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Original article

Synthesis and antiproliferative evaluation of 3-phenylquinolinylchalcone derivatives against non-small cell lung cancers and breast cancers

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ABSTRACT

Certain 3-phenylquinolinylchalcone derivatives were synthesized and evaluated for their antiproliferative activities. Among them, (*E*)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-phenylprop-2-en-1-one (**6a**) and (*E*)-1-(5-bromothiophen-2-yl)-3-(3-(4-methoxyphenyl)quinolin-2-yl)prop-2-en-1-one (**11**) were identified as potential lead compounds for further development. Compound **6a** was active against the growth of H1299 and SKBR-3 with IC_{50} values of 1.41 and 0.70 μM respectively which was more active than the positive topotecan (IC_{50} values of 6.02 and 8.91 μM respectively). Compound **11** exhibited an IC_{50} value of less than 0.10 μM against the growth of MDA-MB231, and non-cytotoxic to the normal mammary epithelial cell (H184B5F5/M10). Mechanism studies indicated that compound **11** induced cell cycle arrest at G2/M phase followed by activation of caspase-3, cleavage of PARP, and consequently caused the cell death.

1. Introduction

Chalcones are natural products which have displayed a wide variety of biological activities, including anti-malarial, anti-protozoal, anti-inflammatory, immunomodulatory, nitric oxide inhibition, tyrosinase inhibition, and anticancer activities [1–9]. Due to their abundance in plants and ease of synthesis, the chalcone class of compounds have attracted extensive studies. On the other hand, quinoline skeleton is one of the key building elements for a large number of natural and synthetic heterocycles which possess a wide variety of biological effects such as antimalarials, bactericidal, antitumor, anti-inflammatory, antiproliferative, and antiviral activities [10–20]. Therefore, a series of chalcone derivatives in which an aryl moiety was replaced with quinoline nucleus have been synthesized and evaluated for their biological activities [21,22].

Combretastatin A-4 (**1**; Fig. 1) is a natural product with potent anticancer activity [23–27]. Its mechanism of action involves reversible, high affinity binding in the colchicine site of tubulin [28]. The novel anticancer properties exhibited by **1** have led to the extensive structural optimization [29–33]. Recently, we have synthesized certain 2,3-diarylquinoline derivatives whose structures are resemble to **1** in which the ethylene bridge was replaced with quinoline. Among them, 6-fluoro-2,3-bis{4-[2-(piperidin-1-yl)ethoxy]phenyl}quinoline (**2**) which possesses the aminoalkyl side chain, was one of the most active derivative against the growth of Hep 3B, H1299, and MDA-MB-231 with a GI_{50} value of 0.71, 1.46, and 0.72 μM respectively [34].

Herein, we report the synthesis of certain 3-phenylquinolinylchalcone derivatives whose structures can be considered as 2,3-diarylquinoline derivatives in which an α,β-unsaturated carbonyl moiety is inserted between quinoline and the C-2 phenyl group. They can also be considered as hybrids of 3-phenylquinolines and chalcones. These 3-phenylquinolinylchalcone derivatives were evaluated *in vitro* against three non-small cell lung cancer cells (H1299, H460, and A549), three breast cancer cells (MCF-7, MDA-MB-231, and SKBR-3), and a normal mammary epithelial cell (H184B5F5/M10). These cancers are common malignancies in the world, and especially are the leading cause of cancer deaths in Asian countries including Taiwan [35–39].

2. Chemistry

Thermal decarboxylation of 3-(4-methoxyphenyl)-2-methylquinoline-4-carboxylic acid (**3**) [40] gave 3-(4-methoxyphenyl)-2-

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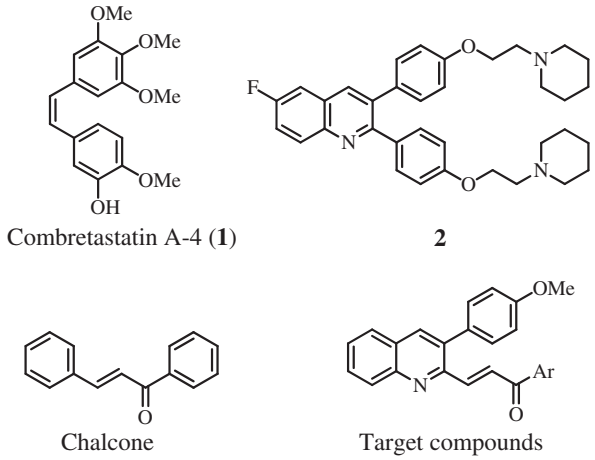


Fig. 1. Structures of combretastin A-4 (**1**), 6-fluoro-2,3-bis{4-[2-(piperidin-1-yl)-ethoxy]phenyl}quinoline (**2**), chalcone, and target compounds.

methylquinoline (**4**) which was then reacted with selenium oxide to afford 3-(4-methoxyphenyl)quinoline-2-carbaldehyde (**5**) as described in Scheme 1. The desired 3-phenylquinolinylchalcones **6**–**16** were synthesized by the Claisen–Schmidt condensation reaction of **5** and appropriately substituted acetophenones as outlined in Scheme 2. Due to the strong electron-withdrawing effect of NO_2 group, (*E*)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-(4-nitrophenyl)prop-2-en-1-one (**7**) can only be synthesized by using acid catalyst while other compounds **6a**–**i** and **8**–**16** were obtained using base catalyst. These 3-phenylquinolinylchalcones were obtained as geometrically pure and with *trans*-configuration (*J* Ha–Hb = 15.50–15.60 Hz) [41] of the sole (*E*)-form isomers.

Structure of **6e** was unambiguously determined by X-ray crystallographical analysis (Fig. 2). The structure was solved and refined by direct methods Shelxs 97 [42] suite of programs. Yellow single crystal (0.25 × 0.20 × 0.20 mm^3) of **6e** was obtained by slow evaporation from $MeOH/CH_2Cl_2$ (30/70) solution: orthorhombic, space group *P b c a*, *a* = 13.6844(5) Å, *b* = 17.8924(7) Å, *c* = 18.0314(7) angst;, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, *V* = 4414.9(3) $Å^3$, *Z* = 8, δ(calcd) = 1.280 Mg m^{-3}, *FW* = 425.46 for $C_{27}H_{23}NO_4$, *F*(000) = 1792. Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC 877658 for compound **6e**. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. (Fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk or via www.ccdc.cam.ac.uk).

3. Results and discussion

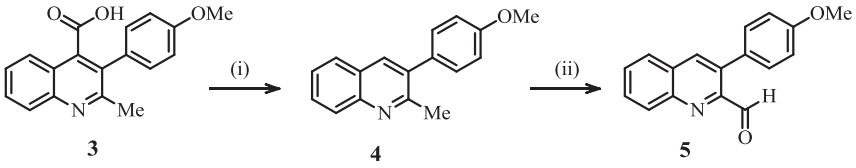
All the synthesized 3-phenylquinolinylchalcone derivatives were evaluated *in vitro* against a panel of six cancer cell lines including three non-small cell lung cancer cells (H1299, H460, and A549), three breast cancer cells (MCF-7, MDA-MB-231, and SKBR-3), and a normal mammary epithelial cell (H184B5F5/M10) using XTT assay [43]. The concentration that inhibited the growth of 50% of cells (IC_{50}) was determined from the linear portion of the curve by calculating the concentration of tested agent that reduced absorbance in treated cells, compared to control cells, by 50%.

The IC_{50} results of these 3-phenylquinolinylchalcone derivatives are summarized in Table 1. 3-(4-Methoxyphenyl)quinoline-2-carbaldehyde (**5**) was inactive while its chalcone derivative, (*E*)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-phenylprop-2-en-1-one (**6a**) was active against the growth of H1299, MDA-MB-231, and SKBR-3 with IC_{50} values of 1.41, 0.86, and 0.70 μM respectively. Although the positive topotecan was active against MDA-MB-231 with an IC_{50} of <0.1 μM, it was only weakly active against H1299 and SKBR-3 with IC_{50} values of 6.02 and 8.91 μM respectively. The antiproliferative activity against H1299 was increased by the introduction of an electron-withdrawing group such as 4-F or 4-NO_2 at C-1 phenyl moiety in which (*E*)-1-(4-fluorophenyl)-3-(3-(4-methoxyphenyl)quinolin-2-yl)prop-2-en-1-one (**6b**) and (*E*)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-(4-nitrophenyl)prop-2-en-1-one (**7**) exhibited IC_{50} values of 0.82 and 0.77 μM respectively.

