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Original article

Synthesis, anticancer and antioxidant activities of 2,4,5-trimethoxy chalcones and analogues from asaronaldehyde: Structure–activity relationship

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ABSTRACT

2,4,5-Trimethoxy chalcones and analogues were synthesized from asaronaldehyde derived from β-asarone. These novel compounds when tested against three human tumour cell lines (MCF-7, SW-982 and HeLa) using MTT assay, revealed that chalcones possessing electron donor groups in para position to carbonyl moiety of phenyl ring A, showed better inhibitory activity (**2**, **3**, **4**, **6**, **7**, **10**, **17**). When evaluated for antioxidant activities, compound **15** exhibited better free radical scavenging property in DPPH assay while compounds **2**, **3**, **5**, **7**, **9**, **10**, **11**, **16**, and **18** showed significant NO scavenging activity. All compounds exhibited very good phenyl hydrazine induced haemolysis of erythrocytes in phenylhydrazine assay. Structure–activity relationship (SAR) study using *in-silico* analysis matched well with *in-vitro* tumour cell inhibitory activity.

1. Introduction

Chalcones (1,3-diaryl-2-propen-1-ones) which are precursors of flavonoids constitute an important group of natural products possessing a wide variety of biological activities [1–17]. Flavonoids which are naturally occurring polyphenols also possess diverse biological applications [18]. The anticancer potential of flavonoids and their biogenetic precursors have been investigated in great length [19,20].

Structure–activity relationship has been reported [21,22] where in the activity of chalcones was found to be dependent on the presence and the position of hydroxyl and methoxy groups in both A and B rings [23,24]. Presence of 2′,4′,5′-trimethoxy groups on the ring A of chalcones favours the antimalarial activity [25,26], while 3′,4′,5′-trimethoxy substitution on ring A inhibits the transport activity of glycoprotein and showed reversal of multidrug resistance (MDR) activity [27,28]. 2′,4′,6′-Trimethoxy and 3′,4′,5′-trimethoxy chalcones showed nitric oxide scavenging and antiproliferation activity [11,29]. Substitution at 2′ and 4′ positions of ring A was essential for breast cancer resistance protein inhibitory activities [30,31]. Fluorine substituted chalcones with methoxy groups showed inhibition of nitric oxide production [32]. Trimethoxy substituted chalcone and stilbene hybrids have been found to show antiplasmodial activity [33].

Antioxidants are molecules capable of inhibiting the oxidation of other molecules and thereby preventing the cell death that occurs due to the release of free radicals. Free radicals such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (•OH), hydrogen peroxide and lipid peroxide radicals have been implicated in a variety of diseases such as asthma, cancer, cardiovascular, diabetes, gastrointestinal inflammation, periodontal disease and other inflammatory processes [34,35]. Antioxidant activity of natural compounds like chalcones and flavonoids is related to a number of different mechanisms such as free radical scavenging, hydrogen donation, singlet oxygen quenching, and metal ion chelation. Antioxidant properties of chalcones are influenced to a great extent by two aryl groups and their substitution patterns. Hydroxyl is one of the key groups to enhance the antioxidant activity due to its easy conversion to phenoxy radical through hydrogen transfer mechanism [36].

In continuation of our work [37], we have synthesized chalcones containing 2,4,5-trimethoxy groups in ring B starting from asaronaldehyde derived from an abundantly available natural source i.e. β-asarone. Here, we report the synthesis and biological activities

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of these trimethoxy chalcones and some of their analogues as anticancer and antioxidant agents. Antioxidant activities were determined using assays viz., DPPH (2,2-diphenyl-1-picrylhydrazyl free radical), NO (nitric oxide scavenging), $PhNHNH_2$ (phenyl hydrazine induced haemolysis of erythrocytes). These compounds were tested for their inhibitory activity against three human tumour cell lines (MCF-7, SW-982 and HeLa) using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The structure–activity relationship (SAR) and biological properties of these newly synthesized molecules were compared to estimate its drug-likeliness and undesired properties. Finally toxicity of these 2,4,5-trimethoxy asarone derivatives were experimentally and theoretically evaluated as safe drug leads.

