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Research paper

Discovery of 3-phenylquinolinylchalcone derivatives as potent and selective anticancer agents against breast cancers

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ABSTRACT

A number of 3-phenylquinolinylchalcone derivatives were synthesized and evaluated *in vitro* for their antiproliferative activities against three breast cancer cell lines (MCF-7, MDA-MB-231, and SKBR-3), and a non-cancer normal epithelial cell line (**H184B5F5/M10**). Among them, (*E*)-3-[3-(4-methoxyphenyl)quinolin-2-yl]-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7**) was active against the growth of MCF-7, MDA-MB-231, and SKBR-3 with IC_{50} values of 1.05, 0.75, and 0.78 μM respectively without significant cytotoxicity to the normal **H184B5F5/M10** cell line and therefore, was selected as a new lead for further mechanism studies. Results indicated that compound **7** inhibited the polymerization of tubulins, induced G2/M cell cycle arrest *via* modulation of the cyclin B1, cdk1 and CDC25. Compound **7** ultimately induced cell apoptosis by the increase of apoptotic protein Bax and the decrease of anti-apoptotic protein Bcl-2. In addition, PARP was cleaved while caspase-3 and -8 activities were induced after the treatment of compound **7** for 24 h in a concentration-dependent manner. Thus, compound **7** induces cell cycle arrest at G2/M phase *via* cleavage of PARP, induces caspase-3 and -8 activities and consequently to cause the cell death. Further study on the structure optimization of **7** is ongoing.

1. Introduction

Combretastatins-A4 (CA-4) is an effective antimitotic agent possessing potent anticancer activity against a panel of cancer cells, including multi-drug resistant cancer cell lines [1–6]. However, only the *cis*-form exhibits strong antitubulin activity, but the *cis*-form tends to isomerize to its inactive *trans*-isomer. Thus, a number of **CA-4** analogs were designed and synthesized in which the ethylene bridge was replaced with various heterocyclic rings with objections to overcome the spontaneous isomerization of *cis*-form to *trans*-isomer. Recently, we have synthesized certain **CA-4** analogs in which the *cis* double bond was replaced with quinoline to arrest the *cis*-conformation [7]. Among them, 6-fluoro-2,3-bis{4-[2-(piperidin-1-yl)ethoxy]phenyl}quinoline (**1**) which possesses the aminoalkyl side chain, was one of the most active derivative against the growth of Hep3B, H1299, and MDA-MB-231 with IC_{50} values of 0.71, 1.46, and 0.72 μM respectively. Furthermore, we have also synthesized certain 3-phenylquinolinylchalcone derivatives whose structures can be considered as **CA-4** analogs in which a α,β-unsaturated carbonyl moiety is inserted between quinoline and the C-2 phenyl group [8]. They can also be considered as chalcone derivatives [9–17] in which an aryl moiety was replaced with quinoline nucleus. Among these 3-phenylquinolinylchalcone derivatives, (*E*)-3-[3-(4-methoxyphenyl)quinolin-2-yl]-1-phenylprop-2-en-1-one (**2**) 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 [8]. The present report intends to establish the antiproliferative structure–activity relationships (SAR) of 3-phenylquinolinylchalcone derivatives and to identify more potential anticancer drug candidates by the introduction of various

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substituents, such as trimethoxy group and aminoalkyl side chains, on the lead compound **2** (Fig. 1). Introduction of aminoalkyl side chains on the furoquinoline pharmacophore of anticancer CIL-102 [18–21] to improve anticancer profiles, i.e., compound **3**, has also been previously reported [22]. These substituted 3-phenylquinolinylchalcone derivatives were evaluated *in vitro* against three breast cancer cell lines (MCF-7, MDA-MB-231, and SKBR-3) which are common malignancies in the world, and especially are the leading cause of cancer deaths in Asian countries including Taiwan [23–27]. The normal mammary epithelial cell (**H184B5F5/M10**) was also evaluated to identify potential anticancer drug candidates which inhibit only the growth of cancer cells but not normal mammary cells.

2. Chemistry

Claisen–Schmidt condensation reaction of 3-(4-methoxyphenyl) quinolin-2-carbaldehyde (**4**) and 3,4,5-trimethoxyacetophenones gave (*E*)-3-[3-(4-methoxyphenyl)quinolin-2-yl]-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7**) as outlined in Scheme 1. Compounds **8** and **9** were synthesized by the treatment of **4** with 4-[2-(piperidin-1-yl)ethoxy]acetophenone [28] and 4-[2-(pyrrolidin-1-yl)ethoxy]acetophenone respectively. Reaction of 4-hydroxyacetophenone and 3-chloro-*N*,*N*-dimethylpropanamine followed by the addition of **4** under basic conditions afforded compound **10**. Preparation of compounds **3**, **5**, and **6** were previously described [8].

Pfitzinger reaction of 4-methoxyphenylacetone with 5-fluoroisatin (**11**) and 5-methoxyisatin (**12**) afforded 6-fluoro-3-(4-methoxyphenyl)-2-methylquinoline-4-carboxylic acid (**13**) and its 6-methoxy analog **14** respectively as described in Scheme 2. Compounds **13** and **14** respectively, were subjected to thermal decarboxylation followed by oxidation with selenium oxide to afford their respective aldehydes **17** and **18**. The desired quinolinylchalcones **19a–g** and **20a–g** were synthesized by a base catalyzed Claisen–Schmidt condensation reaction of **17** and **18** respectively with appropriately substituted acetophenones. This method is attractive since it specifically generates solely (*E*)-form isomer. From ^{1}H NMR spectra, all quinolinylchalcones were found to be *trans* based on the coupling constant between the vinylic system of the propenone moiety (J = 14.8–15.2 Hz) [8].

Compound **7** was further converted to its epoxy-chalcone derivative **21** by the treatment of H_2O_2 in alkaline solution, cyclopropane derivative **22** by Corey-Chaykovsky cyclopropanation (TMSOI/NaOH(aq) in TBAB as phase transfer catalyst), and saturated dihydro derivative **23** by hydrogenation (H_2, Pd/C) (Scheme 3). These compounds were also evaluated for their antiproliferative activities.

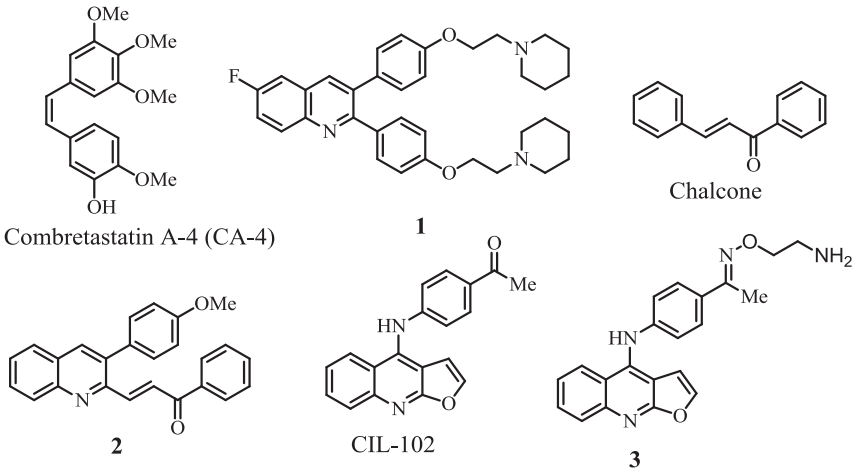
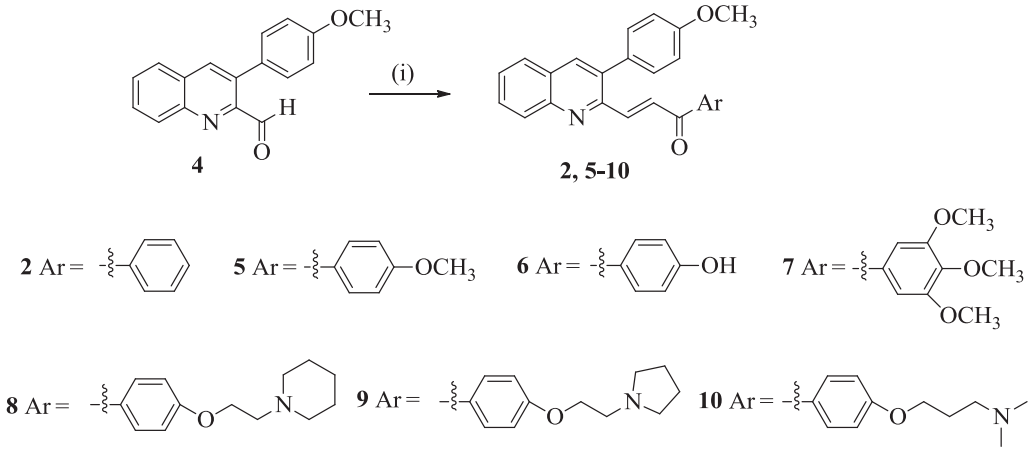
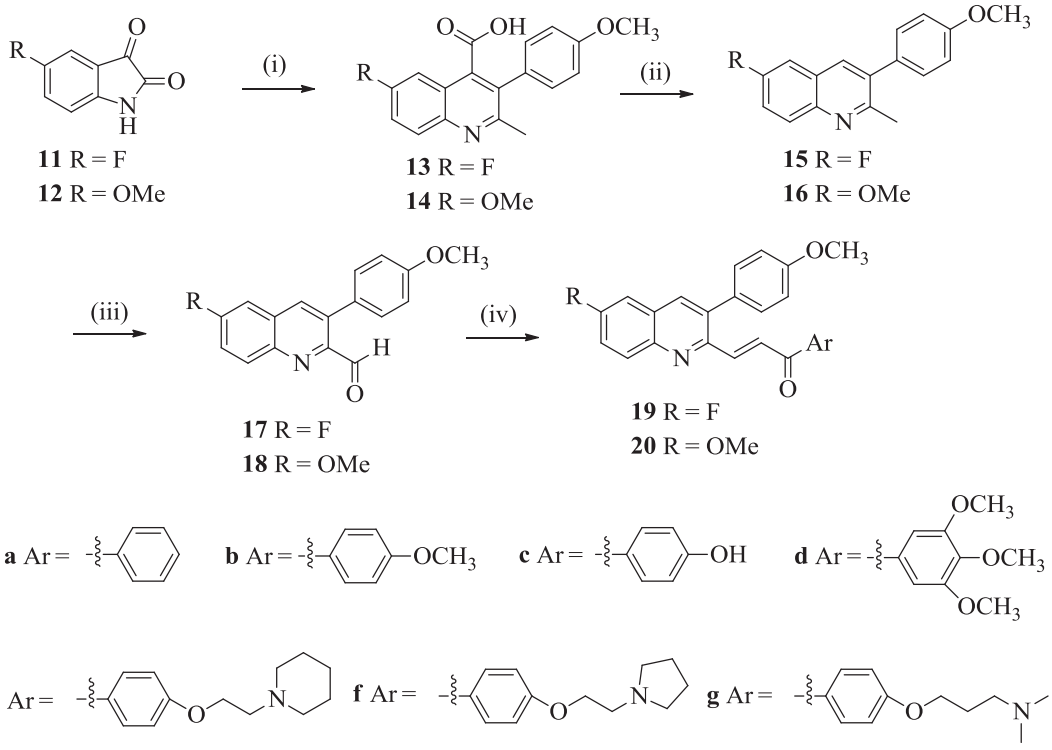


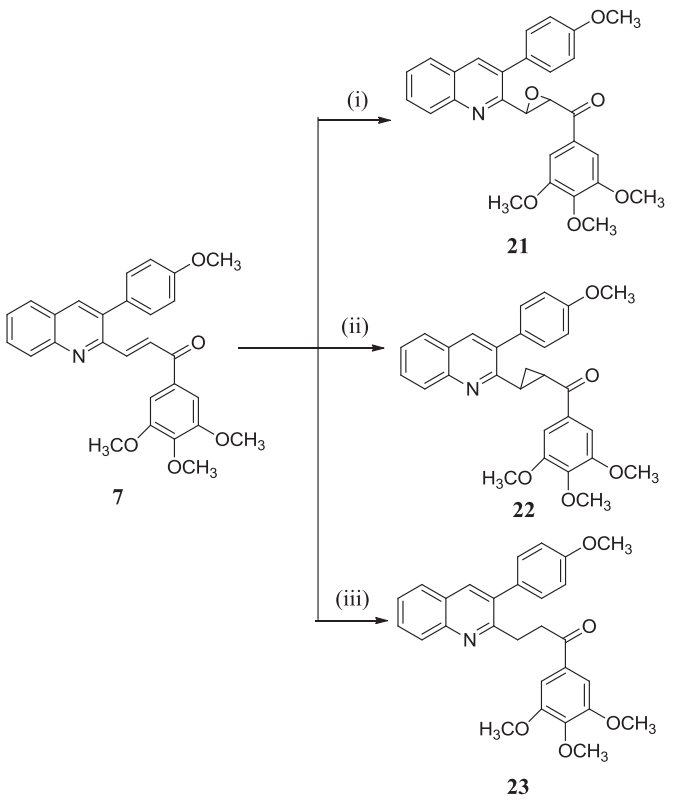
Fig. 1. Structures of CA-4, 6-fluoro-2,3-bis{4-[2-(piperidin-1-yl)ethoxy]phenyl}quinoline (**1**), (*E*)-3-[3-(4-methoxyphenyl)quinolin-2-yl]-1-phenylprop-2-en-1-one (**2**), chalcone, CIL-102, and **3**.