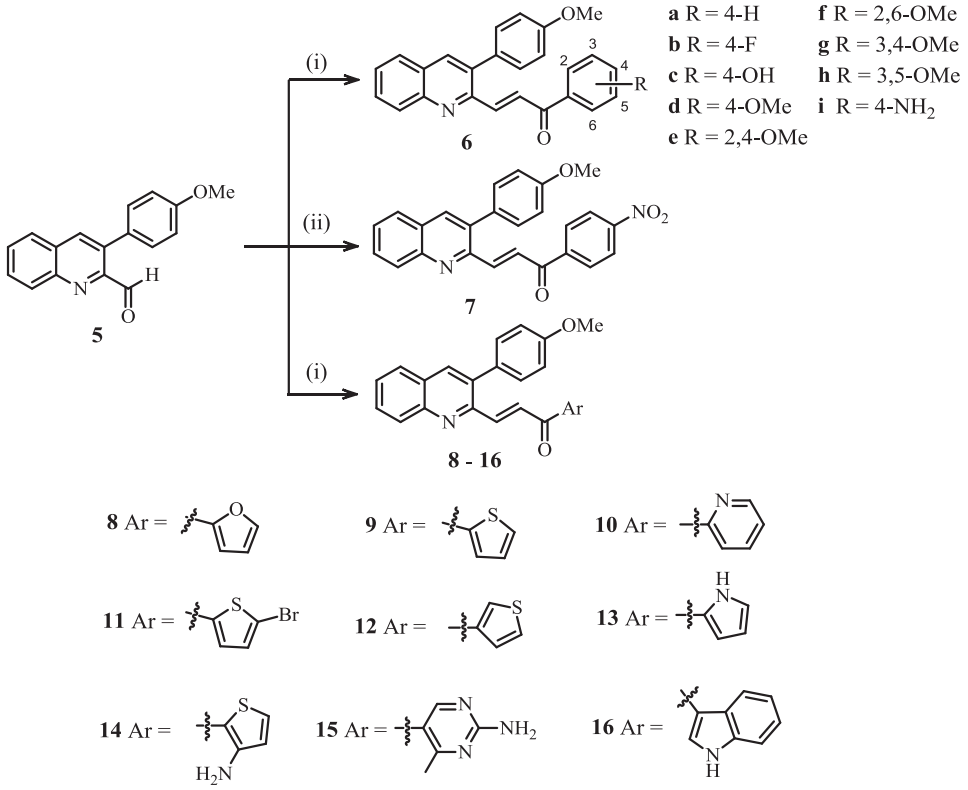
However, introduction of an electron-donating group such as 4-OH, 4-OMe, or 4-NH_2 at C-1 phenyl moiety decreased antiproliferative activity in which compounds **6c**, **6d**, and **6i** were inactive against all the cell lines tested. For the dimethoxyphenyl derivatives **6e**–**h**, 2,4-dimethoxy derivative **6e**, 2,6-dimethoxy derivative **6f**, and 3,4-dimethoxy derivative **6g** were inactive while 3,5-dimethoxy derivative **6h** was active against the growth of H1299, MDA-MB-231, and SKBR-3 with IC_{50} values of 0.93, 0.92, and 0.47 μM respectively which is approximately equal potent to the parent compound **6a**. Substitution of an electron-donating group at *ortho* or *para* position of the phenyl moiety is unfavorable to the antiproliferative activity while the influence of *meta* substitution was not apparent.

In order to establish the structure–activity relationship, C-1 phenyl group was replaced with various heterocyclic rings. The replacement of C-1 phenyl with furan-2-yl, thiophen-2-yl, or pyridin-2-yl ring enhanced antiproliferative activity in which (*E*)-1-(furan-2-yl)-3-(3-(4-methoxyphenyl)quinolin-2-yl)prop-2-en-1-one (**8**), (*E*)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-(thiophen-2-yl)prop-2-en-1-one (**9**), and (*E*)-3-(3-(4-methoxyphenyl) quinolin-2-yl)-1-(pyridin-2-yl)prop-2-en-1-one (**10**) were more active than **6a**. Substitution of a 5-Br group at the furan moiety improved antiproliferative activity against MDA-MB-231 in which (*E*)-1-(5-bromothiophen-2-yl)-3-(3-(4-methoxyphenyl)quinolin-2-yl)prop-2-en-1-one (**11**) exhibited an IC_{50} value of less than 0.10 μM. Compound **11** was also active against the growth of H1299, MCF-7, and SKBR-3 with an IC_{50} value of 0.71, 0.91, and 0.52 μM respectively. (*E*)-3-(3-(4-Methoxyphenyl)quinolin-2-yl)-1-(thiophen-3-yl)prop-2-en-1-one (**12**) was less active than **9** indicated the furan-3-yl substituent at C-1 position was less favorable than the furan-2-yl substituent. Replacement of the thiophen-3-yl group with the pyrrol-3-yl resulted in a loss of antiproliferative activity in which compound **12** was active against the growth of H1299, MDA-MB-231, and SKBR-3 with an IC_{50} value of less than 1.22 μM respectively while **13** was inactive.

Although compound **9** was active against the growth of most of the cancer cells tested, its 3-amino derivative **14** was inactive. Both



Scheme 1. Reagents and conditions: (i) Dowtherm A, 280 °C, 4 h; (ii) SeO_2, 100 °C, 2 h.



Scheme 2. Reagents and conditions: (i) KOH, EtOH, rt, 2 h; (ii) catalytic H_2SO_4, HOAc, reflux, 4 h.

pyrimidine derivative **15** and indole derivative **16** were inactive. Compound **11** was found to be especially active (IC_{50} < 0.10 μM) against the growth of MDA-MB-231 and was inactive (IC_{50} = 9.38 μM) against normal mammary epithelial cell (H184B5F5/M10), and therefore, was selected for further mechanism studies. MDA-MB231 cells were treated with 0.1–5.0 μM of compound **11** for 12, 24, 36 and 48 h and the number of viable cells was counted by XTT method.

Compound **11** inhibited the growth of MDA-MB231 cells in a dose- and time-dependent manner as shown in Fig. 3A. In addition, it was found to be less toxic on normal breast H184B5F5/M10 cells as shown in Fig. 3B. Compound **11** was also selected for further evaluation on its effects of cell cycle distribution by flow cytometric analysis. It induced cell cycle arrest in a concentration dependent manner as shown in Fig. 4 and Table 2. The proportion of cells was decreased in the G1 and accumulated in G2/M phase after 24 h treatment of **11**, while the hypodiploid (sub-G0/G1 phase) cells increased (Table 2).

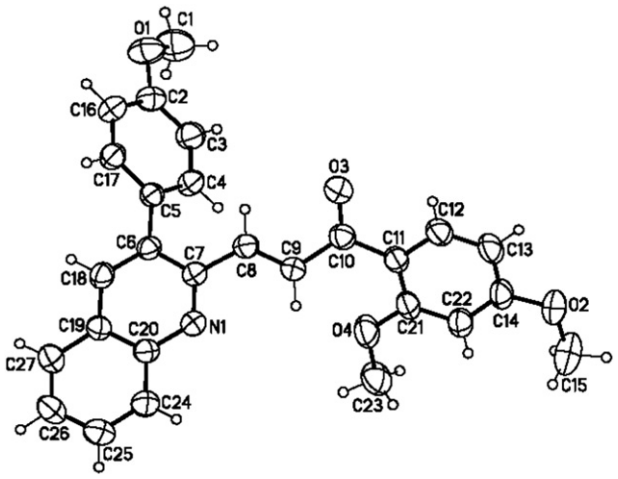


Fig. 2. ORTEP view of (*E*)-1-(2,4-dimethoxyphenyl)-3-(3-(4-methoxyphenyl)quinolin-2-yl)prop-2-en-1-one (**6e**).

Apoptosis can be characterized by morphological and biochemical changes in the cell nucleus, including chromatin condensation and nuclear shrinking. Morphological changes of cells treated with **11** can be visually observed with light microscopy (Fig. 5). We found that the MDA-MB231 cells treated with **11** at 0.5 μM for 24 h became shrinked. Such morphological changes were not apparent in the control cells.