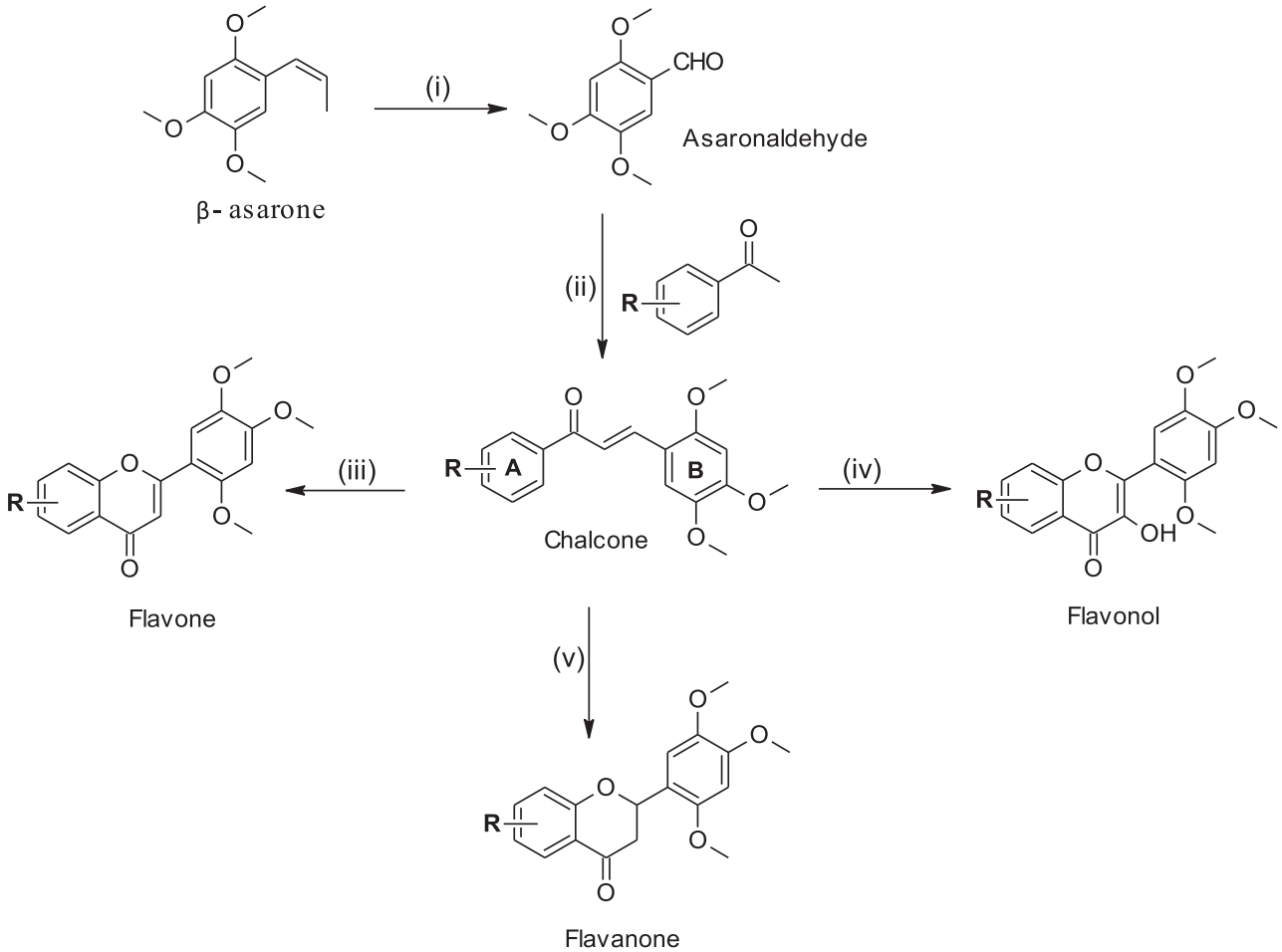
2. Results and discussion

2.1. Chemistry

Acorus calamus (sweet flag) is a widespread, semi-aquatic plant grown in temperate to sub-temperate regions. β-Asarone ((*Z*)-2,4,5-trimethoxy-1-propenylbenzene) is a major active principle (70–80%) found in *A. calamus* oil. β-Asarone was oxidized with $KMnO_4/NaHCO_3$ to get 2,4,5-trimethoxy benzaldehyde (i.e. asaronaldehyde, Scheme 1). The asaronaldehyde was reacted with various commercially available acetophenones in ethanol/aq. KOH at room temperature. After completion, the reaction mass was poured into ice water and acidified with dil HCl to get the desired 2,4,5-trimethoxy chalcones (Scheme 1, **1**–**20**) in good yields ranging from 60 to 96% (Table 1). Chalcones were recrystallized from ethanol/methanol and structures were established with the help of NMR (nuclear magnetic resonance) and mass spectral studies. Coupling constants (*J* values) of vinylic protons in ^1H NMR indicated that compounds **1**–**20** were trans (*E*) isomers ($J_{trans\ c=c}$ = 14–16 Hz). Chalcones with 2′-OH in ring A (**6**, **13** and **15**) were cyclised to get the corresponding flavonoids viz., flavones (**21**–**23**), flavonols (**24**, **25**), and flavanone (**26**). All these compounds were subjected to biological testing and compared their potency with their precursors.

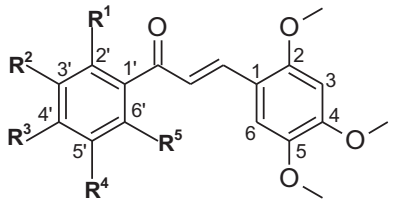
2.2. Biological evaluation

All 26 synthesized compounds i.e. 2,4,5-trimethoxy chalcones and analogues were evaluated for their *in-vitro* anticancer activity against the three human cancer cell lines viz., HeLa (human cervical cancer), SW-982 (human synovial sarcoma), and MCF-7 (human breast cancer) in comparison with β-asarone and using pristimerin (triterpene quinone methide) as a positive control (Table 2). Chalcones were found to have better activity than the cyclised flavones, flavanone and flavonols. Compounds **2**, **7** and **17** having Cl, F, Br groups at 4′ position are relatively more potent and it may be due to electromeric effect contributed by halogen groups being situated at para position to carbonyl group of the phenyl ring. This point is

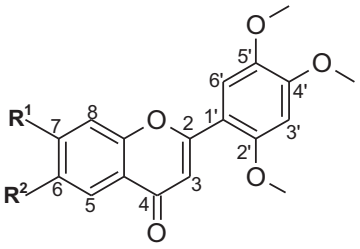


Scheme 1. General procedure for synthesis of chalcones, flavones, flavonols and flavanone. (i) $KMnO_4/NaHCO_3$, r.t.; (ii) aq. KOH (40%), C_2H_5OH, r.t., with corresponding acetophenone; (iii) $DMSO/I_2$, 1–2 h, reflux; (iv) H_2O_2 (30%), NaOH (8 M) in EtOH, 2 h, r.t.; (v) glacial AcOH, 45 h, reflux.

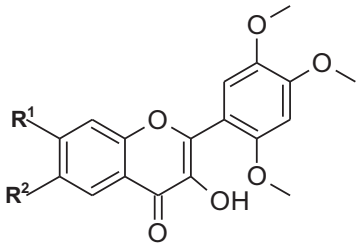
Table 1
2,4,5-Trimethoxy chalcones and analogues **1**–**26**.



