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Synthesis and anti breast cancer activity of biphenyl based chalcones

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ABSTRACT

A series of (2*E*,2′*E*)-1,1′-(3-hydroxy-5-methylbiphenyl-2,6-diyl)-bis(3-pheylprop-2-ene-1-ones (**5**–**33**) were prepared by the reaction of 1,3-diacetyl biphenyls (**1**–**4**) with different aldehydes in presence of catalytic amount of solid KOH in ethanol in excellent yields. The compounds were evaluated for anticancer activity against human breast cancer MCF-7 (estrogen responsive proliferative breast cancer model) and MDA-MB-231 (estrogen independent aggressive breast cancer model) cell lines, HeLa (cervical cancer) cell line, and human embryonic kidney (HEK-293) cells. Most of the compounds preferentially inhibited the growth of the aggressive human breast cancer cell lines, MDA-MB-231 in the range of 4.4–30 μM. The two compounds **9** and **29** proved to be better anticancer agents than the standard drug tamoxifen against the MDA-MB-231 cell lines. Mode of action of these compounds was established to be apoptosis, cell cycle arrest and loss of mitochondrial membrane potential.

1. Introduction

1,3-Diaryl-2-propen-1-ones, commonly known as chalcones are prominent secondary metabolites and precursors of flavonoids and isoflavonoids in plants. Structurally in such compounds two phenyl rings are flanked by 2-propenone moiety and this arrangement makes them 'privileged structure' as described by Evans et al.[1] The 'enone' moiety is present in many biologically active molecules and it is considered to be primarily responsible for eliciting the biological response in such molecule. Chalcones in general are reported to exhibit various pharmacological activities such as anticancer, antimalarial, antiinflammatory, immunomodulatory, antibacterial, immunosuppressive, antiprotozan, trypanocidal, and leismanicidal properties.[2–8] Licochalcone-A, a natural product, isolated from the licorice root, is known to have a wide variety of anticancer effects.[9] They have recently been reported as antiproliferative and antitumor agents and interest in this class of molecules in identifying potent anticancer agents is renewed. These molecules with enone moiety inhibit several enzymes which render them the therapeutic potential. The ease of preparation, the potential of oral administration,[10–13] and safety[13] also support the feasibility of chalcone-based compounds to be used as chemotherapeutic agents. Tremendous amount of work is reported on the synthesis, bio-evaluations, and mechanism of action of these compounds including their interference in microtubule formation[14–24] and many cellular signaling pathways,[25–27] such as nuclear factor kappa B (NF-κB) inhibition and inhibition of IKBα Kinaseβ (IKKβ). Any alteration in the 3-carbon propenone moiety is known to lose the biological activities, the two aromatic rings, therefore have extensively been modified to get a variety of biologically active chalcones and few of them are shown in Figure 1. A number of chalcones with hydrophobic moieties and hydrophilic substituents attached to the aromatic rings such as adamantanyl and steroidal substituents were prepared and showed potent anticancer activity against different cancer cell lines.[28] Recently, we have reported α,α′-(*EE*)-bis(benzylidene)-cycloalkanones as potent antitubercular and based on reports that such compounds may act as anticancer agents as well.[29–31] We thought to synthesize hitherto unreported chalcones and see their anticancer effects. Herein we have synthesized yet another type of novel chalcones based on a 1,3-diacetyl biphenyl system with an extra phenyl-2-propenone moiety (Fig. 2), which is a pharmacophore in this class of compounds, and subsequently evaluated their anticancer activities against human breast cancer. The mode of action of the two potent compounds was also established.

2. Results and discussion

2.1. Chemistry

The starting pentasubstituted 1,3-diacetyl biphenyls (**1**–**4**) were prepared by reaction of different aromatic aldehydes and

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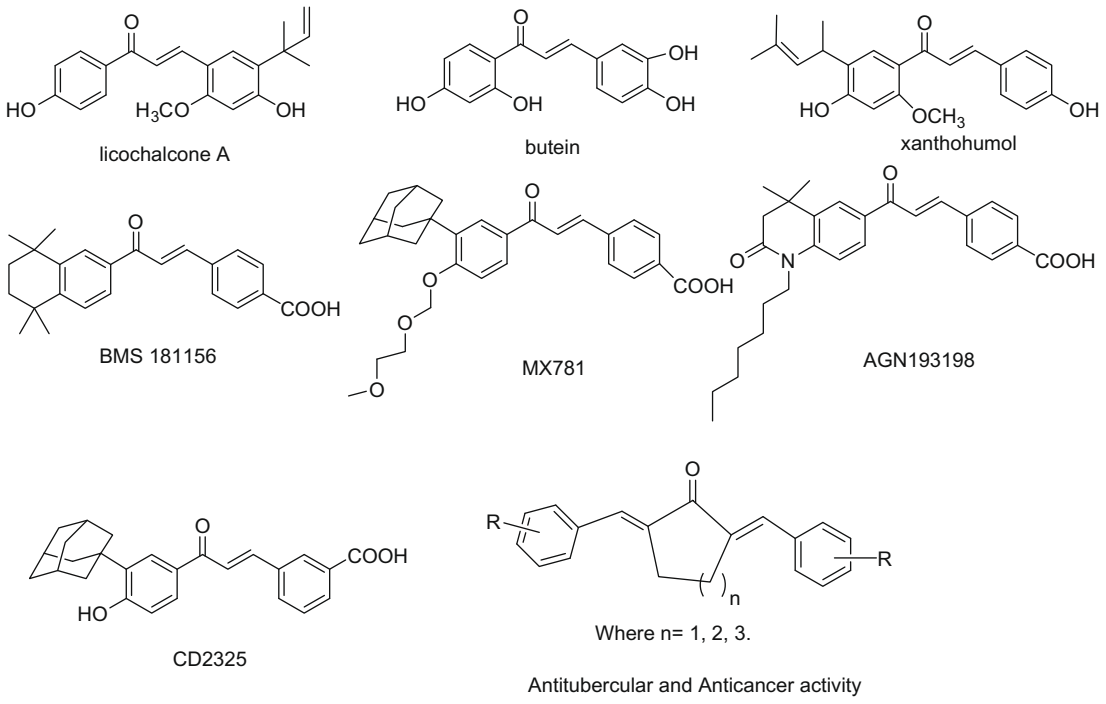


Figure 1. Chemical structure of some biologically active chalcone.

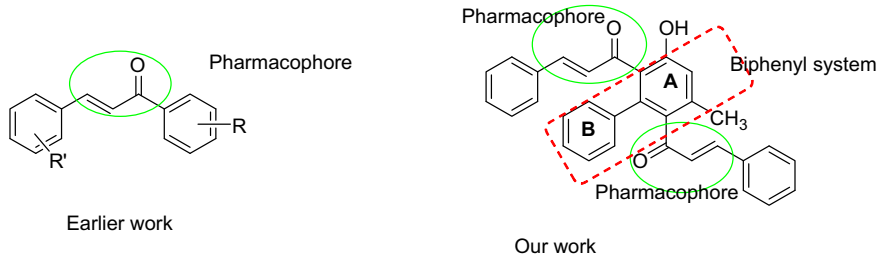
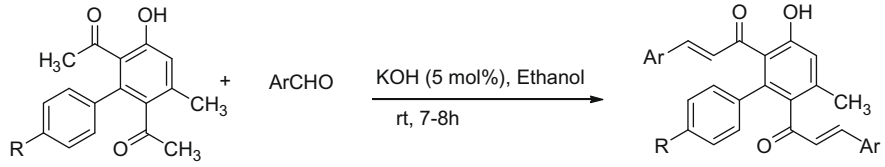


Figure 2. Earlier prepared chalcones and designed molecules with two propenone moieties as pharmacophore.

acetylacetone in presence of piperidine followed by iodine catalyzed oxidative aromatization of the intermediate cyclohexanones as recently reported by us.[32] The reaction of the biphenyls (**1–4**) separately with different aldehydes in the presence of catalytic amount of solid KOH in ethanol resulted in the title compounds **5–33** in excellent yields (Scheme 1, Table 1). The structures of these compounds were established on the basis of their spectroscopic data and microanalyses. The IR spectra of the compounds, in general, exhibited the absorption band at around 3450 cm^{-1} indicating the presence of phenolic hydroxyl group, alkene C–H stretching vibration at around 3021 cm^{-1}, absorption band of conjugated carbonyl group at around 1638–1640 cm^{-1}. The ESMS (mass spectra) of the compounds showed $[M+H]^+$ peaks corresponding to their molecular formulae. The NMR spectra (^1H and ^{13}C) are consistent with the proposed structures. The *E* geometry of both the olefinic bonds was decided on the basis of the coupling constant (*J* value) of 15.5 Hz proving the *trans*-relationship between the two olefinic protons. The ^1H NMR spectrum of a prototype of these chalcones, compound **5**, displayed exchangeable O–H proton signal as a singlet at δ 11.84, while the olefinic proton adjacent to phenyl group appeared as doublet at δ 7.51 having a *J* value of 15.54 Hz. Aromatic proton and another olefinic proton adjacent to phenyl ring were observed as multiplet at δ 7.33–6.92. The other two olefinic protons adjacent to the two carbonyl groups appeared



1- R = H
2- R = Br
3- R = OCH_2Ph
4- R = Cl

5-33

Scheme 1. Synthesis of (2*E*,2′*E*)-1,1′-(3-hydroxy-5-methylbiphenyl-2,6-diyl)-bis(3-pheylprop-2-ene-1-ones) (**5**–**33**).

