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Synthesis and antiproliferative evaluation of 6-arylindeno[1,2-*c*]quinoline derivatives

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ABSTRACT

A number of 6-arylindeno[1,2-*c*]quinoline derivatives were synthesized and evaluated for their antiproliferative activities against the growth of five cancer cell lines including human hepatocelluar carcinoma (Hep G2, Hep 3B and Hep2.2.1), non-small cell lung cancer (A549 and H1299), and normal diploid embryonic lung cell line (MRC-5). The preliminary results indicated that 9-(3-(dimethylamino)propoxy)-6-(4-(3-(dimethylamino)propoxy)phenyl)-2-fluoro-11*H*-indeno[1,2-*c*]quinolin-11-one (**14c**) was the most potent with GI_{50} values of 0.61, 0.67, 0.59, and 0.72 μM against the growth of Hep G2, Hep 3B, Hep 2.2.1, and H1299 cells, respectively. Results have also shown that 2,9-bis(3-(dimethylamino)propoxy)-6-(4-(3-(dimethylamino)propoxy)phenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**17**), which exhibited GI_{50} of 0.60 and 0.68 μM against the growth of Hep G2 and A549, respectively, was more active than the positive topotecan and irinotecan. Compound **17** was less toxic than topotecan against the growth of normal cell (MRC-5) and therefore, was selected for further evaluation. Results indicated that compound **17** induce cell cycle arrest in G2/M phase, DNA fragmentation, and disrupt the microtubule network in A549 cells. The apoptotic induction may through the cleavage of PARP.

1. Introduction

Quinoline ring is found in a wide variety of biologically active compounds and is frequently condensed with various heterocycles.[1–12] For example, camptothecin which bears a quinoline moiety, is an anticancer alkaloid isolated from *Camptotheca acuminate*.[1,2] Camptothecin is a prototypical topoisomerase I (top I) inhibitor with poor water solubility. Subsequent introduction of hydrophilic side chains led to the discovery of topotecan and irinotecan which are currently used as anticancer drugs. However, several drawbacks such as easy opening of the lactone ring and the development of drug resistance have caused an urgent need in search of alternative top I inhibitors which are chemically more stable.[13,14] Recently, a number of condensed quinoline derivatives have been synthesized and evaluated for their top I inhibitory activities. We have also synthesized certain indeno[1,2-*c*]quinoline derivatives (**1a–d**) which were found to be more potent than camptothecin against the growth of human stomach adenocarcinoma (AGS) and non-small cell lung cancer (A549).[12] In continuation of our study to explore more potent anticancer drug candidates, we described herein the preparation of certain 6-arylindeno[1,2-*c*]quinoline derivatives (Fig. 1) and their evaluation in vitro against a panel of five cancer cell lines including three human hepatocelluar carcinoma cells (Hep G2, Hep 3B and Hep2.2.1) and two non-small cell lung cancer cells (A549 and H1299). Hepatocellular carcinoma (HCC) and lung cancer are common malignancies in the world, and are the leading cause of cancer deaths in Asian countries including Taiwan.[15,16] Chronic hepatitis B and C, especially in cirrhotic stage, are associated with the vast majority of cases of HCC.[15,17] Because of the high prevalence of hepatitis B and C virus infection, HCC has been the leading cause of death in Taiwan since 1984.[15,18]

2. Chemistry

The Pfitzinger reaction of indolin-2,3-dione (isatin) and 1,2-bis(4-methoxyphenyl)ethanone under basic conditions gave 2,3-bis(4-methoxyphenyl)quinoline-4-carboxylic acid (**2**)[19] as described in Scheme 1. Treatment of **2** with $POCl_3$ and $AlCl_3$ afforded 9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**5**) which was then reacted with 48% HBr to give 6-(4-hydroxyphenyl)-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**8**) in a fairly good overall yield. 2-Fluoro-9-hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**9**) and its 2-methoxy counterpart **10** were, respectively prepared under the same reaction

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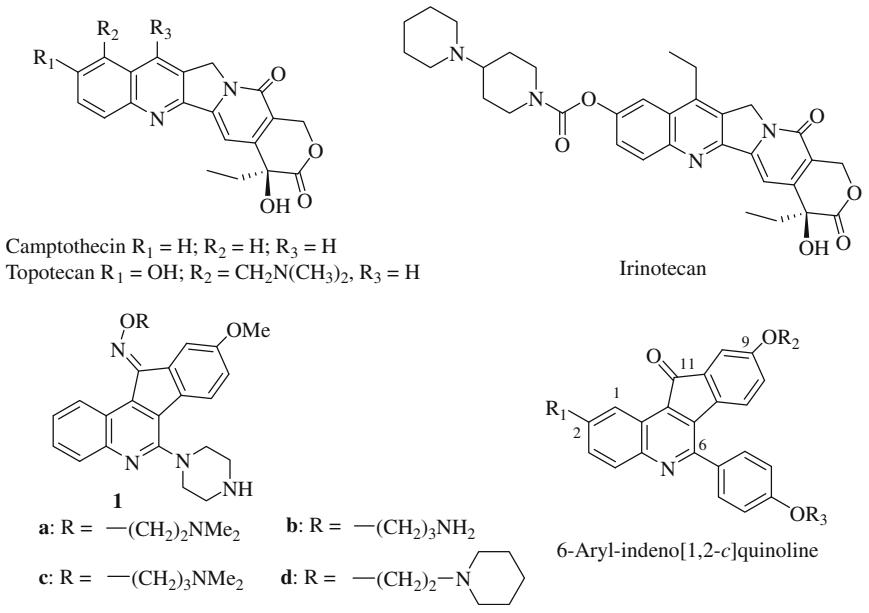
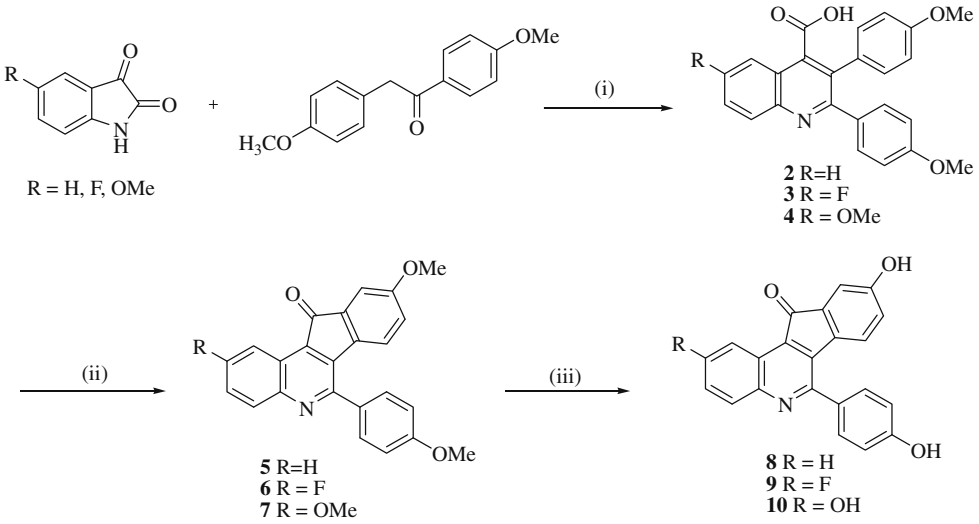


Figure 1. Chemical structures of camptothecin and indeno[1,2-*c*]quinoline derivatives.



Scheme 1. Reagents and conditions: (i) KOH, EtOH, 80 °C, 48 h; (ii) $POCl_3$, $AlCl_3$, 150 °C, 24 h; (iii) 48% HBr, HOAc, reflux, 48 h.

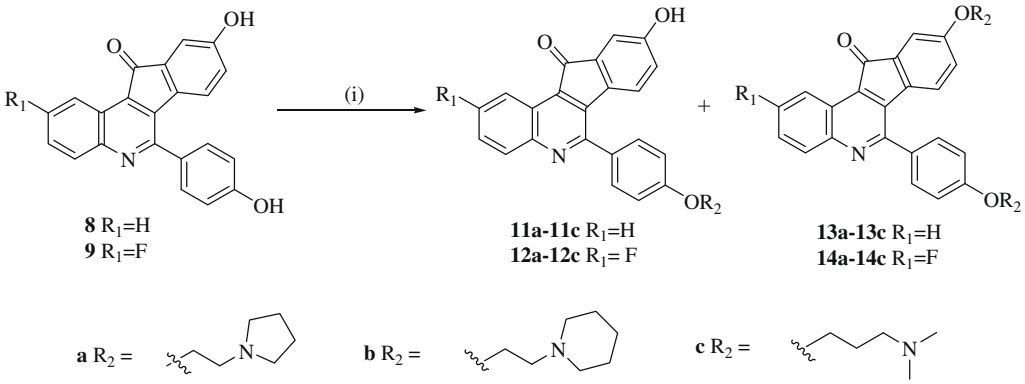
conditions from **6** and **7** which in turn were obtained from 5-fluoroisatin and 5-methoxyisatin, respectively.