1 - 20



21 - 23



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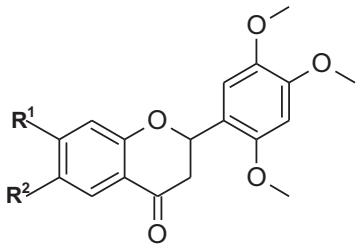

26

Compounds	R^1	R^2	R^3	R^4	R^5	% Yield[a]	Ref.
1	H	NO_2	H	H	H	82	N
2	H	H	Cl	H	H	89	[26]
3	H	H	OCH_3	H	H	85	[33]
4	H	H	CH_3	H	H	92	N
5	H	H	H	H	H	95	N
6	OH	H	OH	H	H	60	[31]
7	H	H	F	H	H	90	N
8	H	H	Morphilino	H	H	85	N
9	H	H	Imidazolyl	H	H	68	N
10	H	NO_2	Cl	H	H	75	N
11	H	H	NO_2	H	H	85	[26]
12	H	CF_3	H	H	H	89	N
13	OH	H	H	Cl	H	79	N
14	H	OCH_3	OH	H	H	86	N
15	OH	H	H	OH	H	65	N
16	OCH_3	H	OCH_3	H	OCH_3	96	N
17	H	H	Br	H	H	93	[26]
18	H	$CH_2C_6H_5$	H	$CH_2C_6H_5$	H	88	N
19	H	H	OH	H	H	83	N
20	OAc	H	H	OAc	H	95	N
21	OH	H	–	–	–	52	N
22	–	OH	–	–	–	49	N
23	–	Cl	–	–	–	54	N
24	–	Cl	–	–	–	53	N
25	OH	H	–	–	–	35	N
26	H	OH	–	–	–	46	N

N – new compound.
[a] Percentage yield of isolated compound.

corroborated by the fact that when NO_2 group (electron withdrawing group) at para position as in **11** did not show significant activity on these three cell lines. Expectedly, compound 3′-nitro, 4′-chloro chalcone (**10**) was most prominent among all, showing better activity as could be explained. Morphilino and imidazolyl substitution at para position as in **8** and **9** did not exhibit any significant shift in activity. 2′,4′-Dihydoxy compound (hydroxyl groups being ortho, para to carbonyl) (**6**) showed higher activity than 2′,5′-dihydoxy chalcone (**15**). Likewise compounds with methoxy and methyl group at 4′ position (**3**, **4**) showed higher activity.

Antioxidant activities of all compounds were evaluated by means of DPPH (2,2-diphenyl-1-picrylhydrazyl free radical), NO (nitric oxide scavenging) and $PhNHNH_2$ (for phenyl hydrazine induced haemolysis of erythrocytes) assays (Table 3). Compound **15** showed superior activity over other molecules in DPPH assay

Table 2
Anticancer activity of 2,4,5-trimethoxy chalcones and analogues (**1**–**26**).

Compounds	IC_{50}[a] (μM)		
	HeLa[b]	SW-982[c]	MCF-7[d]
1	129.9 ± 4.64	101.40 ± 7.39	>150
2	36.54 ± 4.82	58.54 ± 2.58	29.48 ± 4.01
3	37.64 ± 3.92	60.46 ± 0.48	33.87 ± 3.91
4	42.16 ± 3.26	81.08 ± 2.89	33.04 ± 6.62
5	>150	>150	>150
6	37.64 ± 3.75	32.42 ± 1.32	33.76 ± 8.44
7	28.6 ± 3.05	32.49 ± 2.53	29.96 ± 5.67
8	>150	>150	>150
9	>150	>150	>150
10	16.48 ± 1.54	20.57 ± 0.52	16.35 ± 5.99
11	>150	>150	>150
12	>150	>150	>150
13	>150	>150	>150
14	>150	>150	95.53 ± 16.3
15	>150	>150	>150
16	108.08 ± 3.75	96.47 ± 3.82	51.36 ± 6.05
17	31.67 ± 3.54	38.17 ± 0.91	18.26 ± 2.99
18	26.89 ± 3.55	69.94 ± 8.83	23.29 ± 5.28
19	–	154.9 ± 0.34	182.31 ± 4.37
20	57.90 ± 3.33	78.52 ± 9.03	63.67 ± 4.9
21	>150	>150	>150
22	>150	>150	>150
23	>150	>150	>150
24	>150	>150	>150
25	>150	>150	>150
26	>150	>150	>150
A	126.11 ± 6.82	>150	129.35 ± 8.12
B	3.42 ± 0.53	1.28 ± 0.21	0.87 ± 0.04

A: β-asarone.
B: pristimerin (positive control).
[a] IC_{50}: each data represent mean ± S.D. from three different test results in triplicate and expressed as the concentration of test compound which inhibits the cell growth by 50%.
[b] HeLa: human cervical cancer.
[c] SW-982: human synovial sarcoma.
[d] MCF-7: human breast cancer.

indicating better free radical scavenging capacity. All compounds exhibited nitric oxide scavenging activity compared to ascorbic acid. Also all the compounds inhibited phenyl hydrazine induced haemolysis of erythrocytes revealing their ability to scavenge most of the free radicals generated. Here again these compounds showed better activity in comparison with standard molecule, α-tocopherol.

3. Structure–activity relationship using *in silico* analysis

Structure–activity relationship is an important factor in determining compounds with undesired effects or low bioactivity. Osiris Property Explorer [38] is one such knowledge based activity prediction tool which predicts drug likeliness, drug score and undesired properties such as mutagenic, tumorigenic, irritant and reproductive effect of novel compounds based on chemical fragment data of available drugs and non-drugs as reported. Based on these calculations (Table 4), chalcones **6**, **13**, **15** and **16** have been predicted to show better activity. These observations correlated well with *in vitro* results for compounds **6** and **16**, thus matching the prediction levels to the extent of 50%.

4. Conclusion

2,4,5-Trimethoxy chalcones and analogues were synthesized from corresponding acetophenones and asaronaldehyde derived from naturally occurring β-asarone. A few flavones, flavanone and flavonols from corresponding chalcones were also prepared. When

Table 3
Antioxidant activities of all 26 compounds.