Scheme 1. Reagents and conditions: (i) substituted acetophenone, KOH, EtOH, rt, 2hr.



Scheme 2. Reagents and conditions: (i) 4-methoxyphenylacetone, KOH, EtOH, 80 °C, 48hr; (ii) Dowtherm A, 280 °C, 4hr; (iii) SeO_2, 100 °C, 2hr; (iv) substituted acetophenone, KOH, EtOH, rt, 2hr.



Scheme 3. Reagents and conditions: (i) H_2O_2, MeOH, K_2CO_3, rt, 3hr; (ii) TMSOI, TBAB, NaOH; (iii) H_2, Pd/C, 3hr.

3. Results and discussion

3.1. Inhibition of cell proliferation

All the synthesized 3-phenylquinolinylchalcone derivatives were evaluated *in vitro* against three breast cancer cells (MCF-7, MDA-MB-231, and SKBR-3), and a non-cancer normal epithelial cell line (**H184B5F5/M10**) using XTT (sodium 3′-[1-(phenylamino-carbonyl)-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate) assay [29]. 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. Although our first lead compound, (*E*)-3-[3-(4-methoxyphenyl)quinolin-2-yl]-1-phenylprop-2-en-1-one (**2**) [8] was active against the growth of MDA-MB-231 and SKBR-3 with IC_{50} values of 0.86 and 0.70 μM respectively, it was also exhibited a weak cytotoxicity to the non-cancer normal epithelial cell line (**H184B5F5/M10**) with an IC_{50} values of 8.56 μM. Introduction of the methoxy *i.e.* compound **5** or hydroxyl substituent *i.e.* compound **6** on the para-position of C-2 aryl moiety decreased the antiproliferative activity against all the cancer cells tested. However, aminoalkoxy derivatives **8**, **9**, and **10** exhibited comparable or even more potent antiproliferative activities than that of the parent compound **2** against all cell lines tested. Although 3,4,5-trimethoxy derivative **7** was more active than **2** against the growth of MCF-7 (1.05 v.s. 4.04 μM), it was not cytotoxic against the normal mammary epithelial cell (**H184B5F5/M10**) with an IC_{50} value of >10 μM.

For the 6-fluoro substituted derivatives, **19a**–**g**, the antiproliferative activity decreased in an order of **19e**, **19f**, **19g** > **19a** > **19b**, **19c** > **19d** indicated that the introduction of

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

Compd	Cell lines			
	MCF-7	MDA-MB-231	SKBR-3	**H184B5F5/M10**
2	4.04 ± 0.18	0.86 ± 0.07	0.70 ± 0.14	8.56 ± 0.32
5	7.38 ± 0.12	7.54 ± 0.05	9.28 ± 0.04	>10
6	6.71 ± 0.07	6.35 ± 0.09	3.88 ± 0.11	5.69 ± 0.13
7	1.05 ± 0.22	0.75 ± 0.05	0.78 ± 0.07	>10
8	0.85 ± 0.11	0.68 ± 0.02	0.83 ± 0.04	4.66 ± 0.16
9	0.74 ± 0.06	0.76 ± 0.03	0.91 ± 0.07	3.63 ± 0.12
10	0.81 ± 0.16	0.70 ± 0.22	0.80 ± 0.05	7.67 ± 0.11
19a	4.33 ± 0.03	0.76 ± 0.11	0.91 ± 0.07	10.10 ± 0.85
19b	9.72 ± 2.41	8.03 ± 0.15	8.80 ± 0.11	>10
19c	6.75 ± 0.25	8.38 ± 0.37	8.31 ± 0.27	9.87 ± 0.15
19d	>10	>10	7.66 ± 0.05	>10
19e	0.78 ± 0.09	0.74 ± 0.13	0.78 ± 0.14	7.13 ± 0.17
19f	0.76 ± 0.09	0.64 ± 0.09	0.80 ± 0.14	2.08 ± 0.26
19g	0.71 ± 0.06	0.65 ± 0.05	0.73 ± 0.11	7.39 ± 0.38
20a	7.53 ± 0.29	7.92 ± 0.09	9.36 ± 0.16	10.04 ± 0.61
20b	>10	8.76 ± 0.42	10.22 ± 0.15	>10
20c	6.71 ± 0.06	6.88 ± 0.12	9.35 ± 0.25	8.35 ± 0.17
20d	>10	>10	7.62 ± 0.03	>10
20e	0.77 ± 0.05	0.66 ± 0.04	0.37 ± 0.03	8.41 ± 0.13
20f	0.76 ± 0.11	0.72 ± 0.03	0.82 ± 0.12	6.80 ± 0.18
20g	1.02 ± 0.07	0.67 ± 0.15	0.41 ± 0.12	8.82 ± 0.71
21	>10	9.05 ± 0.88	>10	>10
22	>10	>10	>10	>10
23	>10	>10	>10	>10
Topo	5.97 ± 1.03	<0.1	8.91 ± 3.03	>10
Taxol	0.11[a]	0.41[a]	0.10 ± 0.05[b]	ND[c]
CIL-102	0.31 ± 0.07	ND	ND	0.95 ± 0.16

[a] Ref. [30].
[b] Ref. [31].
[c] No data.

aminoalkoxy side chains increased while methoxy or hydroxyl group decreased antiproliferative activity especially trimethoxy group which was inactive against all cell lines tested. The same structure–activity relationship (SAR) was observed for the 6-methoxy substituted derivatives, **20a**–**g**, in which the antiproliferative activity decreased in an order of aminoalkoxy derivatives **20e**, **20f**, **20g** > unsubstituted derivative **20a** > methoxy **20b** or hydroxyl derivatives **20c** > trimethoxy derivative **20d**. Less antiproliferative activity of **20a** than that of **2** indicated the substitution at C-6 position is unfavorable especially the electron-donating group such as OMe.

Among these newly synthesized 3-phenylquinolinylchalcone derivatives, (*E*)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7**) was active against the growth of MCF-7, MDA-MB-231, and SKBR-3 with IC_{50} values of 1.05, 0.75, and 0.78 μM respectively without significant cytotoxicity to the normal **H184B5F5/M10**cell line. Thus, compound **7** was selected as a lead compound and was further converted to its ethylene oxide derivative **21**, cyclopropane derivative **22**, and saturated dihydro derivative **23** for antiproliferative evaluation. Our results indicated compounds **21**–**23** were inactive, implied that the unsaturated ketone is crucial for antiproliferative activities. Compound **7** was then selected for further mechanism studies due to its potent inhibition against the growth of cancer cells but was non-cytotoxic to the normal **H184B5F5/M10** cell line.

3.2. Compound 7 induced G2/M cell cycle arrest and morphological changes

Cell cycle analysis of compound **7** was performed with MDA-MB-231 cells. CIL-102, a known inhibitor of tubulin polymerization, was used as a positive control. Compound **7** induced cell cycle arrest in a concentration- and time-dependent manners as shown in Fig. 2 and Table 2. The presence of 10 μM of CIL-102 for 12 h led to cell cycle arrest in the G2/M phase. In the presence of compound **7** at 1 μM and 5 μM for 12 h, 28% and 36% respectively of the cells were also arrested in G2/M phase followed by apoptosis as indicated by a concentration- and time-dependent increase of cells in the sub G1 phase, suggesting that compound **7** affects tubulin polymerization. Significant morphological changes were also observed by the incubation of MDA-MB-231 cells with increasing concentrations of compound **7** for 24 h as shown in Fig. 3.

3.3. Compound 7 caused disruption of microtubule networks

G2/M cell cycle arrest was extensively reported to occur in apoptosis induced by microtubule-interfering agents [30–32]. Due to the similar pharmacological mechanisms of compound **7** and CIL-102, we hypothesized that compound **7** might also inhibit tubulin polymerization. To assure this hypothesis, microtubule organization and mitosis in response to compounds **7** and **23** (as a negative control) treated cells were initially examined by immunofluorescent microscopy. After the treatment of compound **7**, cells were found to exhibit the filament-like structure and reduce microtubule extent in the cytoplasm (Fig. 4). The effect of compound **7** on the dynamics of microtubule assembly was also examined at the cellular level. As shown in Fig. 5, the inhibited polymerization of tubulin was observed in MDA-MB-231 cells treated with compound **7** or CIL-102 (10 μM) for 24 h compared with the control cells. Compound **7** showed a decrease in the polymer form of tubulin in a dose dependent manner.

3.4. Compound 7 induced apoptosis through regulation of Bcl-2 proteins and caspase activation

In order to assure compound **7**-mediated inhibition of cell growth is due to the induction of apoptosis, we analyzed the effect of compound **7** on the expression of the Bcl-2 family, the anti-apoptotic Bcl-2 and the proapoptotic Bad and Bax. The proteins of the Bcl-2 family play a major role in controlling apoptosis through the regulation of mitochondrial processes and the release of mitochondrial proapoptotic molecules important for the cell death pathway [33]. As shown in Fig. 6, compound **7** did not significantly affect the expression of the apoptotic protein Bad, however, it increased the apoptotic protein Bax and decreased the anti-apoptotic protein Bcl-2. To determine whether compound **7** preferentially kills cancer cells by apoptosis, procaspase-3, procaspase-8, and PARP were evaluated in compound **7**-treated MDA-MB-231 cells by western blotting. Caspases are the family of cysteine proteases and they mediate apoptotic pathway in mammalian [34]. PARP, a nuclear poly (ADP-ribose) polymerase, is involved in DNA repair predominantly in response to environmental stress, and is important for the maintenance of cell viability [35]. Our results have shown that PARP was cleaved while caspase-3 and -8 activities were induced after the treatment of compound **7** for 24 h in a concentration-dependent manner (Fig. 6). Thus, compound **7** induces cell cycle arrest at G2/M phase *via* cleavage of PARP, induces caspase-3 and -8 activities, and consequently caused the cell death.