To understand the mechanism of **11**-induced apoptosis, we examined the changes of the intracellular proteins related to apoptosis, such as caspase-3, and PARP in cells treated with **11**. Caspases play a crucial role in apoptotic cell death. Caspase-3 in particular, could be activated by the proteolytic processing of pro-caspase 3 in response to exogenous apoptosis inducers [44]. Caspase-3 is an executioner caspase whose activation leads to the cleavage of key cellular proteins including DNA repair enzyme poly-(ADP-ribose) polymerase (PARP). PARP is involved in DNA repair predominantly in response to environmental stress, and is important for the maintenance of cell viability [45]. Our results indicated that caspase-3 was activated and PARP was cleaved from 116 kDa intact form into 85 kDa fragment after the treatment of **11** for 24 h in a concentration-dependent manner (Fig. 6). Thus, compound **11** induced cell cycle arrest at G2/M phase followed by activation of caspase-3, cleavage of PARP, and consequently caused the cell death.

4. Conclusion

We have synthesized a number of 3-phenylquinolinylchalcone derivatives and evaluated *in vitro* for their activities against

Table 1
Antiproliferative activities of 3-phenylquinolinylchalcones (IC_{50}, μM).

Compd.	Cell lines						
	H1299	H460	A549	MCF-7	MDA-MB-231	SKBR-3	H184B5F5/M10
5	>10	>10	>10	>10	>10	>10	>10
6a	1.41 ± 0.13	6.67 ± 0.14	7.41 ± 0.08	4.04 ± 0.18	0.86 ± 0.07	0.70 ± 0.14	8.56 ± 0.32
6b	0.82 ± 0.06	6.99 ± 0.58	8.32 ± 0.21	5.45 ± 0.21	0.83 ± 0.05	0.81 ± 0.09	>10
6c	8.97 ± 0.86	6.93 ± 0.07	7.44 ± 0.21	6.71 ± 0.07	6.35 ± 0.09	3.88 ± 0.11	5.69 ± 0.13
6d	6.42 ± 0.13	>10	8.50 ± 0.59	7.38 ± 0.12	7.54 ± 0.05	9.28 ± 0.04	>10
6e	3.77 ± 1.24	7.04 ± 0.05	7.99 ± 0.72	6.26 ± 0.06	3.21 ± 0.21	2.26 ± 0.08	6.11 ± 0.11
6f	4.71 ± 0.15	6.63 ± 0.08	7.41 ± 0.51	4.52 ± 0.04	5.63 ± 0.09	3.83 ± 0.14	4.47 ± 0.06
6g	1.05 ± 0.08	7.54 ± 0.38	8.86 ± 1.34	6.03 ± 0.88	4.79 ± 0.50	3.26 ± 0.26	7.24 ± 0.90
6h	0.93 ± 0.17	7.52 ± 0.37	9.52 ± 0.38	3.23 ± 0.05	0.92 ± 0.57	0.47 ± 0.62	6.66 ± 0.95
6i	6.51 ± 0.10	7.40 ± 0.06	7.53 ± 0.19	6.92 ± 0.03	7.51 ± 0.07	8.561 ± 0.16	9.29 ± 0.12
7	0.77 ± 0.13	6.47 ± 0.19	6.31 ± 0.31	4.11 ± 0.04	0.84 ± 0.07	0.59 ± 0.21	6.10 ± 0.15
8	0.73 ± 0.08	6.43 ± 0.04	6.13 ± 0.05	0.71 ± 0.04	0.63 ± 0.02	0.64 ± 0.14	6.24 ± 0.33
9	0.72 ± 0.06	5.44 ± 0.55	0.92 ± 0.16	1.03 ± 0.05	0.41 ± 0.03	0.80 ± 0.04	7.03 ± 0.23
10	0.66 ± 0.12	6.26 ± 0.15	6.14 ± 0.04	0.81 ± 0.08	0.74 ± 0.03	0.37 ± 0.09	4.14 ± 0.36
11	0.71 ± 0.03	5.17 ± 0.18	6.25 ± 0.09	0.91 ± 0.09	<0.1	0.52 ± 0.05	9.38 ± 0.22
12	1.22 ± 0.14	7.08 ± 0.15	8.21 ± 0.96	6.01 ± 0.07	1.06 ± 0.12	0.66 ± 0.24	6.39 ± 0.18
13	>10	>10	>10	>10	>10	>10	>10
14	>10	>10	>10	>10	>10	>10	>10
15	>10	>10	>10	>10	>10	>10	>10
16	>10	8.01 ± 1.61	>10	>10	>10	3.45 ± 0.03	>10
Top[a]	6.02 ± 0.20	7.29 ± 1.01	4.81 ± 0.72	5.97 ± 1.03	<0.1	8.91 ± 3.03	>10

[a] Top: topotecan.

a panel of six cancer cell lines and a normal mammary epithelial cell. Results indicated that 3-(4-methoxyphenyl)quinoline-2-carbaldehyde (**5**) was inactive while its chalcone derivative, (*E*)-3-(3-(4-methoxyphenyl)-quinolin-2-yl)-1-phenylprop-2-en-1-one (**6a**) was active against the growth of H1299 and SKBR-3 with IC_{50} values of 1.41 and 0.70 μM respectively. The antiproliferative activity against H1299 was increased by the introduction of an electron-withdrawing group (such as 4-F or 4-NO_2) but was decreased by the introduction of an electron-donating group (such as 4-OH, 4-OMe, or 4-NH_2) at the C-1 phenyl moiety. In order to establish the structure–activity relationship, C-1 phenyl group was replaced with various heterocyclic rings. The replacement of C-1 phenyl with furan-2-yl, thiophen-2-yl, or pyridin-2-yl ring enhanced antiproliferative activity. Among them, (*E*)-1-(5-bromothiophen-2-yl)-3-(3-(4-methoxyphenyl)quinolin-2-yl)prop-2-en-1-one (**11**) was the most active which exhibited an IC_{50} value of less than 0.10 μM against the growth of MDA-MB-231 and was

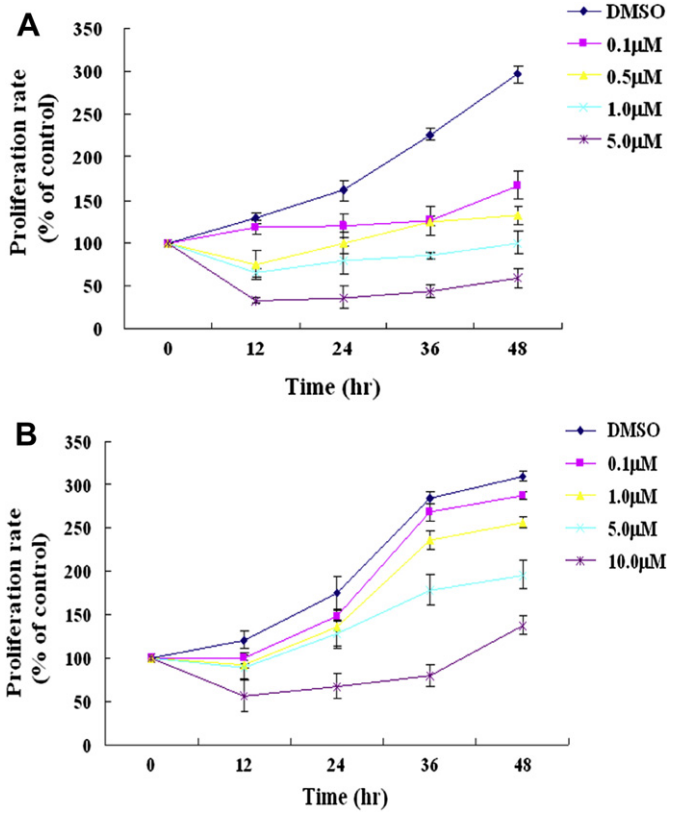


Fig. 3. Compound **11** suppresses the viability of breast cancer cell line MDA-MB-231 (A), and the normal mammary epithelial cell line H184B5F5/M10 (B). MDA-MB-231 cells were treated with DMSO, 0.1, 0.5, 1.0 and 5.0 μM of **11**; H184B5F5/M10 cells were treated with DMSO, 0.1, 1.0, 5.0 and 10.0 μM of **11**. At 12, 24, 36 and 48 h after treatment, 50 μL XTT reaction solution were added to each well for an additional 4 h of incubation. The absorbance at 492 nm was measured on a microtiter plate reader. The results represent the mean ± SD ($n = 4$).

