

Synthesis and antiproliferative evaluation of 2,3-diarylquinoline derivatives†

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A number of 2,3-diarylquinoline derivatives were synthesized and evaluated for antiproliferative activities against the growth of six cancer cell lines including human hepatocellular carcinoma (Hep G2 and Hep 3B), non-small cell lung cancer (A549 and H1299), and breast cancer (MCF-7 and MDA-MB-231) cell lines. The preliminary results indicated that 6-fluoro-2,3-bis{4-[2-(piperidin-1-yl)ethoxy]phenyl}quinoline (**16b**) was one of the most active compounds against the growth of Hep 3B, H1299, and MDA-MB-231 with a GI₅₀ value of 0.71, 1.46, and 0.72 μM respectively which was more active than tamoxifen. Further investigations have shown that **16b** induced cell cycle arrest at G2/M phase followed by DNA fragmentation *via* an increase in the protein expression of Bad, Bax and decrease in Bcl-2, and PARP which consequently cause cell death.

Introduction

The 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 antimicrobial, antitumor, and antiviral activities.^{1–11} Camptothecin is one of the examples which bears a quinoline moiety and is an anticancer alkaloid isolated from *Camptotheca acuminata*.¹² We have also synthesized certain iminoindeno[1,2-*c*]quinoline (**1**) and 6-arylideno[1,2-*c*]quinoline (**2**) derivatives which were found to be more potent than camptothecin against the growth of human cancer cell lines.^{12,13} In continuation of our study to explore more potent anticancer drug candidates, we describe herein the preparation of certain 2,3-diarylquinoline derivatives whose structures can be related to 6-arylideno[1,2-*c*]quinoline (**2**) in which the carbonyl bridge at C-11 was eliminated (Fig. 1). The structures of these 2,3-diarylquinoline derivatives also resemble tamoxifen in which the substituted ethylene bridge was replaced with quinoline, and combretastatin A-4^{14–17} in which the ethylene bridge was replaced with quinoline. These 2,3-diarylquinoline derivatives were evaluated *in vitro* against a panel of six cancer cell lines including two human hepatocellular carcinoma (Hep G2 and

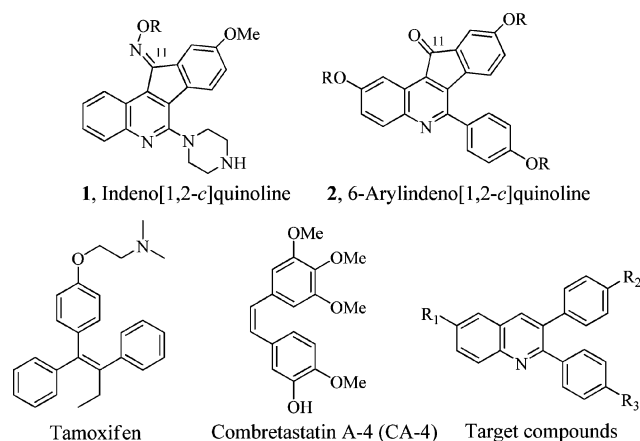


Fig. 1 Structures of indeno[1,2-*c*]quinolines **1**, 6-arylideno[1,2-*c*]quinolines **2**, tamoxifen, combretastatin A-4 (CA-4), and targeted compounds.

Hep 3B), two non-small cell lung cancer (A549 and H1299), and two breast cancer (MCF-7 and MDA-MB-231) cell lines. These cancers are common malignancies in the world, and are the leading cause of cancer deaths in Asian countries including Taiwan.^{18–22}

Results and discussion

Chemistry

Pfitzinger reaction of 5-fluoroindolin-2,3-dione (**3a**) and deoxybenzoin (**4a**) under basic conditions gave 6-fluoro-2,3-bisphenylquinoline-4-carboxylic acid (**5**) as described in Scheme 1. Accordingly, 6-methoxy-2,3-bisphenylquinoline-4-carboxylic acid (**7**) was obtained by the reaction of 5-methoxyindolin-2,3-dione (**3b**) and deoxybenzoin (**4a**). Preparation of compounds **6** and **8**

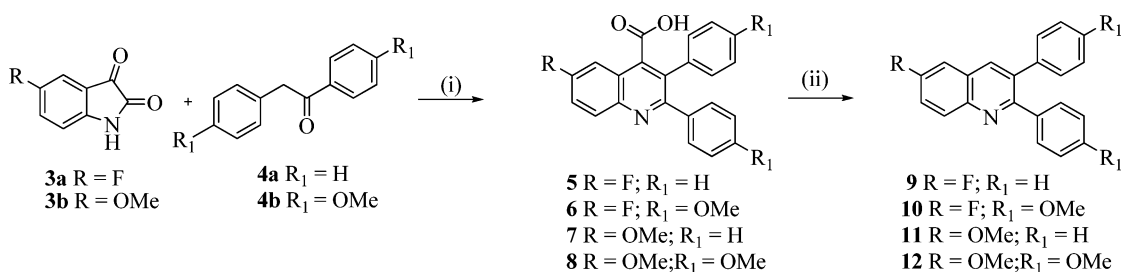
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Scheme 1 Reagents and conditions: (i) KOH, EtOH, 80 °C, 48 h; (ii) PhOPh, 250 °C, 4 h.

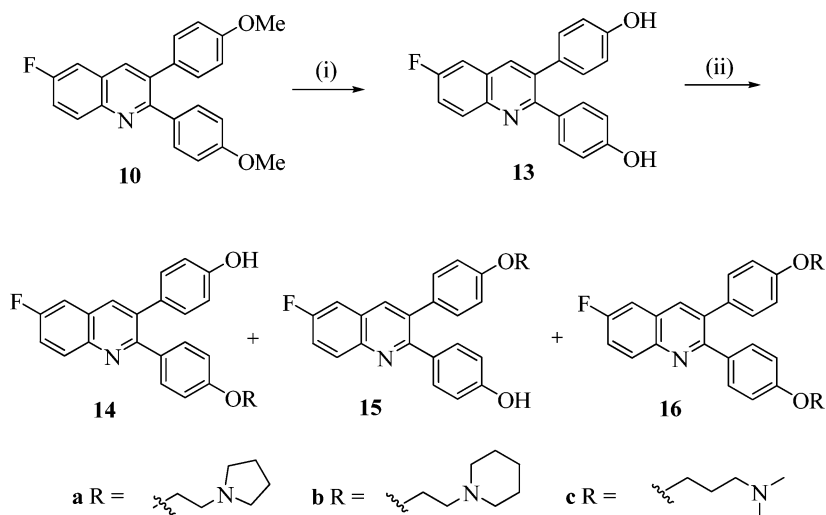
were previously reported.¹³ 2,3-Diarylquinoline derivatives **9–12** were synthesized in a fairly good yield by heating their respective carboxylic acids **5–8** in diphenyl ether at 250 °C.

Demethylation of 6-fluoro-2,3-bis(4-methoxyphenyl)quinoline (**10**) with HBr gave 6-fluoro-2,3-bis(4-hydroxyphenyl)quinoline (**13**) which was then alkylated with *N*-(2-chloroethyl)pyrrolidine to afford a mixture of 4-{6-fluoro-2-[4-(2-(pyrrolidin-1-yl)ethoxy)phenyl]quinolin-3-yl}phenol (**14a**), 4-{6-fluoro-3-[4-(2-(pyrrolidin-1-yl)ethoxy)phenyl]quinolin-2-yl}phenol (**15a**), and 6-fluoro-2,3-bis{4-[2-(pyrrolidin-1-yl)ethoxy]-phenyl}quinoline (**16a**) in a yield of 27%, 33% and 39% respectively, as depicted in Scheme 2.

The structural assignment of the alkylated products **14a**, **15a**, and **16a** was determined by 2D Nuclear Overhauser Effect (2D NOESY) experiments, as shown in Fig. 2. Compound **14a** was assigned as a monoalkylated product at the C-2 phenyl moiety by the correlation between OCH₂ ($\delta_{\text{H}} = 4.11$ ppm)/*meta*-H of 2-phenyl ($\delta_{\text{H}} = 6.88$ ppm); *ortho*-H of 3-phenyl ($\delta_{\text{H}} = 7.07$ ppm)/4-H ($\delta_{\text{H}} = 8.27$ ppm) (Fig. 2(A)). Compound **15a** was assigned as a monoalkylated product at the C-3 phenyl moiety by the correlation between OCH₂ ($\delta_{\text{H}} = 4.09$ ppm)/*meta*-H of 3-phenyl ($\delta_{\text{H}} = 6.92$ ppm); *meta*-H ($\delta_{\text{H}} = 6.92$ ppm)/*ortho*-H ($\delta_{\text{H}} = 7.17$ ppm); and *ortho*-H of 3-phenyl ($\delta_{\text{H}} = 7.17$ ppm)/4-H ($\delta_{\text{H}} = 8.27$ ppm) (Fig. 2(B)). From the 2D NOESY spectrum of the dialkylated product **16a**, similar correlations between OCH₂ ($\delta_{\text{H}} = 4.08$ ppm)/*meta*-H of 3-phenyl ($\delta_{\text{H}} = 6.92$ ppm), *meta*-H of 3-phenyl ($\delta_{\text{H}} = 6.92$ ppm)/*ortho*-H of 3-phenyl ($\delta_{\text{H}} = 7.17$ ppm), and *ortho*-H of 3-

phenyl ($\delta_{\text{H}} = 7.17$ ppm)/4-H ($\delta_{\text{H}} = 8.28$ ppm) were observed (Fig. 2(C)). By comparison of ¹H NMR spectra for **14a** and **15a**, down-field shifts were observed for the alkylation of the C-3 phenyl moiety in which *ortho*-H ($\delta_{\text{H}} = 7.07$ ppm) and *meta*-H ($\delta_{\text{H}} = 6.74$ ppm) proton signals of **14a** shifted down-field to *ortho*-H ($\delta_{\text{H}} = 7.17$ ppm) and *meta*-H ($\delta_{\text{H}} = 6.92$ ppm) proton signals respectively of **15a**. Accordingly, down-field shifts were observed for the alkylation of the C-2 phenyl moiety in which *ortho*-H ($\delta_{\text{H}} = 7.21$ ppm) and *meta*-H ($\delta_{\text{H}} = 6.68$ ppm) proton signals of **15a** shifted down-field to *ortho*-H ($\delta_{\text{H}} = 7.34$ ppm) and *meta*-H ($\delta_{\text{H}} = 6.88$ ppm) proton signals respectively of **14a**. Similar phenomena were observed for the comparison of **14a** and **16a** in which *ortho*-H ($\delta_{\text{H}} = 7.07$ ppm) and *meta*-H ($\delta_{\text{H}} = 6.74$ ppm) proton signals of **14a** shifted down-field to *ortho*-H ($\delta_{\text{H}} = 7.17$ ppm) and *meta*-H ($\delta_{\text{H}} = 6.92$ ppm) proton signals respectively of **16a**. A mixture of **14b**, **15b**, and **16b** was obtained from **13** by alkylation with *N*-(2-chloroethyl)piperidine while a mixture of **14c**, **15c**, and **16c** was obtained from **13** by alkylation with 3-chloro-*N,N*-dimethylpropanamine.