3.5. Modulation of the expression of cell cycle-regulatory proteins

Molecular analysis of human cancers has revealed that cell cycle regulators are frequently mutated in most common malignancies [36]. Cdc25 is sequestered in the cytoplasm by 14-3-3 proteins, which prevents activation of cyclin B1/CDK1 by Cdc25 and results in G2/M arrest [37]. Western blot analysis showed that the treatment of cells with compound 7 resulted in a significant dose-dependent decrease in the protein levels of CDC25, cdk1, and cyclin B1 (Fig. 7). These results suggested that compound **7**

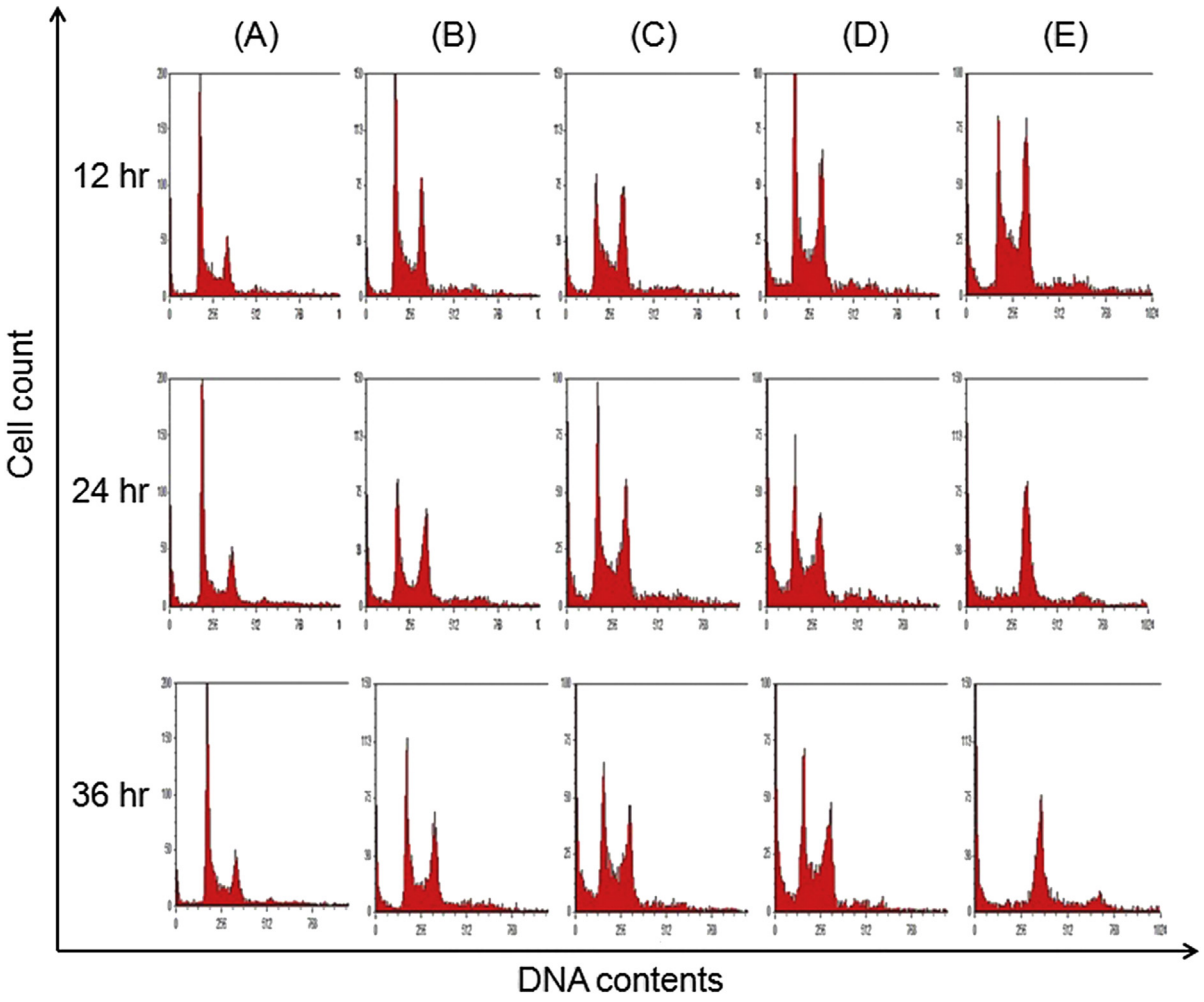


Fig. 2. Flow cytometric analysis of MDA-MB-231 cells. MDA-MB-231 cells were treated with DMSO (A), compound **7** at 1 μM (B), 5 μM (C) or 10 μM (D) or CIL-102 at 10 μM (E) for the indicated times the cells and analyzed for propidium iodide-stained DNA content by flow cytometry. The percentage of cells in each cell cycle phase was quantified (Table 2).

Table 2
Effects of **7** and CIL-102 on MDA-MB-231 cell cycle progression.

Times (hr)	compd	Concentration (μM)	Cell cycle distribution (%)[a]			
			Sub G1	G1	S	G2/M
12	**7**	DMSO	1.7 ± 0.6	67.6 ± 3.2	13.3 ± 2.5	17.4 ± 1.8
		1	2.6 ± 1.2	52.3 ± 2.8	16.8 ± 3.2	28.3 ± 1.7
		5	4.5 ± 3.1	41.8 ± 4.8	17.0 ± 1.9	36.7 ± 3.3
		10	10.1 ± 3.8	45.3 ± 2.1	14.8 ± 2.7	29.8 ± 1.5
	CIL-102	10	8.8 ± 3.7	36.5 ± 3.3	20.1 ± 2.8	34.6 ± 1.7
24	**7**	DMSO	2.2 ± 0.8	69.8 ± 2.1	15.8 ± 1.9	14.4 ± 2.9
		1	6.8 ± 2.3	42.5 ± 4.8	14.6 ± 3.5	36.1 ± 3.6
		5	13.2 ± 2.7	43.5 ± 2.6	11.5 ± 2.7	31.8 ± 3.5
		10	21.9 ± 4.2	32.4 ± 2.4	12.8 ± 1.7	32.9 ± 2.6
	CIL-102	10	11.2 ± 2.8	3.9 ± 1.2	5.8 ± 2.1	79.1 ± 4.7
36	**7**	DMSO	2.7 ± 1.1	68.7 ± 2.1	14.8 ± 1.9	13.8 ± 1.9
		1	10.5 ± 3.8	49.8 ± 2.0	13.8 ± 2.7	25.9 ± 4.1
		5	19.8 ± 2.8	38.2 ± 4.8	17.9 ± 2.5	24.1 ± 3.6
		10	27.8 ± 4.2	26.2 ± 2.7	14.7 ± 2.6	31.3 ± 1.8
	CIL-102	10	12.8 ± 1.5	3.8 ± 1.1	3.2 ± 1.8	80.2 ± 3.9

regulated G2/M cell cycle arrest *via* modulation of the cyclin B1, cdk1 and CDC25.

3.6. Molecular docking of 3-phenylquinolinylchalcone derivatives

Listed in Table 3 are the docking scores of the studied compounds accommodated in the GTP and colchicine binding sites. The docking scores in the GTP site column suggest that GTP (Libdock score = 155.3) is superior to the studied compounds (Libdock scores ranging from 119.7 to 124.2) when filling in the GTP binding pocket. In contrast, the docking scores in the colchicine binding site column shows that the studied compounds (Libdock scores ranging from 117.9 to 134.0) can fit better into colchicine binding site than colchicine does (Libdock

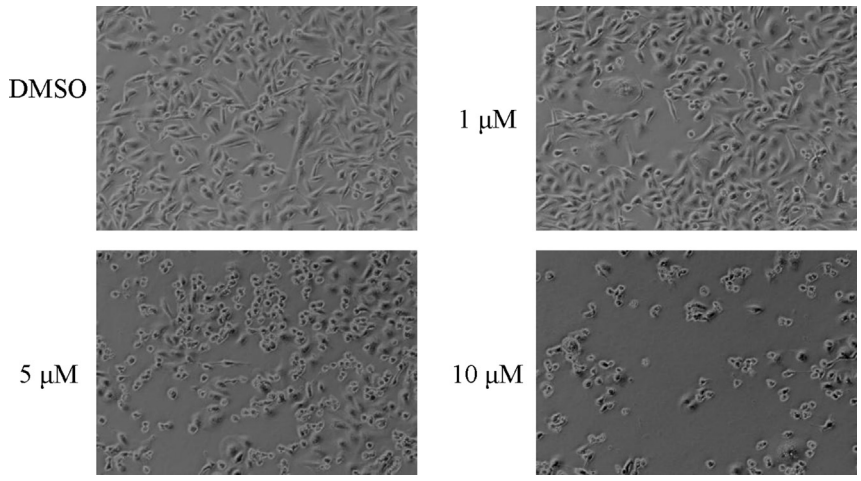


Fig. 3. Induction of morphological change in MDA-MB-231 cells. Cells were treated with DMSO or compound **7** (1–10 μM) for 24 h at 37 °C and photographed under a microscope.

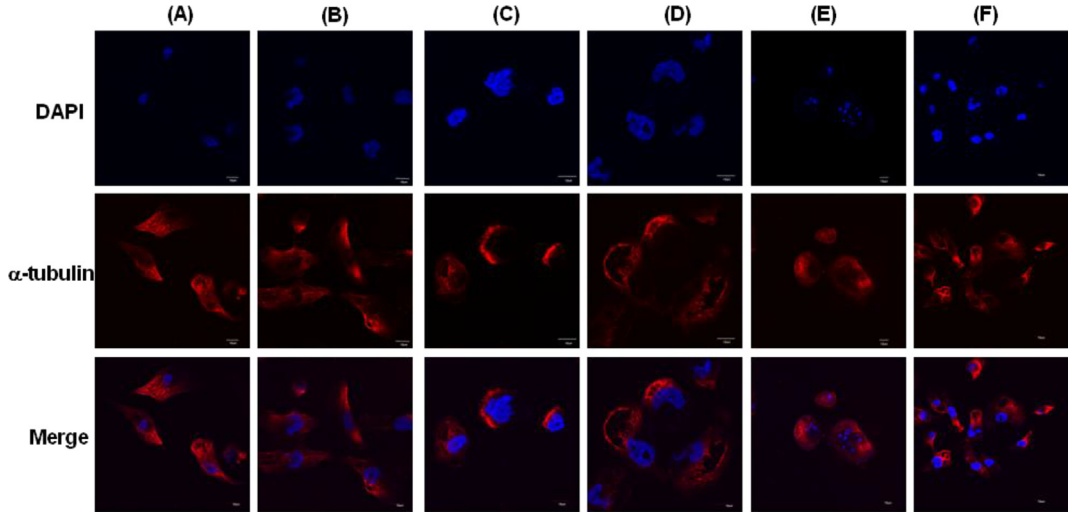


Fig. 4. Microtubule effect of compound **7**. The confocal laser scanning micrograph shows a merged image double-labeled with DAPI and α-tubulin antibodies. MDA-MB-231 cells were incubated with DMSO (A), compound **7** at 1 μM (B), 5 μM (C) 10 μM (D), **CIL-102** at 1 μM (E) or **23** at 10 μM (F) for 24 h. The cellular microtubule network was analyzed by an Olympus FV10i laser confocal system using monoclonal anti-α-tubulin antibody, FITC -conjugated mouse anti-human antibody, and DAPI as described in Section 5. Scale bar, 10 μm.