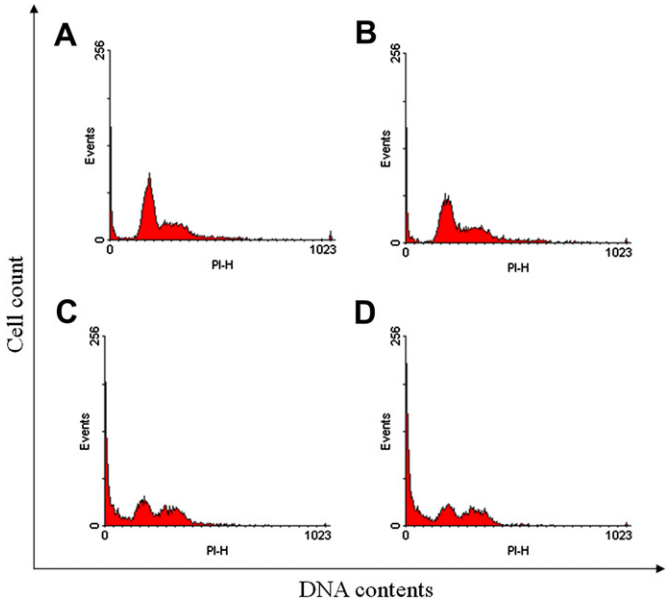


Fig. 4. Flow cytometric analysis of MDA-MB-231 cells. Cells were treated with DMSO (A), compound **11** at 0.1 μM (B), 0.5 μM (C) or 1.0 μM (D) in MB-231 cells; 24 h later the cells were harvested, fixed, and stained with propidium iodide as described in Experimental Section prior to analysis by flow cytometry. The percentage of cells in each cell cycle phase was quantified (Table 2).

Table 2
Effects of **11** on MDA-MB-231 cell cycle progression.

Concentration (μM)	Cell cycle distribution (%)[a]			
	Sub G1	G1	S	G2/M
DMSO	3.3 ± 1.5	63.1 ± 2.7	12.4 ± 2.1	22.1 ± 3.1
0.1	8.1 ± 2.1	54.3 ± 3.4	13.1 ± 3.5	25.7 ± 1.7
0.5	23.5 ± 2.4	39.6 ± 1.9	10.1 ± 2.9	28.3 ± 1.8
1.0	33.4 ± 3.2	30.1 ± 1.7	7.2 ± 2.4	30.1 ± 1.5

[a] Values representative mean ± SD from three experiments.

non-cytotoxic to the normal mammary epithelial cell (H184B5F5/M10) with an IC_{50} value of 9.38 μM. Compound **11** was also active against the growth of H1299, MCF-7, and SKBR-3 with an IC_{50} value of 0.71, 0.91, and 0.52 μM respectively. Mechanism studies indicated that compound **11** induced cell cycle arrest at G2/M phase followed by activation of caspase-3, cleavage of PARP, and consequently caused the cell death. Further studies on the structure optimization are ongoing.

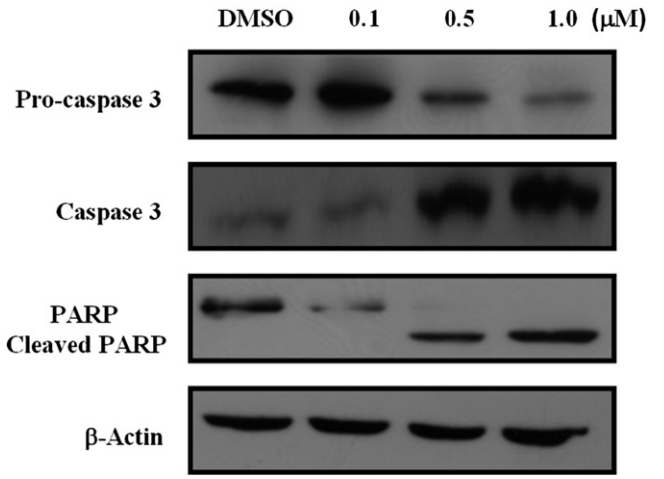


Fig. 6. Immunoblot analysis of the expression of pro-caspase 3, caspase-3, and PARP in MDA-MB-231 cells. Cells were incubated with compound **11** (0.1–1.0 μM) and the expression of the apoptosis-related proteins pro-caspase 3, caspase-3, and PARP was determined at 24 h by western blot. Protein loading was normalized to the expression of β-actin. A representative experiment is shown of three performed.

5. Experimental

5.1. General

Melting points were determined on a Electrothermal IA9100 melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Varian Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Taiwan University using Heraeus CHN–O Rapid EA, and all values are within ±0.4% of the theoretical compositions.

5.1.1. 3-(4-Methoxyphenyl)-2-methylquinoline (*4*)

3-(4-Methoxyphenyl)-2-methylquinoline-4-carboxylic acid (**3**, 1.47 g, 5.0 mmol) in 10 mL Dowtherm® A was heated at 280 °C for 4 h (TLC monitoring). After cooled to room temperature, hexane (50 mL) was added and the resulting precipitate was collected, purified by flash chromatography on silica gel (hexane/CH_2Cl_2 1/1), and crystallized from EtOH to give **4** (0.97 g, 78%) as a brown solid. Mp: 71–72 °C (EtOH). ^1H NMR ($CDCl_3$): 2.68 (s, 3H, CH_3), 3.88 (s, 3H, OMe), 6.99–7.03 (m, 2H, 3′-H), 7.32–7.35 (m, 2H, 2′-H), 7.47–7.51 (m, 1H, 6-H), 7.66–7.70 (m, 1H, 7-H), 7.78 (d, 1H, J = 8.4 Hz, 8-H), 7.94 (s, 1H, 4-H), 8.06 (d, 1H, J = 8.4 Hz, 5-H). ^{13}C NMR ($CDCl_3$): 24.60, 55.33, 113.84 (2C), 125.96, 126.93, 127.37, 128.34, 129.16, 130.34 (2C), 132.20, 135.39, 136.02, 146.86, 127.68, 159.14. Anal. Calcd for $C_{17}H_{15}NO$: C 81.90, H 6.06, N 5.62; found: C 81.92, H 6.18, N 5.50.

5.1.2. 3-(4-Methoxyphenyl)quinoline-2-carbaldehyde (*5*)

A mixture **4** (0.75 g, 3.0 mmol) and selenium dioxide (0.66 g, 6.0 mmol) in 1,4-dioxane (50 mL) was heated at 100 °C for 2 h (TLC monitoring). After cooling, the mixture was treated with 5% $NaHCO_3$ aqueous (80 mL), extracted with CH_2Cl_2 (50 mL × 3), the organic layer was collected, dried over $MgSO_4$, and evaporated. The crude product was crystallized with EtOH to give **5** (0.62 g, 78%) as a yellow solid. Mp: 106–107 °C (EtOH). ^1H NMR ($CDCl_3$): 3.89 (s, 3H, OMe), 7.01–7.05 (m, 2H, 3′-H), 7.35–7.39 (m, 2H, 2′-H), 7.67–7.71

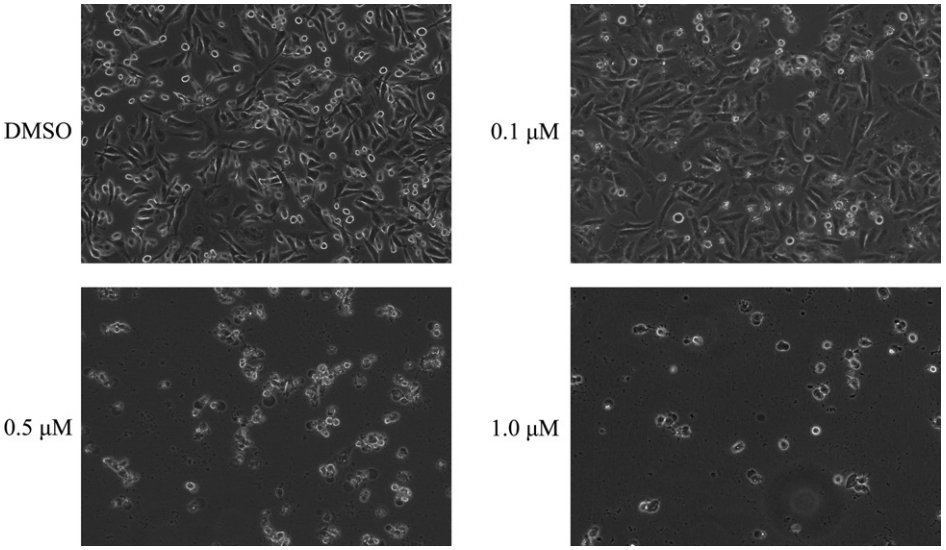


Fig. 5. Induction of morphological change in MDA-MB-231 cells. Cells were treated with DMSO or compound **11** (0.1–1.0 μM) for 24 h at 37 °C and photographed under a microscope.