Table 1
Synthesis of biphenyl chalcones (**5–33**) with different biphenyls and aromatic aldehydes

Entry	R	Ar	Product	Time (h)	Isolated yield (%)
1	H	Phenyl	**5**	7	93
2	H	4-Bromophenyl	**6**	7	92
3	H	4-Fluorophenyl	**7**	7	93
4	H	4-Chlorophenyl	**8**	7	94
5	H	3-Chlorophenyl	**9**	8	92
6	H	2-Chlorophenyl	**10**	8	93
7	H	4-Benzyloxy-phenyl	**11**	7	94
8	H	4-Methoxyphenyl	**12**	7	94
9	H	3,4-Dimethoxy-phenyl	**13**	8	92
10	H	1-Napthyl	**14**	8	92
11	H	2-Napthyl	**15**	8	94
12	Br	Phenyl	**16**	7	92
13	Br	4-Bromophenyl	**17**	7	94
14	Br	4-Fluorophenyl	**18**	8	92
15	Br	4-Chlorophenyl	**19**	7	94
16	Br	4-Benzyloxy-phenyl	**20**	8	92
17	Br	4-Methoxyphenyl	**21**	7	91
18	OCH_2Ph	Phenyl	**22**	8	90
19	OCH_2Ph	4-Bromophenyl	**23**	8	93
20	OCH_2Ph	4-Fluorophenyl	**24**	7	95
21	OCH_2Ph	4-Chlorophenyl	**25**	7	93
22	OCH_2Ph	4-Benzyloxyphenyl	**26**	7	92
23	OCH_2Ph	4-Methoxy phenyl	**27**	8	91
24	OCH_2Ph	3,4-Dimethoxy phenyl	**28**	8	93
25	Cl	Phenyl	**29**	7	92
26	Cl	4-Chlorophenyl	**30**	7	95
27	Cl	2-Chlorophenyl	**31**	8	91
28	Cl	1-Napthyl	**32**	7	92
29	Cl	2-Napthyl	**33**	7	93

as doublet at δ 6.29 and 6.27, respectively, with *J* value of 16.12 and 15.52 Hz. The protons of the methyl group appeared as singlet at δ 2.36.

In the ^{13}C NMR spectrum, the two carbonyl carbons appeared at δ 198.2 and 195.9, while the quaternary aromatic carbon attached to the hydroxyl group was observed at δ 162.4. The olefinic carbons were visible at δ 145.2, 142.6, 128.3, and 126.7 ppm. The aromatic quaternary carbons (ArC) were observed at their usual chemical shifts of δ 162.4, 143.0, 140.9, 139.6, 135.0, 134.7, 133.2, and 120.2 ppm whereas the tertiary aromatic carbons (ArCH) appeared at δ 131.2, 130.8, 130.6, 129.7, 129.1, 128.9, 128.7, 128.5, and 119.3 ppm. The methyl carbon was visible at δ 20.8. Almost similar patterns were observed in ^1H NMR and ^{13}C NMR spectra of other compounds **6–33** of the series.

2.2. Biology

2.2.1. Cell growth inhibitory activity in breast cancer cells

The above compounds **5–33** were evaluated against breast cancer cell lines MCF-7 (estrogen responsive proliferative breast cancer model) and MDA-MB-231 (estrogen independent aggressive breast cancer model), HeLa (cervical cancer) cell line, and human embryonic kidney (HEK-293) cells using MTT assay to assess cell proliferation. The results are shown in Table 2. As evident from the results almost all of the compounds except compounds **10**, **12**, **13**, **21**, **22**, and **33** were active at one or the other concentration against one or the other cell lines. Among the active compounds, the compounds **5**, **8**, **9**, **16**, and **29** were found to inhibit growth of MDA-MB-231 cells at IC_{50} significantly better than the standard anticancer agent, tamoxifen. Out of these five potent anticancer compounds, two compounds **9** and **29** exhibited relatively lesser toxicity in non-malignant cell line, HEK-293.

Table 2
Cell growth inhibitory effect of compounds (**5–33**) and their IC_{50} values (in μM) in different cell lines

S.No.	Compound	MCF-7 ± SD value	MDA-MB231 ± SD value	Hela ± SD value	HEK293 ± SD value
1	**5**	10 ± 0.02	8.5 ± 0.06	Inactive	Inactive
2	**6**	10 ± 0.01	10 ± 0.035	33.5 ± 0.045	10 ± 0.089
3	**7**	20 ± 0.006	20 ± 0.034	30 ± 0.03	Inactive
4	**8**	10 ± 0.01	7.9 ± 0.02	30 ± 0.07	10 ± 0.13
5	**9**	20 ± 0.02	4.67 ± 0.01	22 ± 0.021	15 ± 0.02
6	**10**	Inactive	Inactive	Inactive	Inactive
7	**11**	10 ± 0.02	10 ± 0.023	20 ± 0.02	30 ± 0.021
8	**12**	Inactive	Inactive	Inactive	Inactive
9	**13**	Inactive	Inactive	Inactive	Inactive
10	**14**	30 ± 0.03	Inactive	Inactive	Inactive
11	**15**	31.5 ± 0.035	30 ± 0.031	Inactive	Inactive
12	**16**	10 ± 0.022	8 ± 0.032	30 ± 0.02	30 ± 0.02
13	**17**	15 ± 0.03	18 ± 0.05	30 ± 0.01	30 ± 0.03
14	**18**	20 ± 0.04	22.3 ± 0.056	Inactive	20 ± 0.01
15	**19**	20 ± 0.02	15 ± 0.04	32 ± 0.021	25 ± 0.01
16	**20**	20 ± 0.023	20 ± 0.01	Inactive	25 ± 0.11
17	**21**	Inactive	Inactive	Inactive	Inactive
18	**22**	Inactive	Inactive	Inactive	Inactive
19	**23**	30 ± 0.08	30 ± 0.036	Inactive	20(0.02)
20	**24**	28 ± 0.02	15 ± 0.034	25 ± 0.043	30 ± 0.01
21	**25**	Inactive	30(0.045)	Inactive	Inactive
22	**26**	26 ± 0.04	20 ± 0.01	Inactive	30 ± 0.04
23	**27**	30 ± 0.05	12 ± 0.03	28 ± 0.02	28 ± 0.01
24	**28**	30 ± 0.021	22 ± 0.02	Inactive	30 ± 0.02
25	**29**	15 ± 0.06	4.4 ± 0.02	Inactive	5 ± 0.04
26	**30**	28 ± 0.032	30 ± 0.03	Inactive	Inactive
27	**31**	30 ± 0.02	29 ± 0.06	Inactive	30 ± 0.05
28	**32**	30 ± 0.02	30 ± 0.01	Inactive	30 ± 0.02
29	**33**	Inactive	Inactive	Inactive	Inactive
30	Tamoxifen	8.9 ± 0.02	10 ± 0.02	12.5 ± 0.01	10 ± 0.06

A closure look into the structure activity relationship indicates that out all the compounds (phenyl ring **A** common to all), compounds having no substituent in the phenyl (naphthyl) ring **B** or the phenyl rings (**C**) of the two cinnamoyl parts (compounds **5**, **14**, and **15**) do not show any significant activity against the Hela and HEK cell lines at the tested concentration. Although compound **5** displayed significant activity against MCF-7 and MDA-MB-231 cell lines, yet the other two compounds **14** and **15** with unsubstituted naphthyl moieties did not show any significant inhibition despite the presence of the required phenyl propenone moieties, indicating that substituents in the phenyl (naphthyl) rings are important. The methoxy substituents in the phenyl rings (compounds **12**, **13**, and **21**) of the cinnamoyl phenyls offer no advantage in improving the activity against different cell lines screened. However, compounds with benzyloxy substituents (compounds **11** and **20**) showed activity against MCF7, MDA-MB231, and HEK cell lines. Compound **20** was inactive against Hela cells. The substitution of the phenyl ring **A** at the *para*-position with *Br* (compound **16**) as compared to unsubstituted compound **5** resulted in significant improvement in the inhibitory activities against the above four cell lines and was better than tamoxifen in inhibiting the MDB-MB231 cell line. Among the halogens as substituents in the phenyl ring of the cinnamoyl moiety both the nature and position of substituents affect the biological activities. The trend in inhibitory activities was found to be *Cl* > *Br* > *F* and *m*- > *p* > *o*. Two of the above compounds **9** and **29** with 4-chloro substituents in the phenyl rings **C** and **B**, respectively, were better than the standard drug tamoxifen against the MDB-MB231 cell line. The other halogens at different positions resulted in compounds which did not display significant improvement in the activities as compared to tamoxifen. Further, as compared to MCF-7 and HEK-293, these two compounds inhibited growth of MDA-MB-231 cell lines comparatively at lower concentrations. This may indicate that

these compounds have more specific activity for inhibiting growth of MDA-MB-231 cells.

Compounds **9** and **29** induced cell death significantly in a dose dependent manner in MDA-MB231 cells as shown in Figure 3.