Compounds	IC_{50} values[a] (μg/ml)		
	DPPH	NO	$PhNHNH_2$
1	4.309	2.348	2.209
2	4.593	2.014	2.298
3	4.394	2.070	2.178
4	4.325	2.181	2.236
5	4.506	2.012	2.225
6	4.368	2.763	2.373
7	4.710	1.963	2.216
8	4.269	2.078	2.121
9	4.757	1.972	2.481
10	4.472	1.990	2.151
11	4.754	1.982	2.337
12	5.153	2.085	2.302
13	5.073	2.320	2.233
14	4.310	2.462	2.163
15	2.653	2.444	2.103
16	5.023	1.977	2.134
17	4.318	2.224	2.300
18	5.002	2.092	2.167
19	4.293	2.502	2.285
20	4.650	2.370	2.327
21	4.808	2.273	2.147
22	4.487	2.142	2.141
23	4.973	2.145	2.125
24	4.680	2.106	2.144
25	4.496	2.330	2.154
26	4.566	2.434	2.140
Std	3.039[b]	2.632[b]	3.658[c]

[a] Values are the mean of triplicate of three independent experiments.
[b] Standard ascorbic acid.
[c] Standard α-tocopherol.

Table 4
Drug likeliness properties of all 26 compounds according to Osiris property Explorer tool [38].

Compounds	Mol. wt	*C* log *P*	Drug-likeness	Drug-score	Toxicity risks[a]			
					M[b]	T[c]	I[d]	R[e]
1	343.30	3.06	−4.49	0.22	(−)	(+)	(+)	(+)
2	332.70	3.81	4.34	0.39	(−)	(+)	(+)	(+)
3	328.30	3.09	2.51	0.45	(−)	(+)	(+)	(+)
4	312.30	3.51	1.59	0.4	(−)	(+)	(+)	(+)
5	298.30	3.19	1.97	0.44	(−)	(+)	(+)	(+)
6	330.30	2.59	2.01	0.78	(+)	(+)	(+)	(+)
7	316.30	3.25	2.81	0.43	(−)	(+)	(+)	(+)
8	383.40	2.58	3.89	0.44	(−)	(+)	(+)	(+)
9	364.40	2.53	3.52	0.33	(−)	(+)	(+)	(+)
10	377.70	3.68	−5.56	0.14	(−)	(+)	(+/−)	(+)
11	343.30	3.06	−9.54	0.22	(−)	(+)	(+)	(+)
12	366.30	3.95	−8.35	0.19	(−)	(+)	(+)	(+)
13	348.30	3.51	2.68	0.68	(+)	(+)	(+)	(+)
14	344.30	2.79	2.51	0.46	(−)	(+)	(+)	(+)
15	330.30	2.59	2.12	0.79	(+)	(+)	(+)	(+)
16	388.40	2.88	2.53	0.71	(+)	(+)	(+)	(+)
17	377.20	3.89	−0.6	0.25	(−)	(+)	(+)	(+)
18	510.50	5.72	−2.99	0.09	(−)	(+)	(+)	(+)
19	314.30	2.89	2.91	0.47	(−)	(+)	(+)	(+)
20	414.1	3.05	2.48	0.64	(+)	(+)	(+)	(+)
21	328.30	2.98	2.49	0.78	(+)	(+)	(+)	(+)
22	328.30	2.98	0.61	0.66	(+)	(+)	(+)	(+)
23	346.70	3.98	2.99	0.64	(+)	(+)	(+)	(+)
24	362.70	3.3	2.75	0.67	(+)	(+)	(+)	(+)
25	344.0.3	1.21	0.33	0.39	(+)	(+)	(+)	(+)
26	330.30	2.79	2.12	0.78	(+)	(+)	(+)	(+)

[a] Ranking as (+) no bad effect, (+/−) medium bad effect, (−) bad effect.
[b] M (mutagenic effect).
[c] T (tumorigenic effect).
[d] I (irritant effect).
[e] R (reproductive effect).

these compounds were subjected to *in-vitro* testing against three human tumour cell lines (MCF-7, SW-982 and HeLa) using MTT assay, chalcones that are having electron donor groups in para position to carbonyl moiety of phenyl ring A, showed better inhibitory activity (**2**, **3**, **4**, **6**, **7**, **10**, **17**). When tested for antioxidant activities, compound **15** showed very good free radical scavenging activity in DPPH assay. All compounds exhibited significant NO scavenging activity and showed good phenyl hydrazine induced haemolysis of erythrocytes. Structure–activity relationship (SAR) study has been made and also compared *in-vitro* and *in-silico* results which matched well with each other.

5. Experimental

5.1. Chemistry

Melting points were recorded on an Acro melting point apparatus using a calibrated thermometer. Thin layer chromatography (TLC) and column chromatography (CC) were performed with [TLC silica gel 60 F_{254}, Merck] and silica gel (Kieselgel 60, 230–400 mesh, Merck) respectively. Chromatograms were developed using hexane–EtOAc (8:2, v/v) and chloroform–MeOH (9:1, v/v). IR spectra were recorded on Thermo-Nicolet instrument in KBr discs. Mass spectra were recorded using GCMS-QP2010S (direct probe) and on a QTOF micro™ AMPS MAX 10/6A system. PMR spectra and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 with TMS (tetra methylsilane) as an internal standard on a Bruker AG spectrometer and chemical shifts were recorded in δ units.

5.1.1. Synthesis of 2,4,5-trimethoxy benzaldehyde from β-asarone

β-Asarone ((*E*)-2,4,5-trimethoxy-1-propenylbenzene) was converted into 2,4,5-trimethoxy benzaldehyde by using $KMnO_4$/$NaHCO_3$ [39].

5.1.2. General procedure for synthesis of chalcones via Claisen–Schmidt condensation (**1**–**20**)

To a solution of 2,4,5-trimethoxy benzaldehyde (4 mmol) and appropriate acetophenone (4 mmol) in C_2H_5OH (25 ml), 40% of aqueous KOH (2 mmol) was added. The reaction mixture was stirred at r.t. till completion of reaction (monitored by TLC). Then the reaction mass was poured into ice water and neutralized with aqueous 10% HCl solution. The precipitate was filtered, washed with excess of water, dried and recrystallized from methanol to obtain pure chalcones. All structures were confirmed by mass and NMR spectra as discussed below.

5.1.2.1. (*E*)-1-(3-Nitrophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**1**).

Brown solid, $C_{18}H_{17}NO_6$, mp 185–188 °C.