(m, 1H, 6-H), 7.79–7.83 (m, 1H, 7-H), 7.90 (d, 1H, J = 8.4 Hz, 8-H), 8.19 (s, 1H, 4-H), 8.31 (d, 1H, J = 8.4 Hz, 5-H), 10.27 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$): 55.35, 113.99 (2C), 127.51, 128.97, 129.07, 129.36, 130.30, 130.49, 130.77 (2C), 135.52, 138.47, 146.89, 149.90, 159.70, 192.58. Anal. Calcd for $C_{17}H_{13}NO_2$: C 77.55, H 4.98, N 5.32; found: C 77.45, H 5.06, N 5.26.

5.2. General procedure for the preparation of quinolinyl chalcones **6a–e** and **8–16**

Compound **5** (0.53 g, 2.0 mmol) and appropriate acetophenone (2.0 mmol) were stirred at 0 °C for 15 min. Aqueous solution of KOH (6 equiv) was added and the mixture was stirred at room temperature for 12 h (TLC monitoring). After the reaction reached completion, the resulting mixture was added 1 M HCl until pH 3 resulted and extracted with ethyl acetate (50 mL × 3). The organic layer was collected, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified and crystallized with EtOH to give quinolinyl chalcones **6a–i** and **8–16**.

5.2.1. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-phenylprop-2-en-1-one (**6a**)

Yield 92%. Mp: 147–148 °C (EtOH). ^{1}H NMR ($CDCl_3$): 3.89 (s, 3H, OMe), 7.02–7.06 (m, 2H, 3′-H), 7.35–7.39 (m, 2H, 2′-H), 7.49–7.62 (m, 4H, 6- & ArH), 7.73–7.77 (m, 1H, 7-H), 7.83 (d, 1H, J = 8.4 Hz, 8-H), 8.02 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.11–8.14 (m, 3H, 4- & Ar–H), 8.19 (d, 1H, J = 8.4 Hz, 5-H), 8.39 (d, 1H, J = 15.2 Hz, CH=CH–CO). ^{13}C NMR ($CDCl_3$): 55.38, 114.176 (2C), 127.36, 127.46 (2C), 128.24, 128.59 (2C), 128.77 (2C), 129.62, 129.77, 130.43, 131.04 (2C), 132.99, 136.24, 136.92, 137.90, 141.30, 147.19, 151.51, 159.56, 190.32. Anal. Calcd for $C_{25}H_{19}NO_2$: C 82.15, H 5.25, N 3.83; found: C 82.07, H 5.16, N 3.63.

5.2.2. (E)-1-(4-Fluorophenyl)-3-[3-(4-methoxyphenyl)quinolin-2-yl]prop-2-en-1-one (**6b**)

Yield 44%. Mp: 153–154 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 7.02–7.06 (m, 2H, Ar–H), 7.15–7.21 (m, 2H, Ar–H), 7.35–7.38 (m, 2H, Ar–H) 7.58 (m, 1H, 6-H), 7.76 (m, 1H, 7-H), 7.83 (d, 1H, J = 8.0 Hz, 8-H), 8.02 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.11 (s, 1H, 4-H), 8.13–8.20 (m, 3H, 5- and Ar–H), 8.36 (d, 1H, J = 15.2 Hz, CH=CH–CO). ^{13}C NMR (100 MHz, DMSO-d_6): 55.39, 114.20 (2C), 115.72 (2C, J = 22.0 Hz), 126.92, 127.48, 127.52, 128.27, 129.62, 129.80, 130.41, 131.04 (2C), 131.39 (2C, J = 9.1 Hz), 134.31, 136.28, 136.95, 141.50, 147.21, 151.38, 159.62, 165.75 (J = 253.9 Hz), 188.67. Anal. Calcd for $C_{25}H_{18}FNO_2$: C 78.31, H 4.73, N 3.65; found: C 78.37, H 4.74, N 3.49.

5.2.3. (E)-1-(4-Hydroxyphenyl)-3-[3-(4-methoxyphenyl)quinolin-2-yl]prop-2-en-1-one (**6c**)

Yield 75%. Mp: 239–240 °C (EtOH). ^{1}H NMR (DMSO-d_6): 3.87 (s, 3H, OMe), 6.92–6.95 (m, 2H, Ar–H), 7.12–7.16 (m, 2H, Ar–H), 7.43–7.47 (m, 2H, Ar–H), 7.65–7.69 (m, 1H, 6-H), 7.76 (d, 1H, J = 14.8 Hz, CH=CH–CO), 7.82–7.86 (m, 1H, 7-H), 7.97–8.00 (m, 2H, Ar–H), 8.04 (d, 1H, J = 8.4 Hz, 8-H), 8.16 (d, 1H, J = 8.4 Hz, 5-H), 8.28 (d, 1H, J = 14.8 Hz, CH=CH–CO), 8.36 (s, 1H, 4-H), 10.54 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): 55.28, 114.15 (2C), 115.63 (2C), 127.06, 127.61, 127.85, 127.93, 128.81, 129.00, 130.02, 130.20, 131.12 (2C), 131.19 (2C), 135.65, 137.08, 139.35, 146.51, 150.78, 159.20, 162.49, 187.34. Anal. Calcd for $C_{25}H_{19}NO_3 \cdot 1H_2O$: C 75.17, H 5.30, N 3.51; found: C 74.97, H 5.22, N 3.16.

5.2.4. (E)-1-(4-Methoxyphenyl)-3-[3-(4-methoxyphenyl)quinolin-2-yl]prop-2-en-1-one (**6d**)

Yield 85%. Mp: 160–161 °C (EtOH). ^{1}H NMR ($CDCl_3$): 3.89 (s, 3H, OMe), 3.90 (s, 3H, OMe), 6.97–7.04 (m, 4H, Ar–H), 7.35–7.39 (m, 2H, Ar–H), 7.55–7.59 (m, 1H, 6-H), 7.72–7.77 (m, 1H, 7-H), 7.83 (d, 1H, J = 8.4 Hz, 5-H), 8.00 (d, 1H, J = 14.8 Hz, CH=CH–CO), 8.10 (s, 1H, 4-H), 8.12–8.16 (m, 2H, Ar–H), 8.19 (d, 1H, J = 8.4 Hz, 8-H), 8.39 (d, 1H, J = 14.8 Hz, CH=CH–CO). ^{13}C NMR ($CDCl_3$): 55.37, 55.47, 113.81 (2C), 114.17 (2C), 127.35 (2C), 127.46, 128.19, 129.57, 129.71, 130.50, 130.95, 131.04 (2C), 131.13 (2C), 136.20, 136.89, 140.52, 147.18, 151.74, 159.54, 163.57, 188.55. Anal. Calcd for $C_{26}H_{21}NO_3$: C 78.95, H 5.36, N 3.54; found: C 78.63, H 5.32, N 3.49.

5.2.5. (E)-1-(2,4-Dimethoxyphenyl)-3-[3-(4-methoxyphenyl) quinolin-2-yl]prop-2-en-1-one (**6e**)

Yield 83%; mp 151.9–152.3 °C (EtOH). ^{1}H NMR ($CDCl_3$): 3.87 (s, 3H, OMe), 3.89 (s, 3H, OMe), 3.94 (s, 3H, OMe), 6.49 (d, 1H, J = 2.4 Hz, Ar–H), 6.54 (dd, 1H, J = 8.8, 2.4 Hz, Ar–H), 7.01–7.03 (m, 2H, Ar–H), 7.36–7.39 (m, 2H, Ar–H), 7.55 (m, 1H, 6-H), 7.72 (m, 1H, 7-H), 7.78–7.82 (m, 2H, 5-H and Ar–H), 7.88 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.07 (s, 1H, 4-H), 8.14 (d, 1H, J = 8.4 Hz, 8-H), 8.30 (d, 1H, J = 15.2 Hz, CH=CH–CO). ^{13}C NMR ($CDCl_3$): 55.37, 55.52, 55.75, 98.60, 105.18, 114.11 (2C), 122.15, 127.12, 127.40, 128.08, 129.50, 129.67, 130.70, 131.02 (2C), 132.62, 133.03, 136.12, 136.67, 138.72, 147.23, 152.27, 159.49, 160.85, 164.35, 190.27. Anal. Calcd for $C_{27}H_{23}NO_4$: C 76.22, H 5.45, N 3.29; found: C 76.28, H 5.46, N 3.16.