2.2.2. Compounds 9 and 29 induced apoptosis analyzed by flow cytometry

As the two compounds **9** and **29** were the most potent compounds of the series with IC_{50} values of 4.67 and 4.44 μM, respectively, against MDA-MB-231 cells, we investigated whether the inhibition of cell growth is associated with physiological apoptosis or non-specific necrosis. The ability of compounds **9** and **29** to induce apoptosis was measured by Annexin V-FITC (fluorescein isothiocyanate) and propidium iodide (PI) labeling of MDA-MB231 after treatment with 5 μM for 24 h. Compounds **9** and **29** showed significant (p <0.05) increase in early apoptotic cells at 5 μM concentration indicating significant induction of apoptosis as compared to untreated control in MDA-MB-231 cells (Fig. 4).

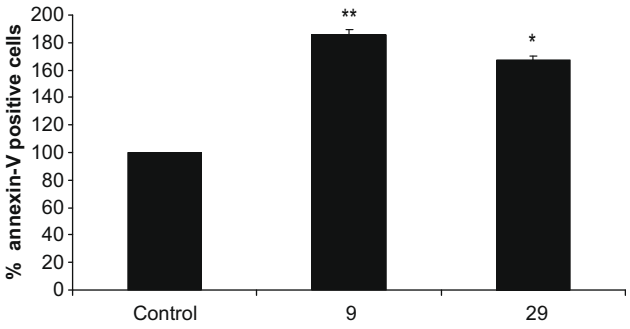


Figure 4. Apoptosis analysis in MDA-MB231 cells: MDA-MB-231 cells were treated with **9** and **29** compounds at 5 μM and their effects on Annexin-V positive cells were measured with flow cytometry.

2.2.3. Compounds 9 and 29 altered cell cycle

The tumor suppressor gene p53 is a multifunctional protein responsible for maintaining genomic integrity and its mutation is known to cause tumors in human.[33] In response to DNA damage, aberrant growth signals, or chemotherapeutic drugs, p53 activates signaling for DNA repair initially, and in failure of DNA repair, induces apoptosis and/or cell cycle arrest.[34–36] The p53 exerts its control on apoptosis by interacting with other important apoptotic molecules, including members of the bcl-2 family.[37] Data from MTT assay showed that compounds **9** and **29** induced significant inhibition of breast cancer cells. We tried to investigate whether this inhibition of cell growth was due to cell cycle arrest. For this, we studied cell cycle distribution of compound treated cells with propidium iodide-labeling and then fluorescence-activated cell sorting analysis. Results showed that compounds **9** and **29** at 5 μM concentration resulted in an increase of sub-G1 phase and decrease of cells in S phase of cell cycle as compared with untreated control (Fig. 5). Further, we analyzed the relative mRNA expression level of cell cycle regulatory gene Cyclin A1 and important apoptotic pathway gene such as bax, bcl-2, p21, and p53. Both the compounds **9** and **29** reduced expression level of cyclinA1, bcl-2 and increased bax, p53, and p21 (Fig. 6).

2.2.4. Compounds 9 and 29 induced loss of mitochondrial membrane potential and caspase activation

A decline of the mitochondrial membrane potential $\Delta\Psi_m$ is an early event in the process of mitochondrial-mediated cell death. Therefore, we determined $\Delta\Psi_m$ of compounds **9** and **29** treated MDA-MB-231 cells at 24 h. We used the mitochondrial membrane potential-sensitive probe JC-1, which forms monomers (green fluorescence) at low membrane potential and J-aggregates (red fluorescence) at higher membrane potential. The ratio between the red and the green signals is indicative of the $\Delta\Psi_m$. Compounds **9** and **29** caused decrease in 590/530 nm ratio indicating loss of mitochondrial membrane potential in MDA-MB231 cells (Fig. 7).

Caspases, the serine proteases are involved in downstream of apoptotic pathway which upon activation leads to DNA fragmentation. Therefore, we were interested to measure the caspase in the cells treated with the compounds **9** and **29** using homogenous caspase assay. A 5 μM concentration of the compounds **9** and **29** significantly induced basal caspase level in MDA-MB-231 cells (Fig. 8).

3. Conclusion

In our study, we have prepared and characterized a series of chalcones based on biphenyl system with two cinnamoyl moieties as pharmacophores. Few of these compounds inhibited the cell growth of human breast cancer cell lines, MCF-7, and MDA-MB-231. Two of the compounds **9** and **29** appeared to be promising lead against aggressive breast cancer. This inhibition of cell growth is due to significant reduction in cells at S phase of cell cycle and increase in sub-diploid apoptotic population of MDA-MB-231 cells. Changes in mRNA expression level measured by real time PCR showed that compounds **9** and **29** increased level of p53, p21, and bax. The compounds led to caspase activation by and cause DNA fragmentation in MDA-MB231 cells. In short, the active bis-

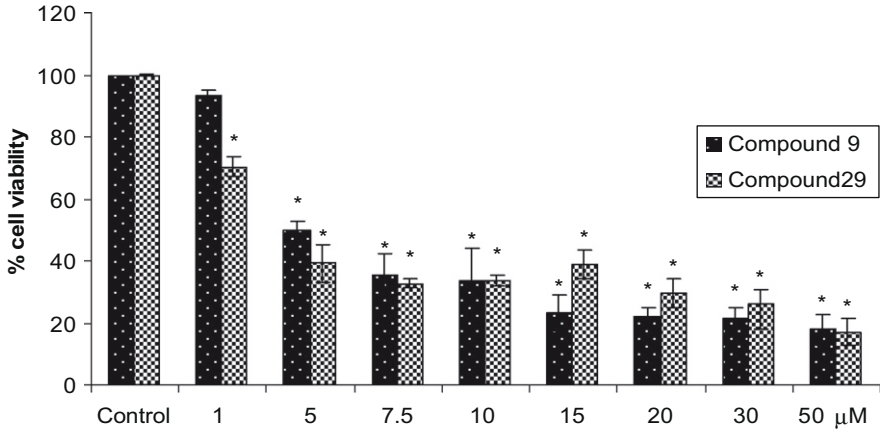


Figure 3. Effect of compounds **9** and **29** on MDA-MB 231 cell proliferation. MDA-MB 231 cells were cultured in 10% FBS medium and treated with 0, 1, 5, 7.5, 10, 15, 20, 30, and 50 μM of compounds **9** and **29** for 24 h and cell viability was monitored by MTT assay. The percent viable cells were calculated in comparison to untreated cells taken as 100%. Values were expressed as mean ± SD of three independent experiments, each performed in triplicate (p <0.05).

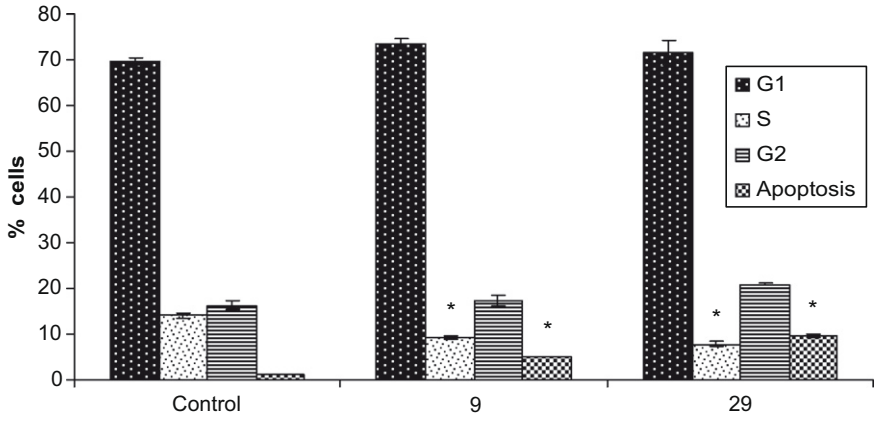


Figure 5. Analysis of cell cycle in MDA-MB231 cells: MDA-MB-231 cells were treated with 5 μM of compounds **9** and **29** and their effect on phases of cell cycle was analyzed by flow cytometry using PI stain.

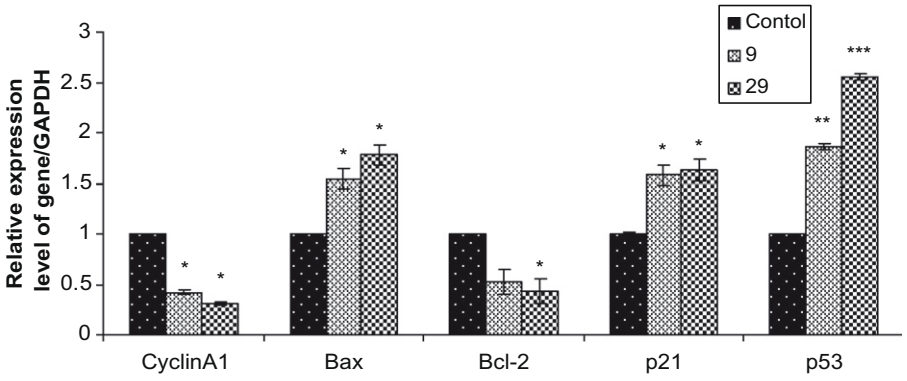
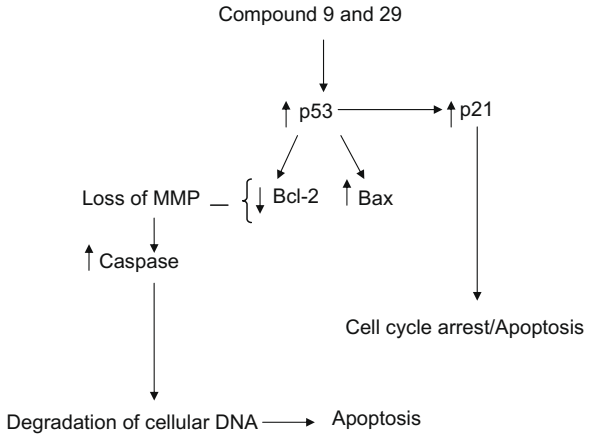


Figure 6. Compounds **9** and **29** alters relative mRNA expression level of various genes such as cyclin A1, Bax, Bcl-2, p21, and p53, analyzed by real-time PCR.

chalcones cause mitochondrial-mediated apoptosis in MDA-MB-231 cells. These studies led to identification of new prototypes for further optimization and development to get novel compounds for the treatment of aggressive breast cancer.