Alkylation of **8** with *N*-(2-chloroethyl)pyrrolidine gave a mixture of monoalkylated 9-hydroxy-6-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**11a**) and dialkylated 9-(2-(pyrrolidin-1-yl)ethoxy)-6-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**13a**) in a yield of 33% and 41%, respectively as depicted in Scheme 2. Structure of **11a** was assigned by the NOESY spectrum in which correlations between OCH_2 (δ_H = 4.13 ppm) and *meta*-H (δ_H = 7.13 ppm) were observed (Fig. 2). Under the same reaction conditions, the mixture of **11b** and **13b** was obtained from **8** and *N*-(2-chloroethyl)piperidine; and the mixture of **11c** and **13c** was obtained from **8** and 3-chloro-*N*,*N*-dimethylpropanamine. Structure of dialkylated product **13b** was confirmed by the NOESY spectrum in which correlations of OCH_2 (δ_H = 4.12 ppm)/*meta*-H (δ_H = 7.08 ppm); OCH_2 (δ_H = 4.24 ppm)/8-H (δ_H = 6.70 ppm); and OCH_2 (δ_H = 4.24 ppm)/10-H (δ_H = 7.20 ppm) were observed (Fig. 2). Accordingly, the mixtures of **12a–c** and **14a–c** were obtained by the alkylation of **9** with *N*-(2-chloroethyl)pyrrolidine, *N*-(2-chloroethyl)piperidine, and 3-chloro-*N*,*N*-dimethylpropanamine, respectively.

Treatment of 2,9-dihydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**10**) with *N*-(2-chloroethyl)pyrrolidine, *N*-(2-chloroethyl)piperidine, and 3-chloro-*N*,*N*-dimethylpropanamine, respectively afforded trialkylated products **15–17** as described in Scheme 3.

3. Results and discussion

All the synthesized 6-arylindeno[1,2-*c*]quinoline derivatives were evaluated in vitro against a panel of five cancer cell lines including three human hepatocelluar carcinoma cells (Hep G2, Hep 3B and Hep2.2.1) and two non-small cell lung cancer cells (A549 and H1299) using XTT (2,3-bis [2-methyloxy-4-nitro-5-sulfophenyl]-



Scheme 2. Reagents and conditions: (i) NaH, alkyl halides, DMF, 80 °C, 25 min.

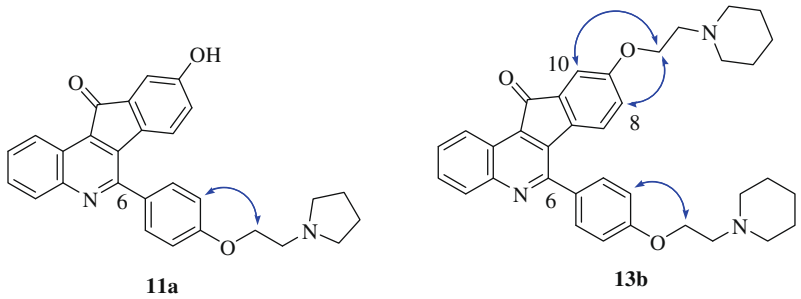
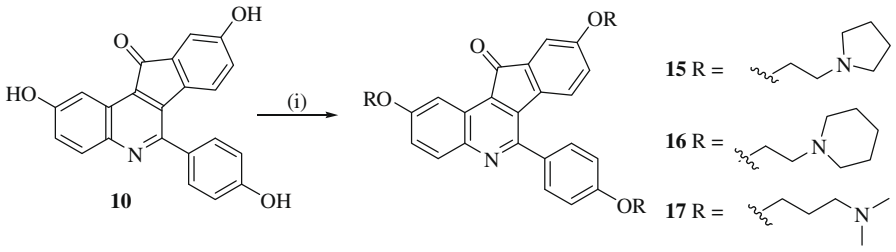


Figure 2. NOESY correlations of compounds **11a** and **13b**.

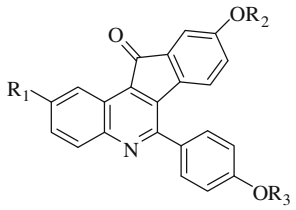


Scheme 3. Reagents and conditions: (i) NaH, alkyl halides, DMF, 80 °C, 60 min.

2*H*-tetrazolium-5-carboxanilide) assay.[20] Tetrazolium salts (e.g., MTT, MTS, XTT) have been used for many years to distinguish living cells from dead ones. They are cleaved to form a formazan dye only by metabolic active cells. XTT is reduced in the cells to formazan by mitochondrial succinate dehydrogenase. The normal diploid embryonic lung cell line (MRC-5) was also evaluated since a potential anticancer drug candidate should selectively affect only tumor cells and not somatic cells. The concentration that inhibited the growth of 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%. The GI_{50} results of 6-arylindeno[1,2-*c*]quinoline-11-one derivatives are summarized in Table 1. 9-Methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**5**) was inactive against all the cancer cells tested with GI_{50} of >10 μM in each case. Introduction of a fluoro or a methoxy group on the C-2 position enhanced antiproliferative activity against the growth of H1299 cancer cell in which compounds **6** and **7** exhibited GI_{50} of 6.44 and 7.84 μM, respectively. With an exception of the H1299 cancer cell, 2,9-dihydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**10**) which exhibited GI_{50} values of 5.51, 6.61, and 4.98 μM against the growth of Hep G2, Hep 2.2.1, and A549, respectively, was more active than its trimethoxy counterpart **7**. Therefore, the substituents of hydrogen-bond donating groups are more favorable than that of hydrogen-bond accepting groups for the 6-arylindeno[1,2-*c*]quinoline pharmacophore. The same trend was observed in which dihydroxy derivatives **8** and **9** are more active than their respective dimethoxy counterparts **5** and **6**. For the C-2 unsubstituted 6-arylindeno[1,2-*c*]quinoline derivatives, introduction of an aminoalkyl side chain on the 6-aryl moiety enhanced antiproliferative activity in which each of compounds **11a**–**c** was more active than compound **8**. Comparable activities of **11a**, **11b** and **11c** indicated no difference in the antiproliferative activity for various aminoalkyl side chains such as pyrrolidin-1-yl ethyl, piperidin-1-yl ethyl, and acyclic dimethylaminopropyl group. Further introduction of an aminoalkyl side chain on the C-9 position did not enhanced antiproliferative activity in which activities of monoaminoalkylated 6-arylindeno[1,2-*c*]quinolines **11a**–**c** were comparable with their respective diaminoalkylated derivatives **13a–c**.

For the C-2 fluoro-substituted 6-arylindeno[1,2-*c*]quinoline derivatives, introduction of an aminoalkyl side chain on the 6-aryl moiety enhanced antiproliferative activity in which each of compounds **12a**–**c** was more active than compound **9**. Further introduction of a pyrrolidin-1-yl ethyl side chain on the C-9 position

Table 1
Antiproliferative activity of 6-arylindeno[1,2\-*c*]quinoline-11-one derivatives (GI_{50}, μM)



