrated to one fourth of its original volume and acidified with 5% aqueous HCl (70–100 mL). In case the product separated out as solid, it was filtered to yield the crude product, otherwise the acidic solution was extracted with ethyl acetate. The crude product was flash column chromatographed affording pure 1,3-diarylpropenones **9**–**19** and **21**–**23** in 48 to 76% yield.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(4-chlorophenyl)propenone (9). The crude product was flash column chromatographed over silica gel using chloroform:*n*-hexane (3:7) as eluting solvent to

afford propenone **9** as an oil (892 mg) in 62.9% yield. R_f: 0.35 (chloroform:*n*-hexane, 3:7); EIMS, *m*/*z* (% rel. int.): 418 $[M+2]^+$(7), 416 $[M]^+$(19), 401 (10), 362 (7), 294 (11), 279 (12), 263 (29), 239 (100), 223 (36), and 209 (10); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.68 and 1.78 (6H, 2s, 3H each, C-4″H and C-5″H), 3.34 (2H, d, J = 8.0 Hz, C-1″H), 3.83, 3.87, and 3.95 (9H, 3s, 3H each, 3×OCH_3), 5.20 (1H, t, J = 8.0 Hz, C-2″H), 7.39 (2H, d, J = 8.5 Hz, C-3H and C-5H), 7.56 (2H, d, J = 8.5 Hz, C-2H and C-6H), 7.76 (1H, d, J = 15.5 Hz, H_α), 7.83 (1H, d, J = 15.5 Hz, H_β), and 13.18 (1H, s, chelated OH); ^{13}C NMR (62.97 MHz, $CDCl_3$): δ 17.69 (C-5″), 25.67 (C-1″), 29.58 (C-4″), 60.90, 60.91 and 61.90 (3×OCH_3), 115.00 and 119.00 (C-1′ and C-3′), 122.28 (C-2″), 127.26 (C-α), 129.12 and 129.43 (C-2, C-3, C-5, and C-6), 130.00 (C-4), 131.50 (C-3″), 133.50 (C-1), 138.50 (C-5′), 141.41 (C-ß), 148.50 (C-2′), 158.00 (C-6′), 158.92 (C-4′) and 193.46 (C=O); IR (KBr) ν_{max}: 2926, 2853, 1743, 1630, 1559, 1403, 1341, 1279, 1042, and 825 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(3-chlorophenyl)propenone (10). The crude product was flash column chromatographed using chloroform:*n*-hexane (3:7) as eluent to yield the desired compound **10** as an oil (723 mg) in 51.0% yield. R_f: 0.25 (chloroform:*n*-hexane, 3:7); EIMS, *m*/*z* (% rel. int.): 418 $[M+2]^+$ (38), 416 $[M]^+$ (100), 401 (30), 361 (15), 263 (70), 223 (35), 220 (10), 205 (10), 177 (12), 149 (11) and 44 (14); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.69 and 1.79 (6H, 2s, 3H each, C-4″H and C-5″H), 3.35 (2H, d, J = 6.1 Hz, C-1″H), 3.86, 3.91, and 3.97 (9H, 3s, 3H each, 3×OCH_3), 5.29 (1H, brs, C-2″H), 7.36–7.61 (4H, m, C-2H, C-4H, C-5H and C-6H), 7.74 (1H, d, J = 15.7 Hz, H_α), 7.85 (1H, d, J = 15.6 Hz, H_β), and 13.16 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.77 (C-5″), 22.47 (C-1″), 29.68 (C-4″), 61.01, 61.15 and 62.07 (3×OCH_3), 110.50 (C-3′), 119.50 (C-1′), 122.51 (C-2″), 126.70 (C-α), 127.98, 128.38, 130.10, and 130.23 (C-2, C-4, C-5, and C-6), 131.78 (C-3″), 135.07 (C-5′), 134.50 (C-1), 139.00 (C-3), 141.20 (C-β), 148.00 (C-2′), 154.00 (C-6′), 159.00 (C-4′) and 194.00 (C=O); IR (Nujol) ν_{max}: 2924, 2361, 1750, 1620, 1550, 1458, 1376, 1018, and 668 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(3-bromophenyl)propenone (11). The crude product was column chromatographed using chloroform:*n*-hexane (3:7) as eluent to yield the desired product as an orange solid (786 mg) in 50.0% yield; mp 101–102 °C. R_f: 0.27 (chloroform:*n*-hexane, 3:7); EIMS, *m*/*z* (% rel. int.): 462 $[M+2]^+$(95), 460 $[M]^+$(96), 445 (40), 419 (18), 405 (21), 278 (17), 263 (100), 235 (35), 223 (56), 177 (14), and 102 (10); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65 and 1.79 (6H, 2s, 3H each, C-4″H, and C-5″H), 3.34 (2H, d, J = 7.0 Hz, C-1″H), 3.85, 3.89, and 3.93 (9H, 3s, 3H each, 3×OCH_3), 5.20 (1H, t, J = 7.0 Hz, C-2″H), 7.27–7.60 (4H, m, C-2H, C-4H, C-5H and