Summary of anticancer effects of compounds **9** *and* **29**

4. Experimental

4.1. Chemistry

Commercially available reagent grade chemicals were used as received. All reactions were followed by TLC on E. Merck Kieselgel 60 F_{254}, with detection by UV light, spraying a 20% $KMnO_4$ aq solution. Column chromatography was performed on silica gel (100–200 mesh E. Merck). IR spectra were recorded as thin films or in KBr solution with a Perkin–Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-200 in $CDCl_3$ and $CDCl_3 + CCl_4$. Chemical shift values are reported in ppm relative to TMS (tetramethylsilane) as internal reference, unless otherwise stated; s (singlet), d (doublet), m (multiplet); *J* in hertz. ESI mass spectra were performed using Quattro II (Micromass). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer.

4.1.1. General procedure for the synthesis of biphenyl based chalcones (5–33)

To a stirring solution of the biphenyl (1 mmol) and aromatic aldehyde (2.1 mmol) in minimum amount of ethanol, solid KOH (5 mol %) was added. The reaction mixture was stirred at ambient temperature till the disappearance of the starting material. After

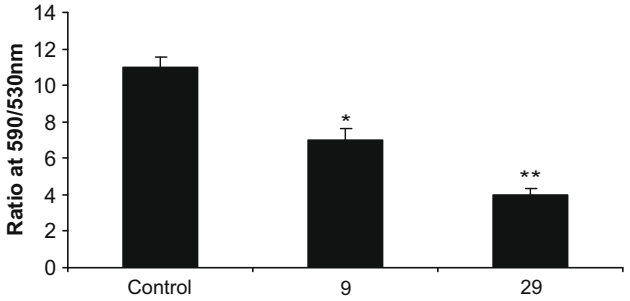


Figure 7. Loss of mitochondrial membrane potential in MDA-MB-231 cells: Compounds **9** and **29** at 5 μM for 24 h in MDA-MB-231 cells decreased ratio at 590/530 nm.

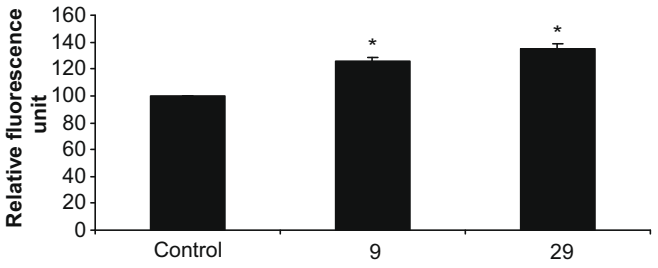


Figure 8. Induction of caspase in MDA-MB-231. Cells were treated with compounds **9** and **29** for 24 h at 5 μM concentrations, and then incubated with DEVD-rhodamine-110. Fluorescence from the activated caspases-mediated release of rhodamine-110 was measured at 560 nm. Data presented are mean of duplicates from three independent experiments.

completion of reaction (TLC), the reaction mixture was neutralized with dil. HCl (30%). The ethanol from the reaction mixture was evaporated under reduced pressure and the reaction mixture was partitioned between chloroform and water. The chloroform layer was separated and washed with water, dried over anhydrous sodium sulfate (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by column chromatography over SiO_2 (60–120 mesh) using appropriate hexane/ethylacetate as eluent to give the respective chalcones.

4.1.2. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-phenylprop-2-en-1-one) (5)

It was obtained as yellow solid, mp 148–150 °C, in 93% yield; R_f = 0.6 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3619, 3450, 3021, 2360, 1635, 1563, 1216, 1039, 762, 671; ^1H NMR (200 MHz, $CDCl_3 + CCl_4$): δ = 11.84 (s, 1H, OH), 7.51 (d, J = 15.5 Hz, 1H, =CH), 7.33–7.14 (m, 13H, 12 × ArH and =CH), 7.00–6.92 (m, 4H, ArH), 6.37, (d, J = 16.1 Hz, 1H, =CH), 6.27 (d, J = 15.5 Hz, 1H, =CH), 2.32 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$): δ = 198.2, 195.9 (2 × CO), 162.4, 145.2, 143.0, 142.6, 140.9, 139.6, 135.0, 134.7, 133.2, 131.2, 130.8, 130.6, 129.2, 129.1, 128.9, 128.7, 128.5, 128.3, 126.7, 120.2, 119.3, 20.8). MS (ESI^+): m/z: 445[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{24}O_3$: C, 83.76; H, 5.44. Found: C, 83.69; H, 5.40.

4.1.3. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-bromophenyl)prop-2-en-1-one) (6)

It was obtained as yellow solid, mp 188–190 °C, in 93% yield; R_f = 0.6 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3842, 3445, 3021, 2361, 1638, 1216, 765, 670; ^1H NMR (200 MHz, $CDCl_3 + CCl_4$): δ = 11.80 (s, 1H, OH), 7.42–7.17 (m, 10H, 8 × ArH and =CH), 7.09–7.05 (m, 2H, ArH), 6.95 (m, 1H, ArH), 6.92–6.80 (m, 3H, ArH), 6.30–6.15 (m, 2H, =CH), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$): δ = 197.6, 195.5 (2 × CO), 162.6, 143.4, 141.0, 140.9, 139.6, 133.9, 133.5, 133.1, 132.4, 132.3, 131.2, 129.9, 129.7, 129.3, 129.1, 128.6, 127.2, 125.2, 125.1, 120.0, 119.4, 20.9; MS (ESI^+): m/z: 601[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{22}O_3Br_2$: C, 61.82; H, 3.68. Found: C, 61.81; H, 3.65.

4.1.4. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-fluorophenyl)prop-2-en-1-one (7)

It was obtained as yellow solid, mp 152–154 °C, in 93% yield; R_f = 0.6 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3862, 3429, 3021, 2359, 1639, 1515, 1216, 1043, 766, 671; ^1H NMR (200 MHz, $CDCl_3 + CCl_4$): δ = 11.82 (s, 1H, OH), 7.46 (d, J = 15.5 Hz, 1H, =CH), 7.32–7.21 (m, 7H, 6 × Ar-H and =CH), 7.00–6.84 (m, 8H, ArH), 6.27 (d, J = 16.1 Hz, 1H, =CH), 6.17 (d, J = 15.5 Hz, 1H, =CH), 2.32 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$): δ = 197.8, 195.6 (2 × CO), 162.5, 161.9, 143.7, 143.1, 141.2, 140.8, 139.7, 133.3, 133.2, 131.2, 130.9, 130.8, 130.6, 130.4, 130.3, 129.2, 129.1, 128.6, 128.0, 126.5, 120.1, 119.3, 116.6, 116.4, 116.2, 116.0, 20.8; MS (ESI^+): m/z: 481[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{22}O_3F_2$: C, 77.49; H, 4.61. Found: C, 77.38; H, 4.57.

4.1.5. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-chlorophenyl)prop-2-en-1-one) (8)

It was obtained as yellow solid, mp 190–192 °C, in 94% yield; R_f = 0.6 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3857, 3020, 2358, 1640, 1518, 1216, 764, 671; ^1H NMR (200 MHz, $CDCl_3 + CCl_4$): δ = 11.87 (s, 1H, OH), 7.45 (d, J = 15.5 Hz, 1H, =CH), 7.31–7.13 (m, 11H, 10 × Ar-H and =CH), 6.97–6.87 (m, 4H, ArH), 6.33 (d, J = 16.1 Hz, 1H, =CH), 6.23 (d, J = 15.5 Hz, 1H, =CH), 2.31 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$): δ = 198.2, 195.9 (2 × CO), 162.5, 143.8, 143.4, 141.1, 139.5, 136.9, 136.7, 133.4, 133.2, 133.1, 131.2, 129.8, 129.6, 129.5, 129.4, 129.3, 129.2, 128.5, 127.1, 120.1, 119.3, 20.9; MS (ESI^+): m/z: 513[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{22}O_3Cl_2$: C, 72.52; H, 4.32. Found: C, 72.50; H, 4.28.

4.1.6. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(3-chlorophenyl)prop-2-en-1-one) (9)

It was obtained as yellow solid, mp 190–192 °C, in 92% yield; R_f = 0.6 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3780, 3435, 3021, 2926, 2357, 1596, 1217, 1020, 770, 671; ^1H NMR (200 MHz, $CDCl_3 + CCl_4$): δ = 11.92 (s, 1H, OH), 7.42–7.12 (m, 12H, 10 × ArH and =CH), 6.96–6.85 (m, 4H, ArH), 6.34 (d, 1H, J = 16.5 Hz, =CH), 6.24 (d, 1H, J = 15.7 Hz, =CH), 2.32 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$): δ = 197.5, 195.4 (2 × CO), 162.8, 143.4, 143.1, 141.0, 140.7, 139.5, 136.9, 136.5, 135.3, 135.1, 133.1, 131.2, 130.6, 130.4, 130.3, 130.1, 129.6, 129.4, 129.3, 128.3, 128.0, 127.9, 127.0, 126.4, 119.9, 119.5, 20.9; MS (ESI^+): m/z: 513[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{22}O_3Cl_2$: C, 72.52; H, 4.32. Found: C, 72.50; H, 4.29.