IR (KBr): 2932, 2839, 1650, 1566, 1523, 1473, 1400, 1350, 1261, 1215, 1145, 1026, 979, 840 cm^{-1}; ^{1}HNMR ($CDCl_3$, 200 MHz): δ 3.92 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 6.53 (1H, s, H-3), 7.13 (1H, s, H-6), 7.45 (1H, d, J = 15.6 Hz, –CH=CH–CO), 7.69 (1H, dd, J = 7.9 Hz), 8.16 (1H, d, J = 15.6 Hz, –CH=CH–CO), 8.32 (1H, d, J = 7.8 Hz), 8.41 (1H, d, J = 7.8 Hz), 8.81 (1H, br s). GC–MS (*m*/*z*) = 343 $[M]^+$ (28), 312 (100), 282 (10), 266 (25), 150 (20).

5.1.2.2. (*E*)-1-(4-Methylphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**4**).

Yellow solid, $C_{19}H_{20}O_4$, mp 130–132 °C.

IR (KBr): 3433, 2931, 2835, 1651, 1608, 1585, 1505, 1465, 1434, 1407, 1299, 1207, 1176, 1033, 821 cm^{-1}; ^{1}HNMR ($CDCl_3$, 200 MHz): δ 2.43 (3H, s, CH_3), 3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.52 (1H, s, H-3), 7.13 (1H, s, H-6), 7.28 (2H, d, J = 8.2 Hz), 7.45 (1H, d, J = 15.8 Hz, –CH=CH–CO), 7.92 (2H, d, J = 8.2 Hz), 8.08 (1H, d, J = 15.8 Hz, –CH=CH–CO). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 190.82 (C=O), 154.99, 152.85, 143.67, 143.37, 140.0, 136.58, 129.53, 128.90, 120.59, 115.99, 111.9, 97.34, 56.95 (OCH_3), 56.69 (OCH_3), 56.40 (OCH_3). GC–MS (*m*/*z*) = 312 $[M]^+$ (12), 281 (100), 265 (18), 237 (14), 119 (32).

5.1.2.3. (*E*)-1-Phenyl-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**5**).

Yellow solid, $C_{18}H_{18}O_4$, mp 107–109 °C.

IR (KBr): 2931, 2839, 1647, 1630, 1558, 1512, 1458, 1400, 1261, 1211, 1137, 1028, 979, 810 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 6.52 (1H, s, H-3), 7.13 (1H, s, H-6), 7.46–7.56 (3H, m), 7.45 (1H, d, J = 15.8 Hz, –CH=CH–CO), 7.98–8.04 (2H, m), 8.09 (1H, d, J = 15.8 Hz, –CH=CH–CO). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 191.37 (C=O), 155.06, 152.94, 143.63, 140.48, 139.18, 132.68, 128.84, 128.77, 120.50, 115.80, 111.77, 97.21, 56.92 (OCH_3), 56.68 (OCH_3), 56.41 (OCH_3). GC–MS (*m*/*z*) = 298 $[M]^+$ (16), 267 (100), 251 (8), 223 (8), 105 (26).

5.1.2.4. (*E*)-1-(4-Flurophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**7**).

Yellow solid, $C_{18}H_{17}FO_4$, mp 224–227 °C.

IR (KBr): 2835, 1654, 1573, 1504, 1469, 1407, 1296, 1153, 1022, 986, 829 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.90 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.52 (1H, s, H-3), 7.15 (1H, s, H-6), 7.15–7.2 (2H, m), 7.42 (1H, d, J = 15.7 Hz, –CH=CH–CO), 8.00–8.07 (2H, m), 8.08 (1H, d, J = 15.7 Hz, –CH=CH–CO). ^{13}C NMR ($CDCl_3$, 50 MH_Z): δ 189.68 (C=O), 155.11, 153.02, 143.64, 140.69, 135.50, 135.44, 131.38, 131.20, 120.02, 116.07, 115.69, 115.64, 111.85, 97.17, 56.91 (OCH_3), 56.65 (OCH_3), 56.40 (OCH_3). GC–MS (*m*/*z*) = 316 $[M]^+$ (14), 285 (100), 269 (8), 241 (10), 170 (8), 123 (68).

5.1.2.5. (*E*)-1-(4-Morphilinophenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**8**).

Yellow solid, $C_{22}H_{25}NO_5$, mp 206–209 °C.

IR (KBr): 3421, 2839, 1639, 1604, 1573, 1508, 1446, 1407, 1299, 1211, 1126, 1026, 929, 821 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.32 (4H, m), 3.84–3.87 (4H, m), 3.89 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.52 (1H, s, H-3), 6.89–6.94 (2H, m), 7.13 (1H, s, H-6), 7.48 (1H, d, J = 15.7 Hz, –CH=CH–CO), 7.98–8.0 (2H, m), 8.07 (1H, d, J = 15.7 Hz, –CH=CH–CO). GC–MS (*m*/*z*) = 383 $[M]^+$ (8), 352 (100), 336 (10), 294 (10), 190 (8), 149 (10).

5.1.2.6. (*E*)-1-(4-Imidazolylphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**9**).

Orangish yellow solid, $C_{21}H_{20}N_2O_4$, mp 189–192 °C.

IR (KBr): 3541, 2835, 1639, 1600, 1558, 1508, 1434, 1411, 1288, 1207, 1126, 1026, 983, 829 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.91 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.53 (1H, s, H-3), 7.13 (1H, s, H-6), 7.46 (1H, d, J = 15.7 Hz, –CH=CH–CO), 7.36–7.54 (4H, m), 8.08–8.16 (3H, m), 8.08 (1H, d, J = 15.7 Hz, –CH=CH–CO). ^{13}C NMR ($CDCl_3$, 50 MH_Z): δ 190.04 (C=O), 160.24, 155.03, 152.83, 143.74, 140.05, 132.02, 131.42, 120.55, 116.16, 115.74, 112.11, 97.47, 57.04 (OCH_3), 56.80 (OCH_3), 56.46 (OCH_3). GC–MS (*m*/*z*) = 364 $[M]^+$ (18), 333 (100), 317 (10), 289 (8), 171 (10), 143 (10).