C-6H), 7.73 (1H, d, J = 15.6 Hz, H_α), 7.85 (1H, d, J = 15.6 Hz, H_β), and 13.16 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.68 (C-5″), 22.56 (C-1″), 29.23 (C-4″), 60.71, 60.88 and 61.02 (3×OCH_3), 111.14 (C-3′), 119.02 (C-1′), 122.32 (C-2″), 123.39 (C-3), 126.51 (C-α), 127.80, 128.16, 129.93, and 130.08 (C-2, C-4, C-5, and C-6), 131.58 (C-3″), 134.85 (C-1), 137.13 (C-5′), 141.03 (C-ß), 153.49 (C-2′), 158.66 (C-6′), 158.94 (C-4′), and 193.26 (C=O); IR (KBr) ν_{max}: 2925, 2361, 1633, 1561, 1468, 1418, 1338, 1146, 1116, 1045, and 979 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl-3-(4-bromophenyl)propenone (12). The crude compound was flash column chromatographed using chloroform:*n*-hexane (1:4) as eluent to afford compound **12** as an oil (904 mg) in 57.6% yield. R_f: 0.37 (chloroform:*n*-hexane, 3:7); EIMS, *m*/*z* (% rel. int.): 462 $[M+2]^+$(23), 460 $[M]^+$(26), 446 (29), 418 (15), 407 (14), 279 (5), 278 (25), 263 (100), 235 (32), 223 (52), 177 (14), 149 (14), and 44 (35); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.69 and 1.79 (6H, 2s, 3H each, C-4″H, and C-5″H), 3.35 (2H, d, J = 6.9 Hz, C-1″H), 3.85, 3.88 and 3.96 (9H, 3s, 3H each, 3×OCH_3), 5.22 (1H, t, J = 6.9 Hz, C-2″H), 7.50 (2H, d, J = 8.5 Hz, C-3H, and C-5H), 7.56 (2H, d, J = 8.5 Hz, C-2H and C-6H), 7.75 (1H, d, J = 15.6 Hz, H_α), 7.91 (1H, d, J = 15.6 Hz, H_β), and 13.18 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.81 (C-5″), 22.49 (C-1″), 29.72 (C-4″), 61.00, 61.14 and 62.05 (3×OCH_3), 111.32 (C-3′), 119.19 (C-1′), 122.46 (C-2″), 124.49 (C-4), 127.57 (C-α), 129.29 (C-3 and C-5), 131.72 (C-3″), 132.21 (C-2 and C-6), 134.34 (C-1), 138.84 (C-5′), 142.97 (C-ß), 153.57 (C-2′), 158.70 (C-6′), 159.08 (C-4′), and 193.43 (C=O); IR (KBr) ν_{max}: 2928, 1632, 1558, 1487, 1464, 1417, 1403, 1338, 1277, 1145, 1046, and 982 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(3-bromo-4-fluorophenyl)propenone (13). The crude product was flash column chromatographed using chloroform:*n*-hexane (3:7) as an eluent to afford **13** as an orange solid (835 mg) in 51.2% yield; mp 85–86 °C. R_f: 0.31 (chloroform:*n*-hexane, 3:7); EIMS, *m*/*z* (% rel. int.): 480 $[M+2]^+$(25), 478 $[M]^+$(25), 462 (44), 446 (11), 434 (30), 305 (12), 263 (100), 235 (41), 223 (90), 120 (38) and 69 (22); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68 and 1.79 (6H, 2s, 3H each, C-4″H and C-5″H), 3.35 (2H, d, J = 6.7 Hz, C-1″H), 3.85, 3.89 and 3.96 (9H, 3s, 3H each, 3 x OCH_3), 5.20 (1H, t, J = 6.7 Hz, C-2″H), 7.12–7.18 (1H, m, H-5), 7.51–7.55 (1H, m, H-6), 7.70 (1H, d, J = 15.6 Hz, H_α), 7.78–7.83 (2H, m, H-2 and H_β) and 13.12 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.71 (C-5″), 22.59 (C-1″), 29.27 (C-4″), 60.51, 60.91, and 62.01 (3×OCH_3), 109.79 (C-3), 111.12 (C-3′), 116.91 (C-5), 119.12 (C-1′), 122.29 (C-2″), 127.79 (C-2), 128.90 (C-α), 129.00 (C-1), 131.97 (C-3″), 133.04 (C-6), 138.74 (C-5′), 139.97 (C-β), 153.44 (C-2′), 158.25, and 158.83 (C-4 and C-6′), 161.59 (C-4′), and 193.42 (C=O); IR (Nujol) ν_{max}: 2924, 2400, 1610, 1569, 1493, 1461, 1377, 1339, 1257, 1145, and 1048 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(3,4-dichlorophenyl)propenone (14). The compound was purified by flash column chromatography using chloroform:*n*-hexane (2:3) as eluent to afford **14** as a yellow solid (734 mg) in 47.8% yield; mp 78 °C. R_f: 0.43 (chloroform:*n*-hexane, 2:3); EIMS, *m*/*z* (% rel. int.): 455 $[M+4]^+$ (22), 453 $[M+2]^+$ (64), 451 $[M]^+$ (85), 436 (65), 408 (48), 396 (52), 264 (89), 235 (68), 223 (90), 199 (53), 149 (63), 83 (40), 69 (93), 57 (71) and 43