4.1.7. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(2-chlorophenyl)prop-2-en-1-one (10)

It was obtained as yellow solid, mp 172–174 °C, in 93% yield; R_f = 0.6 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3880, 3381, 2926, 2364, 1851, 1634, 1572, 1198, 977, 768, 579; ^1H NMR (200 MHz, $CDCl_3 + CCl_4$): δ = 11.82 (s, 1H, OH), 7.93 (d, J = 15.5 Hz, 1H, =CH), 7.40–6.97 (m, 14H, 13 × Ar-H and =CH), 6.62–6.58 (m, 1H, ArH), 6.31 (d, J = 12.2 Hz, 1H, =CH), 6.23 (d, J = 11.6 Hz, 1H, =CH), 2.33 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$): δ = 198.0, 195.4 (2 × CO), 162.6, 143.3, 140.9, 140.8, 139.6, 137.9, 135.8, 135.4, 133.2, 133.0, 131.6, 131.4, 131.2, 130.4, 129.2, 129.1, 128.8, 127.9, 127.2, 126.9, 120.0, 119.4, 20.9; MS (ESI^+): m/z: 513[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{22}O_3Cl_2$: C, 72.52; H, 4.32. Found: C, 72.46; H, 4.23.

4.1.8. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-benzyloxy)phenyl)prop-2-en-1-one) (11)

It was obtained as yellow solid, mp 163–165 °C, in 94% yield; R_f = 0.4 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3856, 3449, 3021, 2362, 1635, 1524, 1216, 1039, 764, 670; ^1H NMR (200 MHz, $CDCl_3 + CCl_4$): δ = 11.92 (s, 1H, OH), 7.48–7.17 (m, 18H, 16 × ArH and =CH), 6.97–6.76 (m, 8H, ArH), 6.27 (d, 1H, J = 16.0 Hz, =CH), 6.14 (d, 1H, J = 15.4 Hz, =CH), 5.04 (s, 2H, OCH_2), 5.03 (s, 2H, OCH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$): δ = 198.3, 195.8 (2 × CO), 162.3, 161.2, 161.1, 145.2, 142.7, 142.4, 140.7, 139.8, 136.7, 133.4, 131.1, 130.5, 130.3, 129.0, 128.9, 128.5, 128.0, 127.7, 127.6, 126.4, 124.8, 120.3, 119.2, 115.5, 115.3, 70.3, 20.8; MS (ESI^+): m/z: 657[M+H]$^+$. Elemental Anal. Calcd for $C_{45}H_{36}O_5$: C, 82.29; H, 5.52. Found: C, 82.10; H, 5.50.

4.1.9. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-methoxyphenyl)prop-2-en-1-one) (12)

It was obtained as yellow solid, mp 172–174 °C in 94% yield; R_f = 0.4 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3756, 3020, 2360, 1598, 1513, 1216, 1170, 1034, 761, 671; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.91 (s, 1H, OH), 7.48 (d, 1H, J = 15.4 Hz, =CH), 7.31–7.17 (m, 7H, 6 × ArH and =CH), 6.96–6.68 (m, 8H, ArH), 6.26 (d, 1H, J = 16.1 Hz, =CH), 6.13 (d, 1H, J = 15.4 Hz, =CH), 3.79 (s, 6H, 2 × OCH_3), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.3, 195.8 (2 × CO), 162.3, 162.0, 161.9, 145.3, 142.7, 142.4, 140.7, 139.8, 133.3, 131.1, 130.5, 130.2, 128.9, 127.8, 127.3, 126.2, 124.7, 120.3, 119.1, 114.6, 114.4, 55.5, 20.8; MS (ESI^+): m/z: 505[M+H]$^+$. Elemental Anal. Calcd for $C_{33}H_{28}O_5$: C, 78.55; H, 5.59. Found: C, 78.51; H, 5.49.

4.1.10. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis (3-(4 methoxyphenyl)prop-2-en-1-one) (13)

It was obtained as yellow solid, mp 90–92 °C, in 92% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3779, 3019, 2363, 1630, 1593, 1512, 1262, 1140, 1023, 759, 667; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.93 (s, 1H, OH), 7.49 (d, 1H, J = 15.4 Hz, =CH), 7.31–7.24 (m, 5H, 4 × ArH and =CH), 6.94–6.70 (m, 7H, ArH), 6.36 (s, 1H, ArH), 6.26 (d, 1H, J = 16.0 Hz, =CH), 6.10 (d, 1H, J = 15.3 Hz, =CH), 3.85 (s, 6H, 2 × OCH_3), 3.81 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.3, 195.6 (2 × CO), 162.3, 151.8, 149.5, 149.4, 145.7, 142.8, 140.6, 140.0, 133.2, 131.2, 128.8, 128.0, 127.5, 126.4, 124.6, 124.2, 123.3, 120.2, 119.2, 111.2, 111.1, 109.9, 109.4, 56.2, 56.1, 20.8; MS (ESI^+): m/z: 565[M+H]$^+$. Elemental Anal. Calcd for $C_{35}H_{32}O_7$: C, 74.45; H, 5.71. Found: C, 74.39; H, 5.67.

4.1.11. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis (3-(naphthalen-1-yl)prop-2-en-1-one) (14)

It was obtained as yellow solid, mp 180–181 °C, in 92% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3776, 3052, 2927, 2365, 1630, 1560, 1344, 1127, 974, 773; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.83 (s, 1H, OH), 8.40 (d, J = 15.2 Hz, 1H, =CH), 8.04 (d, J = 7.3 Hz, 1H, ArH), 7.86–7.77 (m, 6H, 5 × ArH and =CH), 7.45–7.17 (m, 12H, Ar-H), 7.00 (s, 1H, ArH), 6.76–6.72 (d, J = 7.2 Hz, 1H, ArH), 6.43–6.30 (m, 2H, =CH), 2.39 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 197.9, 195.7, 162.4, 143.1, 141.7, 140.9, 139.8, 134.0, 133.6, 132.1, 132.0, 131.7, 131.6, 131.0, 130.9, 129.2, 129.1, 129.0, 127.2, 126.6, 126.5, 125.6, 125.4, 123.7, 123.6, 120.4, 119.4, 21.0; MS (ESI^+): m/z: 545[M+H]$^+$. Elemental Anal. Calcd for $C_{39}H_{28}O_3$: C, 86.01; H, 5.18. Found: C, 85.91; H, 5.15.

4.1.12. (2*E*,2′*E*)-1,1′-(3-Hydroxy-5-methylbiphenyl-2,6-diyl)bis (3-(naphthalen-2-yl)prop-2-en-1-one) (15)

It was obtained as yellow solid, mp 184–185 °C, in 94% yield; R_f = 0.4 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3409, 3052, 2378, 1721, 1632, 1565, 1358, 1259, 1168, 977, 817, 747; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.99 (s, 1H, OH), 7.77–6.91 (m, 22H, 20 × Ar-H and =CH), 6.50, (d, J = 16.1 Hz, 1H, =CH), 6.38 (d, J = 15.4 Hz, 1H, =CH), 2.37 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.2, 195.8 (2 × CO), 162.6, 145.4, 143.1, 142.6, 141.0, 139.8, 134.7, 134.6, 133.6, 133.3, 132.6, 132.2, 131.3, 130.7, 130.6, 129.2, 129.1, 129.0, 128.9, 128.7, 128.4, 128.1, 127.7, 127.6, 127.1, 127.0, 126.9, 124.1, 123.7, 120.2, 119.3, 20.9.); MS (ESI^+): m/z: 545[M+H]$^+$. Elemental Anal. Calcd for $C_{39}H_{28}O_3$: C, 86.01; H, 5.18. Found: C, 85.89; H, 5.15.

4.1.13. (2*E*,2′*E*)-1,1′-(4′-Bromo-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-phenylprop-2-en-1-one) (16)

It was obtained as yellow solid, mp 168–170 °C in 92% yield; R_f = 0.6 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3417, 3021, 2364, 1592, 1432, 1216, 1096, 764, 671; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 12.00 (s, 1H, OH), 7.54–7.43 (m, 3H, 2 × ArH and =CH), 7.30–7.17 (m, 10H, 9 × ArH and =CH), 7.01–6.91 (m, 4H, ArH), 6.46 (d, J = 16.2 Hz, 1H,=CH), 6.17 (d, J = 15.4 Hz, 1H, =CH), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.1, 195.5 (2 × CO), 162.8, 146.1, 143.1, 143.0, 139.4, 138.5, 134.8, 134.5, 133.0, 132.7, 132.2, 131.1, 130.9, 129.3, 129.2, 128.7, 128.6, 128.5, 126.5, 123.9, 119.8, 119.7, 20.7). MS (ESI^+): m/z: 523[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{23}BrO_3$: C, 71.13; H, 4.43. Found: C, 71.06; H, 4.40.