Treatment of 6-methoxy-2,3-diphenylquinoline (**11**) with HBr gave 2,3-diphenylquinolin-6-ol (**17**) which was then alkylated with *N*-(2-chloroethyl)pyrrolidine to afford 2,3-diphenyl-6-[2-(pyrrolidin-1-yl)ethoxy]quinoline (**19a**) as described in Scheme 3. Accordingly, compounds **19b** and **19c** were obtained by the alkylation of **17** with *N*-(2-chloroethyl)piperidine, and 3-chloro-*N,N*-dimethylpropanamine respectively. Under the same reaction conditions, 6-methoxy-2,3-bis(4-methoxyphenyl)quinoline (**12**)



Scheme 2 Reagents and conditions: (i) 48% HBr, HOAc, reflux, 48 h; (ii) NaH, alkyl halides, DMF, 80 °C, 25 min.

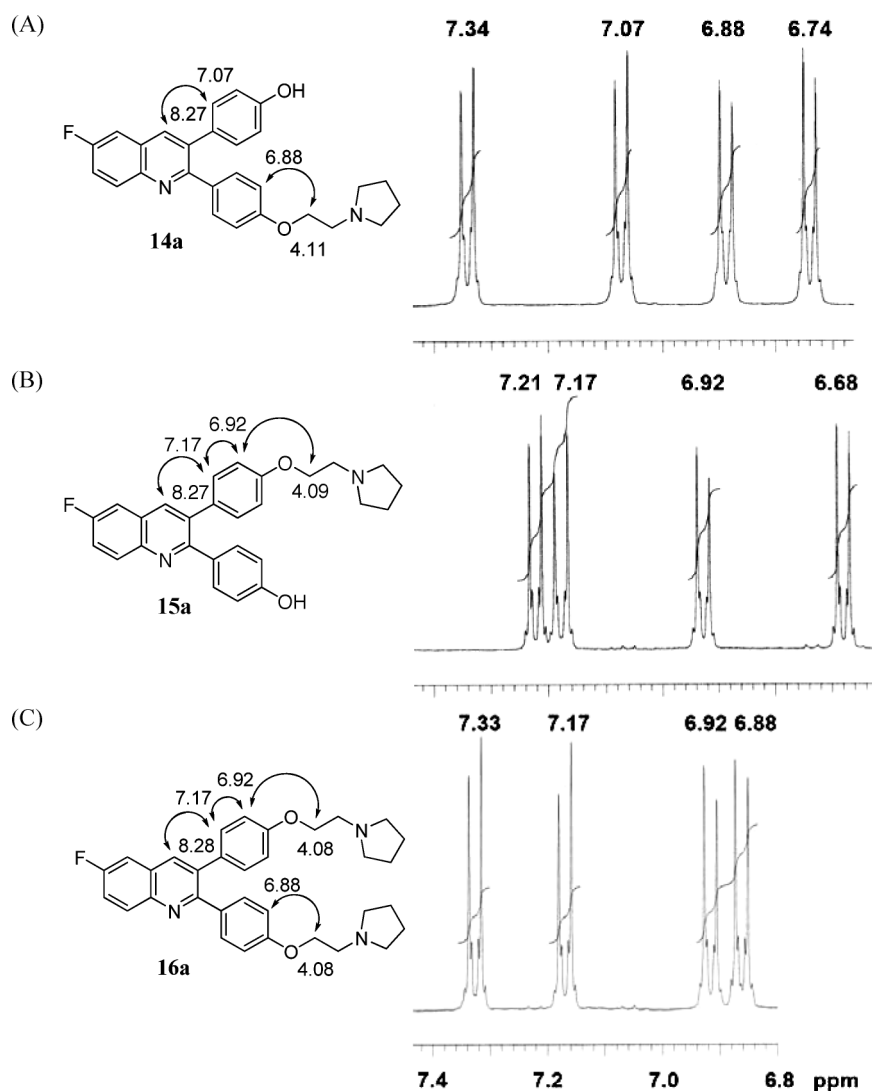


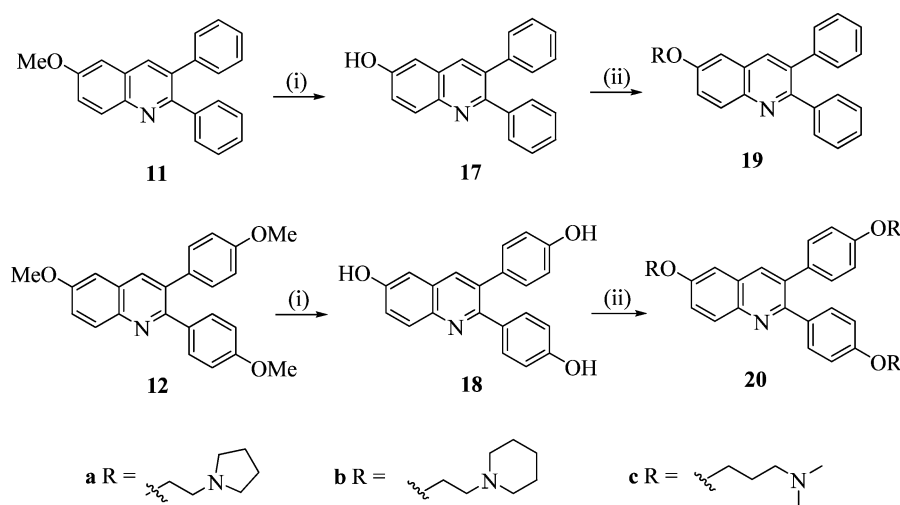
Fig. 2 Comparative studies on the chemical shifts for 2D NOESY correlations and ¹H NMR signals of the 2,3-diaryl aromatic part (δ 6.60–7.40 ppm) of **14a** (A), **15a** (B), and **16a** (C).

was demethylated to give 2,3-dihydroxyphenylquinolin-6-ol (**18**) which was then treated with *N*-(2-chloroethyl)pyrrolidine, *N*-(2-chloroethyl)piperidine, and 3-chloro-*N,N*-dimethylpropanamine respectively to afford trialkylated products **20a–c** respectively.

Antiproliferation activity

All the synthesized 2,3-diarylquinoline derivatives were evaluated *in vitro* against a panel of six cancer cell lines including two human hepatocellular carcinoma cells (Hep G2 and Hep 3B), two non-small cell lung cancer cells (A549 and H1299) and two breast cancer cells (MCF-7 and MDA-MB-231) using XTT (sodium 3'-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate) assay.²³ The concentration that inhibited the growth of 50% of cells (GI₅₀) 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 GI₅₀ results of 2,3-diarylquinoline derivatives are summarized in Table 1. For the C-6 fluoro-substituted 2,3-diarylquinoline derivatives, introduction of the methoxy or hydroxyl substituents in the *para*-position of the C-2 and C-3 aryl moieties did not improve antiproliferative activity, in that 6-fluoro-2,3-diphenylquinoline (**9**), its methoxy derivatives **10**, and hydroxy derivatives **13** were inactive against all the cancer cells tested with GI₅₀ of > 50 μ M in each case. Further introduction of an aminoalkyl side chain in the *para*-position of the C-2 aryl enhanced antiproliferative activity in that the five membered (pyrrolidin-1-yl)ethyl derivative **14a**, the six-membered (piperidin-1-yl)ethyl derivative **14b**, and the acyclic (dimethylamino)propyl derivative **14c** exhibited GI₅₀ values of < 10.59 μ M in each case. Introduction of an aminoalkyl side chain in the *para*-position of the C-3 aryl resulted in selective antiproliferative activity against the growth of Hep 3B cancer cells. Among them, 4-{6-fluoro-3-[4-(2-(piperidin-1-yl)ethoxy)phenyl]quinolin-2-yl}phenol (**15b**) was more active than its (dimethylamino)propyl counterpart **15c**,



Scheme 3 Reagents and conditions: (i) 48% HBr, HOAc, reflux, 48 h; (ii) NaH, alkyl halides, DMF, 80 °C, 40 min.

which in turn is more active than the (pyrrolidin-1-yl)ethoxy counterpart **15a** against the growth of Hep 3B, with GI_{50} values of 0.74, 1.40, and 3.07 μM respectively. Further introduction of the second piperidin-1-yl ethyl side chain enhanced antiproliferative activity against certain specific cancer cells, in which 6-fluoro-2,3-bis{4-[2-(piperidin-1-yl)ethoxy]phenyl}quinoline (**16b**) was especially active against the growth of Hep 3B, H1299, and MDA-MB-231 with GI_{50} values of 0.71, 1.46 and 0.72 μM respectively. Compound **16b** and its (dimethylamino)propyl counterpart **16c** exhibited comparable inhibitory activities towards Hep 3B, H1299, and MDA-MB-231, while the (pyrrolidin-1-yl)ethyl counterpart **16a** was much less active. Therefore, a side chain of six-membered (piperidin-1-yl)ethyl or acyclic (dimethylamino)propyl is more favorable than that of five-membered (pyrrolidin-1-yl)ethyl for the 2,3-diphenylquinoline pharmacophore. Both compounds **16b** and **16c** were found to be more active than the positive tamoxifen against the growth of Hep 3B, H1299 and MDA-MB-231.

Regarding the C-6 methoxy-substituted 2,3-diarylquinoline derivatives, 6-methoxy-2,3-diphenylquinoline (**11**) was inactive. Introduction of the methoxy substituents in the *para*-position of both the C-2 and C-3 aryl moieties did not improve antiproliferative activity, in that compound **12** was inactive against all the cancer cell lines tested. However, their C-6 hydroxyl counterparts, **17** and **18**, were weakly active against the growth of Hep 3B with GI_{50} values of 9.48 and 12.03 μM respectively. For the C-6 aminoalkoxy-substituted 2,3-diarylquinoline derivatives, a side chain of six-membered (piperidin-1-yl)ethyl **19b** or acyclic (dimethylamino)propyl **19c** is more favorable than that of five-membered (pyrrolidin-1-yl)ethyl **19a** against the growth of Hep G2. Further introduction of aminoalkyl side chains in the *para*-position of both the C-2 and C-3 aryl moieties enhanced antiproliferative activity against the growth of Hep 3B, in that the five membered (pyrrolidin-1-yl)ethyl derivative **20a**, the six-membered (piperidin-1-yl)ethyl derivative **20b**, and the acyclic (dimethylamino)propyl derivative **20c** exhibited GI_{50} values of 2.18, 1.01, and 1.37 μM respectively.