(100); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.67 and 1.78 (6H, 2s, C-4″H and C-5″H), 3.33 (2H, d, J = 7.0 Hz, C-1″H), 3.84, 3.88, and 3.95 (9H, 3s, 3H each, 3×OCH_3), 5.18 (1H, t, J = 7.0 Hz, C-2″H), 7.43–7.45 (3H, m, C-2H, C-5H and C-6H), 7.67 (1H, d, J = 15.8 Hz, H_α), 7.87 (1H, d, J = 15.5 Hz, H_β), and 13.11 (1H, s, chelated OH); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 17.66 (C-5″), 22.30 (C-1″), 25.64 (C-4″), 60.88, 61.02, and 61.96 (3×OCH_3), 111.05 (C-3′), 119.06 (C-1′), 122.20 (C-2″), 127.23 (C-α), 128.45, 129.59 and 130.79 (C-2, C-5, and C-6), 131.69 (C-3″), 133.13, 133.91, and 135.31 (C-1, C-3, and C-4), 138.69 (C-5′), 139.82 (C-β), 153.41 (C-2′), 158.72 (C-6′), 158.95 (C-4′), and 192.99 (C=O); IR (KBr) ν_{max}: 2927, 1633, 1603, 1565, 1469, 1405, 1335, 1276, 1200, 1143, 1112, 1096, 1049, and 979 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(3,4-dimethoxyphenyl)propenone (15). The compound was purified by flash column chromatography using ethyl acetate:*n*-hexane (1:4) as eluent to afford **15** as a semi-solid (877 mg) in 58.3% yield. R_f: 0.28 (ethyl acetate: *n*-hexane, 1:4); EIMS, *m*/*z* (% rel. int.): 442 $[M]^+$ (26), 441 (100), 426 (17), 398 (13), 277 (15), 262 (64), 235 (16), 223 (48), 149 (20), 131 (14), and 69 (49); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70, and 1.80 (6H, 2s, 3H each, C-4″H, and C-5″H), 3.36 (2H, d, J = 6.3 Hz, C-1″H), 3.86, 3.91, and 3.95 (15H, 3s, 5×OCH_3), 5.22 (1H, t, J = 6.3 Hz, C-2″H), 6.92 (1H, d, J = 8.1 Hz, C-5H), 7.05–7.24 (2H, m, C-2H, and C-6H), 7.81 (2H, s, H_α, and H_β) and 13.32 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.81 (C-5″), 22.51 (C-1″), 25.78 (C-4″), 55.97, 60.96, 61.14, 61.58, and 62.09 (5×OCH_3), 109.41 (C-1′), 110.51, and 111.32 (C-2, and C-5), 118.54 and 119.32 (C-1 and C-3′), 122.60 (C-α), 123.00 (C-2″), 124.67 (C-6), 129.17 (C-3″), 141.65 (C-5′), 143.58 (C-β), 149.34 (C-3), 151.42 (C-4), 153.48 (C-2′), 156.55 (C-6′), 158.31 (C-4′), and 193.45 (C=O); IR (KBr) ν_{max}: 2931, 2852, 1626, 1556, 1512, 1463, 1343, 1265, 1139, and 1046 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(2,5-dimethoxyphenyl)propenone (16). The crude product was flash column chromatographed using ethyl acetate:*n*-hexane (1:9) as eluent to yield the desired compound as an oil (912 mg) in 60.6% yield. R_f: 0.15 (ethyl acetate:*n*-hexane, 1:9); EIMS, *m*/*z* (% rel. int.): 442 $[M]^+$ (64), 428 (29), 412 (30), 400 (25), 388 (32), 279 (35), 263 (79), 235 (38), 223 (100), 200 (30), 191 (30), 149 (55), and 95 (27); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.69, and 1.79 (6H, 2s, 3H each, C-4″H, and C-5″H), 3.35 (2H, d, J = 7.1 Hz, C-1″H), 3.81, 3.83, 3.84 and 3.95 (15H, 4s, 5×OCH_3), 5.22 (1H, t, J = 7.1 Hz, C-2″H), 6.86–6.95 (2H, m, C-3H, and C-4H), 7.19 (1H, d, J = 2.8 Hz, C-6H), 7.95 (1H, d, J = 15.8 Hz, H_α), 8.11 (1H, d, J = 15.7 Hz, H_β), and 13.30 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.77 (C-5″), 22.47 (C-1″), 25.74 (C-4″), 55.84, 56.16, 60.98, 