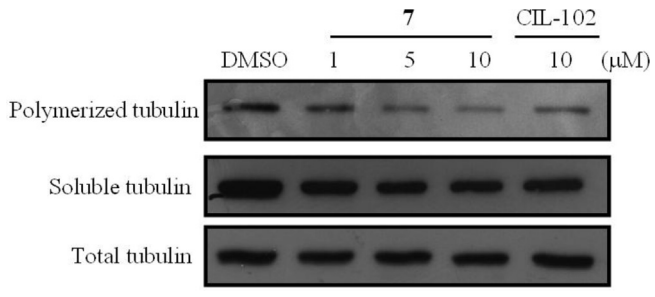


Fig. 5. Effect of **7** on tubulin polymerization in MDA-MB-231 cells. Cytosolic (soluble tubulin) and cytoskeletal (polymerized tubulin) tubulin fractions were extracted from Hep3B cells treated with compound **7** (1–10 μM) for 24 h. CIL-102 (10 μM) was included as controls. Cells were lysed to separate cytosolic and cytoskeletal fractions as described in Materials and methods. Both polymerized tubulin and unpolymerized ones were loaded on SDS-PAGE. After electrophoresis and transfer to PVDF membrane, α-tubulin was visualized by Western blot analysis. Western blot data presented are representative of those obtained in at least three separate experiments.

score = 98.9). Furthermore, the correlation coefficient is positive (0.876) when the docking scores and pIC_{50} values (in H1299 column, Table 3) in colchicine binding site are plotted in Fig. 8(a) for linear relationship, whereas the correlation coefficient is negative (−0.737) for the GTP site docking scores against the pIC_{50} values (in H1299 column, Table 3) plotted in Fig. 8(b). Accordingly, we conclude that the studied compounds are more likely to bind to the colchicine binding site. Shown in Fig. 9a–e are docking results of compounds **7** and **20d**, respectively. Leu255 provides aliphatic-π interaction with the quinoline moiety in compound **7**. Such an interaction is also found in compound **20d**, but is on the 3-(4-methoxyphenyl) moiety due to the docking position shift caused by the 6-methoxy group attached on the quinoline. In compound **7**, the positively charged amino acid residue Lys352 provides cation-π interaction on the 3,4,5-trimethoxyphenyl moiety. Although such an interaction is not present in compound **20d** docking result, with a refined simulation where the structural flexibility are conferred upon the amino acid residues surrounding the docked compound,

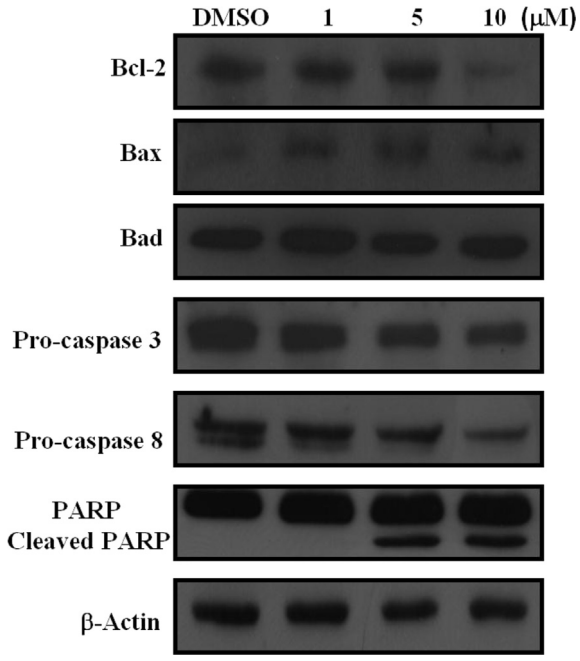


Fig. 6. Effects of apoptosis-associated proteins levels in MDA-MB-231 cells. Cells were cultured with DMSO or 1–10 μM of **7** for 24 h. Then, the cells were harvested and lysed for the detection of protein expression with specific antibody by Western blot analysis. Western blot data presented are representative of those obtained in at least three separate experiments.

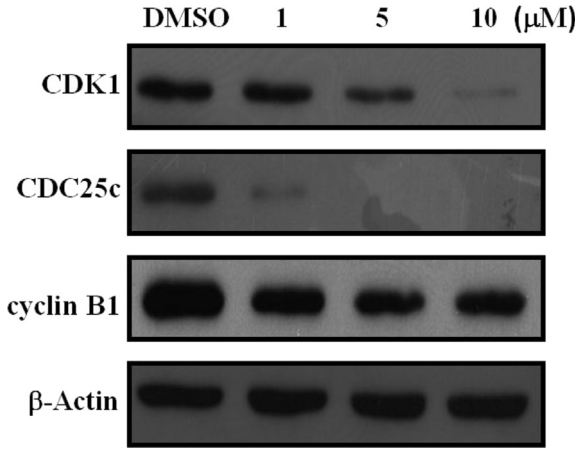


Fig. 7. Effect of **7** on the expression of cell cycle regulators. MDA-MB-231 cells were incubated in the vehicle (DMSO) or **7** (1–10 μM) for 24 h. Then, the cells were harvested and lysed for the detection of protein expression with specific antibody by Western blot analysis. The data are representative of three independent experiments.

Table 3
The tubulin docking results of GTP binding site and CN binding site.

Name	Libdockscore		pIC_{50}	
	GTP site	CN site	MRC-5	H1299
7	119.6	133.9	5.12	6.12
20d	116.5	129.5	5.32	6.02
21x	122.2	118.0	5.16	5.10
21y	124.2	123.1	5.16	5.10
GTP	155.3	–	–	–
CN	–	98.9	–	–

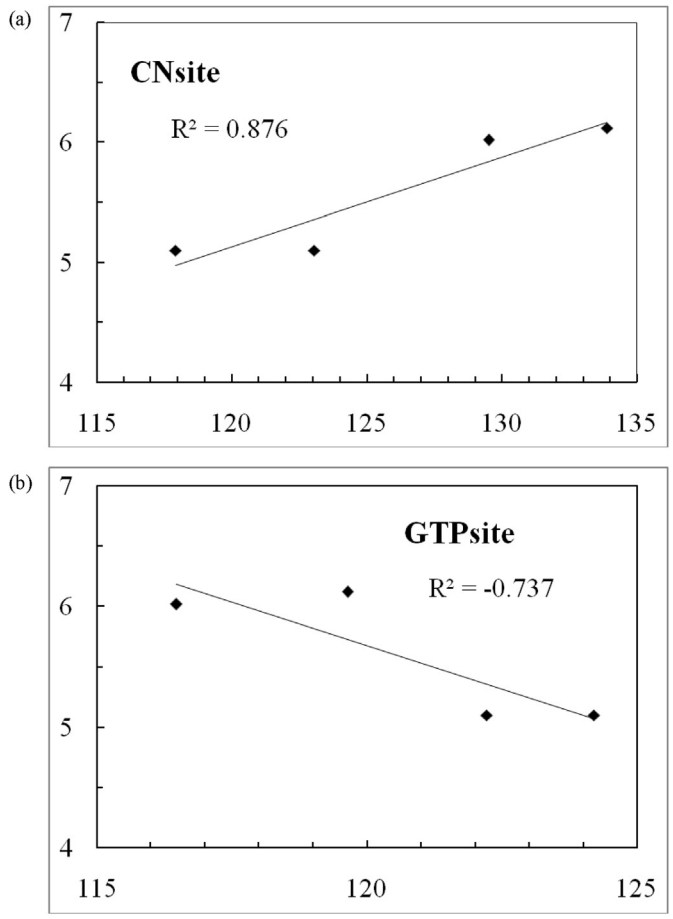


Fig. 8. The correlations of activity (pIC_{50}) versus docking score (Libdockscore) at colchicines binding site (a) and GTP site (b).

compound **20d** can be docked into colchicine binding site in the better manners so that the cation-π interaction may show. Because compound **21** is racemic mixture (labeled as **21x** and **21y**), we also investigated the preference of (*2S*, *3R*)-**21x** or (*2R*, *3S*)-**21y** form to the colchicine binding site. As shown in Figs. S1 and S2, compared to compound **21x**, compound **21y** has more interaction when binding to colchicine site, in accordance with the docking scores 123.1 and 118.0 for **21y** and **21x**, respectively.

4. Conclusion

A number of 3-phenylquinolinylchalcone derivatives were synthesized and evaluated *in vitro* for their antiproliferative activities. Among them, compound **7** was active against the growth of MCF-7, MDA-MB-231, and SKBR-3 with IC_{50} values of 1.05, 0.75, and 0.78 μM respectively without significant cytotoxicity to the normal cells and therefore, was selected as a new lead for further mechanism studies. Results indicated that compound **7** inhibited the polymerization of tubulins, induced G2/M cell cycle arrest *via* modulation of the cyclin B1, cdk1 and CDC25. Compound **7** ultimately induced cell apoptosis by the increase of apoptotic protein Bax and the decrease of anti-apoptotic protein Bcl-2. In addition, PARP was cleaved while caspase-3 and -8 activities were induced after the treatment of compound **7** for 24 h in a concentration-dependent manner (Fig. 10).

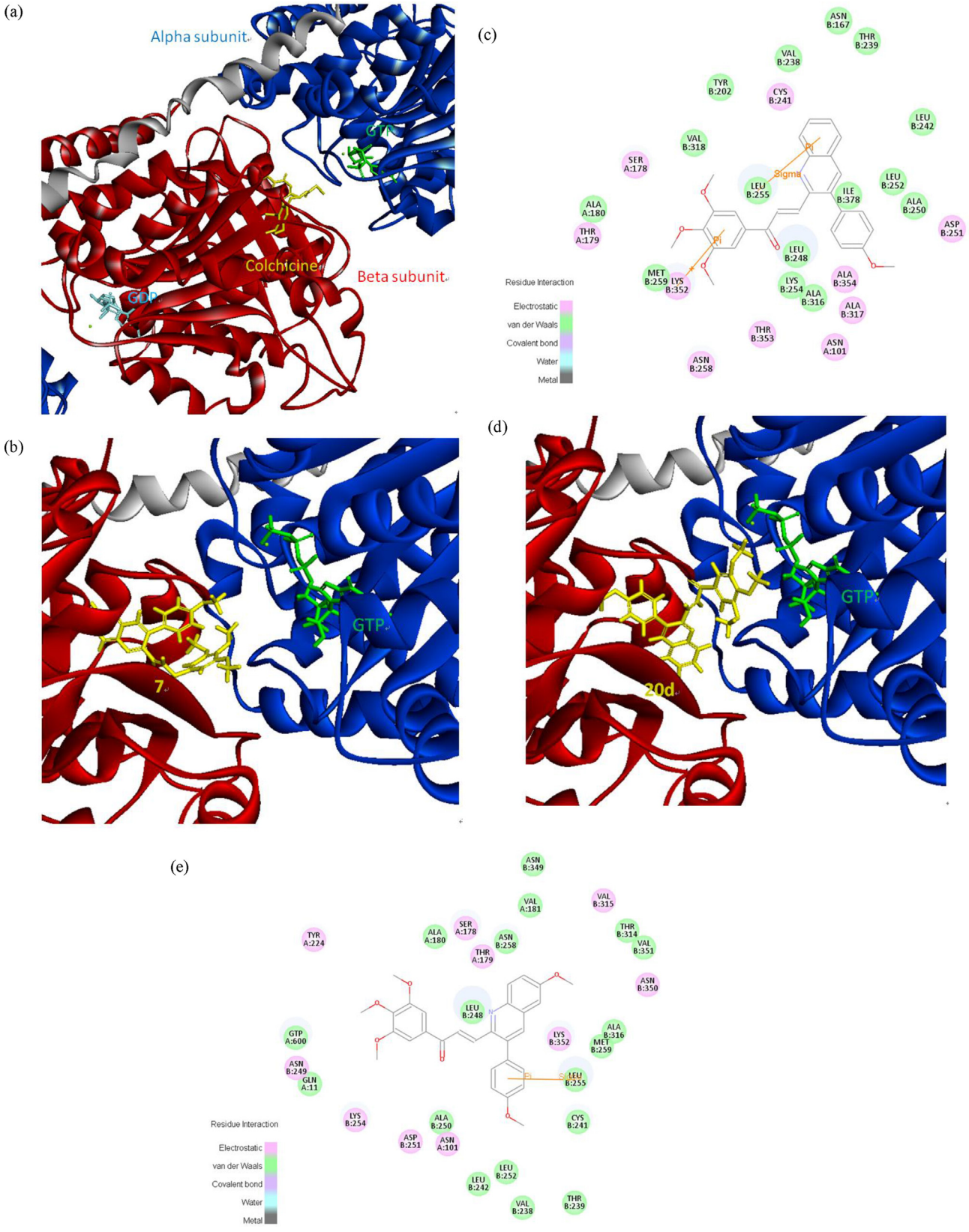


Fig. 9. (a) The tubulin structure (PDB ID: 1SA0). The GTP, colchicine, and GDP were labeled by green, yellow, and blue, respectively. (b and d) The docking pose of **7** and **20d**. (c and e) The docking pose of **7** and **20d** were showed by 2D diagram.