Among these C-6 substituted 2,3-diarylquinoline derivatives, compounds **15b**, **15c**, **16b**, **16c**, **20b**, and **20c** were able to

inhibit the growth of Hep 3B with a GI_{50} value of less than 1.40 μM in each case. Among them, compound **16b** exhibited a GI_{50} value of 0.72 μM against the growth of MDA-MB-231, which was 90-fold more active than the positive tamoxifen. Therefore, compound **16b** was selected for further evaluation of its effect on the MDA-MB-231 cell cycle distribution by flow cytometric analysis. Fig. 3 and Table 2 showed the cell cycle arrest induced by **16b** is in a concentration- and time-dependent manner. The proportion of cells slightly decreased in the G1 and accumulated in G2/M phase after 12 or 24 h treatment, while the hypodiploid (sub-G0/G1 phase) cells increased. The images obtained by immunofluorescence microscopy enabled indirect evaluation of the effect of **16b** on the microtubule network. The microtubule network in control cells displayed intact organization and arrangement. However, when cells were exposed to various concentrations of **16b** for 24 h, it exhibited filament-like structure and reduced microtubule extent in the cytoplasm (Fig. 4). The microtubule network shrank significantly at 1.0 μM and was disrupted thoroughly at 10.0 μM . The morphological changes of apoptosis include membrane blebbing, cell shrinkage, chromatin condensation, and formation of apoptotic bodies.²⁴ Fig. 5 also demonstrates the incubation of the MDA-MB-231 cells with different concentrations of **16b** for 24 h results in significant morphological changes. Cleavage of DNA at the internucleosomal linker sites yielding DNA fragments in multiple fragments (180–200 bp) was regarded as a biochemical hallmark of apoptosis.²⁵ The appearance of such fragments resulted in a ladder formation when fragmented DNA from **16b**-treated cells was separated by agarose gel electrophoresis (Fig. 6). The cells in sub-G0/G1 phase indicate the cells are undergoing DNA fragmentation and cell death. To explore the effect of compound **16b** on apoptosis-related proteins, Bcl-2 family proteins (Bcl-2, Bax, and Bad) and PARP were evaluated in **16b**-treated MDA-MB-231 cells by Western blotting. Bcl-2 is the first identified member of a large family of apoptosis-regulating proteins, consisting of blockers (such as Bcl-2) and promoters (such as Bax and Bad) of cell death.^{26,27} 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.²⁸ Our

Table 1 Antiproliferative activity of 2,3-diarylquinoline derivatives [GI₅₀, μM]^a

Cpd	R ₁	R ₂	R ₃	Cell lines					
				Hep G2	Hep 3B	A549	H1299	MCF-7	MDA-MB-231
9	F	H	H	> 50	> 50	> 50	> 50	> 50	> 50
10	F	OMe	OMe	> 50	> 50	> 50	> 50	> 50	> 50
11	OMe	H	H	> 50	> 50	> 50	> 50	> 50	> 50
12	OMe	OMe	OMe	> 50	> 50	> 50	> 50	> 50	> 50
13	F	OH	OH	> 50	> 50	> 50	> 50	> 50	> 50
14a	F		OH	6.62 ± 0.05	6.27 ± 0.31	6.59 ± 0.12	10.59 ± 0.24	7.79 ± 0.06	7.36 ± 0.02
14b	F		OH	6.39 ± 0.06	6.43 ± 0.10	10.28 ± 3.87	10.06 ± 0.73	10.27 ± 2.45	6.33 ± 0.06
14c	F		OH	6.49 ± 0.03	5.95 ± 0.02	6.53 ± 0.01	7.46 ± 0.15	6.46 ± 0.09	7.23 ± 0.01
15a	F	OH		6.50 ± 0.08	3.07 ± 0.07	6.37 ± 0.09	7.92 ± 0.22	6.35 ± 0.02	6.92 ± 0.07
15b	F	OH		6.43 ± 0.05	0.74 ± 0.06	6.42 ± 0.02	7.22 ± 0.16	6.72 ± 0.16	6.13 ± 0.03
15c	F	OH		6.43 ± 0.01	1.40 ± 0.05	6.56 ± 0.05	6.71 ± 0.09	6.47 ± 0.05	6.18 ± 0.03
16a	F			6.50 ± 0.06	6.45 ± 0.08	6.58 ± 0.05	6.58 ± 0.04	6.50 ± 0.07	6.21 ± 0.01
16b	F			6.27 ± 0.02	0.71 ± 0.03	6.72 ± 0.81	1.46 ± 0.01	6.26 ± 0.01	0.72 ± 0.09
16c	F			6.20 ± 0.10	0.63 ± 0.03	6.69 ± 0.08	1.53 ± 0.10	6.61 ± 0.08	0.97 ± 0.06
17	OH	H	H	> 50	9.48 ± 2.16	> 50	> 50	> 50	10.42 ± 0.74
18	OH	OH	OH	> 50	12.03 ± 0.95	> 50	> 50	> 50	> 50
19a		H	H	> 50	6.47 ± 0.13	> 50	9.06 ± 0.08	8.31 ± 0.59	9.21 ± 0.27
19b		H	H	12.12 ± 2.68	6.11 ± 0.03	> 50	10.17 ± 0.91	10.06 ± 1.58	7.64 ± 0.27
19c		H	H	7.25 ± 0.12	6.21 ± 0.07	8.74 ± 2.16	8.46 ± 2.47	8.35 ± 0.51	7.65 ± 0.28
20a				6.79 ± 0.58	2.18 ± 0.10	6.55 ± 0.07	6.47 ± 0.07	6.36 ± 0.01	6.67 ± 0.05
20b				6.27 ± 0.05	1.01 ± 0.32	6.40 ± 0.01	5.75 ± 0.03	6.52 ± 0.02	5.54 ± 0.75
20c				6.38 ± 0.04	1.37 ± 0.04	6.55 ± 0.03	5.90 ± 0.02	6.54 ± 0.04	6.19 ± 0.02
Tamoxifen				51.40 ± 3.51	7.12 ± 0.03	57.98 ± 0.41	74.58 ± 1.48	15.27 ± 3.21	64.42 ± 3.52

^a The concentration that inhibited the growth of 50% of cells (GI₅₀) 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%. Values are the average of three separate determinations.

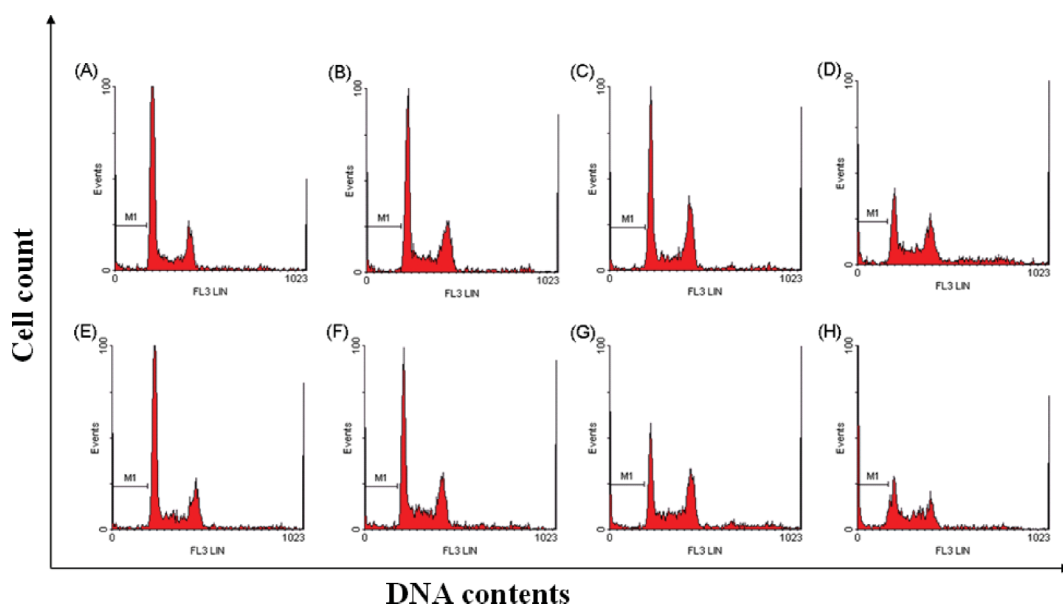


Fig. 3 Flow cytometric analysis of MDA-MB-231 cells. Cells were treated with DMSO (A), 1.0 μM (B), 5.0 μM (C) or 10.0 μM (D) for 12 h; DMSO (E), 1.0 μM (F), or 5.0 μM (G), or 10.0 μM (H) for 24 h of **16b**; cells were harvested, fixed, and stained with propidium iodide as described in the Experimental section. The percentage of cells in each cell cycle phase was quantified (Table 2).