61.11, and 62.04 (5×OCH_3), 111.00, 112.59, and 113.70 (C-3, C-3′ and C-4), 117.00 (C-6), 119.10 (C-1′), 122.66 (C-1), 124.50 (C-α), 127.56 (C-2″), 131.65 (C-3″), 138.41 (C-ß), 139.00 (C-5′), 153.00, and 153.68 (C-2, C-2′ and C-5), 158.00 (C-6′), 159.08 (C-4′), and 194.09 (C=O); IR (KBr) ν_{max}: 3421, 2937, 2361, 1692, 1627, 1583, 1503, 1463, 1418, 1325, 1128, and 1046 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(3,4,5-trimethoxyphenyl)propenone (17). The compound **17** was purified by flash column chromatography using ethyl acetate:*n*-hexane (3:17) as eluent as an oil (895 mg) in 55.7% yield. R_f: 0.15 (ethyl acetate: *n*-hexane, 1:9); EIMS, *m*/*z* (% rel. int.): 472 $[M]^+$ (33), 263 (41), 223 (43), 197 (28), 181 (43), 149 (33), 97 (31), 83 (38), 69 (83), 57 (74), and 43 (100); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.67 and 1.77 (6H, 2s, 3H each, C-4″H, and C-5″H), 3.33 (2H, d, J = 7.0 Hz, C-1″H), 3.83, 3.88, 3.91, 3.92, 3.93, and 3.96 (18H, 6s, 3H each, 6×OCH_3), 5.19 (1H, t, J = 7.0 Hz, C-2″H), 6.84 (2H, s, C-2H, and C-6H), 7.75 (1H, d, J = 13.1 Hz, H_α), 7.76 (1H, d, J = 13.1 Hz, H_β), and 13.25 (1H, s, chelated OH); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 17.65 (C-5″), 22.31 (C-1″), 25.63 (C-4″), 56.04, 56.13, 60.84, 61.01, and 62.02 (6×OCH_3), 105.52 (C-2, and C-6), 106.58 (C-1), 111.20 (C-3′), and 119.08 (C-1′), 122.28 (C-2″), 125.88 (C-α), 131.60 (C-3″), 138.66 (C-5′), 140.17 (C-4), 143.21 (C-β), 153.33 (C-2′, C-3, and C-5), 158.32 and 158.88 (C-4′ and C-6′), and 193.24 (C=O); IR (KBr) ν_{max}: 2937, 1692, 1583, 1504, 1463, 1418, 1325, 1128, 1045, and 1005 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl] - 3 - (3,4 - methylenedioxyphenyl)propenone (18). The crude product was flash column chromatographed using chloroform:*n*-hexane (9:11) as eluent to afford the desired compound as a yellowish-orange solid (997 mg) in 68.8% yield; mp 64–65 °C. R_f: 0.15 (chloroform: *n*-hexane, 2:3); EIMS, *m*/*z* (% rel. int.): 426 $[M]^+$ (54), 411 (27), 383 (21), 371 (14), 278 (8), 263 (100), 235 (35), 223 (53), 205 (21), 195 (8), 175 (24), 148 (38), 135 (29) 117 (26), 89 (47), and 77 (18); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68 and 1.79 (6H, 2s, 3H each, C-4″H and C-5″H), 3.35 (2H, d, J = 6.9 Hz, C-1″H), 3.85, 3.89, and 3.95 (9H, 3s, 3H each, 3×OCH_3), 5.23 (1H, t, J = 6.9 Hz, C-2″H), 6.02 (2H, s, OCH_2O), 6.85 (1H, d, J = 7.9 Hz, C-5H), 7.13 (1H, dd, J = 1.4 and 8.0 Hz, C-6H), 7.15 (1H, d, J = 1.0 Hz, C-2H), 7.76 (2H, s, H_α and H_β) and 13.28 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.81(C-5″), 22.50 (C-1″), 25.78 (C-4″), 60.99, 61.14, and 62.05 (3×OCH_3), 101.61 (OCH_2O), 106.68 (C-2), 108.69 (C-5), 111.44 (C-3′), 122.58 (C-1′), 123.23 (C-2″), 124.90, and 125.19 (C-α and C-6), 129.43 (C-1), 131.63 (C-3″), 138.84 (C-5′), 143.23 (C-β), 148.45 and 149.80 (C-3 and C-4), 153.55 (C-2′), 158.35 (C-6′), 159.01 (C-4′), 193.45 (C=O); IR (KBr) ν_{max}: 2933, 2361, 1627, 1557, 1447, 1255, 1146, 1043, and 982 cm^{-1}.