5.2.6. (E)-1-(2,6-Dimethoxyphenyl)-3-[3-(4-methoxyphenyl) quinolin-2-yl]prop-2-en-1-one (**6f**)

Yield 73%; mp 173.1–173.5 °C (EtOH). ^{1}H NMR ($CDCl_3$): 3.75 (s, 6H, OMe ×2), 3.87 (s, 3H, OMe), 6.53 (d, 2H, J = 8.4 Hz, Ar–H), 6.92–6.99 (m, 2H, Ar–H), 7.23–7.30 (m, 3H, Ar–H), 7.54 (m, 1H, 6-H), 7.59 (d, 1H, J = 15.6 Hz, CH=CH–CO), 7.67 (d, 1H, J = 15.6 Hz, CH=CH–CO), 7.69–7.73 (m, 1H, 7-H), 7.79 (d, 1H, J = 8.0 Hz, 5-H), 8.04 (s, 1H, 4-H), 8.12 (d, 1H, J = 8.4 Hz, 8-H). ^{13}C NMR ($CDCl_3$): 55.40, 55.84 (2C), 103.62, 103.94 (2C), 113.67, 113.91 (2C), 127.35 (2C), 128.08, 129.64, 129.73, 130.50, 130.68, 130.95 (2C), 133.66, 135.86, 136.60, 141.38, 147.25, 151.59, 157.48, 159.37 (2C), 195.17. Anal. Calcd for $C_{27}H_{23}NO_4$: C 76.22, H 5.45, N 3.29; found: C 76.19, H 5.48, N 3.23.

5.2.7. (E)-1-(3,4-Dimethoxyphenyl)-3-[3-(4-methoxyphenyl) quinolin-2-yl]prop-2-en-1-one (**6g**)

Yield 78%; mp 189.8–190.0 °C (EtOH). ^{1}H NMR ($CDCl_3$): 3.87 (s, 3H, OMe), 3.96 (s, 3H, OMe), 3.98 (s, 3H, OMe), 6.95 (d, 1H, J = 8.4 Hz, Ar–H), 7.02–7.05 (m, 2H, Ar–H), 7.35–7.39 (m, 2H, Ar–H), 7.57 (m, 1H, 6-H), 7.66 (d, 1H, J = 2.0 Hz, Ar–H), 7.75 (m, 1H, 7-H), 7.82–7.86 (m, 2H, 5-H and Ar–H), 8.01 (d, 1H, J = 14.8 Hz, CH=CH–CO), 8.11 (s, 1H, 4-H), 8.19 (d, 1H, J = 8.4 Hz, 8-H), 8.39 (d, 1H, J = 14.8 Hz, CH=CH–CO). ^{13}C NMR ($CDCl_3$): 55.37, 56.02, 56.08, 110.01, 110.81, 114.18 (2C), 123.69, 127.18, 127.37, 127.48, 128.21, 129.57, 129.72, 130.52, 131.05 (2C), 131.18, 136.20, 136.89, 140.58, 147.21, 149.23, 151.77, 153.48, 159.59, 188.48. Anal. Calcd for $C_{27}H_{23}NO_4 \cdot 0.1\ H_2O$: C 75.90, H 5.47, N 3.28; found C 75.69, H 5.50, N 3.23.

5.2.8. (E)-1-(3,5-Dimethoxyphenyl)-3-[3-(4-methoxyphenyl) quinolin-2-yl]prop-2-en-1-one (**6h**)

Yield 87%; mp 162.1–162.5 °C (EtOH). ^{1}H NMR ($CDCl_3$): 3.87 (s, 6H, OMe ×2), 3.89 (s, 3H, OMe), 6.68 (t, 1H, J = 2.4 Hz, Ar–H), 7.02–7.06 (m, 2H, Ar–H), 7.25–7.26 (m, 2H, Ar–H), 7.35–7.39 (m, 2H, Ar–H), 7.58 (m, 1H, 6-H), 7.75 (m, 1H, 7-H), 7.83 (d, 1H, J = 8.0 Hz, 5-H), 8.00 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.11 (s, 1H, 4-H), 8.19 (d, 1H, J = 8.4 Hz, 8-H), 8.29 (d, 1H, J = 15.2 Hz, CH=CH–CO). ^{13}C NMR ($CDCl_3$): 55.37, 56.63 (2C), 105.40, 106.61 (2C), 114.20 (2C), 127.45, 127.48 (2C), 128.24, 129.68, 129.77, 130.48, 130.92, 131.05 (2C), 136.22, 136.90, 139.94, 141.51, 147.22, 151.51, 159.60, 160.90 (2C), 190.02. Anal. Calcd for $C_{27}H_{23}NO_4 \cdot 0.1\ H_2O$: C 75.90, H 5.47, N 3.28; found C 75.78, H 5.48, N 3.16.

*5.2.9. (E)-1-(4-Aminophenyl)-3-[3-(4-methoxyphenyl)quinolin-2-yl]prop-2-en-1-one (**6i**)*

Yield 40%; mp 198.2–201 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.88 (s, 3H, OMe), 4.20 (br s, 2H, NH2), 6.68–6.71 (m, 2H, Ar–H), 7.01–7.05 (m, 2H, Ar–H), 7.35–7.39 (m, 2H, Ar–H), 7.56 (m, 1H, 6-H), 7.73 (m, 1H, 7-H), 7.82 (d, 1H, J = 8.4 Hz, 5-H), 7.97 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.00–8.04 (m, 2H, Ar–H), 8.09 (s, 1H, 4-H), 8.19 (d, 1H, J = 8.4 Hz, 8-H), 8.37 (d, 1H, J = 15.2 Hz, CH=CH–CO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.37, 113.89 (2C), 114.16 (2C), 127.22, 127.45, 127.67, 128.13, 128.46, 129.55, 129.63, 130.61, 131.40 (2C), 131.41 (2C), 136.15, 136.83, 139.74, 147.20, 151.25, 152.02, 159.51, 187.91. Anal. Calcd for $C_{25}H_{20}N_2O_2$: C 78.93, H 5.30, N 7.36; found: C 78.67, H 5.27, N 7.01.

*5.2.10. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-(4-nitrophenyl)prop-2-en-1-one (**7**)*

A solution **5** (0.53 g, 2.0 mmol), 4-nitroacetophenone (0.33 g , 2.00 mmol) and 99% H_2SO_4 (0.2 mL) in acetic acid (15 mL) were refluxed for 4 h. The cooled mixture was triturated with water and the resulting precipitate was collected and crystallized with EtOH to give 0.38 g (46%) of **7** as a yellow solid. mp 214.7–216.5 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.90 (s, 3H, OMe), 7.03–7.07 (m, 2H, Ar–H), 7.34–7.38 (m, 2H, Ar–H), 7.60 (m, 1H, 6-H), 7.77 (m, 1H, 7-H), 7.84 (d, 1H, J = 8.0 Hz, 5-H), 8.05 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.14 (s, 1H, 4-H), 8.19 (d, 1H, J = 8.4 Hz, 8-H), 8.22–8.25 (m, 2H, Ar–H), 8.33–8.37 (m, 3H, CH=CH–CO and Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.41, 114.19, 114.23 (2C), 123.82 (2C), 126.35, 127.52, 127.82, 128.41, 129.65 (2C), 129.98, 130.20, 131.04 (2C), 136.43, 137.08, 142.63, 143.13, 147.20, 150.16, 150.82, 159.70, 188.83. Anal. Calcd for $C_{25}H_{18}N_2O_4 \cdot 0.5H_2O$: C 71.59, H 4.56, N 6.68; found: C 71.63, H 4.22, N 6.66.