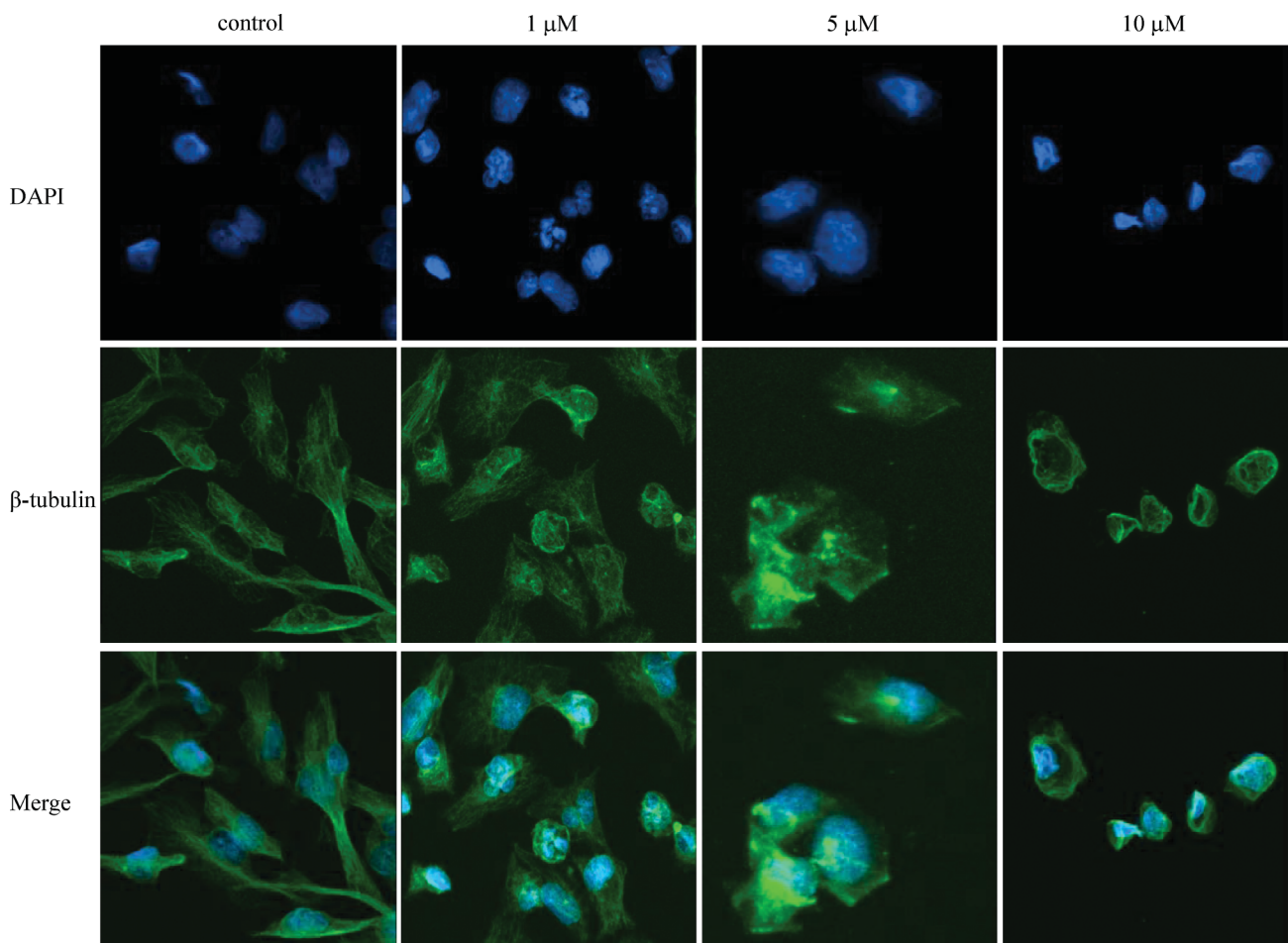
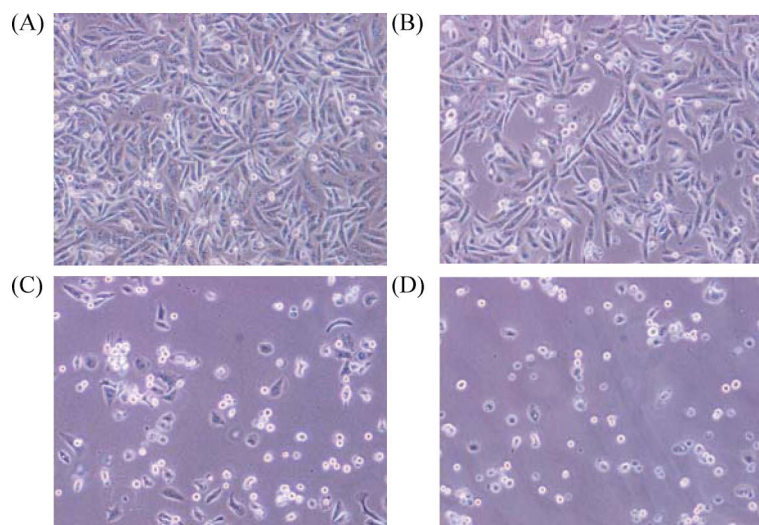
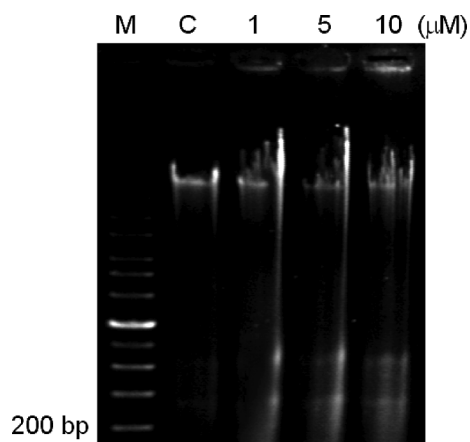


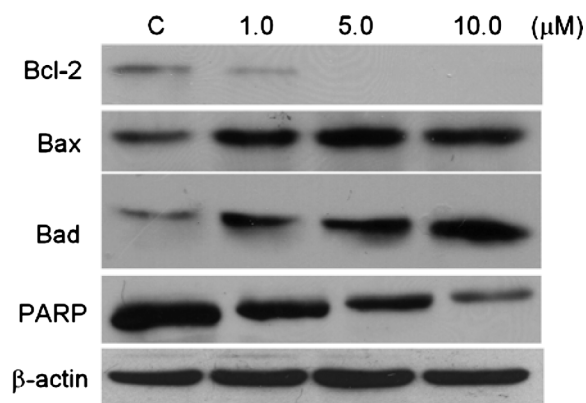
Fig. 4 Microtubule effect of compound **16b**. The confocal laser scanning micrograph shows a merged image double-labeled with DAPI and β-tubulin antibodies. MDA-MB-231 cells were fixed after the treatment of **16b** for 24 h followed by immunofluorescence analysis using anti-β-tubulin antibody, FITC-conjugated secondary antibody, and DAPI staining as described in the Experimental section.

Table 2 Effects of **16b** on MDA-MB-231 cell cycle progression

Time (h)	Concentration (μM)	Cell cycle distribution (%)			
		Sub G1	G1	S	G2/M
12	DMSO	1.9	72.6	18.1	7.4
12	1.0	2.3	69.8	18.4	9.5
12	5.0	3.2	58.2	13.5	25.1
12	10.0	8.1	46.2	16.1	29.6
24	DMSO	2.7	70.4	17.6	9.3
24	1.0	6.2	59.9	18.6	15.3
24	5.0	15.3	45.9	17.1	21.7
24	10.0	20.9	38.4	14.1	26.6

**Fig. 5** Induction of morphological change in MDA-MB-231 cells. Cells were treated with DMSO (A), compound **16b** at 1 μM (B), 5 μM (C) or 10 μM (D) for 24 h; at 37 $^{\circ}\text{C}$ and photographed under a microscope (100 \times).**Fig. 6** Agarose gel electrophoresis for detecting DNA fragmentation in MDA-MB-231 cells treated with compound **16b** for 24 h.

results indicated that **16b** increased the protein expression of Bad and Bax, but decreased expression of Bcl-2 and PARP (Fig. 7). Thus, compound **16b** induces cell cycle arrest at the G2/M phase, followed by DNA fragmentation *via* an increase in the protein expression of Bad and Bax but a decrease in expression of Bcl-2 and PARP, which consequently cause cell death.

**Fig. 7** Effects of **16b** on the expression of Bcl-2, Bax, Bad, and PARP in MDA-MB-231 cells. Exponentially growing MDA-MB-231 cells were treated with the indicated concentrations of **16b** for 24 h. Cell lysates were prepared and protein levels of Bcl-2, Bax, Bad, and PARP were determined by Western blotting analysis. β -Actin was used to confirm equal protein loading.

Conclusions

A number of 2,3-diarylquinoline derivatives were synthesized and evaluated for antiproliferative activities against the growth of Hep G2, Hep 3B, A549, H1299, MCF-7, MDA-MB-231 cancer cell lines. Among these C-6 substituted 2,3-diarylquinoline derivatives,

compounds **15b**, **15c**, **16b**, **16c**, **20b**, and **20c** were able to inhibit the growth of Hep 3B with a GI_{50} value of less than 1.40 μM in each case. Therefore, a side chain of six-membered (piperidin-1-yl)ethyl or acyclic (dimethylamino)propyl is more favorable than that of five-membered (pyrrolidin-1-yl)ethyl for the 2,3-diphenylquinoline pharmacophore. Compound **16b** was one of the most active 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. Further investigations have shown that **16b** induced cell cycle arrest at the G2/M phase followed by DNA fragmentation *via* an increase in the protein expression of Bad and Bax but a decrease in expression of Bcl-2 and PARP, which consequently causes cell death. Compound **16b** was selected as a new lead for potential anticancer drug candidates. Its structural optimization is ongoing.

Experimental section

General

Melting points were determined on an Electrothermal IA9100 melting point apparatus and are uncorrected. The ultraviolet-visible (UV-VIS) absorption spectra were recorded on a Jasco V570 spectrometer. 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 $\pm 0.4\%$ of the theoretical compositions.

6-Fluoro-2,3-diphenylquinoline-4-carboxylic acid (5). A mixture of 5-fluoroisatin (**3a**, 3.30 g, 20 mmol), deoxybenzoin (**4a**, 4.71 g, 24 mmol), and KOH (3.37 g, 60 mmol) in EtOH was heated at 80 $^\circ\text{C}$ 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 ice-cold aqueous phase was acidified to pH 1 with 37% HCl, and the precipitate was collected, washed with H_2O , and crystallized from EtOH to give **5** (5.49 g, 80%). M.p. 317–318 $^\circ\text{C}$. R_f 0.51 (MeOH– CH_2Cl_2 1 : 5). UV (MeOH): λ_{max} nm (log ϵ) 231 (4.67), 328 (3.71). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 7.21–7.33 (m, 10H, Ar–H), 7.54 (dd, 1H, $J = 9.6, 3.2$ Hz, 5-H), 7.82 (ddd, 1H, $J = 9.6, 8.8, 3.2$ Hz, 7-H), 8.25 (dd, 1H, $J = 9.2, 5.2$ Hz, 8-H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 108.12 ($J = 22.7$ Hz), 120.63 ($J = 25.0$ Hz), 122.78 ($J = 10.6$ Hz), 127.60 (2C), 127.83, 128.02 (2C), 129.70 (2C), 130.03 (2C), 130.45, 132.54 ($J = 9.8$ Hz), 136.79, 139.67 (2C), 140.99 ($J = 5.3$ Hz), 143.74, 157.97, 160.53 ($J = 245.6$ Hz), 167.74. Anal. calcd for $\text{C}_{22}\text{H}_{14}\text{FNO}_2$: C 76.96, H 4.11, N 4.08; found: C 76.64, H 4.10, N 4.37.

6-Methoxy-2,3-diphenylquinoline-4-carboxylic acid (7). From 5-methoxyisatin (**3b**) and deoxybenzoin (**4a**) as described for the preparation of **5** in 85% yield. M.p. 321–322 $^\circ\text{C}$ (EtOH). R_f 0.54 (MeOH– CH_2Cl_2 1 : 5). UV (MeOH): λ_{max} nm (log ϵ) 230 (4.63), 218 (4.61). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 3.91 (s, 3H, OCH_3), 7.10 (d, 1H, $J = 2.8$ Hz, 5-H), 7.19–7.32 (m, 10H, Ar–H), 7.53 (dd, 1H, $J = 9.2, 2.8$ Hz, 7-H), 8.08 (d, 1H, $J = 9.6$ Hz, 8-H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 55.57, 102.41, 122.76, 123.05, 127.51 (2C), 127.60, 127.65, 127.93 (2C), 129.66 (2C), 129.76, 130.08 (2C), 131.07, 137.19, 139.97, 140.26, 142.65, 155.63, 158.23, 168.19. Anal. calcd for $\text{C}_{23}\text{H}_{17}\text{NO}_3$: C 77.73, H 4.82, N 3.94; found: C 77.55, H 4.78, N 3.98.