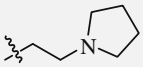
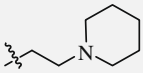
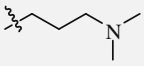
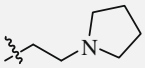
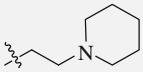
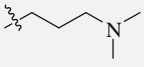
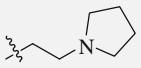
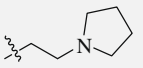
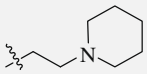
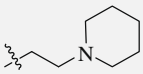
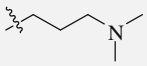
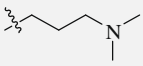
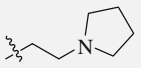
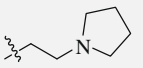
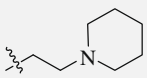
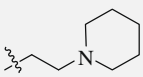
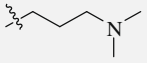
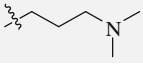
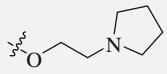
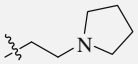
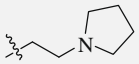
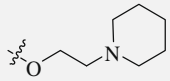
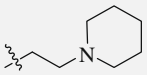
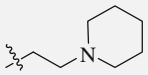
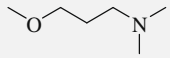
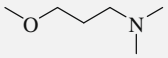
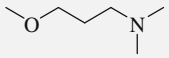
Compd	R_1	R_2	R3	Cell line (GI_{50}, μM)					
				Hep G2	Hep 3B	Hep 2.2.1	A549	H1299	MRC-5
5	H	Me	Me	>10	>10	>10	>10	>10	>10
6	F	Me	Me	>10	>10	>10	>10	6.44 ± 0.06	>10
7	OMe	Me	Me	>10	12.53 ± 3.58	>10	>10	7.84 ± 0.26	10.24 ± 3.09
8	H	H	H	9.12 ± 0.35	>10	>10	>10	>10	>10
9	F	H	H	>10	9.01 ± 0.78	>10	>10	5.88 ± 0.07	>10
10	OH	H	H	5.51 ± 0.20	12.15 ± 3.81	6.61 ± 0.19	4.98 ± 0.05	10.44 ± 0.05	10.33 ± 3.55
11a	H	H		4.28 ± 0.02	3.40 ± 0.01	6.06 ± 0.06	4.33 ± 0.01	7.05 ± 0.01	3.16 ± 0.01
11b	H	H		4.30 ± 0.04	5.40 ± 0.01	6.40 ± 0.01	5.06 ± 0.05	6.55 ± 0.02	3.10 ± 0.02
11c	H	H		5.59 ± 0.05	5.34 ± 0.05	6.82 ± 1.16	5.87 ± 0.01	6.39 ± 0.10	4.61 ± 0.05
12a	F	H		6.10 ± 0.03	6.34 ± 0.02	5.69 ± 0.01	6.63 ± 0.02	5.25 ± 0.02	6.02 ± 0.01
12b	F	H		4.78 ± 0.01	6.61 ± 0.01	6.03 ± 0.06	6.00 ± 0.02	5.32 ± 0.03	5.91 ± 0.01
12c	F	H		5.92 ± 0.01	6.72 ± 0.02	5.62 ± 0.01	6.35 ± 0.01	6.13 ± 0.01	4.75 ± 0.01
13a	H			4.03 ± 0.02	3.46 ± 0.02	6.15 ± 0.03	3.75 ± 0.01	6.95 ± 0.01	5.31 ± 0.02
13b	H			4.32 ± 0.03	1.35 ± 0.01	6.24 ± 0.04	5.68 ± 0.03	6.38 ± 0.06	4.34 ± 0.04
13c	H			4.35 ± 0.01	3.05 ± 0.01	5.39 ± 0.06	3.07 ± 0.01	5.35 ± 0.01	3.68 ± 0.02
14a	F			4.92 ± 0.01	6.24 ± 0.03	6.11 ± 0.59	6.23 ± 0.03	4.30 ± 0.02	5.15 ± 0.02
14b	F			2.82 ± 0.02	0.66 ± 0.02	3.00 ± 0.01	6.24 ± 0.03	0.79 ± 0.20	4.52 ± 0.01
14c	F			0.61 ± 0.01	0.67 ± 0.01	0.59 ± 0.04	3.67 ± 0.02	0.72 ± 0.01	1.86 ± 0.02

Table 1 (*continued*)

Compd	R_1	R_2	R3	Cell line (GI_{50}, μM)					
				Hep G2	Hep 3B	Hep 2.2.1	A549	H1299	MRC-5
15				0.62 ± 0.01	1.73 ± 0.01	3.44 ± 0.04	0.67 ± 0.01	0.37 ± 0.07	0.71 ± 0.07
16				0.70 ± 0.01	5.02 ± 0.24	6.07 ± 0.01	0.81 ± 0.01	6.26 ± 0.01	4.64 ± 0.01
17				0.60 ± 0.01	4.44 ± 0.01	4.25 ± 0.02	0.68 ± 0.03	5.18 ± 0.09	6.20 ± 0.06
Topotecan				3.83 ± 0.15	0.22 ± 0.01	4.95 ± 0.08	5.98 ± 0.26	>10	4.25 ± 0.81
Irinotecan				5.94 ± 0.75	4.73 ± 0.21	>10	>10	>10	77.53 ± 1.82

did not enhanced antiproliferative activity in which activity of diaminoalkylated derivative **14a** was comparable with its monoaminoalkylated derivative **12a**. However, introduction of the second piperidin-1-yl ethyl side chain on the C-9 position enhanced antiproliferative activity against certain specific cancer cells in which compound **14b** was especially active against the growth of Hep 3B and H1299 with GI_{50} of 0.66 and 0.79 μM, respectively. Introduction of the second dimethylaminopropyl side chain on the C-9 position significantly enhanced antiproliferative activity against all cancer cells in which compound **14c** was much more active than **12c**. Compound **14b** was found to be more selective and less cytotoxic than **14c** against the growth of normal diploid embryonic lung cell line (MRC-5).

For the trisubstituted 6-arylindeno[1,2-*c*]quinoline derivatives, the substituted aminoalkyl side chains play important roles in the antiproliferative activities in which activities against cancer cells decreased in an order pyrrolidin-1-yl ethyl **15** > acyclic dimethylaminopropyl **17** > piperidin-1-yl ethyl **16**. Although compound **15** was the most potent among these 6-arylindeno[1,2-*c*]quinoline derivatives, it was non-selective and highly cytotoxic against the normal MRC-5. Compound **17**, which exhibited GI_{50} of 0.60 and 0.68 μM against the growth of Hep G2 and A549, respectively, was more active than the positive topotecan and irinotecan. In addition, compound **17** was found to be less toxic than **15** and topotecan against the growth of MRC-5. Therefore, compound **17** and its trimethoxy substituted 6-arylindeno[1,2-*c*]quinoline analog **7** (as a negative control) were selected for further evaluation on their effects of A549 cell cycle distribution by flow cytometric analysis. Compound **7** had no effect on cell cycle progression even at a concentration of 10.0 μM while compound **17** induced cell cycle arrest in a concentration-dependent manner as shown in Figure 3 and Table 2. The proportion of cells was slightly decreased in the S and accumulated in G2/M phase after 24 h treatment of **17**, while the hypodiploid (sub-G0/G1 phase) cells increased. The morphological changes of apoptosis include membrane blebbing, cell shrinkage, chromatin condensation, and formation of apoptotic bodies.[21] Stain of **17**-treated A549 cells with DAPI clearly showed apoptotic bodies as shown in Figure 4. The images obtained by immunofluorescence microscopy evaluate the effect of compound **17** on microtubule network indirectly. The microtubule network in control cells displayed intact organization and arrangement. However, when cells were exposed to various concentrations of **17** 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. Significant morphological changes were also observed by the incubation of A549 cells with different concentrations of **17** for 24 h as shown in Figure 5. 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.[22] The appearance of such fragments resulted in a ladder formation evidently when fragmented DNA from **17**-treated cells was separated by agarose gel electrophoresis (Fig. 6). Cells in sub-G0/G1 phase indicate they are undergoing the DNA fragmentation and cell death. To examine the effect of compound **17** on apoptosis related protein, cleavage of PARP was evaluated in **17**-treated A549 cells by western blotting. 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.[23] Our results have shown that PARP was cleaved from 116 kDa intact form into 85 kDa fragment after the treatment of **17** in a concentration-dependent manner (Fig. 7). Thus, compound **17** induces cell cycle arrest at G2/M phase followed by DNA fragmentation *via* cleavage of PARP and consequently to cause the cell death.

4. Conclusion

A number of 6-arylindeno[1,2-*c*]quinoline derivatives were synthesized and evaluated for antiproliferative activities against the growth of cancer cell lines including Hep G2, Hep 3B, Hep 2.2.1, A549, H1299, and normal cell line MRC-5. Among them, 2,9-bis(3-(dimethylamino) propoxy)-6-(4-(3-(dimethylamino)propoxy)phenyl)-11*H*-indeno[1,2-*c*]-quinolin-11-one (**17**), which exhibited GI_{50} of 0.60 and 0.68 μM against the growth of Hep G2 and A549, respectively, was more active than the positive topotecan and irinotecan. Compound **17** was less toxic than topotecan against the growth of normal cell (MRC-5) and therefore, was selected for further evaluation. Results indicated that **17** induce cell cycle arrest at G2/M phase, DNA fragmentation, and disrupt the microtubule network in A549 cells. The apoptotic induction may through the cleavage of PARP. In vivo studies on compound **17** are currently under investigation.