*5.2.11. (E)-1-(Furan-2-yl)-3-[3-(4-methoxyphenyl)quinolin-2-yl] prop-2-en-1-one (**8**)*

Yield 41%; mp 164.6–165.3 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 6.61 (dd, 1H, J = 1.6, 3.6 Hz, furanyl–H), 7.02–7.06 (m, 2H, Ar–H), 7.34–7.38 (m, 2H, Ar–H), 7.42 (dd, 1H, J = 0.8, 3.6 Hz, furanyl–H), 7.58 (m, 1H, 6-H), 7.68 (dd, 1H, J = 0.8, 1.6 Hz, furanyl–H), 7.75 (m, 1H, 7-H), 7.83 (d, 1H, J = 8.4 Hz, 5-H), 8.06 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.11 (s, 1H, 4-H), 8.18 (d, 1H, J = 8.4 Hz, 8-H), 8.22 (d, 1H, J = 15.2 Hz, CH=CH–CO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.40, 112.48, 114.21 (2C), 118.30, 126.99, 127.46, 127.49, 128.27, 129.64, 129.76, 130.39, 131.06 (2C), 136.27, 136.93, 140.64, 146.94, 147.19, 151.36, 153.71, 159.62, 177.98. Anal. Calcd for $C_{23}H_{17}NO_3$: C 77.73, H 4.82, N 3.94; found: C 77.52, H 4.79, N 3.87.

*5.2.12. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-(thiophen-2-yl)prop-2-en-1-one (**9**)*

Yield 94%; mp 167.4–167.8 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 7.02–7.06 (m, 2H, Ar–H), 7.20 (dd, 1H, J = 3.6, 4.8 Hz, thiophenyl–H), 7.35–7.38 (m, 2H, Ar–H), 7.58 (m, 1H, 6-H), 7.70 (dd, 1H, J = 1.2, 4.8 Hz, thiophenyl–H), 7.76 (m, 1H, 7-H), 7.83 (d, 1H, J = 8.0 Hz, 5-H), 8.01 (dd, 1-H, J = 1.2, 3.6 Hz, thiophenyl–H), 8.03 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.11 (s, 1H, 4-H), 8.19 (d, 1H, J = 8.4 Hz, 8-H), 8.24 (d, 1H, J = 15.2 Hz, CH=CH–CO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.40, 114.22 (2C), 127.21, 127.49 (2C), 128.28 (2C), 129.62, 129.78, 130.39, 131.06 (2C), 132.53, 134.29, 136.31, 136.94, 140.69, 145.72, 147.20, 151.37, 159.61, 182.28. Anal. Calcd for $C_{23}H_{17}NO_2S$: C 74.37, H 4.61, N 3.77; found: C 74.04, H 4.49, N 3.60.

*5.2.13. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-(pyridin-2-yl) prop-2-en-1-one (**10**)*

Yield 20%; mp 143.8–144.4 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.90 (s, 3H, OMe), 7.02–7.06 (m, 2H, Ar–H), 7.37–7.41 (m, 2H, Ar–H), 7.49 (ddd, 1H, J = 1.2, 4.8, 7.6 Hz, pyridinyl–H), 7.57 (m, 1H, 6-H), 7.74 (m, 1H, 7-H), 7.82 (dd, 1H, J = 1.6, 8.0 Hz, 5-H), 7.87 (dd, 1H, J = 1.6, 7.6 Hz, pyridinyl–H), 8.10–8.16 (m, 3H, 4-, pyridinyl–H and CH=CH–CO), 8.23 (d, 1H, J = 8.4 Hz, 8-H), 8.79–8.81 (m, 1H, pyridinyl–H), 8.96 (d, 1H, J = 15.2 Hz, CH=CH–CO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.39, 114.13 (2C), 122.87, 126.91, 126.94, 127.38, 127.42, 128.23, 129.64, 129.88, 130.57, 131.11 (2C), 136.24, 136.75, 136.91, 141.58, 147.28, 149.03, 151.71, 154.19, 159.54, 190.06. Anal. Calcd for $C_{24}H_{18}N_2O_2$: C 78.67, H 4.95, N 7.65; found: C 78.42, H 4.82, N 7.59.

*5.2.14. (E)-1-(5-Bromothiophen-2-yl)-3-[3-(4-methoxyphenyl) quinolin-2-yl]prop-2-en-1-one (**11**)*

Yield 77%; mp 169.3–169.6 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 7.02–7.06 (m, 2H, Ar–H), 7.16 (d, 1H, J = 4.0 Hz, thiophenyl–H), 7.33–7.37 (m, 2H, Ar–H), 7.58 (m, 1H, 6-H), 7.73–7.78 (m, 2H, 7- and thiophenyl–H), 7.82 (d, 1H, J = 8.0 Hz, 5-H), 8.02 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.11 (s, 1H, 4-H), 8.14 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.18 (d, 1H, J = 8.4 Hz, 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.40, 114.21 (2C), 123.35, 126.02, 127.49, 127.59, 128.30, 129.57, 129.85, 130.26, 131.04 (2C), 131.44, 132.58, 136.35, 136.99, 141.15, 147.16, 147.22, 151.10, 159.62, 181.12. Anal. Calcd for $C_{23}H_{16}BrNO_2S$: C 61.34, H 3.58, N 3.11; found: C 61.37, H 3.54, N 2.98.

*5.2.15. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-(thiophen-3-yl)prop-2-en-1-one (**12**)*

Yield 51%; mp 163.5–164 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 7.02–7.06 (m, 2H, Ar–H), 7.35–7.38 (m, 3H, Ar- and thiophenyl–H), 7.58 (m, 1H, 6-H), 7.71 (dd, 1H, J = 1.6, 5.2 Hz, thiophenyl–H), 7.75 (m, 1H, 7-H), 7.83 (dd, 1H, J = 1.2, 8.0 Hz, 5-H), 8.01 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.11 (s, 1H, 4-H), 8.19 (d, 1H, J = 8.4 Hz, 8-H), 8.23 (d, 1H, J = 15.2 Hz, CH=CH–CO), 8.31 (dd, 1H, J = 1.2, 2.8 Hz, thiophenyl–H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.39, 114.19 (2C), 126.41, 127.46, 127.48, 127.52, 128.15, 128.24, 129.57, 129.78, 130.42, 131.04 (2C), 132.92, 136.25, 136.93, 140.65, 143.14, 147.19, 151.49, 159.59, 183.89. Anal. Calcd for $C_{23}H_{17}NO_2S$: C 74.37, H 4.61, N 3.77; found: C 74.06, H 4.56, N 3.61.

*5.2.16. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-(1H-pyrrol-2-yl)prop-2-en-1-one (**13**)*

Yield 70%; mp 165.3–167.5 °C (EtOH). ^{1}H NMR (400 MHz, DMSO): 3.86 (s, 3H, OMe), 6.31 (m, 1H, pyrrolyl–H), 7.12–7.16 (m, 2H, Ar–H), 7.22–7.24 (m, 2H, pyrrolyl–H), 7.43–7.46 (m, 2H, Ar–H), 7.65 (m, 1H, 6-H), 7.77 (d, 1H, J = 14.8 Hz, CH=CH–CO), 7.84 (m, 1H, 7-H), 8.04 (d, 1H, J = 7.6 Hz, 5-H), 8.10 (d, 1H, J = 14.8 Hz, CH=CH–CO), 8.16 (d, 1H, J = 7.6 Hz, 8-H), 8.36 (s, 1H, 4-H), 12.05 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO): 55.27, 110.53, 114.15 (2C), 117.58, 127.11, 127.50, 127.75, 127.90, 128.06, 128.98, 130.13 (2C), 131.09 (2C), 132.90, 135.52, 137.05, 137.52, 146.51, 150.89, 159.18, 177.41. Anal. Calcd for $C_{23}H_{18}N_2O_2$: C 77.95, H 5.12, N 7.90; found: C 77.98, H 5.10, N 7.86.