5.1.2.7. (*E*)-1-(3-Nitro,4-chlorophenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**10**).

Dark orange solid, $C_{18}H_{16}ClNO_6$, mp 156–159 °C.

IR (KBr): 2835, 1658, 1577, 1515, 1465, 1407, 1292, 1207, 1126, 1026, 991, 837 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.81 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 6.52 (1H, s, H-3), 7.10 (1H, s, H-6), 7.38 (1H, d, J = 15.6 Hz, –CH=CH–CO), 7.68 (1H, d, J = 8.4 Hz), 8.11–8.18 (1H, dd, J = 2 & J = 8.4 Hz), 8.14 (1H, d, J = 15.6 Hz, –CH=CH–CO), 8.46 (1H, d, J = 2 Hz). ^{13}C NMR ($CDCl_3$, 50 MH_Z): δ 187.68 (C=O), 155.68, 153.79, 148.31, 143.76, 142.86, 138.67, 132.80, 132.55, 131.02, 125.67, 118.55, 115.18, 112.12, 97.0, 57.0 (OCH_3), 56.67 (OCH_3), 55.48 (OCH_3). GC–MS (*m*/*z*) = 377 $[M]^+$ (10), 346 (100), 316 (10), 300 (26), 288 (8), 184 (14), 138 (16).

5.1.2.8. (*E*)-1-(3-Triflurophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**12**).

Yellow solid, $C_{19}H_{17}F_3O_4$, mp 134–137 °C.

IR (KBr): 2930, 2835, 1658, 1577, 1515, 1467, 1438, 1292, 1203, 1157, 114, 1026, 938, 844 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.91 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.53 (1H, s, H-3), 7.12 (1H, s, H-6), 7.42 (1H, d, J = 15.7 Hz, −CH=CH−CO), 7.63 (1H, m), 7.81 (1H, br d, J = 7.7 Hz), 8.12 (1H, d, J = 15.7 Hz, −CH=CH−CO), 8.21 (2H, m). GC−MS (m/z) = 366 $[M]^+$ (20), 335 (100), 291 (8), 277 (10), 173 (20), 145 (28).

*5.1.2.9. (E)-1-(2-Hydroxy,5-chlorophenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**13**).* Orange solid, $C_{18}H_{17}ClO_5$, mp 160–163 °C.

IR (KBr): 3422, 2930, 2902, 2833, 1640, 1563, 1496, 1417, 1266, 1187, 1081, 1011 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.94 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 6.53 (1H, s, H-3), 6.97 (1H, d, J = 9.0 Hz), 7.13 (1H, s, H-6), 7.41 (1H, dd, J = 2.5, 9.0 Hz), 7.50 (1H, d, J = 15.4 Hz, −CH=CH−CO), 7.87 (1H, d, J = 2.5 Hz), 8.25 (1H, d, J = 15.4 Hz, −CH=CH−CO),13.0 (1H, s, OH). GC−MS (m/z) = 348 $[M]^+$ (45), 317 (100), 194 (24), 179 (22), 155 (26), 127 (15).

*5.1.2.10. (E)-1-(3-Methoxy,4-hydroxyphenyl)-3-(2,4,5-trimethoxyphe nyl)prop-2-en-1-one (**14**).* Light orange solid, $C_{19}H_{20}O_6$, mp 181–183 °C.

IR (KBr): 3050, 2950, 2835, 1648, 1593, 1515, 1465, 1442, 1380, 1357, 1299, 1253, 1215, 1126, 1026, 972, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz); δ 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.10 (1H, s), 6.53 (1H, s, H-3), 6.99 (1H, d, J = 4.4 Hz), 7.18 (1H, s, H-6), 7.48 (1H, d, J = 15.7 Hz, −CH=CH−CO), 7.70–7.63 (2H, m), 8.07 (1H, d, J = 15.7 Hz, −CH=CH−CO). GC−MS (m/z) = 344 $[M]^+$ (16), 313 (100), 298 (15), 207 (42), 194 (24), 167 (67), 152 (22).

*5.1.2.11. (E)-1-(2,5-Dihydroxyphenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**15**).* Brick red solid, $C_{18}H_{20}O_6$, mp 218–221 °C.

IR (KBr): 3421, 2935, 1635, 1558, 1508, 1469, 1407, 1280, 1215, 1176, 1122, 1022, 897, 840 cm^{-1}. ^{13}C NMR (DMSO-d_6, 50 MHz): δ 193.75 (C=O), 155.37, 155.18, 153.82, 149.70, 143.75, 139.89, 124.54, 121.08, 118.58, 115.23, 114.93, 112.62, 98.38, 56.95 (2 × OCH_3), 56.33 (OCH_3) (identified as diacetoxy compound, **20**). GC−MS (m/z) = 330 $[M]^+$ (54), 299 (100), 194 (70), 179 (56), 151 (40), 136 (30).

*5.1.2.12. (E)-1-(2,4,6-Trimethoxyphenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**16**).* Light yellow solid, $C_{21}H_{24}O_7$, mp 132–134 °C.

IR (KBr): 2842, 1650, 1604, 1515, 1465, 1407, 1257, 1207, 1153, 1122, 1026, 945, 813 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.81 (3H, s, OCH_3), 3.76 (6H, s, 2 × OCH_3), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.16 (2H, s), 6.47 (1H, s, H-3), 6.89 (1H, d, J = 16.0 Hz, −CH=CH−CO), 7.04 (1H, s, H-6), 7.65 (1H, d, J = 16.0 Hz, −CH=CH−CO). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 195.10 (C=O), 162.50, 159.09, 154.51, 152.64, 143.75, 139.97, 127.64, 116.09, 112.78, 111.45, 97.54, 91.28, 56.87 (OCH_3), 56.43 (2 × OCH_3), 56.32 (2 × OCH_3), 55.80 (OCH_3). GC−MS (m/z) = 388 $[M]^+$ (10), 357 (100), 341 (8), 195 (13), 180 (14), 151 (12), 137 (10).

*5.1.2.13. (E)-1-(3,5-Dibenzyloxyphenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**18**).* Yellow solid, $C_{32}H_{30}O_6$, mp 154–156 °C.