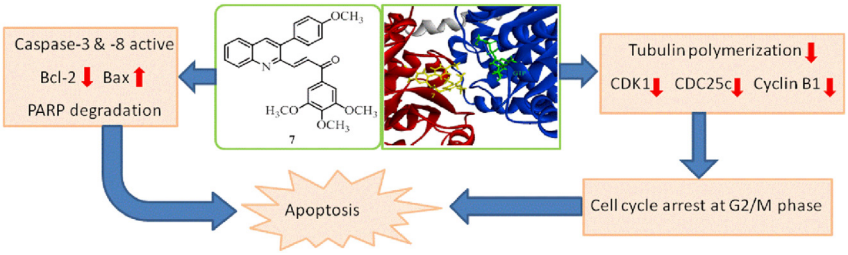


Fig. 10. A proposed model illustrates the molecular mechanism and the overall possible signaling pathways for **7**-induced G2/M arrest and apoptosis in MDA-MB-231 cells.

5. Experimental

5.1. General

Melting points were determined on a Electrothermal IA9100 melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H and ^{13}C) spectra were recorded on a Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography (TLC) was performed on silica gel 60 F-254 plates purchased from E. Merck and Co.. ESI-MS were recorded on a Bruker Daltonics Apex II 30e. 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. Purity analysis of compound was detected by high performance liquid chromatography (HPLC) system. The HPLC (Hitachi) consisted of an L-2100 pump, L-2200 autosampler, L-2420 UV–vis detector and the analysis was carried out using the Mightysil RP-18 GP column (250 × 4.6 mm i.d., 5 μm), and the temperature was set at room temperature and the mobile phase was composed of methanol and 0.02 M $NaHCO_3$ (93:7). The flow rate was 1 mL/min and the wavelength of detection was set at 282 nm.

5.1.1. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (7)

Compound **4** (0.53 g, 2.0 mmol) and 3′,4′,5′-trimethoxyacetophenone (0.42 g, 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 2 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 **7** in yield 90%. Mp. 170.6–171.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 3.95 (s, 6H, OMe × 2), 3.96 (s, 3H, OMe), 7.02–7.06 (m, 2H, 3′-H), 7.36–7.39 (m, 4H, 2′-H and 2″-H), 7.57–7.61 (m, 1H, 6-H), 7.74–7.78 (m, 1H, 7-H), 7.85 (d, 1H, J = 8.0 Hz, 5-H), 8.03 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.12 (s, 1H, 4-H), 8.11 (d, 1H, J = 8.4 Hz, 8-H), 8.31 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.37, 56.40 (2C), 60.96, 106.35 (2C), 114.18 (2C), 127.03, 127.50 (2C), 128.24, 129.54, 129.81, 130.44, 131.03 (2C), 133.21, 136.24, 136.99, 141.34, 142.71, 147.17, 151.59, 153.12 (2C), 159.60, 188.96. ESI (m/z) 455.97 $[M+1]^+$. Anal. calcd for $C_{28}H_{25}NO_5$: C 73.82, H 5.53, N 3.08 found C 73.74, H 5.52, N 3.01. Purity: 98.47% (as determined by RP-HPLC, t_r = 5.31 min).

5.1.2. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-{4-[2-(piperidin-1-yl)-ethoxy]phenyl}-prop- 2-en-1-one (8)

Compound **8** was obtained from **4** and 4′-[2-(pyrrolidin-1-yl) ethoxy]acetophenone [28] as described for **7** in 46% yield. Mp. 50.0–50.4 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.44–1.47 (m, 2H, piperidinyl-H), 1.58–1.63 (m, 4H, piperidinyl-H), 2.51–2.52 (m, 4H, piperidinyl-H), 2.79 (t, 2H, J = 6.0 Hz, $OCH_2\underline{CH_2}$), 3.86 (s, 3H, OMe), 4.17 (t, 2H, J = 6.0 Hz, $O\underline{CH_2}CH_2$), 6.95–7.03 (m, 4H, 3′ and 3″-H), 7.33–7.36 (m, 2H, 2′-H), 7.52–7.56 (m, 1H, 6-H), 7.70–7.74 (m, 1H, 7-H), 7.79 (d, 1H, J = 8.0 Hz, 5-H), 7.99 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.06 (s, 1H, 4-H), 8.09–8.13 (m, 2H, 2″-H), 8.18 (d, 1H, J = 8.4 Hz, 8-H), 8.38 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 24.00, 25.75 (2C), 54.94 (2C), 55.26, 57.60, 66.06, 114.07 (2C), 114.29 (2C), 127.24, 127.25, 127.37, 128.08, 129.47, 129.62, 130.38, 130.81, 130.94 (2C), 131.01 (2C), 136.08, 136.78, 140.38, 147.07, 151.60, 159.43, 162.74, 188.38. ESI (m/z) 493.09 $[M+1]^+$. Anal. calcd for $C_{32}H_{32}N_2O_3 \cdot 0.25H_2O$: C 77.18, H 6.60, N 5.62; found C 76.99, H 6.76, N 5.35. Purity: 98.99% (t_r = 6.10 min).

5.1.3. (E)-3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-{4-[2-(pyrrolidin-1-yl)-ethoxy]phenyl}-prop-2-en-1-one (9)

Compound **9** was obtained from **4** and 4-[2-(piperidin-1-yl) ethoxy]acetophenone [28] as described for **7** in 63% yield. Mp. 106.6–107.7 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 2.16 (br s, 4H, pyrrolidinyl-H), 3.42 (br s, 4H, pyrrolidinyl-H), 3.52 (t, 2H, J = 5.6 Hz, $OCH_2\underline{CH_2}$), 3.89 (s, 3H, OMe), 4.62 (t, 2H, J = 5.6 Hz, $O\underline{CH_2}CH_2$), 6.99–7.06 (m, 4H, 3″-H and 3′-H), 7.35–7.38 (m, 2H, 2′-H), 7.55–7.59 (m, 1H, 6-H), 7.73–7.77 (m, 1H, 7-H), 7.83 (d, 1H, J = 8.0 Hz, 5-H), 8.00 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.11–8.14 (m, 3H, 4-H and 2″-H), 8.16 (d, 1H, J = 8.4 Hz, 8-H), 8.39 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 23.20 (2C), 53.76, 54.38 (2C), 55.38, 67.71, 114.15 (2C), 114.36 (2C), 127.06, 127.42, 127.44, 128.20, 129.54, 129.76, 130.42, 131.02 (2C), 131.21 (2C), 131.96, 136.17, 136.92, 140.87, 147.14, 151.53, 159.54, 161.05, 188.42. ESI (m/z) 479.05 $[M+1]^+$. Anal. calcd for $C_{31}H_{30}N_2O_3 \cdot 1.5H_2O$: C 73.64, H 6.58, N 5.54; found C 73.54, H 6.21, N 5.44. Purity: 99.01% (t_r = 5.77 min).

5.1.4. (E)-1-{4-[3-(Dimethylamino)propoxy]phenyl}-3-[3-(4-methoxyphenyl)quinolin-2-yl]prop-2-en-1-one (10)

A mixture of 4-hydroxyacetophenone (3.0 mmol), 3-chloro-*N*,*N*-dimethylpropanamine HCl (3.0 mmol), K_2CO_3 (1.0 mmol) in 30 mL of EtOH was refluxed for 8 h (TLC monitoring). The solvent was removed in vacuo and the residue was reacted with compound **4** as described for **7** in 58% yield. Mp. 79.1–80.2 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 2.35–2.38 (m, 2H, $OCH_2\underline{CH_2}CH_2$), 2.78 (s, 6H, NMe_2), 3.14 (t, 2H, J = 8.0 Hz, $OCH_2CH_2\underline{CH_2}$), 3.89 (s, 3H, OMe), 4.17 (t, 2H, J = 5.6 Hz, $O\underline{CH_2}CH_2CH_2$), 6.95–6.97 (m, 2H, 3″-H), 7.02–7.04 (m, 2H, 3′-H), 7.35–7.37 (m, 2H, 2′-H), 7.55–7.59 (m, 1H, 6-H), 7.73–7.76 (m, 1H, 7-H), 7.83 (d, 1H, J = 8.0 Hz, 5-H), 8.00 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.10–8.12 (m, 3H, 4-H and 2″-H), 8.19 (d, 1H, J = 8.4 Hz, 8-H), 8.39 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 24.89, 43.44 (2C), 55.36, 55.73, 65.07, 114.13 (2C), 114.17 (2C), 127.16, 127.38, 127.43, 128.17, 129.48, 129.74, 130.42, 131.00 (2C), 131.13 (2C), 131.32, 136.14, 136.89, 140.64, 147.12, 151.56, 159.51, 162.17, 188.45. ESI (m/z) 467.02 $[M+1]^+$. Anal. calcd for

$C_{30}H_{30}N_2O_3 \cdot 0.5H_2O$: C 75.76, H 6.57, N 5.89; found C 75.88, H 6.93, N 6.28. Purity: 96.27% (t_r = 6.16 min).

5.1.5. 6-Fluoro-3-(4-methoxyphenyl)-2-methylquinoline-4-carboxylic acid (**13**)

A mixture of 5-fluoroisatin (**11**, 6.60 g, 40 mmol), 4-methoxyphenylacetone (7.88 g, 48 mmol) and KOH (6.74 g, 120 mmol) in EtOH (200 mL) was refluxed for 48 h (TLC monitoring). Evaporation of the solvent afforded a residue which was dissolved in H_2O (50 mL), and the solution was washed twice with Et_2O (30 mL). The aqueous phase was acidified to pH 1 with 37% HCl in ice bath, and the precipitate was collected, washed with H_2O and recrystallized with EtOH to give **13** (11.58 g, 93%) as a white solid. Mp. 269 °C (Dec) (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.45 (s, 3H, 2-Me), 3.83 (s, 3H, OMe), 7.05–7.08 (m, 2H, 3′-H), 7.29–7.32 (m, 2H, 2′-H), 7.45 (dd, 1H, J = 9.6, 2.8 Hz, 5-H), 7.73 (ddd, 1H, J = 9.2, 8.4, 2.8 Hz, 7-H), 8.12 (dd, 1H, J = 9.2, 5.6 Hz, 8-H). ^{13}C NMR (100 MHz, DMSO-d_6): 24.43, 55.15, 108.07, 113.82 (2C), 119.78, 122.49, 128.17, 130.49 (2C), 131.27, 131.59, 139.63, 143.39, 157.60, 159.03, 159.92, 167.79. Anal. calcd for $C_{18}H_{14}FNO_3$: C 69.45, H 4.53, N 4.50; found C 69.45, H 4.59, N 4.57.

5.1.6. 6-Methoxy-3-(4-methoxyphenyl)-2-methylquinoline-4-carboxylic acid (**14**)

Compound **14** was obtained from **12** as described for **13** in 84% yield. Mp. 278.1 °C (Dec) (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.40 (s, 3H, 2-Me), 3.82 (s, 3H, OMe), 3.87 (s, 3H, 6-OMe), 7.03–7.06 (m, 3H, 3′-H and 5-H), 7.27–7.29 (m, 1H, 2′-H), 7.45 (d, 1H, J = 8.0 Hz, 7-H), 7.95 (d, 1H, J = 8.4 Hz, 8-H). ^{13}C NMR (100 MHz, DMSO-d_6): 24.16, 55.19, 55.52, 102.71, 113.81 (2C), 121.98, 122.80, 129.21, 130.22, 130.60 (2C), 139.38, 142.29, 155.06, 157.53, 158.95, 168.38. Anal. calcd for $C_{19}H_{17}NO_4 \cdot 0.2H_2O$: C 69.80, H 5.36, N 4.28; found C 69.78, H 5.44, N 4.16.