5. Experimental

5.1. General

Melting points were determined on a Electrothermal IA9100 melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Varian Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on Silica Gel 60 F-254 plates purchased from E.

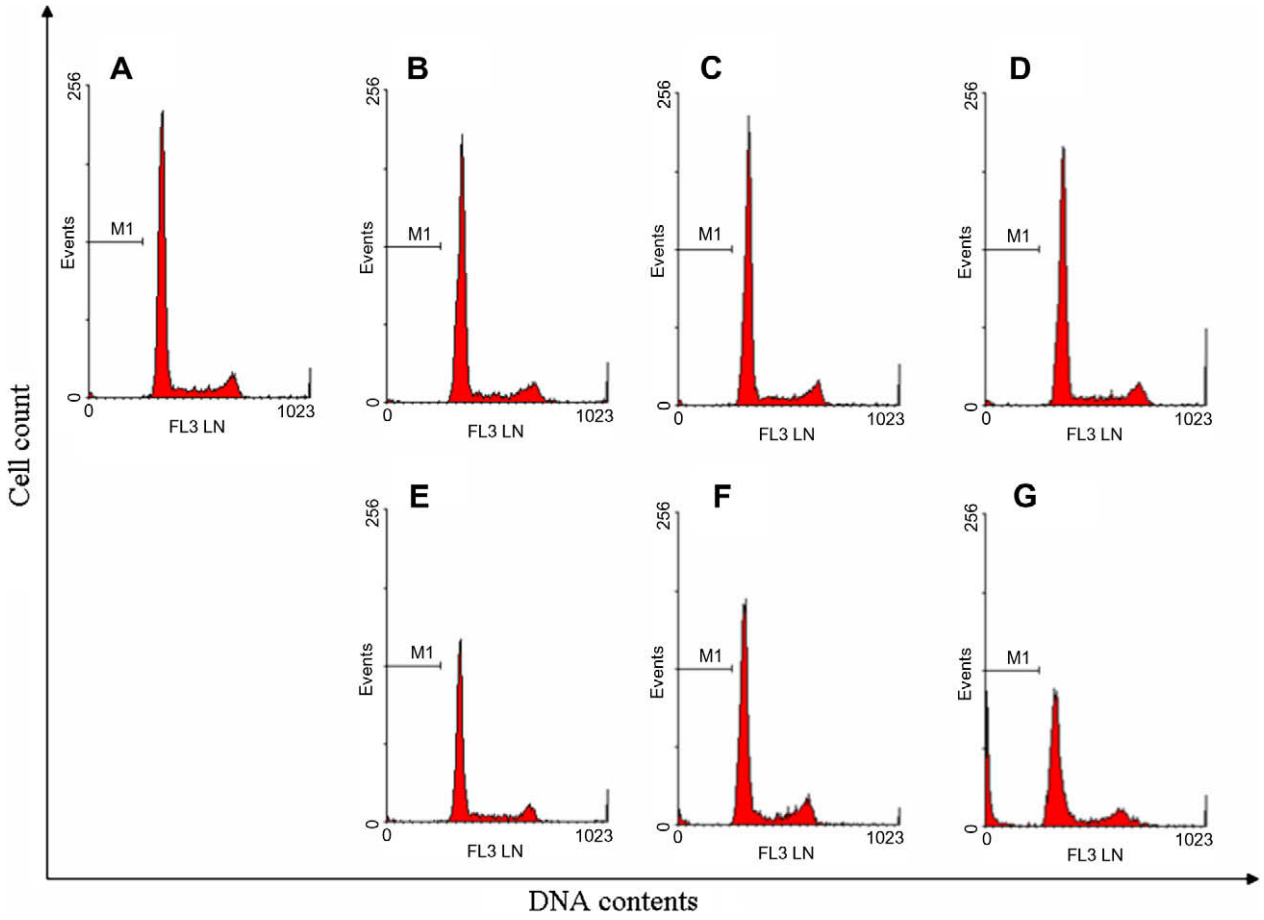


Figure 3. Flow cytometric analysis of A549 cells. Cells were treated with DMSO (A), compound **7** at 1.0 μM (B), 5.0 μM (C) or 10.0 μM (D); and compound **17** at 0.1 μM (E), 1.0 μM (F) or 5.0 μM (G). After the treatment for 24 h, cells were harvested, fixed, and stained with propidium iodide as described in Section 5.2.2. The percentage of cells in each cell cycle phase was summarized in Table 2.

Table 2
Effects of compounds **7** and **17** on A549 cell cycle progression

Compound	Concentration (μM)	Cell cycle distribution (%)			
		Sub G1	G1	S	G2/M
DMSO		0.9	76.2	14.3	9.5
7	1.0	0.9	78.2	13.2	8.6
7	5.0	1.0	77.9	14.3	7.8
7	10.0	1.0	76.9	14.7	8.4
17	0.1	1.5	73.9	16.3	9.8
17	1.0	2.0	75.0	13.6	11.0
17	5.0	17.1	71.9	12.3	15.8

Merck and Co. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Taiwan University using Heraeus CHN-O Rapid EA, and all values are within ±0.4% of the theoretical compositions.

5.1.1. 2,3-Bis(4-methoxyphenyl)quinoline-4-carboxylic acid (2)

A mixture of isatin (2.94 g, 20 mmol), 1,2-bis(4-methoxyphenyl)ethanone (6.15 g, 24 mmol), and KOH (3.37 g, 60 mmol) in EtOH was heated at 80 °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 recrystallized from EtOH to give **2** (3.23 g, 84%) as a white solid. Mp 312–313 °C (lit. 308 °C).[19] ^{1}H NMR (400 MHz, DMSO-d_6): 3.74 and 3.76 (two s, 6H, $OCH_3 \times 2$), 6.82 (m, 2H, Ar-H), 6.90 (m, 2H, Ar-H), 7.14 (m, 2H, Ar-H), 7.30 (m, 2H, Ar-H), 7.70 (m, 1H, 5-H), 7.81–7.86 (m, 2H, 6- and 7-H), 8.11 (d, 1H, 8-H). ^{13}C NMR (100 MHz, DMSO-d_6): 55.08, 55.11, 113.09 (2C), 113.59 (2C), 121.91, 124.78, 127.61, 128.94, 129.23, 129.36, 130.17, 131.25 (2C), 131.33 (2C), 132.37, 141.80, 146.36, 158.01, 158.65, 159.07, 168.33. Anal. Calcd for $C_{24}H_{19}NO_4$: C, 74.79; H, 4.97; N, 3.63. Found: C, 74.41; H, 5.37; N, 3.41.

5.1.2. 6-Fluoro-2,3-bis(4-methoxyphenyl)quinoline-4-carboxylic acid (3)

From 5-fluoroisatin and 1,2-bis(4-methoxyphenyl)ethanone as described for **2**: Yield: 83%. Mp 310–311 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.74 and 3.76 (two s, 6H, $OCH_3 \times 2$), 6.82 (m, 2H, Ar-H), 6.91 (m, 2H, Ar-H), 7.14 (m, 2H, Ar-H), 7.28 (m, 2H, Ar-H), 7.48 (dd, 1H, J = 9.6, 2.8 Hz, 5-H), 7.75–7.80 (ddd, 1H, J = 9.2, 9.2, 2.8 Hz, 7-H), 8.22 (d, 1H, J = 9.2, 5.6 Hz, 8-H), 13.92 (br s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.06, 55.09, 107.94 (J = 23.5 Hz), 113.08 (2C), 113.61 (2C), 120.28 (J = 25.8 Hz), 122.61 (J = 9.8 Hz), 129.05, 129.99, 131.20 (2C), 131.26 (2C), 132.03, 132.33 (J = 9.9 Hz), 141.11 (J = 6.0 Hz), 143.63, 157.63, 158.74, 159.07, 160.30 (J = 243.3 Hz), 167.87. Anal. Calcd for $C_{24}H_{18}FNO_4 \cdot 0.5H_2O$: C, 66.96; H, 4.93; N, 3.25. Found: C, 66.61; H, 5.29; N, 2.88.