6-Fluoro-2,3-diphenylquinoline (9). A mixture of **5** (3.43 g, 10 mmol) in diphenyl ether (15 mL) was heated at 250 $^\circ\text{C}$ for 4 h (TLC monitoring). The mixture was cooled and then 50 mL hexane was added. The precipitate thus formed was collected, washed with ether, and crystallized from EtOH to give **9** (2.04 g, 68%). M.p. 154–155 $^\circ\text{C}$. R_f 0.52 (MeOH– CH_2Cl_2 1 : 200). UV (MeOH): λ_{max} nm (log ϵ) 232 (4.67), 256 (4.54), 330 (3.85). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 7.26–7.39 (m, 10H, Ar–H), 7.72 (ddd, 1H, $J = 8.8, 8.8, 2.8$ Hz, 7-H), 7.87 (dd, 1H, $J = 9.2, 2.8$ Hz, 5-H), 8.15 (dd, $J = 9.2, 5.6$ Hz, 8-H), 8.41 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 110.86 ($J = 22.0$ Hz), 120.03 ($J = 25.8$ Hz), 127.45, 127.55 ($J = 10.6$ Hz), 127.77 (2C), 128.05, 128.32 (2C), 129.51 (2C), 129.74 (2C), 131.61 ($J = 9.1$ Hz), 134.69, 137.21 ($J = 5.3$ Hz), 139.25, 139.93, 143.82, 157.16 ($J = 2.2$ Hz), 159.94 ($J = 244.1$ Hz). Anal. calcd for $\text{C}_{21}\text{H}_{14}\text{FN}$ ·0.1 H_2O : C 83.76, H 4.75, N 4.65; found: C 83.75, H 4.69, N 4.91.

6-Fluoro-2,3-bis(4-methoxyphenyl)quinoline (10). From **6** as described for the preparation of **9**: 67% yield. M.p. 189–190 $^\circ\text{C}$ (EtOH). R_f 0.62 (MeOH– CH_2Cl_2 1 : 100). UV (MeOH): λ_{max} nm (log ϵ) 222 (4.71), 231 (4.70), 272 (4.47), 342 (4.01), 340 (4.01). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 3.74 and 3.75 (two s, 6H, $\text{OCH}_3 \times 2$), 6.84–6.91 (m, 4H, Ar–H), 7.16–7.18 (m, 2H, Ar–H), 7.31–7.33 (m, 2H, Ar–H), 7.64 (ddd, 1H, $J = 9.2, 9.2, 3.2$ Hz, 7-H), 7.78 (dd, 1H, $J = 9.2, 2.8$ Hz, 5-H), 8.08 (dd, 1H, $J = 9.2, 5.6$ Hz, 8-H), 8.27 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 55.11 (2C), 110.69 ($J = 21.2$ Hz), 113.24 (2C), 113.89 (2C), 119.63 ($J = 25.7$ Hz), 127.42 ($J = 10.6$ Hz), 130.62 (2C), 131.14 (2C), 131.42 ($J = 9.1$ Hz), 131.67, 132.38, 134.24, 136.87 ($J = 4.5$ Hz), 143.70, 156.81 ($J = 2.2$ Hz), 158.62, 159.14, 159.75 ($J = 243.3$ Hz). Anal. calcd for $\text{C}_{23}\text{H}_{18}\text{FNO}_2$: C 76.86, H 5.05, N 3.90; found: C 76.57, H 5.05, N 4.20.

6-Methoxy-2,3-diphenylquinoline (11). From **7** as described for the preparation of **9**: 55% yield. M.p. 170–172 $^\circ\text{C}$ (EtOH). R_f 0.43 (MeOH– CH_2Cl_2 1 : 100). UV (MeOH): λ_{max} nm (log ϵ) 260 (4.67), 230 (4.65), 221 (4.63), 340 (3.90), 336 (3.90), 343 (3.90). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 3.91 (s, 3H, OCH_3), 7.22–7.35 (m, 10H, Ar–H), 7.41–7.45 (m, 2H, 7- and 8-H), 7.97 (d, 1H, $J = 8.4$ Hz, 5-H), 8.25 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 55.72, 105.64, 122.62, 127.38, 127.84 (2C), 127.92, 128.10, 128.42 (2C), 129.64 (2C), 129.86 (2C), 130.32, 134.27, 136.62, 139.83, 140.38, 142.82, 155.15, 157.73. Anal. calcd for $\text{C}_{22}\text{H}_{17}\text{NO}$ ·0.2 H_2O : C 83.89, H 5.57, N 4.45; found: C 83.90, H 5.51, N 4.73.

6-Methoxy-2,3-bis(4-methoxyphenyl)quinoline (12). From **8** as described for the preparation of **9**: 62% yield. M.p. 115–116 $^\circ\text{C}$ (EtOH). R_f 0.41 (MeOH– CH_2Cl_2 1 : 100). UV (MeOH): λ_{max} nm (log ϵ) 226 (4.70), 246 (4.67), 271 (4.56), 349 (4.07). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 3.75, 3.77 and 3.91 (three s, 9H, $\text{OCH}_3 \times 3$), 6.83–9.87 (m, 2H, Ar–H), 6.90–6.93 (m, 2H, Ar–H), 7.17–7.20 (m, 2H, Ar–H), 7.30–7.33 (m, 2H, Ar–H), 7.38–7.41 (m, 2H, 5- and 8-H), 7.96 (dd, 1H, $J = 9.6, 0.8$ Hz, 7-H), 8.17 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 55.06, 55.07, 55.52, 105.40, 113.14 (2C), 113.82 (2C), 122.05, 127.81, 130.06, 130.58

(2C), 131.04 (2C), 132.15, 132.75, 133.65, 136.17, 142.58, 154.67, 157.34, 158.45, 158.85. Anal. calcd for $C_{24}H_{21}NO_3$: C 77.61, H 5.70, N 3.77; found: C 77.37, H 5.75, N 4.08.

6-Fluoro-2,3-bis(4-hydroxyphenyl)quinoline (13). A solution of **10** (0.36 g, 1.0 mmol) in 48% HBr (5 mL) was heated at reflux for 48 h. The mixture was cooled and evaporated *in vacuo* to give a residue which was treated with H_2O (50 mL). The crude product was collected and crystallized from MeOH to give **13** (0.31 g, 94%). M.p. 269–270 °C (MeOH). R_f 0.52 (MeOH– CH_2Cl_2 1 : 35). UV (MeOH): λ_{max} nm (log ϵ) 221 (4.70), 273 (4.43), 345 (4.01). 1H NMR (400 MHz, $DMSO-d_6$): 6.66–6.75 (m, 4H, Ar–H), 7.04–7.08 (m, 2H, Ar–H), 7.21–7.24 (m, 2H, Ar–H), 7.63 (ddd, 1H, $J = 8.2, 8.8, 2.8$ Hz, 7-H), 7.78 (dd, 1H, $J = 9.2, 2.4$ Hz, 5-H), 8.07 (dd, 1H, $J = 9.2, 5.6$ Hz, 8-H), 8.24 (s, 1H, 4-H), 9.57 and 9.61 (two s, 2H, OH). ^{13}C NMR (100 MHz, $DMSO-d_6$): 110.60 ($J = 22.0$ Hz), 114.57 (2C), 115.24 (2C), 119.35 ($J = 25.7$ Hz), 127.37 ($J = 10.6$ Hz), 130.16, 130.57 (2C), 130.88, 131.16 (2C), 131.30 ($J = 9.1$ Hz), 134.62, 136.48 ($J = 5.3$ Hz), 143.60, 156.78, 157.22 ($J = 2.3$ Hz), 157.39, 159.64 ($J = 242.5$ Hz). Anal. calcd for $C_{21}H_{14}FNO_2 \cdot 1.0 H_2O$: C 72.20, H 4.62, N 4.01; found: C 72.10, H 4.90, N 4.25.

4-{6-Fluoro-2-[4-(2-(pyrrolidin-1-yl)ethoxy)phenyl]quino-lin-3-yl}-phenol (14a), 4-{6-Fluoro-3-[4-(2-(pyrrolidin-1-yl)ethoxy)phenyl]quinolin-2-yl}phenol (15a) and 6-Fluoro-2,3-bis{4-[2-(pyrrolidin-1-yl)ethoxy]-phenyl}quinoline (16a). To a stirred solution of **13** (0.33 g, 1.0 mmol) in dry DMF (20 mL) was added NaH (60% in oil, 0.50 g) at 0 °C for 1 h. *N*-(2-Chloroethyl)pyrrolidine·HCl (0.51 g, 3 mmol) was added and heated at 80 °C for 25 min. The reaction mixture was partitioned between H_2O (50 mL) and CH_2Cl_2 (50 mL). The organic layer was dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography (MeOH– CH_2Cl_2 1/10) to give three fractions which were evaporated to dryness and the residue was recrystallized from EtOH.

Compound **14a** (0.12 g, 27% yield) was obtained as a white solid. M.p. 113–114 °C (EtOH). R_f 0.44 (MeOH– CH_2Cl_2 1 : 10). UV (MeOH): λ_{max} nm (log ϵ) 220 (4.76), 272 (4.47), 340 (4.01), 343 (3.98). 1H NMR (400 MHz, $DMSO-d_6$): 1.74 (m, 4H, pyrrolidinyl-H), 2.68 (m, 4H, pyrrolidinyl-H), 2.94 (m, 2H, CH_2N), 4.11 (t, 2H, $J = 5.6$ Hz, OCH_2), 6.72–6.76 (m, 2H, Ar–H), 6.87–6.90 (m, 2H, Ar–H), 7.05–7.09 (m, 2H, Ar–H), 7.33–7.35 (m, 2H, Ar–H), 7.65 (ddd, 1H, $J = 8.8, 8.8, 2.8$ Hz, 7-H), 7.80 (dd, 1H, $J = 9.6, 2.8$ Hz, 5-H), 8.08 (dd, 1H, $J = 9.2, 5.6$ Hz, 8-H), 8.27 (s, 1H, 4-H), 9.60 (br s, 1H, OH). ^{13}C NMR (100 MHz, $DMSO-d_6$): 23.02 (2C), 54.00 (2C), 54.08, 66.01, 110.64 ($J = 21.9$ Hz), 113.72 (2C), 115.30 (2C), 119.48 ($J = 25.7$ Hz), 127.48 ($J = 10.6$ Hz), 129.99, 130.60 (2C), 131.14 (2C), 131.38 ($J = 9.9$ Hz), 132.62, 134.62, 136.60 ($J = 4.2$ Hz), 143.60, 156.84, 158.18, 159.73 ($J = 243.3$ Hz). Anal. calcd for $C_{27}H_{25}FN_2O_2 \cdot 1.7 H_2O$: C 70.63, H, 6.23, N 6.10; found: C 70.38, H 5.93, N 6.08.

Compound **15a** (0.15 g, 33%) was obtained as a white solid. M.p. 122–123 °C (EtOH). R_f 0.52 (MeOH– CH_2Cl_2 1 : 10). UV (MeOH): λ_{max} nm (log ϵ) 221 (4.77), 272 (4.46), 343 (4.02). 1H NMR (400 MHz, $DMSO-d_6$): 1.70–1.72 (m, 4H, pyrrolidinyl-H), 2.58 (br s, 4H, pyrrolidinyl-H), 2.85 (t, 2H, $J = 5.6$ Hz, CH_2N), 4.09 (t, 2H, $J = 5.6$ Hz, OCH_2), 6.66–6.70 (m, 2H, Ar–H), 6.92–6.95 (m, 2H, Ar–H), 7.16–7.23 (m, 4H, Ar–H), 7.65 (ddd, 1H, $J = 9.2, 9.2, 3.2$ Hz, 7-H), 7.79 (dd, 1H, $J = 9.2, 2.8$ Hz, 5-H), 8.08 (dd, 1H, $J = 9.2, 5.2$ Hz, 8-H), 8.27 (s, 1H, 4-H), 9.64 (br s, 1H, OH).