4.1.14. (2*E*,2′*E*)-1,1′-(4′-Bromo-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-bromophenyl)prop-2-en-1-one) (17)

It was obtained as yellow solid, mp 221–223 °C in 94% yield; R_f = 0.5 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3426, 3021, 2363, 1633, 1584, 1216, 761, 671; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.94 (s, 1H, OH), 7.46–7.38 (m, 7H, 5 × ArH and =CH), 7.26–7.12 (m, 4H, ArH), 6.97–6.82 (m, 4H, ArH), 6.40 (d, J = 16.1 Hz, 1H, =CH), 6.11 (d, J = 15.4 Hz, 1H, =CH), 2.30 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 197.6, 195.2 (2 × CO), 162.9, 144.3, 143.3, 141.6, 139.4, 138.4, 133.7, 133.3, 132.9, 132.7, 132.6, 132.5, 132.2, 129.9, 128.8, 127.0, 125.6, 125.4, 124.0, 119.8, 119.7, 20.8; MS (ESI^+): m/z: 679[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{21}Br_3O_3$: C, 54.66; H, 3.11. Found: C, 54.50; H, 3.09.

4.1.15. (2*E*,2′*E*)-1,1′-(4′-Bromo-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-fluorophenyl)prop-2-en-1-one) (18)

It was obtained as yellow solid, mp 168–170 °C in 92% yield; R_f = 0.5 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3449, 2929, 2367, 1634, 1506, 1359, 1223, 1160, 829, 773; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.94 (s, 1H, OH), 7.47–7.43 (m, 3H, 2 × ArH and =CH), 7.31–7.15 (m, 4H, 3 × ArH and =CH), 7.04–6.87 (m, 8H, ArH), 6.36 (d, J = 16.1 Hz, 1H, =CH), 6.06 (d, J = 15.4 Hz, 1H, =CH), 2.30 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 197.8, 195.3 (2 × CO), 162.8, 144.6, 143.2, 141.7, 139.3, 138.5, 132.7, 132.2, 130.6, 130.4, 128.1, 126.3, 123.9, 119.8, 116.8, 116.3, 20.8); MS (ESI^+): m/z: 559[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{21}BrF_2O_3$: C, 66.56; H, 3.78. Found: C, 66.50; H, 3.71.

4.1.16. (2*E*,2′*E*)-1,1′-(4′-Bromo-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-chlorophenyl)prop-2-en-1-one) (19)

It was obtained as yellow solid, mp 174–176 °C in 94% yield; R_f = 0.5 (8:2 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3395, 3021, 2363, 1591, 1216, 1096, 766, 671; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.94 (s, 1H, OH), 7.47–7.39 (m, 3H, 2 × ArH and =CH), 7.30–7.15 (m, 8H, 7 × ArH and =CH), 6.97–6.85 (m, 4H, ArH), 6.39 (d, J = 16.1 Hz, 1H, =CH), 6.10 (d, J = 15.4 Hz, 1H, =CH), 2.30 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4); δ = 197.7, 195.2 (2 × CO), 162.9, 144.3, 143.3, 141.6, 138.4, 137.3, 137.0, 133.3, 132.9, 132.7, 132.2, 129.7, 129.6, 129.0, 128.7, 128.5, 126.9, 124.0, 119.8, 20.8; MS (ESI^+): m/z: 591[M+H]$^+$. Elemental Anal. Calcd for $C_{31}H_{21}BrCl_2O_3$: C, 62.86; H, 3.57. Found: C, 62.80; H, 3.43.

4.1.17. (2*E*,2′*E*)-1,1′-(4′-Bromo-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-(benzyloxy)phenyl)prop-2-en-1-one) (20)

It was obtained as yellow solid, mp 192–195 °C in 92% yield; R_f = 0.5 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3752, 3454, 3021, 2364, 1563, 1216, 1017,761; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 12.07 (s, 1H, OH), 7.51–7.16 (m, 17H, 15 × ArH and =CH), 6.98–6.87 (m, 8H, ArH), 6.35 (d, J = 16.1 Hz, 1H, =CH), 6.03 (d, J = 15.3 Hz, 1H, =CH), 5.06 (s, 4H, 2 × OCH_2), 2.30 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.3, 195.4 (2 × CO), 162.6, 161.4, 161.2, 146.1, 143.0, 142.7, 139.3, 138.7, 136.7,

136.6, 133.1, 132.7, 132.1, 130.6, 130.5, 129.0, 128.5, 127.8, 127.7, 127.3, 126.5, 124.5, 123.7, 119.9, 119.6, 115.6, 70.4, 20.7; MS (ESI^+): *m/z*: 735[M+H]$^+$. Elemental Anal. Calcd for $C_{45}H_{35}O_5Br$: C, 73.47; H, 4.80. Found: C, 73.41; H, 4.75.

4.1.18. (2*E*,2′*E*)-1,1′-(4′-Bromo-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-methoxyphenyl)prop-2-en-1-one) (21)

It was obtained as yellow solid, mp 178–180 °C in 91% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3756, 3019, 2364, 1598, 1216, 1168, 1030, 763; ^{1}H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 12.04 (s, 1H, OH), 7.51–7.42 (m, 3H, 2 × ArH and =CH), 7.26–7.16 (m, 4H, 3 × ArH and =CH), 6.99–6.95 (m, 4H, ArH), 6.83–6.76 (m, 4H, ArH), 6.35 (d, *J* = 16.0 Hz, 1H, =CH), 6.03 (d, *J* = 15.2 Hz, 1H, =CH), 3.80 (s, 6H, 2 × OCH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.3, 195.4 (2 × CO), 162.6, 162.2, 162.1, 146.2, 143.1, 142.7, 139.2, 138.7, 133.1, 132.7, 132.0, 130.5, 130.4, 127.6, 127.1, 126.4, 123.6, 119.9, 119.6, 114.7, 55.6, 20.7; MS (ESI^+): *m/z*: 583[M+H]$^+$. Elemental Anal. Calcd for $C_{33}H_{27}O_5Br$: C, 67.93; H, 4.66. Found: C, 67.80; H, 4.55.

4.1.19. (2*E*,2′*E*)-1,1′-(4′-(Benzyloxy)-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-phenylprop-2-en-1-one) (22)

It was obtained as yellow solid, mp 168–169 °C in 90% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3779, 3380, 3021, 2359, 1596, 1216, 762, 671; ^{1}H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.86 (s, 1H, OH), 7.51 (d, *J* = 15.5 Hz, 1H, =CH), 7.28–7.22 (m, 15H, 14 × ArH and =CH), 7.07–7.03 (m, 2H, ArH), 6.94–6.86 (m, 4H, ArH), 6.42 (d, *J* = 16.0 Hz, 1H, =CH), 6.28 (d, *J* = 15.5 Hz, 1H, =CH), 4.84 (s, 2H, OCH_2), 2.32 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.3, 196.0 (2 × CO), 162.5, 159.8, 145.0, 143.0, 142.1, 140.7, 136.7, 135.2, 134.8, 133.3, 132.5, 132.0, 130.8, 130.6, 129.2, 129.0, 128.8, 128.7, 128.6, 128.4, 128.3, 127.5, 126.9, 120.3, 118.9, 115.5, 70.4, 20.8); MS (ESI^+): *m/z*: 551[M+H]$^+$. Elemental Anal. Calcd for $C_{38}H_{30}O_4$: C, 82.89; H, 5.49. Found: C, 82.70; H, 5.45.

4.1.20. (2*E*,2′*E*)-1,1′-(4′-(Benzyloxy)-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-bromophenyl)prop-2-en-1-one) (23)

It was obtained as yellow solid, mp 170–171 °C in 93% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3778, 3376, 2927, 2365, 1632, 1236, 1009, 821, 771, 487; ^{1}H NMR (200 MHz, $CDCl_{3\ +\ CCl4}$): δ = 11.79 (s, 1H, OH), 7.43–7.09 (m, 14H, 12 × ArH and =CH), 6.92–6.88 (m, 6H, ArH), 6.35 (d, *J* = 16.5 Hz, 1H, =CH), 6.24 (d, *J* = 15.6 Hz, 1H, =CH), 4.85 (s, 2H, OCH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 197.8, 195.7 (2 × CO), 162.6, 159.8, 143.4, 143.2, 140.5, 136.5, 134.1, 133.6, 132.6, 132.4, 132.3, 131.8, 129.9, 129.8, 129.0, 128.7, 128.5, 127.6, 127.4, 125.2, 125.0, 120.2, 119.1, 115.4, 109.9, 70.5, 20.9; MS (ESI^+): *m/z*: 707[M+H]$^+$. Elemental Anal. Calcd for $C_{38}H_{28}O_4Br_2$: C, 64.42; H, 3.98. Found: C, 64.39; H, 3.88.

4.1.21. (2*E*,2′*E*)-1,1′-(4′-(Benzyloxy)-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-fluorophenyl)prop-2-en-1-one) (24)

It was obtained as yellow solid, mp 64–65 °C in 93% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3783, 3364, 3167, 2364, 1637, 1507, 1236, 1170, 827, 741, 504; ^{1}H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.78 (s, 1H, OH), 7.44 (d, *J* = 15.4 Hz, 1H, =CH), 7.22 (s, 9H, 8 × ArH and =CH), 7.00–6.85 (m, 10H, ArH), 6.29 (d, *J* = 16.1 Hz, 1H, =CH), 6.17 (d, *J* = 15.4 Hz, 1H, =CH), 4.86 (s, 2H, OCH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 197.5, 195.5 (2 × CO), 166.8, 166.7, 162.6, 161.8, 159.8, 143.2, 143.1, 140.6, 140.5, 136.6, 133.2, 132.5, 132.0, 131.4, 131.1, 131.0, 130.5, 130.4, 130.3, 130.2, 128.9, 128.4, 128.1, 127.4, 126.7, 120.2, 119.0, 116.6, 116.4, 116.1, 116.0, 115.4, 70.4, 20.8; MS (ESI^+): *m/z*: 587[M+H]$^+$. Elemental Anal. Calcd for $C_{38}H_{28}O_4F_2$: C, 77.80; H, 4.81. Found: C, 77.62; H, 4.78.