5.1.7. 6-Fluoro-3-(4-methoxyphenyl)-2-methylquinoline (**15**)

A mixture of **13** (1.55 g, 5.0 mmol) in 10 mL dowtherm was heated in 280 °C for 4 h (TLC monitoring). The mixture was cooled and then hexane (50 mL) was added and stirred at room temperature for 2 h. The resulting precipitate was collected, washed with H_2O, and then purified by flash chromatography on silica gel (hexane/CH_2Cl_2 1/1) and recrystallized from EtOH to give 0.98 g (73%) of **15** as a brown solid. mp. 87.9–88.7 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 2.66 (s, 3H, 2-Me), 3.89 (s, 3H, OMe), 6.99–7.03 (m, 2H, 3′-H), 7.31–7.34 (m, 2H, 2′-H), 7.39 (dd, 1H, J = 9.2, 2.8 Hz, 5-H), 7.44 (ddd, 1H, J = 8.8, 8.4, 2.8 Hz, 7-H), 7.88 (s, 1H, 4-H), 8.05 (dd, 1H, J = 9.6, 5.6 Hz, 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 24.48, 55.34, 110.29, 113.92 (2C), 119.23, 127.47, 130.28 (2C), 130.80, 131.81, 135.33, 136.20, 143.95, 157.04, 159.28, 160.18. Anal. calcd for $C_{17}H_{14}FNO$: C 76.39, H 5.28, N 5.24; found: C 76.25, H 5.38, N 5.18.

5.1.8. 6-Methoxy-3-(4-methoxyphenyl)-2-methylquinoline (**16**)

Compound **16** was obtained from **14** as described for **15** in 70% yield. Mp. 208.1–209.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 2.63 (s, 3H, 2-Me), 3.88 (s, 3H, OMe), 3.92 (s, 3H, 6-OMe), 6.98–7.02 (m, 2H, 3′-H), 7.04 (d, 1H, J = 2.8 Hz, 5-H), 7.31–7.35 (m, 3H, 2′-H and 7-H), 7.84 (s, 1H, 4-H), 7.95 (d, 1H, J = 9.2 Hz, 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 24.22, 55.32, 55.48, 104.94, 113.81 (2C), 121.72, 127.77, 129.78, 130.31 (2C), 132.38, 135.02, 135.60, 142.95, 154.92, 157.38, 159.08. Anal. calcd for $C_{18}H_{17}NO_2 \cdot 0.4H_2O$: C 75.45, H 6.26, N 4.89; found C 75.63, H 6.21, N 4.82.

5.1.9. 6-Fluoro-3-(4-methoxyphenyl)quinoline-2-carbaldehyde (**17**)

A mixture **15** (0.80 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) and then cooled to room temperature. The reaction mixture was filtered through celite to remove the black residue. Evaporation of the solvent afforded a residue which was dissolved in CH_2Cl_2 (200 mL), washed with brine (100 mL), H_2O (100 mL), saturated sodium bicarbonate solution (100 mL) and dried ($MgSO_4$). The crude product was recrystallized with EtOH to give 0.62 g (73%) of **17** as a yellow solid. mp. 116.4–117.2 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 7.01–7.05 (m, 2H, 3′-H), 7.34–7.38 (m, 2H, 2′-H), 7.49 (dd, 1H, J = 8.4, 2.8 Hz, 5-H), 7.58 (ddd, 1H, J = 9.2, 8.4, 2.8 Hz, 7-H), 8.13 (s, 1H, 4-H), 8.33 (dd, 1H, J = 9.2, 5.6 Hz, 8-H), 10.24 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.37, 110.43, 114.08 (2C), 121.02, 128.65, 129.94, 130.78 (2C), 133.33, 136.32, 137.72, 144.06, 149.43, 159.88, 162.23, 192.23. Anal. calcd for $C_{17}H_{12}FNO_2$: C 72.59, H 4.30, N 4.98; found C 72.82, H 4.45, N 4.76.

5.1.10. 6-Methoxy-3-(4-methoxyphenyl)quinoline-2-carbaldehyde (**18**)

Compound **18** was obtained from **16** as described for **17** in 70% yield. Mp. 100.4–102.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 3.97 (s, 3H, 6-OMe), 7.01–7.05 (m, 2H, 3′-H), 7.10 (d, 1H, J = 2.8 Hz, 5-H), 7.35–7.39 (m, 2H, 2′-H), 7.45 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 8.05 (s, 1H, 4-H), 8.20 (d, 1H, J = 9.2 Hz, 8-H), 10.22 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.35, 55.70, 104.30, 113.93 (2C), 123.81, 129.28, 130.57, 130.77 (2C), 132.09, 136.25, 136.86, 143.23, 147.59, 159.66, 160.12, 192.37. Anal. calcd for $C_{18}H_{15}NO_3$: C 73.69, H 5.16, N 4.91; found C 73.63, H 4.91, N 4.70.

5.1.11. General procedure for the preparation of quinolinyl-chalcones, **19a–g** and **20a–g**

Compound **17** (2.0 mmol) and appropriate acetophenone (2.0 mmol) in EtOH (50 mL) were stirred for 15 min, followed by the addition of an aqueous solution of KOH (6 equiv) at 0 °C for 1 h. The mixture was stirred at room temperature overnight. After the reaction reached completion (TLC monitoring), the resulting mixture was concentrated in vacuo, diluted with water (50 mL), acidified with 1 M HCl to pH = 3, extracted with CH_2Cl_2 (50 mL × 3). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 50/1) and recrystallized with EtOH to give quinolinyl-chalcones **19a–g**. Compounds **20a–g** were prepared from **18** (2.0 mmol) and appropriate acetophenone (2.0 mmol) under the same reaction procedures.

5.1.12. (E)-3-[6-Fluoro-3-(4-methoxyphenyl)quinolin-2-yl]-1-phenylprop-2-en-1-one (**19a**)

Yield 90%; Mp. 146.1–147.6 °C (EtOH). ^{1}H NMR (400 MHz,$CDCl_3$): 3.89 (s, 3H, OMe), 7.02–7.06 (m, 2H, 3′-H), 7.34–7.38 (m, 2H, 2′-H), 7.49 (dd, 1H, J = 8.4, 2.8 Hz, 5-H), 7.49–7.62 (m, 4H, 7-H, 3″-H and 4″-H), 7.99 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.04 (s, 1H, 4-H), 8.10–8.13 (m, 2H, 2″-H), 8.19 (dd, 1H, J = 9.2, 5.2 Hz, 8-H), 8.36 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.38, 110.32, 114.24 (2C), 121.25, 127.28, 128.61 (2C), 128.74 (2C), 128.86, 130.03, 131.00 (2C), 132.24, 133.04, 136.20, 136.94, 137.86, 140.98, 144.33, 150.95, 159.71, 161.08, 190.25. ESI (m/z) 383.94 $[M+1]^+$. Anal. calcd for $C_{25}H_{18}FNO_2$: C 78.30, H 4.74, N 3.65; found C 78.32, H 4.76, N 3.58. Purity: 99.34% (t_r = 6.02 min).

5.1.13. (E)-3-[6-Fluoro-3-(4-methoxyphenyl)quinolin-2-yl]-1-(4-methoxyphenyl)prop-2-en-1-one (**19b**)

Yield 87%; Mp. 155.3–157.6 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 3.90 (s, 3H, OMe), 6.98–7.06 (m, 4H, 3″-H and 3′-H), 7.34–7.38 (m, 2H, 2′-H), 7.44 (dd, 1H, J = 8.8, 2.4 Hz, 5-H), 7.52 (ddd, 1H, J = 9.6, 8.8, 3.2 Hz, 7-H), 7.98 (d, 1H, J = 14.8 Hz, 2-CH=CHCO), 8.04 (s, 1H, 4-H), 8.11–8.15 (m, 2H, 2″-H), 8.19 (dd, 1H, J = 9.2, 5.2 Hz, 8-H), 8.36 (d, 1H, J = 14.8 Hz, 2-CH=CHCO). ^{13}C

NMR (100 MHz, $CDCl_3$): 55.39, 55.49, 110.32, 113.84 (2C), 114.20 (2C), 120.19, 127.26, 128.81, 130.10, 130.90, 131.00 (2C), 131.12 (2C), 132.19, 136.19, 136.90, 140.21, 144.32, 151.17, 159.69, 161.02, 163.60, 188.48. ESI (m/z) 435.95 $[M+1]^+$. Anal. calcd for $C_{26}H_{20}FNO_3 \cdot 0.8H_2O$: C 72.68, H 5.11, N 3.26; found C 72.40, H 5.05, N 3.06. Purity: 97.26% (t_r = 5.03 min).

*5.1.14. (E)-3-[6-Fluoro-3-(4-methoxyphenyl)quinolin-2-yl]-1-(4-hydroxyphenyl)prop-2-en-1-one (**19c**)*

Yield 65%; Mp. 231.7–232.3 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 3.87 (s, 3H, OMe), 6.91–6.95 (m, 2H, 3″-H), 7.12–7.16 (m, 2H, 3′-H), 7.42–7.46 (m, 2H, 2′-H), 7.71–7.77 (m, 2H, 7-H and 2-CH=CHCO), 7.84 (dd, 1H, J = 9.6, 2.8 Hz, 5-H), 7.95–7.99 (m, 2H, 2″-H), 8.19–8.28 (m, 2H, 8-H and 2-CH=CHCO), 8.35 (s, 1H, 4-H), 10.52 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.28, 110.79, 114.18 (2C), 115.60 (2C), 120.45, 127.04, 128.56, 128.76, 129.68, 131.06 (2C), 131.18 (2C), 131.99, 136.24, 136.60, 139.07, 143.77, 150.38, 159.30, 160.31, 162.49, 187.24. ESI (m/z) 399.93 $[M+1]^+$. Anal. calcd for $C_{25}H_{18}FNO_3$: C 75.18, H 4.54, N 3.51; found C 74.96, H 4.74, N 3.42. Purity: 99.52% (t_r = 3.15 min).

*5.1.15. (E)-3-[6-Fluoro-3-(4-methoxyphenyl)quinolin-2-yl]-1-(3,4,5-trimethoxyphenyl)-prop-2-en-1-one (**19d**)*

Yield 86%; . Mp. 200.3–200.7 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 3.95 (s, 3H, OMe), 3.96 (s, 6H, OMe × 2), 7.00–7.08 (m, 2H, 3′-H), 7.35–7.37 (m, 4H, 2′-H and 2″-H), 7.45 (dd, 1H, J = 8.8, 2.4 Hz, 5-H), 7.49–7.55 (m, 1H, 7-H), 8.00 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.06 (s, 1H, 4-H), 8.20 (dd, 1H, J = 9.2, 5.2 Hz, 8-H), 8.27 (d, 1H, J = 14.8 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.38, 56.41 (2C), 60.98, 106.37 (2C), 110.36, 114.25 (2C), 120.28, 126.95, 128.88, 130.05, 130.99 (2C), 132.19, 133.16, 136.27, 136.94, 141.04, 142.77, 144.32, 151.01, 153.14 (2C), 159.75, 161.10, 188.90. ESI (m/z) 473.98 $[M+1]^+$. Anal. calcd for $C_{28}H_{24}FNO_5 \cdot 0.2H_2O$: C 70.49, H 5.15, N 2.94; found C 70.64, H 5.17, N 2.88. Purity: 98.13% (t_r = 5.27 min).

*5.1.16. (E)-3-[6-Fluoro-3-(4-methoxyphenyl)quinolin-2-yl]-1-{4-[2-(piperidin-1-yl)ethoxy]-phenyl}prop-2-en-1-one (**19e**)*

Yield 55%; Mp. 198.6–199.2 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43–1.49 (m, 2H, piperidinyl-H), 1.60–1.65 (m, 4H, piperidinyl-H), 2.53 (br s, 4H, piperidinyl-H), 2.81 (t, 2H, J = 6.0 Hz, OCH_2CH_2N), 3.87 (s, 3H, OMe), 4.20 (t, 2H, J = 6.0 Hz, OCH_2CH_2N), 6.97–7.05 (m, 4H, 3′-H and 3″-H), 7.34–7.37 (m, 2H, 2′-H), 7.43 (dd, 1H, J = 8.8, 2.8 Hz, 5-H), 7.51 (ddd, 1H, J = 9.2, 8.8, 2.4 Hz, 7-H), 7.93 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.04 (s, 1H, 4-H), 8.10–8.14 (m, 2H, 2″-H), 8.19 (dd, 1H, J = 9.2, 5.2 Hz, 8-H), 8.36 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 24.10, 25.87 (2C), 55.06 (2C), 55.37, 57.70, 66.19, 110.32, 114.16 (2C), 114.38 (2C), 120.16, 127.24, 128.79, 130.07, 130.86, 130.98 (2C), 131.08 (2C), 132.17, 136.17, 136.88, 140.16, 144.30, 151.16, 159.67, 160.99, 162.87, 188.42. ESI (m/z) 511.03 $[M+1]^+$. Anal. calcd for $C_{32}H_{31}FN_2O_3 \cdot 0.5H_2O$: C: 73.97, H: 6.21, N: 5.39; found C: 73.82, H: 6.48, N: 5.03. Purity: 98.57% (t_r = 6.06 min).