^{13}C NMR (100 MHz, $DMSO-d_6$): 23.10 (2C), 54.02 (2C), 54.23, 66.45, 110.66 ($J = 21.2$ Hz), 114.36 (2C), 114.63 (2C), 119.53 ($J = 25.8$ Hz), 127.33 ($J = 10.6$ Hz), 130.60 (2C), 130.77, 131.18 (2C), 131.33 ($J = 9.0$ Hz), 131.86, 134.22, 136.75 ($J = 5.3$ Hz), 143.70, 157.16 ($J = 2.2$ Hz), 157.44, 157.77, 159.66 ($J = 242.5$ Hz). Anal. calcd for $C_{27}H_{25}FN_2O_2 \cdot 0.5H_2O$: C 74.12, H 5.99, N 6.40; found: C 74.10, H 5.99, N, 6.52.

Compound **16a** (0.21 g, 39%) was obtained as a white oil. R_f 0.52 (MeOH– CH_2Cl_2 1 : 3). UV (MeOH): λ_{max} nm (log ϵ) 219 (4.80), 221 (4.80), 271 (4.52), 340 (4.00), 342 (4.00). 1H NMR (400 MHz, $DMSO-d_6$): 1.67–1.73 (m, 8H, pyrrolidinyl-H), 2.58 (br s, 8H, pyrrolidinyl-H), 2.82–2.86 (m, 4H, CH_2N), 4.06–4.10 (m, 4H, OCH_2), 6.86–6.94 (m, 4H, Ar–H), 7.15–7.19 (m, 2H, Ar–H), 7.31–7.34 (m, 2H, Ar–H), 7.64 (ddd, 1H, $J = 8.8, 8.8, 2.8$ Hz, 7-H), 7.79 (dd, 1H, $J = 9.6, 2.8$ Hz, 5-H), 8.09 (dd, 1H, $J = 8.8, 5.2$ Hz, 8-H), 8.28 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $DMSO-d_6$): 23.09 (4C), 53.97 (4C), 54.13, 54.14, 66.37 (2C), 110.67 ($J = 21.9$ Hz), 113.72 (2C), 114.39 (2C), 119.58 ($J = 25.8$ Hz), 127.42 ($J = 10.6$ Hz), 130.61 (2C), 131.15 (2C), 131.39 ($J = 25.0$ Hz), 131.69, 132.40, 134.19, 136.84 ($J = 4.5$ Hz), 143.71, 156.75, 157.80, 158.31, 159.74 ($J = 243.2$ Hz). Anal. calcd for $C_{33}H_{36}FN_2O_2 \cdot 0.5H_2O$: C 74.13, H 6.98, N 7.86; found: C 73.98, H 7.24, N 7.65.

4-{6-Fluoro-2-[4-(2-(piperidin-1-yl)ethoxy)phenyl]quino-lin-3-yl}-phenol (14b), 4-{6-Fluoro-3-[4-(2-(piperidin-1-yl)ethoxy)phenyl]quinolin-2-yl}phenol (15b) and 6-Fluoro-2,3-bis{4-[2-(piperidin-1-yl)ethoxy]phenyl}quinoline (16b). From **13** and *N*-(2-chloroethyl)piperidine·HCl as described for the preparation of **14b**, **15b**, and **16b**.

Compound **14b** (0.11 g, 25%) was obtained as a white solid. M.p. 113–115 °C (EtOH). R_f 0.50 (MeOH– CH_2Cl_2 1 : 10). UV (MeOH): λ_{max} nm (log ϵ) 222 (4.78), 272 (4.50), 340 (4.02), 343 (4.01). 1H NMR (400 MHz, $DMSO-d_6$): 1.36–1.40 (m, 2H, piperidinyl-H), 1.47–1.53 (m, 4H, piperidinyl-H), 2.45 (br s, 4H, piperidinyl-H), 2.67 (t, 2H, $J = 5.6$ Hz, CH_2N), 4.06 (t, 2H, $J = 5.6$ Hz, OCH_2), 6.71–6.75 (m, 2H, Ar–H), 6.85–6.87 (m, 2H, Ar–H), 7.05–7.07 (m, 2H, Ar–H), 7.31–7.33 (m, 2H, Ar–H), 7.64 (ddd, 1H, $J = 8.8, 8.8, 2.8$ Hz, 7-H), 7.79 (dd, 1H, $J = 9.6, 2.8$ Hz, 5-H), 8.08 (dd, 1H, $J = 9.6, 5.6$ Hz, 8-H), 8.26 (s, 1H, 4-H), 9.59 (br s, 1H, OH). ^{13}C NMR (100 MHz, $DMSO-d_6$): 23.79, 25.40 (2C), 54.34 (2C), 57.25, 65.42, 110.61 ($J = 22.0$ Hz), 113.73 (2C), 115.29 (2C), 119.44 ($J = 25.7$ Hz), 127.45 ($J = 10.6$ Hz), 129.99, 130.58 (2C), 131.10 (2C), 131.37 ($J = 9.1$ Hz), 132.49, 134.61, 136.62 ($J = 5.0$ Hz), 143.60, 156.84 (2C), 158.34, 159.72 ($J = 243.3$ Hz). Anal. calcd for $C_{28}H_{27}FN_2O_2 \cdot 0.8 H_2O$: C 73.60, H 6.31, N 6.13; found: C 73.58, H 6.61, N, 6.00.

Compound **15b** (0.13 g, 30%) was obtained as a white solid. M.p. 124–125 °C (EtOH). R_f 0.62 (MeOH– CH_2Cl_2 1 : 10). UV (MeOH): λ_{max} nm (log ϵ) 221 (4.79), 272 (4.48), 340 (4.02), 342 (4.02). 1H NMR (400 MHz, $DMSO-d_6$): 1.36–1.41 (m, 2H, piperidinyl-H), 1.48–1.53 (m, 4H, piperidinyl-H), 2.45 (br s, 4H, piperidinyl-H), 2.67 (t, 2H, $J = 5.6$ Hz, CH_2N), 4.07 (t, 2H, $J = 5.6$ Hz, OCH_2), 6.66–6.70 (m, 2H, Ar–H), 6.90–6.94 (m, 2H, Ar–H), 7.15–7.24 (m, 4H, Ar–H), 7.65 (ddd, 1H, $J = 8.8, 8.8, 2.8$ Hz, 7-H), 7.79 (dd, 1H, $J = 9.2, 2.8$ Hz, 5-H), 8.08 (dd, 1H, $J = 9.2, 5.6$ Hz, 8-H), 8.27 (s, 1H, 4-H), 9.63 (br s, 1H, OH). ^{13}C NMR (100 MHz, $DMSO-d_6$): 23.83, 25.46 (2C), 54.37 (2C), 57.30, 65.49, 110.63 ($J = 22.0$ Hz), 114.38 (2C), 114.61 (2C), 119.48 ($J = 25.8$ Hz), 127.30 ($J = 10.6$ Hz), 130.55 (2C), 130.76, 131.14 (2C), 131.30 ($J = 9.1$ Hz), 131.80,

134.21, 136.71 ($J = 5.3$ Hz), 143.68, 157.15, 157.41, 157.81, 159.64 ($J = 243.3$ Hz). Anal. calcd for $C_{28}H_{27}FN_2O_2 \cdot 1.0H_2O$: C, 73.01; H, 6.36; N, 6.08; found: C, 73.07; H, 6.29; N, 6.15.

Compound **16b** (0.21 g, 38%) was obtained as a white oil. R_f 0.53 (MeOH– CH_2Cl_2 1 : 4). UV (MeOH): λ_{max} nm (log ϵ) 220 (4.82), 271 (4.50), 340 (3.99). 1H NMR (400 MHz, DMSO- d_6): 1.36–1.40 (m, 4H, piperidiny-H), 1.46–1.52 (m, 8H, piperidiny-H), 2.45 (br s, 8H, piperidiny-H), 2.67 (br s, 4H, CH_2N), 4.05–4.09 (m, 4H, OCH_2), 6.85–6.94 (m, 4H, Ar–H), 7.16–7.19 (m, 2H, Ar–H), 7.31–7.34 (m, 2H, Ar–H), 7.66 (ddd, 1H, $J = 8.8, 8.8, 2.8$ Hz, 7-H), 7.80 (dd, 1H, $J = 9.2, 2.8$ Hz, 5-H), 8.09 (dd, 1H, $J = 9.2, 5.6$ Hz, 8-H), 8.29 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO- d_6): 23.79 (2C), 25.42 (4C), 54.34 (4C), 57.26 (2C), 65.46 (2C), 110.64 ($J = 21.2$ Hz), 113.76 (2C), 114.43 (2C), 119.57 ($J = 25.8$ Hz), 127.39 ($J = 10.6$ Hz), 130.57 (2C), 131.09 (2C), 131.38 ($J = 9.9$ Hz), 131.64, 132.34, 134.20, 136.80 ($J = 5.4$ Hz), 143.68, 156.77, 157.84, 158.35, 1159.72 ($J = 243.3$ Hz). Anal. calcd for $C_{35}H_{40}FN_3O_2 \cdot 0.4 H_2O$: C 74.94, H 7.33, N 7.49; found: C 75.06, H 7.42, N 7.54.

4-{2-[4-(3-(Dimethylamino)propoxy)phenyl]-6-fluoroquinolin-3-yl}phenol (14c), **4-{3-[4-(3-(Dimethylamino)propoxy)phenyl]-6-fluoroquinolin-2-yl}phenol (15c)** and **3,3'-[4,4'-(6-Fluoroquinoline-2,3-diyl)bis(4,1-phenylene)]bis(oxy)-bis(*N,N*-dimethylpropan-1-amine) (16c)**. From **13** and 3-chloro-*N,N*-dimethylpropanamine·HCl as described for the preparation of **14c**, **15c**, and **16c**.

Compound **14c** (0.11 g, 26%) was obtained as a white solid. M.p. 128–130 °C (EtOH). R_f 0.42 (MeOH– CH_2Cl_2 1 : 4). UV (MeOH): λ_{max} nm (log ϵ) 220 (4.74), 272 (4.44), 345 (3.96), 340 (3.96). 1H NMR (400 MHz, DMSO- d_6): 1.87–1.94 (m, 2H, $OCH_2CH_2CH_2$), 2.27 (s, 6H, NMe_2), 2.51–2.54 (m, 2H, CH_2N), 4.01 (t, 2H, $J = 6.4$ Hz, OCH_2), 6.74–6.77 (m, 2H, Ar–H), 6.84–6.88 (m, 2H, Ar–H), 7.05–7.08 (m, 2H, Ar–H), 7.31–7.35 (m, 2H, Ar–H), 7.64 (ddd, 1H, $J = 8.8, 8.8, 2.8$ Hz, 7-H), 7.80 (dd, 1H, $J = 9.2, 2.8$ Hz, 5-H), 8.08 (dd, 1H, $J = 9.2, 5.6$ Hz, 8-H), 8.27 (s, 1H, 4-H), 9.66 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO- d_6): 26.25, 44.54 (2C), 55.30, 65.55, 110.62 ($J = 21.2$ Hz), 113.63 (2C), 115.30 (2C), 119.44 ($J = 25.0$ Hz), 127.45 ($J = 10.6$ Hz), 129.93, 130.55 (2C), 131.11 (2C), 131.36 ($J = 9.9$ Hz), 132.43, 134.62, 136.59 ($J = 5.3$ Hz), 143.59, 156.84 ($J = 2.3$ Hz), 156.89, 158.42, 159.70 ($J = 243.2$ Hz). Anal. calcd for $C_{26}H_{25}FN_2O_2 \cdot 1.6 H_2O$: C 70.13, H 6.38, N 6.29; found: C 69.99, H 6.13, N 6.19.