4.1.22. (2*E*,2′*E*)-1,1′-(4′-(Benzyloxy)-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-chlorophenyl)prop-2-en-1-one) (25)

It was obtained as yellow solid, mp 164–165 °C in 93% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} ^{1}3779, 3409, 3021, 2923, 1595, 1432, 1216, 1018, 929, 761, 671; ^{1}H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.79 (s, 1H, OH), 7.43 (d, *J* = 15.9 Hz, 1H, =CH), 7.24–7.18 (m, 13H, 12 × ArH and =CH), 6.99–6.85 (m, 6H, ArH), 6.34 (d, *J* = 16.3 Hz, 1H, =CH), 6.23 (d, *J* = 15.7 Hz, 1H, =CH), 4.85 (s, 2H, OCH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 197.8, 195.7 (2 × CO), 162.6, 159.8, 143.3, 143.2, 140.7, 140.5, 136.8, 136.5, 133.6, 133.2, 132.6, 131.8, 129.7 129.6, 129.5, 129.3, 129.0, 128.6, 128.5, 127.6, 127.3, 119.1, 115.4, 70.4, 20.9; MS (ESI^+): *m/z*: 619[M+H]$^+$. Elemental Anal. Calcd for $C_{38}H_{28}O_4Cl_2$: C, 73.67; H, 4.56. Found: C, 73.60; H, 4.52.

4.1.23. (2*E*,2′*E*)-1,1′-(4′-(Benzyloxy)-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-(benzyloxy)phenyl)prop-2-en-1-one) (26)

It was obtained as yellow solid, mp 64–65 °C in 92% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3781, 3324, 3036, 2362, 1602, 1505, 1247, 1016, 829, 731, 607; ^{1}H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.92 (s, 1H, OH), 7.46–7.22 (m, 20H, 18 × ArH and =CH), 7.03–6.77 (m, 10H, ArH), 6.30 (d, *J* = 16.0 Hz, 1H, =CH), 6.12 (d, *J* = 15.3 Hz, 1H, =CH), 5.04 (s, 2H, OCH_2), 4.97 (s, 2H, OCH_2), 4.84 (s, 2H, OCH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.0, 195.7 (2 × CO), 162.4, 161.1, 161.0, 159.6, 144.7, 142.6, 141.8, 140.4, 136.9, 136.7, 133.3, 132.5, 132.3, 130.4, 130.3, 128.9, 128.8, 128.4, 128.2, 127.7, 127.6, 127.4, 126.5, 125.1, 120.3, 118.9, 115.5, 115.3, 70.3, 20.8; MS (ESI^+): *m/z*: 763[M+H]$^+$. Elemental Anal. Calcd for $C_{52}H_{42}O_6$: C, 81.87; H, 5.55. Found: C, 81.80; H, 5.53.

4.1.24. (2*E*,2′*E*)-1,1′-(4′-(Benzyloxy)-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(4-methoxyphenyl)prop-2-en-1-one) (27)

It was obtained as yellow solid, mp 69–70 °C in 91% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3783, 2838, 2362, 1628, 1604, 1509, 1248, 1169, 1027, 830, 728, 561; ^{1}H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.95 (s, 1H, OH), 7.49 (d, *J* = 15.4 Hz, 1H, =CH), 7.25 (s, 9H, 8 × ArH and CH), 7.05–6.72 (m, 10H, ArH), 6.34 (d, *J* = 16.1 Hz, 1H, =CH), 6.15 (d, *J* = 15.5 Hz, 1H, =CH), 4.84 (s, 2H, OCH_2), 3.79 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.8, 196.1 (2 × CO), 162.2, 162.1, 161.9, 159.6, 145.4, 142.8, 142.2, 140.6, 136.9, 133.4, 132.4, 132.2, 130.5, 130.4, 128.9, 128.3, 127.9, 127.6, 127.4, 126.4, 124.9, 120.5, 118.8, 115.4, 114.7, 114.5, 70.4, 55.7, 55.6, 20.8; MS (ESI^+): *m/z*: 611[M+H]$^+$. Elemental Anal. Calcd for $C_{40}H_{34}O_6$: C, 78.67; H, 5.61. Found: C, 78.65; H, 5.58.

4.1.25. (2*E*,2′*E*)-1,1′-(4′-(Benzyloxy)-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(3,4-dimethoxyphenyl)prop-2-en-1-one) (28)

It was obtained as yellow solid, mp 138–140 °C in 93% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3783, 2928, 2368, 1629, 1509, 1260, 1136, 1018, 843, 735, 569; ^{1}H NMR (300 MHz, $CDCl_3$ + CCl_4): δ = 11.93 (s, 1H, OH), 7.45 (d, *J* = 11.6 Hz, =CH), 7.25–7.17 (m, 7H, 6 × ArH and =CH), 6.90–6.84 (m, 5H, ArH), 6.75–6.67 (m, 4H, ArH), 6.46 (d, *J* = 1.17 Hz, 1H, ArH), 6.28 (d, *J* = 12.0 Hz, 1H, =CH), 6.10 (d, *J* = 11.5 Hz, 1H, =CH), 4.81 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.81 (s, 6H, 2 × OCH_3), 3.76 (s, 3H, OCH_3), 2.29 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.3, 195.7 (2 × CO), 162.4, 159.5, 151.9, 151.8, 149.6, 149.5, 145.4, 142.8, 142.4, 140.4, 136.6, 133.3, 132.5, 132.3, 128.8, 128.3, 128.2, 127.7, 127.5, 126.6, 124.8, 124.0, 123.3, 120.4, 118.9, 115.2, 111.3, 111.1, 110.1, 109.5, 70.4, 56.2, 56.1, 56.0, 20.8). MS (ESI^+): *m/z*: 671[M+H]$^+$. Elemental Anal. Calcd for $C_{42}H_{38}O_8$: C, 75.21; H, 5.71. Found: C, 75.12; H, 5.69.

4.1.26. (2*E*,2′*E*)-1,1′-(4′-Chloro-3-hydroxy-5-methylbiphenyl-2,-6-diyl)bis(3-phenylprop-2-en-1-one) (29)

It was obtained as yellow solid, mp 160–161 °C in 92% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3780, 3409, 3020, 2359, 1631, 1216, 726, 627; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.92 (s, 1H, OH), 7.53 (d, *J* = 15.4 Hz, 1H, =CH), 7.39–7.26 (m, 12H, 11 × Ar-H and =CH), 7.02–6.91 (m, 4H, ArH), 6.44, (d, *J* = 16.1 Hz, 1H, =CH), 6.18 (d, *J* = 15.5 Hz, 1H, =CH), 2.31 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.1, 195.5 (2 × CO), 162.7, 146.0, 143.1, 139.4, 138.0, 135.7, 134.8, 134.5, 133.1, 132.4, 131.1, 130.9, 129.3, 129.2, 128.6, 128.4, 126.5, 119.9, 119.6, 20.8; MS (ESI^+): *m/z*: 479$[M+H]^+$. Elemental Anal. Calcd for $C_{31}H_{23}O_3Cl$: C, 77.74; H, 4.84. Found: C, 77.69; H, 4.81.

4.1.27. (2*E*,2′*E*)-1,1′-(4′-Chloro-3-hydroxy-5-methylbiphenyl-2,-6-diyl)bis(3-(4-chlorophenyl)prop-2-en-1-one) (30)

It was obtained as yellow solid, mp 162–163 °C in 95% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3776, 3021, 2359, 1592, 1215, 764, 621; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.89 (s, 1H, OH), 7.50 (d, *J* = 15.4 Hz, 1H, =CH), 7.29–7.22 (m, 10H, 9 × Ar-H and =CH), 6.99–6.88 (m, 4H, ArH), 6.40 (d, *J* = 16.2 Hz, 1H, =CH), 6.15 (d, 15.4 Hz, 1H, =CH), 2.34 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 197.7, 195.2 (2 × CO), 162.7, 144.3, 143.3, 141.5, 139.3, 137.9, 137.3, 137.0, 135.8, 133.3, 132.9, 132.5, 129.7, 129.5, 129.3, 128.6, 126.9, 119.8, 20.8; MS (ESI^+): *m/z*: 547$[M+H]^+$. Elemental Anal. Calcd for $C_{31}H_{21}O_3Cl_3$: C, 67.94; H, 3.86. Found: C, 67.90; H, 3.81.