IR (KBr): 3436, 1658, 1585, 1512, 1438, 1292, 1215, 1161, 1126, 1029, 964, 833 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.89 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 5.10 (4H, s, 2 × CH_2), 6.51 (1H, s, H-3), 7.10 (1H, s, H-6), 7.25−−7.28 (3H, m), 7.35–7.46 (10H, m), 7.32 (1H, d, J = 15.8 Hz, −CH=CH−CO), 8.11 (1H, d, J = 15.8 Hz, −CH=CH−CO). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 190.71 (C=O), 160.38, 155.12, 153.06, 143.73, 141.30, 140.61, 136.95, 129.05, 128.55, 128.06, 120.34, 115.9, 111.69, 107.97, 106.97, 106.48, 97.26, 70.78 (2 × ϕ−CH_2), 57.03 (OCH_3), 56.76 (OCH_3), 56.46 (OCH_3). GC−MS (m/z) = 510 $[M]^+$ (16), 479 (50), 419 (4), 269 (6), 91 (100).

*5.1.2.14. (E)-1-(4-Hydroxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**19**).* Orangish yellow solid, $C_{18}H_{18}O_5$, mp 186–189 °C.

IR (KBr): 3448, 2835, 1647, 1604, 1562, 1515, 1465, 1407, 1296, 1207, 1126, 1029, 825 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.13 (1H, s), 6.52 (1H, s, H-3), 6.93 (2H, d, J = 8.6 Hz), 7.12 (1H, s, H-6), 7.46 (1H, d, J = 15.8 Hz, −CH=CH−CO), 7.98 (2H, d, J = 8.6 Hz), 8.09 (1H, d, J = 15.8 Hz, −CH=CH−CO). GC−MS (m/z) = 314 $[M]^+$ (12), 283 (100), 267 (8), 239 (8), 121 (23).

*5.1.2.15. (E)-1-(2,5-Dicetoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**20**).* Dark yellow solid, $C_{22}H_{22}O_8$, mp 148–150 °C.

IR (KBr): 2985, 1759, 1651, 1562, 1515, 1415, 1292, 1180, 1149, 1029, 1002, 855 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 2.2 (3H, s), 2.31 (3H, s), 3.87 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.50 (1H, s, H-3), 7.04 (1H, s), 7.10 (1H, d, J = 16.0 Hz, −CH=CH−CO), 7.18 (1H, s, H-6), 7.23 (1H, d, J = 2.4 Hz), 7.41 (1H, d, J = 2.4 Hz), 7.88 (1H, d, J = 16.0 Hz, −CH=CH−CO). GC−MS (m/z) = 414 $[M]^+$ (10), 383 (13), 341 (12), 299 (15), 194 (10), 137 (8), 43 (100).

*5.1.3. General procedure for synthesis of flavones (**21**–**23**)*

To a solution of 2′-hydroxy-2,4,5-trimethoxy chalcones ((**6**, **15**, **13**) (1 equiv)) in DMSO (10.0 ml), I_2 (catalytical amounts) was added. The reaction mixture was heated to reflux for 1–2 h, cooled and poured into water and extracted into EtOAc (3 × 25.0 ml). The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated to get the product.

*5.1.3.1. 2-(2,4,5-Trimethoxyphenyl)-7-hydroxy chromen-4-one (**21**).* Light orange solid, $C_{18}H_{16}O_6$, mp 228–231 °C.

IR (KBr): 3450, 3089, 2989, 2842, 1630, 1612, 1558, 1519, 1438, 1392, 1285, 1215, 1134, 1022, 975, 848, 817 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz): δ 3.82 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.80–7.02 (4H, m), 7.80–7.87 (2H, m). GC−MS (m/z) = 328 $[M]^+$ (100), 313 (21), 297 (14), 285 (15), 192 (20), 177 (18), 137 (45).

*5.1.3.2. 2-(2,4,5-Trimethoxyphenyl)-6-hydroxy chromen-4-one (**22**).* Light brown solid, $C_{18}H_{16}O_6$, mp 254–256 °C.

IR (KBr): 3136, 2927, 1616, 1566, 1523, 1469, 1365, 1272, 1226, 1149, 1018, 852, 833 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz): δ 3.81 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.85 (1H, s), 7.20–7.65 (5H, m), 9.95 (1H, s, OH). GC−MS (m/z) = 328 $[M]^+$ (100), 313 (20), 297 (12), 285 (16), 192 (18), 177 (17), 137 (50).

*5.1.3.3. 2-(2,4,5-Trimethoxyphenyl)-6-chloro chromen-4-one (**23**).* Light orange solid, $C_{18}H_{15}ClO_5$, mp 181–83 °C.

IR (KBr): 3421, 1639, 1612, 1558, 1438, 1269, 1207, 1153, 1022, 852, 817 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.87 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 6.59 (1H, s, H-3′), 7.17 (1H, s, H-6′), 7.42 (1H, s), 7.47 (1H, d, J = 8.8 Hz), 7.60 (1H, dd, J = 2.5 and 8.8 Hz), 8.17 (1H, d, J = 2.5 Hz). GC−MS (m/z) = 346 $[M]^+$ (100), 331 (26), 303 (24), 192 (18), 177 (17), 155 (44), 149 (22).

*5.1.4. General procedure for synthesis of flavonol (**24**, **25**)*

A suspension of a mixture of powdered 2′-hydroxychalcone (0.10 g, 0.45 mmol) in EtOH, an aq. NaOH solution (8 M, 1.0 ml) and a 30% hydrogen peroxide solution (0.25 g) was stirred at r.t. for 2 h. The crude product was filtered off, washed with water to give flavonol. Recrystallization of the crude product from MeOH gave pure compound.

*5.1.4.1. 2-(2,4,5-Trimethoxyphenyl)-6-chloro,3-hydroxy chromen-4-one (**24**).* Light orange solid, $C_{18}H_{15}ClO_6$, mp 188–190 °C.