*5.1.17. (E)-3-[6-Fluoro-3-(4-methoxyphenyl)quinolin-2-yl]-1-{4-[2-(pyrrolidin-1-yl)ethoxy]-phenyl}prop-2-en-1-one (**19f**)*

Yield 57%; . Mp. 88.0–88.9 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.89–1.93 (m, 4H, pyrrolidinyl-H), 2.84 (br s, 4H, pyrrolidinyl-H), 3.08 (t, 2H, J = 6.0 Hz, OCH_2CH_2N), 3.89 (s, 3H, OMe), 4.30 (t, 2H, J = 6.0 Hz, OCH_2CH_2N), 6.99–7.05 (m, 4H, 3′-H and 3″-H), 7.34–7.39 (m, 2H, 2′-H), 7.44 (dd, 1H, J = 8.8, 2.8 Hz, 5-H), 7.52 (ddd, 1H, J = 9.2, 8.4, 2.4 Hz, 7-H), 7.97 (d, 1H, J = 14.8 Hz, 2-CH=CHCO), 8.04 (s, 1H, 4-H), 8.10–8.14 (m, 2H, 2″-H), 8.19 (dd, 1H, J = 9.2, 5.2 Hz, 8-H), 8.35 (d, 1H, J = 14.8 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 23.42 (2C), 54.59, 54.62 (2C), 55.39, 66.49, 110.31, 114.24 (2C), 114.39 (2C), 120.19, 127.25, 128.82, 130.11, 130.87, 130.99 (2C), 131.12 (2C), 132.21, 136.18, 136.91, 140.27, 144.34, 151.17, 159.71, 161.04, 162.47, 188.45. ESI (m/z) 497.06 $[M+1]^+$. Anal. calcd for $C_{31}H_{29}FN_2O_3 \cdot 0.2H_2O$: C 74.44, H 5.92, N 5.60; found C 74.26, H 6.03, N 5.37. Purity: 97.74% (t_r = 6.18 min).

*5.1.18. (E)-1-{4-[3-(Dimethylamino)propoxy]phenyl}-3-[6-fluoro-3-(4-methoxyphenyl)-quinolin-2-yl]prop-2-en-1-one (**19g**)*

Yield 62%; Mp. 103.5–104.6 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 2.03–2.10 (m, 2H, $OCH_2CH_2CH_2N$), 2.36 (s, 6H, NMe_2), 2.60 (t, 2H, J = 7.2 Hz, $OCH_2CH_2CH_2N$), 3.89 (s, 3H, OMe), 4.12 (t, 2H, J = 5.6 Hz, $OCH_2CH_2CH_2N$), 6.96–7.05 (m, 4H, 3′-H and 3″-H), 7.34–7.38 (m, 2H, 2′-H), 7.44 (dd, 1H, J = 8.8, 2.8 Hz, 5-H), 7.52 (ddd, 1H, J = 9.2, 8.8, 2.4 Hz, 7-H), 7.97 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.04 (s, 1H, 4-H), 8.10–8.14 (m, 2H, 2″-H), 8.18 (dd, 1H, J = 9.2, 5.2 Hz, 8-H), 8.36 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 26.88, 45.05 (2C), 55.37, 56.08, 66.21, 110.3, 114.22 (2C), 114.28 (2C), 120.17, 127.27, 128.82, 130.09, 130.86, 130.99 (2C), 131.11 (2C), 132.17, 136.18, 136.88, 140.17, 144.31, 151.17, 159.68, 161.01, 162.95, 188.45. ESI (m/z) 485.03 $[M+1]^+$. Anal. calcd for $C_{30}H_{29}FN_2O_3 \cdot 0.2H_2O$: C 73.81, H 6.07, N 5.74; found C 73.65, H 6.11, N 5.69. Purity: 95.56% (t_r = 6.29 min).

*5.1.19. (E)-3-[6-Methoxy-3-(4-methoxyphenyl)quinolin-2-yl]-1-phenylprop-2-en-1-one (**20a**)*

Yield 87%; Mp. 130.2–130.7 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 3.95 (s, 3H, 6-OMe), 7.01–7.05 (m, 2H, 3′-H), 7.07 (d, 1H, J = 2.8 Hz, 5-H), 7.34–7.38 (m, 2H, 2′-H), 7.40 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 7.48–7.61 (m, 3H, 3″-H and 4″-H), 7.98 (s, 1H, 4-H), 8.00 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.08 (d, 1H, J = 9.2 Hz, 8-H), 8.10–8.13 (m, 2H, 2″-H), 8.19 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.36, 55.59, 104.47, 114.12 (2C), 123.03, 126.23, 128.55 (2C), 128.71 (2C), 129.43, 130.60, 131.01 (2C), 131.15, 132.87, 135.56, 136.63, 138.02, 141.50, 143.47, 148.93, 158.64, 159.51, 190.34. ESI (m/z) 395.96 $[M+1]^+$. Anal. calcd for $C_{26}H_{21}NO_3$: C 78.97, H 5.35, N 3.54; found C 78.84, H 5.23, N 3.52. Purity: 99.00% (t_r = 5.79 min).

*5.1.20. (E)-3-[6-Methoxy-3-(4-methoxyphenyl)quinolin-2-yl]-1-(4-methoxyphenyl)prop-2-en-1-one (**20b**)*

Yield 82%; Mp. 147.6–148.7 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.96 (s, 3H, 6-OMe), 6.97–7.00 (m, 2H, 3″-H), 7.01–7.05 (m, 2H, 3′-H), 7.07 (d, 1H, J = 2.8 Hz, 5-H), 7.34–7.39 (m, 2H, 2′-H), 7.40 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 7.98 (s, 1H, 4-H), 7.99 (d, 1H, J = 14.8 Hz, 2-CH=CHCO), 8.08 (d, 1H, J = 9.2 Hz, 8-H), 8.12–8.16 (m, 2H, 2″-H), 8.34 (d, 1H, J = 14.8 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 55.60, 55.69, 55.83, 104.76, 114.01 (2C), 114.36 (2C), 123.19, 126.47, 129.61, 130.92, 131.25 (2C), 131.31 (2C), 131.37, 135.80, 136.84, 140.95, 143.72, 149.43, 158.82, 159.74, 163.72, 188.84. ESI (m/z) 426.00 $[M+1]^+$. Anal. calcd for $C_{27}H_{23}NO_4 \cdot 0.2H_2O$: C 75.58, H 5.50, N 3.26; found C 75.32, H 5.52, N 3.17. Purity: 98.86% (t_r = 6.02 min).

*5.1.21. (E)-1-(4-Hydroxyphenyl)-3-[6-methoxy-3-(4-methoxyphenyl)quinolin-2-yl]prop-2-en-1-one (**20c**)*

Yield 68%; Mp. 223.4–223.8 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 3.83 (s, 3H, OMe), 3.89 (s, 3H, 6-OMe), 6.87–6.90 (m, 2H, 3″-H), 7.08–7.11 (m, 2H, 3′-H), 7.36–7.40 (m, 3H, 2′-H and 5-H), 7.44 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 7.70 (d, 1H, J = 14.8 Hz, 2-CH=CHCO), 7.91–7.95 (m, 2H, 2″-H), 8.01 (d, 1H, J = 9.2 Hz, 8-H), 8.17 (s, 1H, 4-H), 8.18 (d, 1H, J = 14.8 Hz, 2-CH=CHCO), 10.46 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.24, 55.62, 105.31, 114.12 (2C), 115.56 (2C), 123.06, 125.84, 128.90, 129.16, 130.18, 130.62, 131.01 (2C), 131.07 (2C), 135.65, 135.98, 139.52, 142.75, 148.09, 158.17, 159.15, 162.36, 187.30. ESI (m/z) 411.96 $[M+1]^+$. Anal. calcd for

$C_{26}H_{21}NO_4 \cdot 0.2H_2O$: C 75.24, H 5.20, N 3.37; found C 74.88, H 5.13, N 3.42. Purity: 99.27% (t_r = 3.27 min).

*5.1.22. (E)-3-[6-Methoxy-3-(4-methoxyphenyl)quinolin-2-yl]-1-(3,4,5-trimethoxyphenyl)-prop-2-en-1-one (**20d**)*

Yield 92%; Mp. 179.4–179.5 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OMe), 3.95 (s, 6H, OMe), 3.96 (s, 3H, OMe), 3.96 (s, 3H, 6-OMe), 7.02–7.05 (m, 2H, 3′-H), 7.08 (d, 1H, J = 2.8 Hz, 5-H), 7.35–7.38 (m, 4H, 2′-H and 2″-H), 7.41 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 8.00 (s, 1H, 4-H), 8.01 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.09 (d, 1H, J = 9.2 Hz, 8-H), 8.26 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (400 MHz, $CDCl_3$): 55.35, 55.60, 56.36 (2C), 60.95, 104.50, 106.23 (2C), 114.11 (2C), 123.10, 125.85, 129.44, 130.56, 131.00 (2C), 131.03, 133.32, 135.67, 136.64, 141.54, 142.53, 143.41, 148.96, 153.07 (2C), 158.68, 159.52, 188.96. ESI (m/z) 486.00 $[M+1]^+$. Anal. calcd for $C_{29}H_{27}NO_6$: C 71.74, H 5.61, N 2.88; found C 71.53, H 5.28, N 2.78. Purity: 98.53% (t_r = 5.19 min).

*5.1.23. (E)-3-[6-Methoxy-3-(4-methoxyphenyl)quinolin-2-yl]-1-{4-[2-(piperi-din-1-yl)eth-oxy]phenyl}prop-2-en-1-one (**20e**)*

Yield 60%; Mp. 98.2–99.1 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.42–1.49 (m, 2H, piperidinyl-H), 1.58–1.66 (m, 4H, piperidinyl-H), 2.54 (m, 4H, piperidinyl-H), 2.81 (t, 2H, J = 6.0 Hz, OCH_2CH_2N), 3.89 (s, 3H, OMe), 3.95 (s, 3H, 6-OMe), 4.20 (t, 2H, J = 6.0 Hz, OCH_2CH_2N), 6.97–7.00 (m, 2H, 3″-H), 7.01–7.05 (m, 2H, 3′-H), 7.07 (d, 1H, J = 2.8 Hz, 5-H), 7.34–7.38 (m, 2H, 2′-H), 7.40 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 7.97 (s, 1H, 4-H), 7.98 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.08 (d, 1H, J = 9.2 Hz, 8-H), 8.10–8.14 (m, 2H, 2″-H), 8.34 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 24.12, 25.87 (2C), 55.06 (2C), 55.38, 55.60, 57.72, 66.20, 104.56, 114.15 (2C), 114.37 (2C), 122.93, 126.31, 129.38, 130.72, 131.25 (2C), 131.06 (2C), 131.16, 135.56, 136.61, 140.69, 143.51, 149.24, 158.61, 159.54, 162.77, 188.96. ESI (m/z) 523.06 $[M+1]^+$. Anal. calcd for $C_{33}H_{34}N_2O_4 \cdot 0.2H_2O$: C: 75.32, H: 6.59, N: 5.32; found C: 75.04, H: 6.56, N: 5.10. Purity: 97.93% (t_r = 6.27 min).