5.1.3. 6-Methoxy-2,3-bis(4-methoxyphenyl)quinoline-4-carboxylic acid (4)

From 5-methoxyisatin and 1,2-bis(4-methoxyphenyl)ethanone as described for **2**: yield: 86%. Mp 305–306 °C. ^{1}H NMR

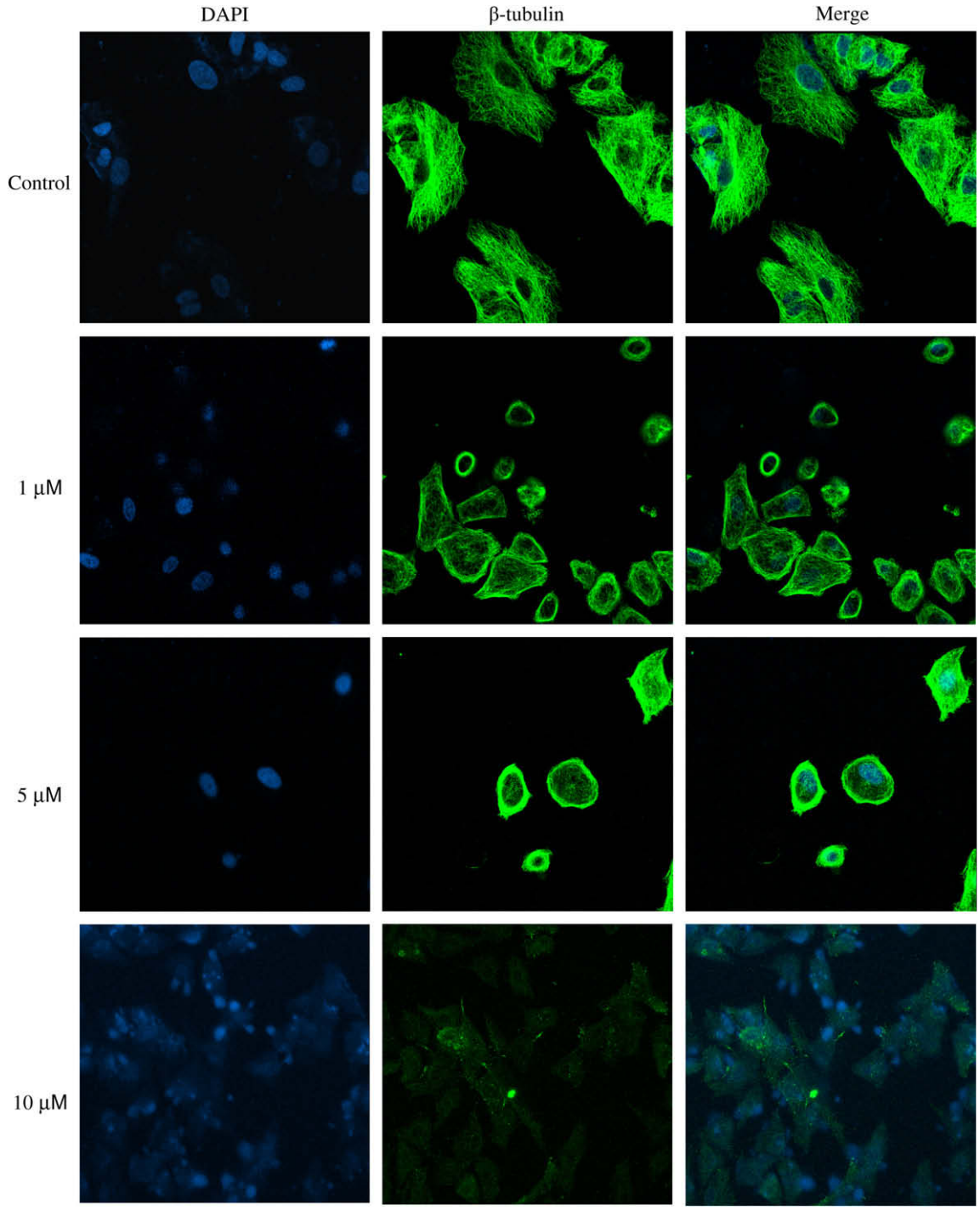


Figure 4. Microtubule effect of compound **17**. The confocal laser scanning micrograph shows a merged image double-labeled with DAPI and β-tubulin antibodies. A549 cells were fixed after the treatment of **17** for 24 h followed by the immunofluorescence analysis using anti-β-tubulin antibody, FITC-conjugated secondary antibody, and DAPI staining as described in Section 5.2.3.

(400 MHz, DMSO-d_6): 3.75, 3.76, and 3.90 (three s, 9H, $OCH_3 \times 3$), 6.81 (m, 2H, Ar-H), 6.90 (m, 2H, Ar-H), 7.06 (d, 1H, J = 2.4 Hz, 5-H), 7.13 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 7.52 (dd, 1H, J = 9.2, 2.8 Hz, 7-H), 8.04 (d, 1H, J = 9.2 Hz, 8-H). ^{13}C NMR (100 MHz, DMSO-d_6): 55.06, 55.07, 55.54, 102.33, 113.03 (2C), 113.56 (2C), 122.49, 122.91, 129.32, 129.51, 130.96, 131.11 (2C), 131.27 (2C), 132.41, 140.48, 142.58, 155.39, 157.98, 158.61, 158.83, 168.39. Anal. Calcd for $C_{25}H_{21}NO_5$: C, 72.28; H, 5.10; N, 3.37. Found: C, 72.41; H, 5.48; N, 3.11.

5.1.4. 9-Methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (5)

A solution of **2** (3.85 g, 10 mmol) in $POCl_3$ (30 mL) was heated at 150 °C for 24 h. After cooling to room temperature, chlorobenzene (30 mL) and $AlCl_3$ (1.33 g, 10 mmol) were added and refluxed for 3 h (TLC monitoring). After cooling, the mixture was poured into ice-water (150 mL). The resulting precipitate thus obtained was collected and then suspended in 5% $NaHCO_3$ aqueous (200 mL) with vigorous stirring for 1 h. The crude solid was then collected,

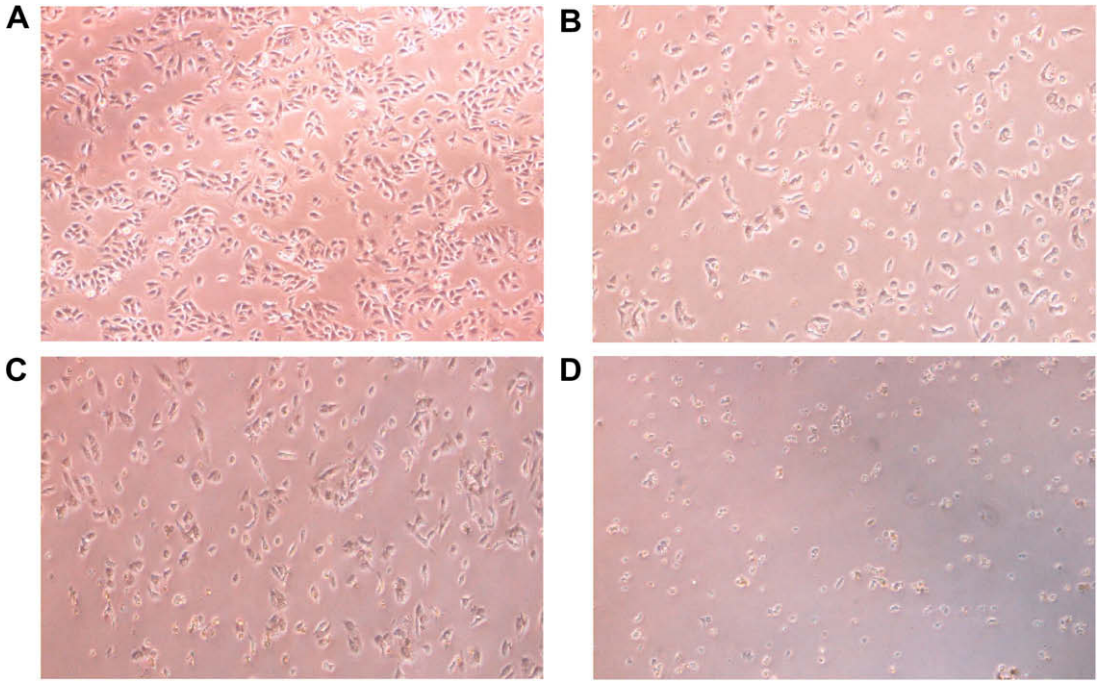


Figure 5. Induction of morphological change in A549 cells. Cells were treated with DMSO (A), compound **17** at 1 μM (B), 5 μM (C) or 10 μM (D) for 24 h at 37 °C and photographed under a microscope.