IR (KBr): 3298, 1608, 1566, 1519, 1469, 1407, 1269, 1211, 1029, 871, 779 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.89 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.0 (3H, s, OCH_3), 6.65 (1H, s, H-3′), 7.11 (1H, s, H-6′), 7.47 (1H, d, J = 9.0 Hz), 7.60 (1H, dd, J = 2.2 and 9.0 Hz), 8.24 (1H, d,

J = 2.2 Hz). GC–MS (m/z) = 362 $[M]^+$(100), 345 (82), 331 (76), 315 (24), 287 (22), 275 (24), 207 (62), 179 (67), 155 (32).

*5.1.4.2. 2-(2,4,5-Trimethoxyphenyl)-3,6-dihydroxy chromen-4-one (**25**).* Light orange solid, $C_{18}H_{16}O_7$, mp 226–229 °C.

IR (KBr): 3286, 1610, 1567, 1521, 1463, 1407, 1265, 1209, 1027, 885, 813 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz): δ 3.72 (3H, s, OCH_3), 3.80 (1H, s, OCH_3), 3.88 (3H, s, OCH_3), 6.84 (1H, s, H-3′), 7.1 (1H, s, H-6′), 7.74 (2H, m), 8.05 (1H, br s), 9.0 (1H, s, OH). GC–MS (m/z) = 344 $[M]^+$ (3), 313 (6), 238 (23), 163 (10), 78 (77), 63 (100).

*5.1.5. General procedure for synthesis of flavanone (**26**)*

2′-Hydroxychalcone (1 g) in glacial AcOH (10 ml) was heated to reflux for 45 h. Then the mixture was poured into cold water and extracted with EtOAc (2 × 25 ml). EtOAc layer was washed with brine and the organic layer dried over $MgSO_4$. Solvent evaporated to dryness. Required product was purified by column chromatography (hexane–EtOAc with increasing polarity).

*5.1.5.1. 2-(2,4,5-Trimethoxyphenyl)-6-hydroxy chroman-4-one (**26**).* Pale yellow solid, $C_{18}H_{18}O_6$, mp 175–177 °C.

IR (KBr): 3450, 3417, 2939, 2839, 1693, 1616, 1523, 1454, 1407, 1207, 1122, 1029, 991, 860 cm^{-1}; ^{1}HNMR ($CDCl_3$, 200 MHz): δ 2.79–3.0 (2H, m), 3.89 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 5.76 (1H, dd, J = 4.2 and 12.0 Hz), 6.07 (1H, br s), 6.55 (1H, s, H-3′), 6.97 (1H, d, J = 8.8 Hz), 7.10 (1H, dd, J = 3.0 and 8.8 Hz), 7.15 (1H, s, H-6′), 7.44 (1H, d, J = 3.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MH_Z): δ 190.34 (C=O), 156.86, 151.08, 150.92, 150.18, 143.68, 125.46, 121.35, 119.74, 119.21, 111.60, 111.16, 97.87, 74.86 (C-2), 57.14 (OCH_3), 56.67 (OCH_3), 56.60 (OCH_3), 44.24 (C-3). GC–MS (m/z) = 330 $[M]^+$ (60), 299 (100), 194 (72), 179 (58), 151 (40), 136 (26).

5.2. Biology

5.2.1. 5.7.1Anticancer activity

The cancerous cell lines were maintained in Dulbecco's Modified Eagle's Medium (Sigma–Aldrich Inc., USA) supplemented with 10% foetal bovine serum (Gibco BRL., USA) in a CO_2 incubator. The cytotoxicity of the compounds was measured by MTT assay [40]. Three different kinds of cancerous cell lines, viz., HeLa (cervical), MCF-7 (breast) and SW-982 (synovial) were plated in a 96-well plate at the density of 10,000 cells per well. After 24 h, the cells were treated with various concentrations of chalcones from 250 μM to 200 nM and pristimerin (triterpene quinine methide) (50 μM–50 nM) as standard. The cells were further 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:

$$\text{Cell death} = 100 - [(\text{test absorbance}/\text{control absorbance}) \times 100]$$

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

5.2.2. Antioxidant activity

5.2.2.1. In-vitro antioxidant activity (DPPH method) [41]. The scavenging reaction between (DPPH•) and an antioxidant (H–A) can be written as:

$$\underset{\text{(Purple)}}{(\text{DPPH})} + (\text{H–A}) \rightarrow \underset{\text{(Yellow)}}{\text{DPPH–H}} + (\text{A})$$

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance's decrease from the DPPH radical to the DPPH–H form. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

1 mg/ml solution of DPPH in methanol was prepared as stock. Different concentrations of the sample were prepared and 100 μl of stock DPPH was added to each concentration and made up to 3 ml with methanol. Absorbance @517 nm was measured after incubating for 15 min and the readings were taken against a blank without the sample. Ascorbic acid at various concentrations was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{Inhibition}(\%) = (1 - A_1/A_0)*100$$

where A_0 is the absorbance of control and A_1 is the absorbance of test substance.

5.2.2.2. Nitric oxide scavenging activity [42]. The procedure of nitric oxide scavenging activity is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.

10 mM of sodium nitroprusside was prepared in phosphate saline buffer (pH 7.4) and 1 ml was added to different concentrations of sample. After incubation at room temperature for 2.5 h, 0.5 ml of Griess reagent was added and absorbance at 546 nm was recorded against a blank. Ascorbic acid was used as the standard. The percentage of inhibition was calculated using the formula.

$$\text{Inhibition}(\%) = (1 - A_1/A_0)*100$$

where A_0 is the absorbance of control and A_1 is the absorbance of test substance.

5.2.2.3. Assay for phenyl hydrazine induced haemolysis of erythrocytes [43]. The erythrocyte suspension (20% PCV) was prepared from rat blood. To the different sample concentrations 1 ml of 0.5 mM phenyl hydrazine, 0.1 ml of 20% erythrocyte suspension were added and made up the volume to 3 ml with phosphate buffer saline. This was incubated at 37 °C for 1 h and centrifuged at 1000 *g* for 10 min. The supernatant was transferred to fresh test tubes and the absorbance was recorded at 540 nm. α-Tocopherol was used as the positive control.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.01.018.

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