1-[2-Hydroxy-3-(3-methyl-2-butenyl)-4,5,6-trimethoxyphenyl]-3-(2-furanyl)propenone (19). The crude product was flash column chromatographed using chloroform:*n*-hexane (3:7) as eluent to afford the compound **19** as an oil (716 mg) in 56.6% yield. R_f: 0.26 (chloroform:*n*-hexane, 3:7); EIMS, *m*/*z* (% rel. int.): 372 $[M]^+$ (22), 371 (100), 356 (29), 328 (21), 316 (33), 263 (77), 235 (25), 223 (73), 205 (12), 177 (12), 121 (22), and 65 (16); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68 and 1.79 (6H, 2s, 3H each, C-4″H and C-5″H), 3.34 (2H, d, J = 6.9 Hz, C-1″H), 3.83, 3.92, and 3.95 (9H, 3s, 3×OCH_3), 5.21 (1H, t, J = 6.9 Hz, C-2″H), 6.51 (1H, dd, J = 1.6 and 3.1 Hz, C-4H), 6.70 (1H, d, J = 3.1 Hz, C-3H), 7.52 (1H, s, C-5H),

7.64 (1H, d, $J=15.3$ Hz, H_α), 7.76 (1H, d, $J=15.3$ Hz, H_β), and 13.37 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.69 (C-5″), 22.38 (C-1″), 25.66 (C-4″), 60.71, 60.89, and 61.79 (3×OCH_3), 111.24 and 112.17 (C-3′ and C-4), 115.59 (C-3), 118.82 (C-1′), 122.48 (C-2″), 124.30 (C-α), 129.53 (C-5), 131.48 (C-3″), 138.68 (C-5′), 144.79 (C-β), 152.06, and 153.53 (C-2′ and C-2), 158.34 (C-6′), 159.48 (C-4′), and 192.97 (C=O); IR (KBr) ν_{max}: 3401, 2932, 1631, 1548, 1463, 1416, 1333, 1281, 1143, 1116, 1046 and 981 cm^{-1}.