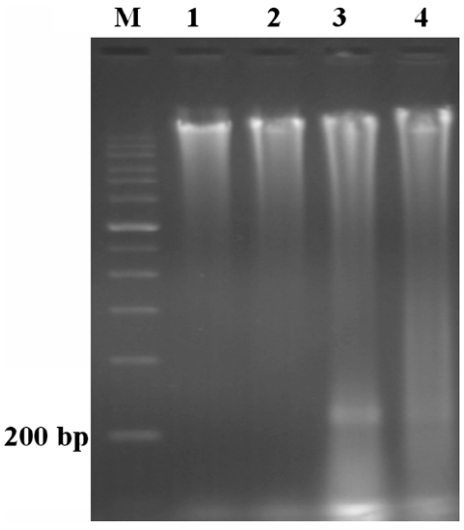


Figure 6. Agarose gel electrophoresis for detecting DNA fragmentation in A549 cells treated with compound **17** for 24 h. Lane M, DNA size marker; lane 1, DMSO; lane 2, 1.0 μM; lane 3, 5.0 μM; lane 4, 10.0 μM.

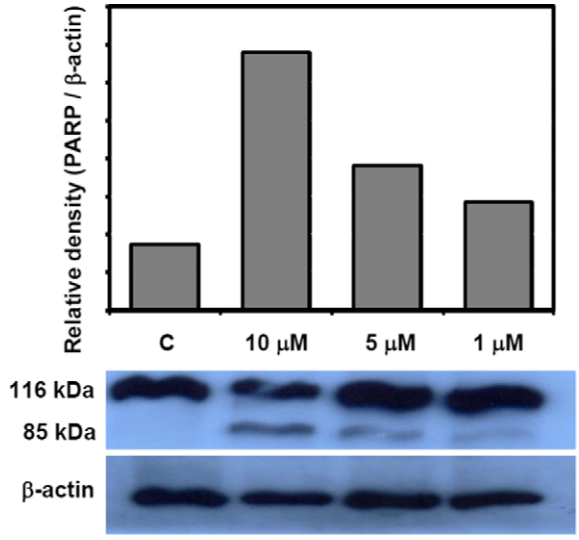


Figure 7. Expression of PARP in A549 cells by the treatment of compound **17**. Exponentially growing cells were treated with **17** for 24 h. Equal amounts of cell lysate were resolved using SDS–PAGE and analyzed by Western blot using anti-PARP antibodies. The blots were re-probed with anti-β-actin antibody to confirm equal protein loading.

purified by column chromatography ($MeOH/CH_2Cl_2$ 1/50), and crystallized from EtOH to give **5** (2.09 g, 57%) as a red solid. Mp: 214–215 °C. ^1H NMR (400 MHz, $CDCl_3$): 3.83 and 3.93 (two s, 6H, 9- and 4′-OCH_3), 6.71 (dd, 1H, J = 8.0, 2.4 Hz, 8-H), 6.84 (d, 1H, J = 8.0 Hz, 7-H), 7.08 (m, 2H, Ar-H), 7.21 (d, 1H, J = 2.4 H, 10-H), 7.56–7.67 (m, 4H, 2-, 3- and Ar-H), 8.04 (m, 1H, 4-H), 8.82 (m, 1H, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.44, 55.72, 110.68, 114.17 (2C), 114.20, 119.10, 122.51, 123.79, 124.47, 129.20, 129.57, 129.76, 130.14 (2C), 134.32, 135.58, 135.61, 136.73, 149.22, 154.96, 160.45, 160.85, 195.27. Anal. Calcd for $C_{24}H_{17}NO_3 \cdot 0.5H_2O$: C, 76.57; H, 4.83; N, 3.72. Found: C, 76.64; H, 4.99; N, 3.58.

5.1.5. 2-Fluoro-9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (6)

From **3** as described for **5**: yield: 83%. Mp 260–261 °C. ^1H NMR (400 MHz, $CDCl_3$): 3.83 and 3.92 (two s, 6H, 9- and 4′-OCH_3), 6.72 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.87 (d, 1H, J = 8.4 Hz, 7-H), 7.10 (m, 2H, Ar-H), 7.21 (d, 1H, J = 2.4 Hz, 10-H), 7.38–7.43 (*m*, 1H, 3-H), 7.65 (m, 2H, Ar-H), 8.07 (m, 1H, 4-H), 8.44 (dd, 1H, J = 9.2, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.46, 55.76, 107.20 (J = 23.4 Hz), 108.07, 110.78, 111.24, 114.23 (2C), 119.25, 120.45

(J = 26.6 Hz), 124.75, 130.15 (2C), 132.00 (J = 9.8 Hz), 135.11, 135.58, 137.45, 154.15, 160.56, 161.11, 162.60 (J = 250.0 Hz), 194.32. Anal. Calcd for $C_{24}H_{16}FNO_3 \cdot 0.3H_2O$: C, 73.75; H, 4.29; N, 3.58. Found: C, 73.47; H, 4.19; N, 3.53.

5.1.6. 2,9-Dimethoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]-quinolin-11-one (7)

From **4** as described for **5**: yield: 74%. Mp 203–204 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.82, 3.92, and 4.00 (three s, 9H, $OCH_3 \times 3$), 6.70 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.85 (d, 1H, J = 8.4 Hz, 7-H), 7.07 (m, 2H, Ar-H), 7.18 (d, 1H, J = 2.4 Hz, 10-H), 7.26 (dd, 1H, J = 9.2, 2.8 Hz, 3-H), 7.62 (m, 2H, Ar-H), 7.92 (d, 1H, J = 9.2 Hz, 4-H), 8.11 (d, 1H, J = 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.42, 55.70, 55.76, 100.47, 110.49, 114.12 (2C), 118.89, 123.45, 123.97, 124.46, 130.18 (2C), 131.05, 132.10, 132.75, 135.59, 135.80, 136.54, 146.11, 151.94, 160.23, 160.32, 160.77, 195.44. Anal. Calcd for $C_{25}H_{19}NO_4$: C, 75.55; H, 4.82; N, 3.52. Found: C, 75.46; H, 4.98; N, 3.41.

5.1.7. 9-Hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (8)

A solution **5** (0.37 g, 1.0 mmol) in 5 mL of 48% HBr was heated at reflux under a nitrogen atmosphere for 48 h (TLC monitoring). The mixture was then cooled and evaporated in vacuo to give a residue which was treated with H_2O (50 mL). The crude product was collected and recrystallized with EtOH to give 0.27 g (78%) of 8 as a red solid. Mp 338–339 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 6.74 (m, 2H, 7- and 8-H), 7.00–7.57 (m, 3H, 10- and Ar-H), 7.55 (m, 2H, Ar-H), 7.68–7.78 (*m*, 2H, 2- and 3-H), 8.02 (d, 1H, J = 8.4 Hz, 4-H), 8.67 (m, 1H, 1-H), 10.18 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 112.31, 115.44 (2C), 120.54, 121.85, 123.11, 124.56, 127.45, 127.70, 129.76, 130.39 (2C), 130.67, 132.62, 134.43, 134.96, 137.07, 146.45, 154.06, 159.02, 159.35, 194.24. Anal. Calcd for $C_{22}H_{13}NO_3 \cdot 0.8H_2O \cdot 0.7HBr$: C, 63.40; H, 3.71; N, 3.36. Found: C, 63.03; H, 3.51; N, 3.29.

5.1.8. 2-Fluoro-9-hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno-[1,2-*c*]quinolin-11-one (9)

From **6** as described for **8**: yield: 98%. Mp 324–325°C. ^{1}H NMR (400 MHz, DMSO-d_6): 6.68–6.74 (m, 2H, 7- and 8-H), 6.94–6.98 (m, 3H, 10- and Ar-H), 7.48 (m, 2H, Ar-H), 7.58 (ddd, 1H, J = 8.8, 8.8, 3.2 Hz, 3-H), 8.00 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.18 (dd, 1H, J = 9.6, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 106.13 (J = 23.5 Hz), 112.26, 115.34 (2C), 120.27 (J = 21.3 Hz), 120.38, 122.20 (J = 12.2 Hz), 124.75, 128.89, 130.21 (2C), 131.69 (J = 9.9 Hz), 132.55, 133.20 (J = 6.0 Hz), 135.02, 137.46, 145.01, 154.06 (J = 2.3 Hz), 159.62, 159.36, 161.78 (J = 247.8 Hz), 194.15. Anal. Calcd for $C_{22}H_{12}FNO_3 \cdot 1H_2O \cdot 0.3HCl$: C, 68.39; H, 3.74; N, 3.63. Found: C, 68.65; H, 4.04; N, 3.38.