4.1.28. (2*E*,2′*E*)-1,1′-(4′-Chloro-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(2-chlorophenyl)prop-2-en-1-one) (31)

It was obtained as yellow solid, mp 190–192 °C in 91% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3779, 3404, 3061, 2923, 2367, 1595, 1564, 1437, 1351, 1312, 1162, 1026, 970, 858, 747, 580, 517; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.91 (s, 1H, OH), 7.94 (d, *J* = 15.4 Hz, 1H, =CH), 7.39–6.99 (m, 13H, 12 × Ar-H and =CH), 6.64–6.60 (d, *J* = 7.4 Hz, 1H, ArH), 6.39 (d, *J* = 16.1 Hz, 1H, =CH), 6.17 (d, *J* = 15.4 Hz, 1H, =CH), 2.32 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.0, 195.2 (2 × CO), 162.8, 143.3, 141.7, 139.4, 138.5, 137.9, 135.7, 135.4, 133.2, 132.8, 131.7, 131.4, 130.9, 130.6, 130.5, 130.1, 129.2, 128.7, 128.1, 128.0, 127.8, 127.5, 127.4, 127.2, 119.8, 20.8; MS (ESI^+): *m/z*: 547$[M+H]^+$. Elemental Anal. Calcd for $C_{31}H_{21}O_3Cl_3$: C, 67.94; H, 3.86. Found: C, 67.88; H, 3.85.

4.1.29. (2*E*,2′*E*)-1,1′-(4′-Chloro-3-hydroxy-5-methylbiphenyl-2,-6-diyl)bis(3-(naphthalen-1-yl)prop-2-en-1-one) (32)

It was obtained as yellow solid, mp 184–185 °C in 923% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3784, 3431, 2923, 1666, 1635, 1593, 1563, 1347, 1166, 973, 773; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 11.95 (s, 1H, OH), 8.44 (d, *J* = 15.3 Hz), 8.07–7.81 (m, 7H, 6 × ArH and =CH) 7.53–7.04 (m, 10H, Ar-H), 6.76–6.73 (m, 1H, ArH), 6.57 (d, *J* = 9.9 Hz, 1H, ArH), 6.52 (d, *J* = 15.8 Hz, 1H, =CH), 6.31 (d, *J* = 15.1 Hz, 1H, =CH), 2.40 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 197.8, 195.4, 162.7, 143.2, 142.5, 139.9, 139.4, 138.2, 135.8, 134.0, 133.4, 132.8, 132.1, 131.9, 131.7, 131.3, 131.0, 129.4, 129.2, 129.1, 128.8, 127.4, 127.3, 126.7, 126.6, 125.7, 123.7, 123.4, 120.1, 119.8, 20.9). MS (ESI^+): *m/z*: 579$[M+H]^+$. Elemental Anal. Calcd for $C_{39}H_{27}O_3Cl$: C, 80.89; H, 4.70. Found: C, 80.81; H, 4.67.

4.1.30. (2*E*,2′*E*)-1,1′-(4′-Chloro-3-hydroxy-5-methylbiphenyl-2,6-diyl)bis(3-(naphthalen-2-yl)prop-2-en-1-one) (33)

It was obtained as yellow solid, mp 179–180 °C in 93% yield; R_f = 0.4 (7:3 hexane/ethylacetate); IR (KBr): ν_{max} in cm^{-1} 3779, 3630, 2923, 2363, 1625, 1550, 1357, 1163, 979, 815, 745, 474; ^1H NMR (200 MHz, $CDCl_3$ + CCl_4): δ = 12.06 (s, 1H, OH), 7.77–7.66 (m, 8H, 6 × ArH and =CH) 7.58–7.40 (m, 6H, Ar-H), 7.32–7.26 (m, 4H, ArH), 7.15–6.93 (m, 3H, ArH), 6.58 (d, *J* = 16.4 Hz, 1H, =CH), 6.31 (d, *J* = 15.4 Hz, 1H, =CH), 2.35 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + CCl_4): δ = 198.3, 195.5 (2 × CO), 162.8, 146.3, 143.2, 143.1, 139.5, 138.1, 135.7, 134.8, 134.7, 133.6, 133.2, 132.5, 132.4, 132.0, 130.9, 130.7, 129.3, 129.1, 129.0, 128.9, 128.6, 128.2, 128.1, 127.9, 127.7, 127.2, 127.0, 126.8, 123.9, 123.6, 120.0, 119.7, 20.8; MS (ESI^+): *m/z*: 579$[M+H]^+$. Elemental Anal. Calcd for $C_{39}H_{27}O_3Cl$: C, 80.89; H, 4.70. Found: C, 80.82; H, 4.66.

4.2. Biology

4.2.1. MTT assay in breast cancer cell line

The antiproliferative activities of the compounds were determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay.[38] 1×10^4 cells/well were seeded in 100 μl DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37 °C in a CO_2 incubator. Compounds, diluted to the desired concentrations in culture medium. After 24 h of incubation, media were removed and to each well 10 μl MTT (5 mg/ml) was added and the plates were further incubated for 4 h. Supernatant from each well was carefully removed, formazon crystals were dissolved in 100 μl of DMSO and absorbance at 540 nm wavelength was recorded.

4.2.2. Flow cytometric evaluation of apoptosis

MDA-MB231 cells (1×10^6) were seeded in six-well plates and allowed to grow overnight. The medium was then replaced with complete medium containing 5 μM final concentrations of compounds 9 and **29** or with vehicle alone (0.001% DMSO) as control. After 24 h treatment, cells from the supernatant and adherent monolayer cells were harvested by trypsinization, washed with PBS at 300×*g*. Then the cells (1×10^5) were stained with Annexin V-FITC and propidium iodide using the Annexin-V-PI apoptosis detection kit (Sigma). Flow cytometry was performed using a FACScan (Becton Dickinson) equipped with a single 488-nm argon laser as described earlier.[39] Annexin V-FITC were analyzed using excitation and emission settings of 488 nm and 535 nm (FL-1 channel); PI, 488 nm and 610 nm (FL-2 channel). Debris and clumps were gated out using forward and orthogonal light scatter. The experiment was repeated three times.

4.2.3. Cell cycle analysis

To determine the effect of compound on the stages of cell cycle, MDA-MB231 cells (1×10^6) were seeded in six-well plates and treated with compounds 29 and **9** at a final concentration of 5 μM for 24 h. After 24 h treatments, both floating and trypsinized adherent cells were collected and fixed with 70% ethanol. After fixation cells were washed with PBS and stained with 50 μg/ml propidium iodide in hypotonic lysis buffer (0.1% sodium citrate, 0.1% Triton X-100) containing DNase-free RNase A for 20 min. Stained cells were analyzed using fluorescence-activated cell sorter caliber (Becton Dickinson).[40]

4.2.3.1. Quantitative Real Time PCR. Total RNA was isolated using Trizol reagent (Invitrogen) following the manufacturer's instructions. Total RNA (1 μg) was digested using DNase I (Invitrogen). The DNase-treated RNA was then reverse transcribed using cDNA synthesis kit (Fermentas). Triplicate samples of cDNA were amplified by PCR using the ABI Prism 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The amplification reaction mixture (25 μl total volume) contained 1 μl cDNA, 20 pmol forward primer (*p21* F primer gaccgattttaccgatccatt, *Bax* F primer atgttttctgacggcaacttc, Bcl-2 F primer agtacctgaaccggcacct, cyclinA F primer aatgggcagtacaggaggac, p53 F primer ctttccacgacggtgaca and GAPDH F primer gctctctgctcctcctgttc, 20 pmol reverse primer (*p21* R primer

aatctctggccgctctttct, *Bax* R primer atcagttccggcaccttg, bcl-2 R primer ggccgtacagttccacaaa, cyclin A R primer ccacagtcaggg-gagtgcttt, p53 R primer tcctccatggcagtgacc and GAPDH R primer acgaccaaatccgttgactc, 12.5 μl iTaq SYBR Green Supermix (Bio-Rad), and 9.5 μl DEPC-H_2O. RRNA (18S) was used as an endogenous control to normalize the quantification of the target transcripts in each sample.

4.2.4. Mitochondrial membrane potential analysis

The effect of compounds 9 and **29** on the mitochondrial membrane potential was determined using JC-1. MDA-MB231 cells (1×10^6) were cultured in six-well plates and allowed to grow overnight. After being treated with **9** and **29** at 5 μM concentration. After 24 h treatment, cells were collected by trypsinization and washed with PBS followed by resuspending in JC-1 (10 mmol/l, Molecular Probes) and incubated at 37 °C for 15 min. Cells were rinsed three times with DMEM high glucose-containing medium without phenol red and suspended in prewarmed medium. The cells were then subjected to flow cytometric analysis on a FACScan (Becton Dickinson) in the FL1, FL2 channel to detect mitochondrial potential.

4.2.4.1. Caspase assay. The homogeneous caspase assay kit (Roche, Germany) that includes caspases 2, 3, 6, 7, 8, 9, and 10 was used to measure caspase activities after apoptosis induction according to manufacturer's instructions. MDA-MB231 cells were cultured under serum-deprived conditions for 4 h. Compound treatment was given for 24 h at 5 μM concentrations. Cells were then incubated with DEVD-rhodamine-110 (tetra-peptide sequence 'aspartic acid–glutamic acid–valine–aspartic acid' recognized by caspases). Upon cleavage of the rhodamine substrate by activated caspases, fluorescence from the released rhodamine-110 was measured.

4.2.5. Data analysis

All data were expressed as mean ± SD with at least three separate experiments. IC50 were determined with linear regression analysis using Microsoft Excel. Statistically significant comparison was calculated using student's *t*-test for unpaired variants. Values of $p < 0.05$ were regarded as statistically significant.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.015.

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