Compound **15c** (0.13 g, 32%) was obtained as yellow solid. M.p. 132–134 °C (EtOH). R_f 0.52 (MeOH– CH_2Cl_2 1 : 8). UV (MeOH): λ_{max} nm (log ϵ) 220 (4.74), 271 (4.42), 343 (3.96), 340 (3.96). 1H NMR (400 MHz, DMSO- d_6): 2.05–2.12 (m, 2H, $OCH_2CH_2CH_2$), 2.73 (s, 6H, NMe_2), 3.10–3.14 (m, 2H, CH_2N), 4.06 (t, 2H, $J = 6.0$ Hz, OCH_2), 6.66–6.70 (m, 2H, Ar–H), 6.91–6.95 (m, 2H, Ar–H), 7.18–7.24 (m, 4H, Ar–H), 7.65 (ddd, 1H, $J = 8.8, 8.8, 2.8$ Hz, 7-H), 7.81 (dd, 1H, $J = 9.6, 2.8$ Hz, 5-H), 8.08 (dd, 1H, $J = 9.2, 5.2$ Hz, 8-H), 8.27 (s, 1H, 4-H), 9.63 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO- d_6): 24.33, 42.69 (2C), 54.43, 64.89, 110.63 ($J = 22.0$ Hz), 114.33 (2C), 114.60 (2C), 119.53 ($J = 25.8$ Hz), 127.28 ($J = 10.6$ Hz), 130.58 (2C), 130.76, 131.12 (2C), 131.31 ($J = 9.1$ Hz), 132.08, 134.13, 136.69 ($J = 5.3$ Hz), 143.68, 157.15, 157.40, 157.59, 159.64 ($J = 243.3$ Hz). Anal. calcd for $C_{26}H_{25}FN_2O_2 \cdot 0.5 H_2O$: C 73.39, H 6.16, N 6.58; found: C 73.22, H 6.04, N 6.68.

Compound **16c** (0.20 g, 39%) was obtained as white oil. R_f 0.63 (MeOH– CH_2Cl_2 /NH₄OH 1 : 5 : 0.01). UV (MeOH): λ_{max} nm (log

ϵ) 220 (4.79), 272 (4.46), 340 (3.97), 342 (3.97). 1H -NMR (400 MHz, DMSO- d_6): 2.08–2.16 (m, 4H, $OCH_2CH_2CH_2 \times 2$), 2.66 and 2.67 (two s, 12H, $NMe_2 \times 2$), 3.04–3.09 (m, 4H, CH_2N), 4.05–4.09 (m, 4H, OCH_2), 6.87–6.96 (m, 4H, Ar–H), 7.18–7.22 (m, 2H, Ar–H), 7.32–7.36 (m, 2H, Ar–H), 7.67 (ddd, 1H, $J = 9.2, 9.2, 3.2$ Hz, 7-H), 7.82 (dd, 1H, $J = 9.6, 2.8$ Hz, 5-H), 8.10 (dd, 1H, $J = 9.2, 5.6$ Hz, 8-H), 8.31 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO- d_6): 24.31 (2C), 42.48 (4C), 54.17 (2C), 65.04, 65.07, 110.71 ($J = 21.3$ Hz), 113.75 (2C), 114.41 (2C), 119.66 ($J = 25.8$ Hz), 127.42 ($J = 10.6$ Hz), 130.64 (2C), 131.15 (2C), 131.25 ($J = 20.5$ Hz), 131.85, 132.55, 134.17, 136.88 ($J = 5.3$ Hz), 143.69, 156.72 ($J = 2.2$ Hz), 157.70, 158.20, 159.74 ($J = 243.2$ Hz). Anal. calcd for $C_{31}H_{36}FN_3O_2 \cdot 0.3 H_2O$: C 73.43, H 7.28, N 8.29; found: C 73.44, H 7.40, N 8.08.

2,3-Diphenylquinolin-6-ol (17). From **11** as described for the preparation of **13**: 94% yield. M.p. 212–213 °C (EtOH). R_f 0.53 (MeOH– CH_2Cl_2 1 : 20). UV (MeOH): λ_{max} nm (log ϵ) 260 (4.61), 228 (4.61), 346 (3.85), 343 (3.85), 340 (3.85). 1H -NMR (400 MHz, DMSO- d_6): 7.23–7.37 (m, 12H, Ar–H), 7.95 (d, 1H, $J = 8.8$ Hz, 8-H), 8.17 (s, 1H, 4-H), 10.11 (s, 1H, OH). ^{13}C -NMR (100 MHz, DMSO- d_6): 108.12, 122.46, 127.08, 127.53, 127.62 (2C), 128.18 (2C), 128.23, 129.51 (2C), 129.69 (2C), 130.24, 133.89, 135.83, 139.86, 140.43, 141.95, 154.20, 155.87. Anal. calcd for $C_{21}H_{15}NO \cdot 0.2 H_2O$: C 83.81, H 5.16, N 4.65; found: C 83.74, H 5.16, N, 4.91.

2,3-Dihydroxyphenylquinolin-6-ol (18). From **12** as described for the preparation of **13**: 97% yield. M.p. 309–310 °C (EtOH). R_f 0.50 (MeOH– CH_2Cl_2 1 : 10). UV (MeOH): λ_{max} nm (log ϵ) 219 (4.62), 245 (4.51), 265 (4.46), 355 (3.91). 1H NMR (400 MHz, DMSO- d_6): 6.64–6.72 (m, 4H, Ar–H), 7.03–7.05 (m, 2H, Ar–H), 7.16–7.19 (m, 3H, 5-H and Ar–H), 7.28 (dd, 1H, $J = 9.2$ and 2.8 Hz, 7-H), 7.85 (d, 1H, $J = 9.2$ Hz, 8-H), 8.01 (s, 1H, 4-H), 9.51 and 9.99 (two br s, 3H, OH). ^{13}C NMR (100 MHz, DMSO- d_6): 108.00, 114.45 (2C), 115.11 (2C), 121.85, 128.07, 130.01, 130.56 (2C), 130.77, 131.01 (2C), 131.45, 133.82, 135.21, 141.74, 154.39, 154.42, 156.49, 156.93. Anal. calcd for $C_{21}H_{15}NO_3 \cdot 0.2 H_2O$: C 75.75, H 4.66, N 4.21; found: C 75.81, H 4.54, N 4.19.

2,3-Diphenyl-6-[2-(pyrrolidin-1-yl)ethoxy]quinoline (19a). To a stirred solution of **17** (0.30 g, 1 mmol) in dry DMF (20 mL) was added NaH (60% in oil, 0.50 g) at 0 °C for 1 h. *N*-(2-Chloroethyl)pyrrolidine·HCl (0.68 g, 4 mmol) was added and the mixture was heated at 80 °C for 1 h. The reaction mixture was partitioned between H₂O (50 mL) and CH_2Cl_2 (50 mL). The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by column chromatography (MeOH– CH_2Cl_2 1/10) and recrystallized from EtOH to give **19a** (0.32 g, 82%) as a white solid. M.p. 116–117 °C (EtOH). R_f 0.40 (MeOH– CH_2Cl_2 1 : 20). UV (MeOH): λ_{max} nm (log ϵ) 229 (4.66), 216 (4.65), 259 (4.56), 340 (3.84). 1H NMR (400 MHz, DMSO- d_6): 1.68–1.72 (m, 4H, pyrrolidiny-H), 2.54–2.58 (m, 4H, pyrrolidiny-H), 2.88 (t, 2H, $J = 6.0$ Hz, CH_2N), 4.23 (t, 2H, $J = 6.0$ Hz, OCH_2), 7.24–7.37 (m, 10H, Ar–H), 7.44 (dd, 1H, $J = 9.2, 2.8$ Hz, 7-H), 7.47 (d, 1H, $J = 2.8$ Hz, 5-H), 7.98 (d, 1H, $J = 9.2$ Hz, 5-H), 8.25 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO- d_6): 23.16 (2C), 54.03 (2C), 54.20, 67.16, 106.24, 122.65, 127.22, 127.68 (2C), 127.96, 128.28 (2C), 129.49 (2C), 129.74 (2C), 130.29, 134.10, 136.35, 136.55, 139.75, 140.28, 142.66, 154.96, 156.77. Anal. calcd for

C₂₇H₂₆N₂O·0.5 H₂O: C 80.36, H 6.74, N 6.94; found: C 80.16; H 6.82, N 6.96.

2,3-Diphenyl-6-[2-(piperidin-1-yl)ethoxy]quinoline (19b).

From **17** and *N*-(2-chloroethyl)piperidine·HCl as described for the preparation of **19a**. Compound **19b** was obtained in 85% yield (0.35 g) as a white oil. *R*_f 0.54 (MeOH–CH₂Cl₂ 1 : 20). UV (MeOH): λ_{max} nm (log ε) 230 (4.69), 216 (4.68), 259 (4.63), 343 (3.90), 340 (3.90). ¹H NMR (400 MHz, DMSO-*d*₆): 1.36–1.40 (m, 2H, piperidinyl-H), 1.48–1.53 (m, 4H, piperidinyl-H), 2.47 (br s, 4H, piperidinyl-H), 2.74 (t, 2H, *J* = 6.0 Hz, CH₂N), 4.21 (t, 2H, *J* = 6.0 Hz, OCH₂), 7.22–7.35 (m, 10H, Ar–H), 7.42 (dd, 1H, *J* = 9.2, 2.8 Hz, 7-H), 7.46 (d, 1H, *J* = 2.8 Hz, 5-H), 7.96 (d, 1H, *J* = 9.2 Hz, 8-H), 8.22 (s, 1H, 4-H). ¹³C NMR (100 MHz, DMSO-*d*₆): 23.89, 25.53 (2C), 54.43 (2C), 57.19, 66.01, 106.37, 122.67, 127.23, 127.70 (2C), 127.98, 128.29 (2C), 129.50 (2C), 129.74 (2C), 130.21, 134.12, 136.46 (2C), 139.76, 140.30, 142.69, 155.01, 156.79. Anal. calcd for C₂₈H₂₈N₂O·0.4 H₂O: C 80.89, H 6.98, N 6.74; found: C 80.88; H, 7.02; N, 6.78.