1-[2-Hydroxy-4,6-dimethoxy-5-(prop-2-enyloxy)phenyl]-3-(3,4,5-trimethoxyphenyl)propenone (21). The crude product was flash column chromatographed using ethyl acetate:petroleum ether (1:4) as eluent to yield an orange crystalline solid (1.12 g) in 76.5% yield; mp 118 °C. R_f: 0.22 (ethyl acetate:petroleum ether, 1:4); EIMS, *m*/*z* (% rel. int.): 430 $[M]^+$ (18), 389 (71), 371 (46), 221 (5), 195 (100), 193 (8), 167 (75), 151 (37), 137 (40), and 121 (17); ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.87, 3.88, 3.89, and 3.90 (15H, 4s, 5×OCH_3), 4.47 (2H, d, $J=8.5$ Hz, C-1″H), 5.20–5.40 (2H, m, C-3″H), 6.04–6.15 (1H, m, C-2″H), 6.27 (1H, s, C-3′H), 6.85 (2H, s, C-2H and C-6H), 7.76 (1H, d, $J=15.5$ Hz, H_α), 7.80 (1H, d, $J=15.5$ Hz, H_β), and 13.72 (1H, s, chelated OH); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 55.95, 56.07, 60.87, and 61.78 (5×OCH_3), 74.45 (C-1″), 96.47 (C-2″), 105.53 (C-2 and C-6), 108.56 (C-3′), 117.69 (C-3″), 125.64 (C-α), 130.76 (C-1), 133.74, and 133.95 (C-1′ and C-5′), 140.18 (C-4), 143.25 (C-β), 153.34 (C-3 and C-5), 154.94 (C-6′), 160.21 (C-2′), 162.68 (C-4′) and 192.54 (C=O); IR (KBr) ν_{max}: 3450, 2934, 1630, 1581, 1561, 1505, 1321, 1273, 1133, and 994 cm^{-1}.

1-[2-Hydroxy-4,6-dimethoxy-5-(prop-2-enyloxy)phenyl]-3-(3,4-methylenedioxyphenyl)propenone (22). The crude compound was purified by flash column chromatography using chloroform:petroleum ether (1:1) as eluent to afford an orange solid (877 mg) in 67.2% yield; mp 98–99 °C. R_f: 0.46 (chloroform:*n*-hexane, 1:1); EIMS, *m*/*z* (% rel. int.): 384 $[M]^+$ (14), 343 (64), 327 (6), 195 (100), 167 (26), 135 (15) and 44 (7); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88, and 3.93 (6H, 2s, 3H each, 2×OCH_3), 4.46 (2H, d, $J=5.7$ Hz, C-1″H), 5.22–5.40 (2H, m, C-3″H), 6.02 (2H, s, OCH_2O), 6.05–6.18 (1H, m, C-2″H), 6.28 (1H, s, C-3′H), 6.86 (1H, d, $J=7.8$ Hz, C-5H), 7.13 (1H, dd, $J=1.5$, and 7.8 Hz, C-6H), 7.16 (1H, d, $J=1.5$ Hz, C-2H), 7.78 (1H, d, $J=15.6$ Hz, H_α) and 7.79 (1H, d, $J=15.6$ Hz, H_β), and 13.81 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 56.05, and 61.90 (2×OCH_3), 74.64 (C-1″), 96.61 (C-2″), 101.61 (OCH_2O), 106.74 (C-2), 108.60 (C-3′), 108.72 (C-5), 117.80 (C-3″), 124.69 (C-α), 125.17 (C-6), 130.02 (C-1), 134.24 (C-1′), 140.00 (C-5′), 143.31 (C-β), 148.00 (C-3), 150.00 (C-4), 155.00 (C-2′), 160.00 (C-6′), 162.83 (C-4′), and 193.00 (C=O); IR (KBr) ν_{max}: 3432, 2926, 1617, 1497, 1359, 1249, 1199, 1104, 977, and 926 cm^{-1}.