*5.2.17. (E)-1-(3-Aminothiophen-2-yl)-3-[3-(4-methoxyphenyl) quinolin-2-yl]prop-2-en-1-one (**14**)*

Yield 77%; mp 171.3–172.5 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.88 (s, 3H, OMe), 6.40 (br s, 2H, NH_2), 6.58 (d, 1H, J = 5.2 Hz, thiophenyl–H), 7.01–7.05 (m, 2H, Ar–H), 7.35–7.39 (m, 2H, Ar–H), 7.40 (d, 1H, J = 5.2 Hz, thiophenyl–H), 7.56 (m, 1H, 6-H), 7.73 (m, 1H, 7-H), 7.82 (d, 1H, J = 8.4 Hz, 5-H), 7.95 (s, 2H, CH=CH–CO), 8.08 (s, 1H, 4-H), 8.19 (d, 1H, J = 8.4 Hz, 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.35, 112.94, 114.13 (2C), 119.71, 127.22, 127.38, 128.07, 129.63 (2C), 130.01, 130.63, 131.01 (2C), 132.99, 136.09, 136.83, 138.34, 147.18, 151.72, 155.84, 159.47, 182.57. Anal. Calcd for $C_{23}H_{18}N_2O_2S$: C 71.48, H 4.69, N 7.25, S 8.30; found: C 71.29, H 4.77, N 7.08, 8.04.

*5.2.18. (E)-1-(2-Amino-4-methylpyrimidin-5-yl)-3-[3-(4-methoxyphenyl)quinolin-2-yl]prop-2-en-1-one (**15**)*

Yield 40%; mp 151.3–152.5 °C (EtOH). ^{1}H NMR (400 MHz, DMSO): 2.51 (s, 3H, CH_3), 3.86 (s, 3H, OMe), 7.12–7.14 (m, 2H, Ar–H), 7.43–7.47 (m, 2H, Ar–H), 7.49 (br s, 2H, NH_2), 7.66 (d, 1H, J = 14.8 Hz, CH=CH–CO), 7.68 (m, 1H, 6-H), 7.82 (M, 1H, 7-H), 8.02 (d, 1H, J = 8.4 Hz, 5-H), 8.05 (d, 1H, J = 14.8 Hz, CH=CH–CO), 8.13 (d, 1H, J = 8.4 Hz, 8-H), 8.36 (s, 1H, 4-H), 8.82 (s, 1H, pyrimidinyl–H). ^{13}C NMR (100 MHz, DMSO): 24.48, 55.29, 114.15 (2C), 119.91, 127.62, 127.83, 127.91, 129.03, 129.38, 129.97, 130.18, 131.10 (2C), 135.59, 137.05, 139.28, 146.49, 150.68, 159.19, 160.68, 163.18, 169.78, 188.15. Anal. Calcd for $C_{24}H_{20}N_4O_2 \cdot 1.5H_2O$: C 68.07, H 5.47, N 13.23; found: C 68.01, H 5.58, N 13.06.

*5.2.19. (E)-1-(1H-Indol-3-yl)-3-[3-(4-methoxyphenyl)quinolin-2-yl]prop-2-en-1-one (**16**)*

Yield 27%; mp 278–279.4 °C (EtOH). ^{1}H NMR (400 MHz, DMSO): 3.88 (s, 3H, OMe), 7.14–7.17 (m, 2H, Ar–H), 7.21–7.29 (m, 2H, indolyl–H), 7.44–7.48 (m, 2H, Ar–H), 7.52 (d, 1H, J = 7.2 Hz, indolyl–H), 7.66 (dd, 1H, J = 1.2, 8.0 Hz, 6-H), 7.79 (d, 1H, J = 14.8 Hz, CH=CH–CO), 7.84 (m, 1H, 7-H), 8.05 (d, 1-H, J = 7.2 Hz, 5-H), 8.18 (d, 1H, J = 8.4 Hz, 8-H), 8.24 (d, 1H, J = 14.8 Hz, CH=CH–CO). 8.28 (dd, 1H, J = 1.6, 7.8 Hz, indolyl–H), 8.35 (s, 1H, 4-H), 8.66 (s, 1H, indolyl–H), 12.20 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO): 55.29, 112.34, 114.16 (2C), 117.59, 121.69, 122.07, 123.33, 125.80, 127.37, 127.72, 127.94, 128.92, 129.53, 130.08, 130.29, 131.09 (2C), 134.96, 135.57, 136.65, 136.97 (2C), 146.56, 151.32, 159.19, 183.40. Anal. Calcd for $C_{27}H_{20}N_2O_2 \cdot 0.2H_2O$: C 79.47, H 5.04, N 6.86; found: C 79.29, H 4.92, N 6.58.

5.3. Pharmacological methods

5.3.1. Antiproliferative assay

Cancer cells (H460, A549, H1299, MCF-7, MDA-MB-231, SKBR-3) and normal mammary epithelial cell (H184B5F5/M10) were purchased from Bioresources Collection and Research Center, Taiwan. Cell lines were maintained in the same standard medium and grown as a monolayer in DMEM (Gibco, USA) and supplemented with 10% fetal bovine serum (FBS) and antibiotics i.e. 100 IU/mL penicillin, 0.1 mg/mL streptomycin and 0.25 μg/mL amphotericin. Culture was maintained at 37 °C with 5% CO_2 in a humidified atmosphere.

Cells (5×10^3 cells/well) were treated as indicated for 72 h in medium containing 10% FBS. Cell viability was quantitated with the use of sodium 3′-[1-(phenylamino-carbonyl)-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate (XTT) colorimetric assay (Biological Industries, Beit-Haemek, Israel). XTT labeling reagent (1 mg/mL) was mixed with electron-coupling reagent, following the manufacturer's instructions, and 50 μL of the mixture was added directly to the cells. The plates were further incubated at 37 °C for 4 h. Color was measured spectrophotometrically in a microtiter plate reader at 492 nm and used as a relative measure of viable cell number. The number of viable cells following treatment was compared to solvent and untreated control cells and used to determine the percent of control growth as ($Ab_{treated}$/$Ab_{control}$) × 100, where Ab represents the mean absorbance (n = 3). The concentration that killed 50% of cells (GI_{50}) was determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells, compared to control cells, by 50%.

5.3.2. Cell cycle analysis

MDA-MB-231 cells treated with DMSO and compound **11** at different concentration (0.1, 0.5, 1.0 μM) for 24 h were harvested, rinsed in PBS, resuspended and fixed in 70% ethanol and store cells at −20 °C in fixation buffer until ready for analysis. Then the pellets were suspended in 1 mL of propidium iodide (PI) solution containing 20 μg/μL of PI, 0.2 mg/mL RNase, and 0.1% (v/v) Trition X-100. Cell samples were incubated at room temperature in the dark for at least 30 min and analyzed by a flow cytometer (Coulter Epics). Data recording was made using Epics software and cell cycle data were analyzed using Multicycle software (coulter).

5.3.3. DNA fragmentation assay

DNA fragmentation was determined by agarose gel electrophoresis. Cells were treated with various concentrations of compound **11** (0.1, 0.5, 1.0 μM) for 24 h and then washed twice with PBS. Total DNA was isolated using a commercial kit (genomic DNA purification kit, Fermentas Life Sciences). DNA agarose electrophoresis was executed at 100 V on a 2.0% agarose gel in 1× TAE buffer (40 mmol/L of Tris, 2 mmol/L of EDTA, 20 mmol/L of acetic acid). DNA ladder marker (0.2–14.0 kb; GeneMark) was added to gel as a reference for the analysis of internucleosomal DNA fragmentation. The gel was stained with ethidium bromide (20 μg/mL) and photographed under ultraviolet illumination.

5.3.4. Immunoblot analysis

After treatment, cells were collected and washed twice with cold PBS. The cells were then lysed in lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, 1 mM EGTA, 1 mM NaVO3, 10 mM NaF, 1 mM DTT, 1 mM PMSF, 25 μg/mL aprotinin, and 25 μg/mL leupeptin) and kept on ice for 30 min. The lysates were then centrifuged at 12,000 g at 4 °C for 20 min; the supernatants were stored at −70 °C until use. The protein concentration was determined by the Bradford method. 20 μg protein was separated by 8–12% SDS-PAGE and transferred onto a PVDF membrane using a glycine transfer buffer (192 mM glycine, 25 mM Tris–HCl, pH 8.8, and 20% methanol [v/v]). After blocking with 5% non-fat dried milk, the membrane was incubated for 2 h with primary antibodies, followed by 30 min with secondary antibodies in milk containing Tris-buffered saline (TBS) and 0.5% Tween. Anti-human-PARP, pro-caspase 3 and caspase-3 antibodies were used at a 1:1000 dilution as the primary antibodies, while horseradish peroxidase-conjugated horse anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) was used at a 1:5000 dilution as the secondary antibody. The membrane was then exposed to X-ray film. Protein bands were detected using the enhanced chemiluminescence blotting detection system (Amersham, USA).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.11.027.

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