5.1.9. 2,9-Dihydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (10)

From **7** as described for **8**: yield: 72%. Mp 326 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 6.70 (dd, 1H, J = 8.4, 2.0 Hz, 8-H), 6.74 (d, 1H, J = 8.4 Hz, 7-H), 6.94 (m, 2H, Ar-H), 6.98 (d, 1H, J = 2.0 Hz, 10-H), 7.25 (dd, 1H, J = 9.2, 2.8 Hz, 3-H), 7.46 (m, 2H, Ar-H), 7.81 (d, 1H, J = 9.2 Hz, 4-H), 7.97 (d, 1H, J = 2.8 Hz, 1-H), 9.84, 10.18, and 10.51 (three br s, 3H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 104.41, 111.91, 115.23 (2C), 120.06, 122.94, 123.45, 124.39, 125.78, 130.17 (2C), 131.12, 133.28, 135.39, 136.47, 144.66, 151.17, 158.04, 158.47, 158.94, 195.09. Anal. Calcd for $C_{22}H_{13}NO_4 \cdot 1.0H_2O \cdot 0.1HCl$: C, 70.08; H, 4.05; N, 3.72. Found: C, 69.79; H, 4.07; N, 3.66.

5.1.10. 9-Hydroxy-6-{4-[2-(pyrrolidin-1-yl)ethoxy]phenyl}-11*H*-indeno[1,2-*c*]quinolin-11-one (11a) and 9-[2-(pyrrolidin-1-yl)ethoxy]-6-{4-[2-(pyrrolidin-1-yl)ethoxy]phenyl}-11*H*-indeno[1,2-*c*]quinolin-11-one (13a)

To a solution of **8** (0.34 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 (0.51 g, 3 mmol) was added and the mixture was 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 separated, dried over $MgSO_4$, and evaporated. The resulting residue was purified by column chromatography (MeOH/CH_2Cl_2 1/10) to give two fractions. The first fraction contained the monosubstituted product **11a** (0.15 g, 33%) as a red solid. Mp 245 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 1.72 (m, 4H, pyrrolininyl-H), 2.59 (m, 4H, pyrrolininyl-H), 2.88 (t, 2H, J = 6.0 Hz, CH_2N), 4.19 (t, 2H, J = 6.0 Hz, OCH_2), 6.67–6.73 (m, 2H, 7- and 8-H), 6.98 (d, 1H, J = 2.0 Hz, 10-H), 7.13 (*m*, 2H, Ar-H), 7.56 (*m*, 2H, Ar-H), 7.63–7.70 (m, 2H, 2- and 3-H), 7.94 (m, 1H, 4-H), 8.63 (m, 1H, 1-H), 10.21 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 23.17 (2C), 54.08 (2C), 54.30, 66.79, 112.23, 114.45 (2C), 120.25, 121.72, 122.97, 124.43, 129.28, 129.43, 129.77, 130.15 (2C), 131.48, 133.15, 133.25, 135.12, 136.63, 148.34, 154.55, 159.09, 159.25, 194.85. Anal. Calcd for $C_{28}H_{24}N_2O_3 \cdot 0.6H_2O$: C, 75.17; H, 5.69; N, 6.26. Found: C, 74.88; H, 5.90; N, 6.52.

The second fraction contained disubstituted product **13a** (0.22 g, 41%) as a red solid. Mp 150–151 °C. ^{1}H NMR (400 MHz, DMSO-d_6+TFA): 1.92–2.16 (m, 8H, pyrrolininyl-H), 3.14–3.28 (m, 4H, $OCH_2N \times 2$), 3.64–3.75 (m, 8H, pyrrolidinyl-H), 4.42 (t, 2H, J = 5.2 Hz, OCH_2), 4.49 (t, 2H, J = 5.2 Hz, OCH_2), 6.90 (d, 1H, J = 8.4 Hz, 7-H), 7.01 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 7.32 (*m*, 2H, Ar-H), 7.40 (d, 1H, J = 2.4 Hz, 10-H), 7.78–7.89 (*m*, 4H, 2-, 3-, and Ar-H), 8.11 (*d*, 1H, J = 8.4 Hz, 4-H), 8.82 (*dd*, 1H, J = 8.4, 1.2 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6+TFA): 22.82 (2C), 22.86 (2C), 53.11, 53.33, 54.30 (2C), 54.38 (2C), 63.92, 64.33, 112.33, 115.33 (2C), 120.68, 122.51, 123.78, 124.66, 127.61, 130.30, 130.58, 130.95 (2C), 131.74, 135.40, 135.44, 135.78, 136.88, 146.88, 153.91, 159.46, 159.65, 193.99. Anal. Calcd for $C_{34}H_{35}N_3O_3 \cdot 0.4H_2O$: C, 75.49; H, 6.68; N, 7.77. Found: C, 75.43; H, 6.56; N, 7.45.

5.1.11. 9-Hydroxy-6-{4-[2-(piperidin-1-yl)ethoxy]phenyl}-11*H*-indeno[1,2-*c*]quinolin-11-one (11b) and 9-[2-(piperidin-1-yl)-ethoxy]-6-{4-[2-(piperidin-1-yl)ethoxy]phenyl}-11*H*-indeno-[1,2-*c*]-quinolin-11-one (13b)

From **7** and *N*-(2-chloroethyl)piperidine·HCl as described for **11a** and **13a**. Compound **11b** was obtained in 30% yield (0.14 g) as a red solid. Mp 249 °C (Dec).^{1}H NMR (400 MHz, DMSO-d_6): 1.37–1.42 (m, 2H, piperidinyl-H), 1.47–1.54 (*m*, 4H, piperidinyl-H), 2.46 (*m*, 4H, piperidinyl-H), 2.70 (t, 2H, J = 5.6 Hz, CH_2N), 4.17 (t, 2H, J = 6.0 Hz, OCH_2), 6.66–6.72 (*m*, 2H, 7- and 8-H), 6.96 (d, 1H, J = 2.4 Hz, 10-H), 7.11 (m, 2H, Ar-H), 7.57–7.69 (m, 4H, 2-, 3-, and Ar-H), 7.92 (m, 1H, 4-H), 8.61 (dd, 1H, J = 8.0, 1.6 Hz, 1-H), 10.21 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 23.98, 25.63 (2C), 54.52 (2C), 57.45, 65.83, 112.26, 114.52 (2C), 120.27, 121.75, 123.00, 124.46, 129.29, 129.45, 129.80, 130.17 (2C), 131.46, 133.15, 133.27, 135.15, 136.66, 148.37, 154.57, 159.14, 159.34, 194.89. Anal. Calcd for $C_{29}H_{26}N_2O_3 \cdot 0.5H_2O \cdot 0.3HCl$: C, 74.02; H, 5.86; N, 5.95. Found: C, 74.17; H, 6.06; N, 5.55.

Compound **13b** was obtained in 47% yield (0.26 g) as a red solid. Mp 167–168 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.42–1.51 (m, 4H, piperidinyl-H), 1.59–1.69 (m, 8H, piperidinyl-H), 2.51–2.59 (m, 8H, piperidinyl-H), 2.77 (t, 2H, J = 5.6 Hz, CH_2N), 2.88 (t, 2H, J = 6.0 Hz, CH_2N), 4.12 (t, 2H, J = 6.0 Hz, OCH_2), 4.24 (t, 2H, J = 6.0 Hz, OCH_2), 6.70 (dd, 1H, J = 8.4, 2.8 Hz, 8-H), 6.83 (d, 1H, J = 8.4 Hz, 7-H), 7.08 (m, 2H, Ar-H), 7.20 (d, 1H, J = 2.4 Hz, 10-H),

7.55–7.65 (m, 4H, 2-, 3-, and Ar-H), 8.03 (m, 1H, 4-H), 8.82 (m, 1H, 1-H). ^{13}C NMR (100 MHz,$CDCl_3$): 24.05 (2C), 25.77 (2C), 25.78 (2C), 54.98 (2C), 55.08 (2C), 57.64, 57.83, 66.03, 66.46, 111.48, 114.84 (2C), 119.63, 122.51, 123.79, 124.48, 129.15, 129.57, 129.72, 130.11 (2C), 132.09, 134.32, 135.54, 135.61, 136.71, 149.23, 154.96, 159.63, 160.06, 195.24. Anal. Calcd for $C_{36}H_{39}N_3O_3\cdot1.0H_2O$: C, 74.57; H, 7.14; N, 7.25. Found: C, 74.50; H, 6.82; N, 7.23.