1-[2-Hydroxy-4,6-dimethoxy-5-(prop-2-enyloxy)phenyl]-3-(2-furanyl)propenone (23). The crude compound was flash column chromatograped using chloroform:*n*-hexane (2:3) as eluent to yield an orange solid (735 mg) in 65.6% yield; mp 100 °C. R_f: 0.37 (ethyl acetate:petroleum ether, 1:4); EIMS, *m*/*z* (% rel. int.): 330 $[M]^+$ (13), 289 (72), 195 (100), 167 (31), 149 (4), 121 (5), 93 (3) and 67 (4); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 and 3.92 (6H, 2s, 3H each, 2×OCH_3), 4.48 (2H, d, $J=5.8$ Hz, C-1″H), 5.22–5.41 (2H, m, C-3″H), 6.07–6.19 (1H, m, C-2″H), 6.28 (1H, s, H-3′), 6.85 (1H, d, $J=3.4$ Hz, C-3H), 7.13 (1H, dd, $J=1.4$ and 3.4 Hz, C-4H), 7.16 (1H, d, $J=1.4$ Hz, C-5H), 7.77 (1H, d, $J=15.5$ Hz, H_α), 7.86 (1H, d, $J=15.5$ Hz, H_β), and 13.74 (1H, s, chelated OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 56.07 and 61.91 (2×OCH_3), 74.64 (C-1″), 96.59 (C-2″), 106.70, and 108.70 (C-3 and C-4), 117.79 (C-3″), 124.62 and 125.17 (C-5 and C-α), 129.95 (C-1′), 133.94 and 134.18 (C-3′ and C-5′), 143.28 (C-β), 149.77 (C-2), 155.22 (C-2′), 160.26 (C-6′), 162.76 (C-4′), and 192.77 (C=O); IR (KBr) ν_{max}: 3448, 2924, 2362, 1618, 1560, 1497, 1359, 1249, 1199, 1105, and 927 cm^{-1}.

General methods for biological activity evaluation

Assay for invasion. The assay consists of three-dimensional confrontations between tumour cells and normal tissue.[43] Fragments of 9-day-old embryonic chick heart were precultured and selected for a diameter of 0.4 mm. These precultured heart fragments (PHF) were confronted individually with an aggregate (diameter of 0.2 mm) of MCF-7/6 cells, first on top of semi-solid agar overnight to allow attachment, and subsequently in liquid medium for 8 days in suspension culture. The cultures were then fixed individually in paraffin and serially sectioned for histological analysis. In order to evaluate the interaction between the MCF-7/6 cells and PHF, the sections were stained with haematoxylin–eosin or with an immunohistochemical technique to reveal chick heart antigens.

Treatments. All compounds were dissolved in dimethyl sulphoxide (DMSO) to give a stock solution of 10^{-1} M concentration from which further dilutions in culture medium were made. For each compound, two different experiments were performed involving two confrontations treated in suspension with 10 μM concentration of the compound for 8 days. Control cultures were treated with solvent alone (DMSO at corresponding concentrations).

Assay for growth. Aggregates of MCF-7/6 cells and PHF were cultured separately in the same conditions as described for the assay for invasion. Their larger (a) and smaller (b) diameters were measured with a microscope (×25) at the start and after 8 days of incubation. The volumes of the cultures were calculated in accordance with the formula $V=0.4\times a\times b^2$.[44] At least six cultures were measured for each treatment.

Assay for inhibition of lipid peroxidation. The reaction mixture in a final volume of 2 mL consisted of 0.025 M Tris–HCl (pH 7.5), microsomes (1 mg protein), 3 mM ADP and 0.15 mM $FeCl_3$. The tubes were pre-incubated for 10 min at 37 °C, followed by the addition of the test compounds added at a concentration of 100 μM in 0.2 mL of DMSO and then incubated again for 10 min at 37 °C. The reaction was started by the addition of 0.5 mM NADPH for initiation of enzymatic lipid peroxidation

and incubated for different intervals. The reaction was terminated by the addition of 0.2 mL of 50% TCA, followed by addition of 0.2 mL of 5 N HCl and 1.6 mL of 30% TBA. The tubes were heated in an oil bath at 95 °C for 30 min, cooled and centrifuged at 3000 rpm. The intensity of the colour of the thiobarbituric acid reactive substance (TBRS) formed was read at 535 nm. The lipid peroxidation was found to be linear up to 15 min under the conditions described here.

Assay of DPPH radical scavenging. A solution of the test compound in methanol (4 mL) at various concentrations ranging from 1 to 400 μM depending on the potency of the inhibitor was added to 1 mL of DPPH solution in methanol (0.15 mM). The contents were vigorously mixed, allowed to stand at 20 °C for 30 min and the absorption was read at 517 nm.

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