3-(2,3-Diphenylquinolin-6-yloxy)-*N,N*-dimethylpropan-1-amine (19c). From **17** and 3-chloro-*N,N*-dimethyl-propanamine·HCl as described for the preparation of **19a**. Compound **19c** was obtained in 86% yield (0.33 g) as a white oil. *R*_f 0.53 (MeOH–CH₂Cl₂ 1 : 10). UV (MeOH): λ_{max} nm (log ε) 231 (4.67), 220 (4.66), 259 (4.64), 345 (3.91), 340 (3.90). ¹H NMR (400 MHz, DMSO-*d*₆): 1.95 (quin, 2H, *J* = 6.8 Hz, OCH₂CH₂CH₂), 2.18 (s, 6H, NMe₂), 2.42 (t, 2H, *J* = 6.8 Hz, CH₂N), 4.16 (t, 2H, *J* = 6.8 Hz, OCH₂), 7.23–7.37 (m, 10H, Ar–H), 7.41–7.45 (m, 2H, 5- and 7-H), 7.97 (d, 1H, *J* = 8.8 Hz, 8-H), 8.26 (s, 1H, 4-H). ¹³C NMR (100 MHz, DMSO-*d*₆): 26.73, 45.13 (2C), 55.61, 66.27, 106.16, 122.60, 127.16, 127.63 (2C), 127.95, 128.22 (2C), 129.46 (2C), 129.69 (2C), 130.15, 134.05, 136.41 (2C), 139.73, 140.27, 142.61, 154.90, 156.90. Anal. calcd for C₂₆H₂₆N₂O·1.2 H₂O: C 77.27, H 7.08, N 6.93; found: C 77.15, H 7.17, N 6.92.

6-[2-(Pyrrolidin-1-yl)ethoxy]-2,3-bis{4-[2-(pyrrolidin-1-yl)ethoxy]phenyl}quinoline (20a). From **18** and *N*-(2-chloroethyl)pyrrolidine·HCl as described for the preparation of **19a**. Compound **20a** was obtained in 80% yield (0.50 g) as a white oil. *R*_f 0.53 (MeOH–CH₂Cl₂/NH₄OH 1 : 5 : 0.02). UV (MeOH): λ_{max} nm (log ε) 213 (4.76), 270 (4.55), 247 (4.54), 347 (3.84), 340 (3.81). ¹H NMR (400 MHz, DMSO-*d*₆): 1.62–1.68 (m, 12H, pyrrolidinyl-H), 2.45–2.53 (m, 12H, pyrrolidinyl-H), 2.72–2.76 (m, 4H, CH₂N), 2.83 (t, 2H, *J* = 5.6 Hz, CH₂N), 3.99–4.03 (m, 4H, OCH₂), 4.17 (t, 2H, *J* = 5.6 Hz, OCH₂), 6.79–6.88 (m, 4H, Ar–H), 7.11–7.14 (m, 2H, Ar–H), 7.26–7.28 (m, 2H, Ar–H), 7.34–7.37 (m, 2H, 5- and 7-H), 7.90 (d, 1H, *J* = 8.8 Hz, 8-H), 8.09 (s, 1H, 4-H). ¹³C NMR (100 MHz, DMSO-*d*₆): 23.14 (4C), 23.16 (2C), 54.02 (4C), 54.22 (2C), 54.31 (2C), 54.34, 66.65 (2C), 67.11, 106.08, 113.60 (2C), 114.29 (2C), 122.23, 127.82, 130.06, 130.55 (2C), 131.05 (2C), 132.11, 132.70, 133.60, 136.12, 142.55, 154.61, 156.51, 157.70, 158.10. Anal. calcd for C₃₉H₄₈N₄O₃·1.1 H₂O: C 73.12, H 7.90, N, 8.74; found: C 72.83, H 8.17, N 8.64.

6-[2-(Piperidin-1-yl)ethoxy]-2,3-bis{4-[2-(piperidin-1-yl)ethoxy]phenyl}quinoline (20b). From **18** and *N*-(2-chloroethyl)piperidine·HCl as described for the preparation of **19a**. Compound **20b** was obtained in 86% yield (0.33 g) as a white oil. *R*_f 0.40 (MeOH–CH₂Cl₂/NH₄OH 1 : 5 : 0.01). UV (MeOH): λ_{max} nm (log ε) 213 (4.80), 224 (4.77), 246 (4.59), 270

(4.56), 345 (3.91), 347 (3.91). ¹H NMR (400 MHz, DMSO-*d*₆): 1.35–1.39 (m, 6H, piperidinyl-H), 1.44–1.54 (m, 12H, piperidinyl-H), 2.36–2.46 (m, 12H, piperidinyl-H), 2.61–2.65 (m, 4H, CH₂N), 2.73 (t, 2H, *J* = 6.0 Hz, CH₂N), 4.02–4.06 (m, 4H, OCH₂), 4.20 (t, 2H, *J* = 6.0 Hz, OCH₂), 6.82–6.91 (m, 4H, Ar–H), 7.13–7.16 (d, 2H, Ar–H), 7.38–7.30 (d, 2H, Ar–H), 7.6–7.41 (m, 2H, 5- and 7-H), 7.91 (d, 1H, *J* = 9.2 Hz, 8-H), 8.12 (s, 1H, 4-H). ¹³C NMR (100 MHz, DMSO-*d*₆): 23.90 (3C), 25.54 (6C), 54.42 (6C), 57.22, 57.35, 57.37, 65.52 (2C), 65.97, 106.20, 113.65 (2C), 114.36 (2C), 122.22, 127.80, 130.02, 130.52 (2C), 131.01 (2C), 132.12, 132.70, 133.59, 136.10, 142.52, 154.61, 156.50, 157.70, 158.10. Anal. calcd for C₄₂H₅₄N₄O₃·0.5 H₂O: C 75.08, H 8.25; N 8.34; found: C 75.18, H 8.07, N 8.37.

3,3'-{4,4'-[6-(3-(Dimethylamino)propoxy)quinoline-2,3-diyl]bis-(4,1-phenylene)}bis-(oxy)bis(*N,N*-dimethylpropan-1-amine) (20c).

From **18c** and 3-chloro-*N,N*-dimethyl-propanamine·HCl as described for the preparation of **19a**. Compound **20c** was obtained in 77% yield (0.45 g) as a white oil. *R*_f 0.32 (MeOH–CH₂Cl₂/NH₄OH 1 : 5 : 0.02). UV (MeOH): λ_{max} nm (log ε) 213 (4.77), 223 (4.74), 271 (4.62), 248 (4.62), 348 (3.92). ¹H NMR (400 MHz, DMSO-*d*₆): 1.79–1.96 (m, 6H, OCH₂CH₂CH₂), 2.13, 2.14 and 2.16 (three s, 18H, NMe₂), 2.31–2.42 (m, 6H, CH₂N), 3.85–4.00 (m, 4H, OCH₂), 4.13 (t, 2H, *J* = 6.4 Hz, OCH₂), 6.81–6.90 (m, 4H, Ar–H), 7.13–7.16 (m, 2H, Ar–H), 7.27–7.30 (m, 2H, Ar–H), 7.35–7.40 (m, 2H, 5- and 7-H), 7.91 (d, 1H, *J* = 8.8 Hz, 8-H), 8.15 (s, 1H, 4-H). ¹³C NMR (100 MHz, DMSO-*d*₆): 26.82, 26.89, 26.91, 45.21 (6C), 55.66, 55.67 (2C), 65.69, 65.72, 66.24, 106.05, 113.60 (2C), 114.29 (2C), 122.25, 127.85, 130.02, 130.57 (2C), 131.04 (2C), 132.06, 132.65, 133.62, 136.13, 142.50, 154.63, 156.67, 157.84, 158.24. Anal. calcd for C₃₆H₄₈N₄O₃·1.8 H₂O: C 70.05, H 8.43, N 9.08; found: C 69.92, H 8.24, N 8.97.

Pharmacological methods

Antiproliferative assay. Cancer cells (Hep G2, Hep 3B, A549, H1299, MCF-7, MDA-MB-231) were purchased from Biore-sources Collection and Research Center, Taiwan. Cell lines were maintained in the same standard medium and grown as a mono-layer in DMEM (Gibco, USA) and supplemented with 10% fetal bovine serum (FBS) and antibiotics *i.e.* 100 IU mL⁻¹ penicillin, 0.1 mg mL⁻¹ streptomycin and 0.25 μg mL⁻¹ amphotericin. Culture was maintained at 37 °C with 5% CO₂ in a humidified atmosphere.

Cells (5 × 10³ cells well⁻¹) were treated as indicated for 72 h in medium containing 10% FBS. Cell viability was quantified 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⁻¹) 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 percentage of control growth as (Ab_{treated}/Ab_{control}) × 100, where Ab represents the mean absorbance (*n* = 3). The concentration that killed 50% of cells (GI₅₀) 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%.²³

Cell cycle analysis. MDA-MB-231 cells treated with DMSO and **16b** at different concentrations (1.0, 5.0, 10.0 μM) for 12 or 24 h were harvested, rinsed in PBS, resuspended and fixed in 70% ethanol and stored at $-20\text{ }^{\circ}\text{C}$ in fixation buffer until ready for analysis. Then the pellets were suspended in 1 mL of propidium iodide (PI) solution containing 20 $\mu\text{g mL}^{-1}$ of PI, 0.2 mg mL^{-1} RNase, and 0.1% (v/v) Triton 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).

Immunofluorescence analysis. MDA-MB-231 cells were seeded on cover glasses in 6-well plates with **16b** (1.0, 5.0, 10.0 μM) treatment for 24 h. After incubation, cells were washed with 1X 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 permeabilized with solution containing 2% FBS and 0.4% Triton X-100 in PBS at room temperature for 15 min. After permeabilization, cells were stained with β -tubulin monoclonal antibody (Santa Cruz 1 : 1000) at $4\text{ }^{\circ}\text{C}$ overnight. After primary antibody incubation, cells were washed with PBS containing 0.2% Triton X-100 three times, and stained with fluorescein isothiocyanate-conjugated anti-mouse IgG antibody (Santa Cruz, 1 : 200 diluted) at room temperature for 1 h. Finally, cells were washed with PBS and stained with DAPI (0.1 mg mL^{-1}) for 5 min at room temperature in the dark. The excess DAPI solution was removed followed by washing with PBS twice. Samples were mounted before analyzing under a fluorescence microscope.

DNA fragmentation assay. DNA fragmentation was determined by agarose gel electrophoresis. Cells were treated with various concentrations of compound **16b** (1.0, 5.0, 10.0 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 1X TAE buffer (40 mmol L^{-1} of Tris, 2 mmol L^{-1} of EDTA, 20 mmol L^{-1} 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 $\mu\text{g mL}^{-1}$) and photographed under ultraviolet illumination.

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 NaVO_3 , 10 mM NaF, 1 mM DTT, 1 mM PMSF, 25 $\mu\text{g mL}^{-1}$ aprotinin, and 25 $\mu\text{g mL}^{-1}$ leupeptin) and kept on ice for 30 min. The lysates were then centrifuged at 12000 g at $4\text{ }^{\circ}\text{C}$ for 20 min; the supernatants were stored at $-70\text{ }^{\circ}\text{C}$ 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-Bcl-2,

Bax, Bad and PARP 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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