*5.1.24. (E)-3-[6-Methoxy-3-(4-methoxyphenyl)quinolin-2-yl]-1-{4-[2-(pyrrolidin-1-yl)eth-oxy]phenyl}prop-2-en-1-one (**20f**)*

Yield 58%; Mp. 66.7–67.1 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.80–1.85 (m, 4H, pyrrolidinyl-H), 2.66–2.67 (m, 4H, pyrrolidinyl-H), 2.96 (t, 2H, J = 6.0 Hz, OCH_2CH_2N), 3.89 (s, 3H, OMe), 3.96 (s, 3H, 6-OMe), 4.21 (t, 2H, J = 6.0 Hz, OCH_2CH_2N), 6.98–7.07 (m, 4H, 3′-H and 3″-H), 7.07 (d, 1H, J = 2.8 Hz, 5-H), 7.34–7.38 (m, 2H, 2′-H), 7.40 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 7.97 (s, 1H, 4-H), 7.98 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.08 (d, 1H, J = 9.2 Hz, 8-H), 8.11–8.14 (m, 2H, 2″-H), 8.34 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 23.46 (2C), 54.70 (2C), 54.82, 55.36, 55.58, 67.17, 104.51, 114.12 (2C), 114.33 (2C), 122.94, 126.24, 129.36, 130.68, 131.01 (2C), 131.05 (2C), 131.09, 131.13, 135.55, 136.59, 140.69, 143.48, 149.19, 158.57, 159.50, 162.73, 188.58. ESI (m/z) 509.02 $[M+1]^+$. Anal. calcd for $C_{32}H_{32}N_2O_4 \cdot 0.4H_2O$: C 74.51, H 6.41, N 5.43; found C 74.58, H 6.59, N 5.10. Purity: 97.86% (t_r = 6.11 min).

*5.1.25. (E)-1-{4-[3-(Dimethylamino)propoxy]phenyl}-3-[6-methoxy-3-(4-methoxyphenyl)-quinolin-2-yl]prop-2-en-1-one (**20g**)*

Yield 53%; Mp. 49.7–51.8 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.97–2.03 (m, 2H, $OCH_2CH_2CH_2N$), 2.28 (s, 6H, NMe_2), 2.50 (t, 2H, J = 7.2 Hz, $OCH_2CH_2CH_2N$), 3.87 (s, 3H, OMe), 3.93 (s, 3H, 6-OMe), 4.09 (t, 2H, J = 6.4 Hz, $OCH_2CH_2CH_2N$), 6.95–7.03 (m, 4H, 3′-H and 3″-H), 7.05 (d, 1H, J = 2.8 Hz, 5-H), 7.33–7.36 (m, 2H, 2′-H), 7.39 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 7.96 (s, 1H, 4-H), 7.99 (d, 1H, J = 15.2 Hz, 2-CH=CHCO), 8.07 (d, 1H, J = 9.2 Hz, 8-H), 8.09–8.13 (m, 2H, 2″-H), 8.33 (d, 1H, J = 15.2 Hz, 2-CH=CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): 27.16, 45.28 (2C), 55.27, 55.49, 56.08, 66.28, 104.45, 114.04 (2C), 114.18 (2C), 122.88, 126.15, 129.28, 130.59, 130.86, 130.94 (2C), 130.98 (2C), 131.02, 135.47, 136.49, 140.58, 143.38, 149.07, 158.48, 159.42, 162.90, 188.46. ESI (m/z) 497.06 $[M+1]^+$. Anal. calcd for $C_{31}H_{32}N_2O_4 \cdot 0.2H_2O$: C 74.44, H 6.53, N 5.60; found C 74.35, H 6.52, N 5.56. Purity: 97.05% (t_r = 5.98 min).

*5.1.26. {3-[3-(4-Methoxyphenyl)quinolin-2-yl]oxiran-2-yl}(3,4,5-trimethoxyphenyl)methan-one (**21**)*

A suspension of **7** (0.45 g, 1 mmol) in MeOH (30 mL) is treated successively with K_2CO_3 (0.33 g, 3 mmol) and 35% aqueous hydrogen peroxide solution (1 mL, 10 mmol), the mixture was stirred at room temperature for 3 h (TLC monitoring). The solvent was removed under reduced pressure and the resulting residue dissolved in CH_2Cl_2 (50 mL), and washed with H_2O (40 mL). The organic phase was separated, washed with brine and dried by $MgSO_4$, then the solvent removed under reduced pressure and recrystallized with EtOH to give compound **21** (0.42 g, 90%). Mp. 151.9–152.9 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 3.86 (s, 3H, OMe), 3.90 (s, 6H, OMe), 3.93 (s, 3H, OMe), 4.26 (d, 1H, J = 2.0 Hz), 5.29 (d, 1H, J = 2.0 Hz), 6.99–7.02 (m, 2H, 2′-H), 7.46–7.79 (m, 4H, 2″-and 2′-H), 7.58–7.62 (m, 1H, 6-H), 7.73–7.77 (m, 1H, 7-H), 7.87 (d, 1H, J = 8.4 Hz, 5-H), 8.13–8.16 (m, 2H, 4- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.36, 56.31 (2C), 58.18, 59.01, 60.97, 106.06 (2C), 114.26, 114.30 (2C), 127.49, 127.56, 127.90, 129.30, 129.52, 129.77, 130.40, 130.79 (2C), 136.01, 136.78, 143.29, 146.92, 151.41, 153.20, 159.66, 192.68. ESI (m/z) 471.96 $[M+1]^+$. Anal. calcd for $C_{28}H_{25}NO_6$: C 71.32, H 5.34, N 2.97; found C 71.30, H 5.43, N 2.76. Purity: 97.53% (t_r = 4.49 min).

*5.1.27. {2-[3-(4-Methoxyphenyl)quinolin-2-yl]cyclopropyl}(3,4,5-trimethoxyphenyl)methan-one (**22**)*

A mixture of compound **7** (0.45 g, 1 mmol), trimethylsulfoxonium iodide (0.26 g, 2 mmol) and tetrabutylammonium bromide (0.06 g, 0.2 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 15 min. 50% aq NaOH (10 mL) solution was added dropwise and the reaction mixture was refluxed at 80 °C for 4 h (TLC monitoring). The reaction mixture was cooled, CH_2Cl_2 (50 mL) was added and washed twice with H_2O (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 100/1) and recrystallized with EtOH to give **22** (0.42 g, 89%) as a gray solid. Mp.: 148.6–150.3 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.86–1.91 and 1.99–2.04 (two m, 2H, cyclopropyl-CH_2), 2.93–2.97 (m, 1H, 2-CHCHCO), 3.59–3.64 (m, 1H, 2-CHCHCO), 3.84 (s, 3H, OMe), 3.89 (s, 6H, OMe), 3.91 (s, 3H, OMe), 6.92–6.96 (m, 2H, 2′-H), 7.33 (s, 2H, 2″-H), 7.34–7.38 (m, 2H, 2′-H), 7.47–7.51 (m, 1H, 6-H), 7.66–7.70 (m, 1H, 7-H), 7.79 (d, 1H, J = 8.0 Hz, 5-H), 7.96 (s, 1H, 4-H), 8.04 (d, 1H, J = 8.4 Hz, 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 20.87, 29.02, 29.44, 55.26, 56.26 (2C), 60.88, 105.74 (2C), 113.98 (2C), 126.02, 126.87, 127.24, 128.54, 129.18, 130.59 (2C), 130.99, 132.95, 135.25, 135.85, 142.39, 146.83, 152.97 (2C), 157.45, 159.22, 197.37. ESI (m/z) 470.01 $[M+1]^+$. Anal. calcd for $C_{29}H_{27}NO_5$: C 74.18, H 5.80, N 2.98; found C 74.12, H 5.66, N 2.77. Purity: 99.45% (t_r = 4.45 min).

*5.1.28. 3-[3-(4-Methoxyphenyl)quinolin-2-yl]-1-(3,4,5-trimethoxyphenyl)propan-1-one (**23**)*

A solution of **7** (0.91 g, 2 mmol) in acetic acid (30 mL) was hydrogenated for 2 h (TLC monitoring) under H_2 with Pd/C (120 mg). The reaction mixture was filtered with celite and the filtrate concentrated in vacuo to give a residual solid, and recrystallized from EtOH to give **23** (0.85 g, 93%) as a white solid. Mp.: 128.1–129.6 °C (EtOH). ^{1}H NMR ($CDCl_3$): 3.94 (t, 2H, J = 6.8 Hz, 2-CH_2CH_2CO), 3.52 (t, 2H, J = 6.8 Hz, 2-CH_2CH_2CO), 3.88 (s, 3H, OMe), 3.90 (s, 6H, OMe), 3.92 (s, 3H, OMe), 7.00–7.03 (m, 2H, 3′-H), 7.28 (s, 2H, 2″-H), 7.38–7.41 (m, 2H, 2′-H), 7.46–7.50 (m, 1H, 6-H),

7.62–7.66 (m, 1H, 7-H), 7.78 (d, 1H, J = 8.0 Hz, 5-H), 7.92 (d, 1H, J = 8.4 Hz, 8-H), 7.94 (s, 1H, 4-H). ^{113}C NMR ($CDCl_3$): 30.69, 36.51, 55.34, 56.26 (2C), 60.94, 105.56 (2C), 113.95 (2C), 126.07, 126.83, 127.39, 128.47, 129.06, 129.47, 130.46 (2C), 131.80, 132.67, 135.34, 136.22, 142.22, 146.81, 152.96, 159.16, 159.20, 198.61. ESI (m/z) 457.98 $[M+1]^+$. Anal. calcd for $C_{28}H_{27}NO_5$: C 73.50, H 5.95, N 3.06; found C 73.60, H 6.01, N 2.97. Purity: 99.39% (t_r = 4.48 min).

5.2. Pharmacological methods

5.2.1. Antiproliferative assay

Cancer cells (H460, A549, H1299, MCF-7, MDA-MB-231, SKBR-3), normal lung cell (MRC-5) 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 amphotercin. 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% [29].

5.2.2. Cell cycle analysis

MDA-MB-231 cells treated with DMSO, compound **7** at different concentration (1, 5, and 10 μM) or CIL-102 (10 μM) for 12, 24 and 36 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).

5.2.3. Immunofluorescence analysis

MDA-MB-231 Cells were seeded on cover glasses in 12-well plates with compound **7** (1–10 μM), CIL-102 (10 μM) or **23** (10 μM) treatment for 24 h. After incubation, cells were washed with 1× PBS twice and fixed in 4% paraformaldehyde for 1 h. Then, cells were washed with PBS containing 0.1 M Glycine for 5 min and permibilized with solution containing 2% FBS and 0.4% TritonX-100 in PBS at room temperature for 15 min. After permibilization, cells were stained with β-tubulin monoclonal antibody (Santa Cruza 1:1000) at 4 °C overnight. After primary antibody incubation, cells were washed with PBS containing 0.2% TritonX-100 three times, and stained with fluorescein isothiocyanate-conjugated anti-mouse IgG antibody (Origene, 1:200 diluted) at room temperature for 1 h. Finally, washed with PBS and stained with DAPI (0.1 mg/ml) for 5 min at room temperature in the dark. Removed the excess DAPI solution and washed with PBS twice. Mounted the samples before analyzing under a fluorescence microscopy.

5.2.4. 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 were 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 8, caspase 8 and Bad 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).

5.3. Molecular modeling

Libdoc was performed by Discovery Studio 3.5 (Accelrys, San Diego, CA, USA) [38–40]. Ligand conformations were generated and adjusted by Solvent Accessible Solvent Area (SASA) methods; polar and non-polar probes were employed to place grid in the protein binding site. The hot spots would be pointed out in the binding site by this step. In libdock, “docking” means that conformations of ligands would be aligned to hot spots by the triplets method. In the scoring step, Broyden-Fletcher-Goldfarb-Shanno (BFGS) pose optimization was achieved and shown on the top of simple pair-wise scores [41]. These results were also called by Libdock scores. In our study, the tubulin structure was retrieved from Protein Data Bank (PDB code: 1SA0) where the GTP site and colchicine binding site are labeled in Fig. 9.

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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.2015.04.054.

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