5.1.12. 6-{4-[3-(Dimethylamino)propoxy]phenyl}-9-hydroxy-11*H*-indeno[1,2-*c*]quinolin-11-one (11c) and 9-[3-(dimethylamino)propoxy]-6-{4-[3-(dimethylamino)propoxy] phenyl}-11*H*-indeno[1,2-*c*]-quinolin-11-one (13c)

From **7** and 3-chloro-*N*,*N*-dimethylpropanamine·HCl as described for **11a** and **13a**. Compound **11c** was obtained in 30% yield (0.13 g) as a red solid. Mp 245 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 1.94 (m, 2H, $OCH_2CH_2CH_2$), 2.23 (s, 6H, NMe_2), 2.48 (t, 2H, J = 7.2 Hz, CH_2N), 4.13 (t, 2H, J = 6.4 Hz, OCH_2), 6.73 (m, 2H, 7- and 8-H), 7.01 (d, 1H, J = 2.0 Hz, 10-H), 7.13 (m, 2H, Ar-H), 7.61–7.73 (*m*, 4H, 2-, 3-, and Ar-H), 7.96 (*dd*, 1H, J = 7.2, 1.2 Hz, 4-H), 8.66 (*m*, 1H, 1-H), 10.21 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.69, 45.02 (2C), 55.59, 65.92, 112.25, 114.41 (2C), 120.29, 121.74, 122.98, 124.45, 129.30, 129.47, 129.81, 130.17 (2C), 131.40, 133.15, 133.28, 135.13, 136.66, 148.35, 154.58, 159.13, 159.42, 194.90. Anal. Calcd for $C_{27}H_{24}N_2O_3\cdot1.0H_2O\cdot0.5HCl$: C, 70.37; H, 5.81; N, 6.08. Found: C, 70.48; H, 5.97; N, 5.93.

Compound **13c** was obtained in 46% yield (0.23 g) as a red solid; Mp 154–155 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 1.91–2.07 (m, 4H, $OCH_2CH_2CH_2$), 2.25, 2.29 (two s, 12H, NMe_2), 2.45 (t, 2H, J = 7.2 Hz, CH_2N), 2.53 (t, 2H, J = 7.2 Hz, CH_2N), 4.05 (t, 2H, J = 6.4 Hz, OCH_2), 4.15 (t, 2H, J = 6.4 Hz, OCH_2), 6.69 (dd, 1H, J = 8.0, 2.4 Hz, 8-H), 6.84 (d, 1H, J = 8.0 Hz, 7-H), 7.08 (m, 2H, Ar-H), 7.20 (d, 1H, J = 2.4 Hz, 10-H), 7.55–7.65 (m, 4H, 2-, 3-, and Ar-H), 8.05 (d, 1H, J = 8.4 Hz, 4-H), 8.82 (m, 1H, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 27.37, 27.54, 45.51 (2C), 45.56 (2C), 56.14, 56.37, 66.40, 66.74, 111.28, 114.72 (2C), 119.60, 122.50, 123.78, 124.50, 129.13, 129.57, 129.69, 130.11 (2C), 131.91, 134.30, 135.48, 135.53, 136.78, 149.22, 155.02, 159.89, 160.29, 195.33. Anal. Calcd for $C_{32}H_{35}N_3O_3\cdot0.5H_2O\cdot0.1HCl$: C, 73.57; H, 6.98; N, 8.05. Found: C, 73.79; H, 7.00; N, 7.76.

5.1.13. 2-Fluoro-9-hydroxy-6-{4-[2-(pyrrolidin-1-yl)ethoxy]-phenyl}-11*H*-indeno[1,2-*c*] quinolin-11-one (12a) and 2-fluoro-9-[2-(pyrrolidin-1-yl)ethoxy]-6-{4-[2-(pyrrolidin-1-yl)ethoxy]-phenyl}-11*H*-indeno-[1,2-*c*]quinolin-11-one (14a)

From **9** and *N*-(2-chloroethyl) pyrrolidine·HCl as described for **11a** and **13a**. Compound **12a** was obtained in 32% yield (0.15 g) as a red solid. Mp 203–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 1.78 (m, 4H, pyrrolidinyl-H), 2.62 (m, 4H, pyrrolidinyl-H), 2.91 (t, 2H, J = 6.0 Hz, CH_2N), 4.20 (t, 2H, J = 6.0 Hz, OCH_2), 6.66–6.72 (m, 2H, 7- and 8-H), 6.95 (d, 1H, J = 2.4 Hz, 10-H), 7.12 (m, 2H, Ar-H), 7.54–7.59 (m, 3H, 3- and Ar-H), 7.98 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.18 (dd, 1H, J = 9.6, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 23.14 (2C), 54.07 (2C), 54.25, 66.66, 106.06 (J = 23.5 Hz), 112.26, 114.44 (2C), 119.91, 120.05 (J = 26.6 Hz), 120.29, 122.20 (J = 12.1 Hz), 124.65, 130.14 (2C), 131.18, 132.29 (J = 9.9 Hz), 132.62, 132.88 (J = 6.8 Hz), 135.08, 137.28, 145.63, 153.89, 159.28 (J = 8.3 Hz), 161.80 (J = 247.0 Hz), 194.29. Anal. Calcd for $C_{28}H_{23}FN_2O_3\cdot0.5HCl$: C, 71.12; H, 5.02; N, 5.92. Found: C, 71.50; H, 5.42; N, 5.82.

Compound **14a** was obtained in 50% yield (0.28 g) as a red solid; mp 163–164 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.78–1.90 (m, 8H, pyrrolidinyl-H), 2.65 (m, 4H, pyrrolindinyl-H), 2.74 (m, 4H, pyrrolindinyl-H), 2.92 (t, 2H, J = 5.6 Hz, OCH_2N), 3.02 (t, 2H, J = 5.6 Hz, OCH_2N), 4.14 (t, 2H, J = 5.6 Hz, OCH_2), 4.26 (t, 2H, J = 5.6 Hz, OCH_2), 6.74 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.86 (d, 1H, J = 8.4 Hz, 7-H), 7.08 (m, 2H, Ar-H), 7.21 (d, 1H, J = 2.4 Hz, 10-H), 7.39 (ddd, 1H, J = 9.2, 9.2, 2.8 Hz, 3-H), 7.61 (m, 2H, Ar-H), 8.04 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.44 (dd, 1H, J = 9.6, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.43 (2C), 23.46 (2C), 54.69 (2C), 54.78 (2C), 54.96, 66.97, 67.50, 107.16 (J = 23.5 Hz), 111.49, 114.84 (2C), 119.72, 120.33 (J = 26.5 Hz), 123.15 (J = 12.1 Hz), 124.74, 130.09 (2C), 131.82, 132.08 (J = 9.9 Hz), 133.91 (J = 6.9 Hz), 135.14, 135.50, 137.37, 146.49, 154.16, 159.63, 160.26, 162.55 (J = 250.0 Hz), 194.71. Anal. Calcd for $C_{34}H_{34}FN_3O_3\cdot0.6HCl$: C, 71.19; H, 6.09; N, 7.33. Found: C, 71.21; H, 6.38; N, 7.09.

5.1.14. 2-Fluoro-9-hydroxy-6-{4-[2-(piperidin-1-yl)ethoxy]-phenyl}-11*H*-indeno[1,2-*c*]quino-lin-11-one (12b) and 2-fluoro-9-[2-(piperidin-1-yl)ethoxy]-6-{4-[2-(piperidin-1-yl) ethoxy]-phenyl}-11*H*-indeno[1,2-*c*]quinolin-11-one (14b)

From **9** and *N*-(2-chloroethyl) piperidine·HCl as described for **11a** and **13a**. Compound **12b** was obtained in 37% yield (0.17 g) as a red solid. Mp 245–246 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 1.43 (m, 2H, piperidinyl-H), 1.57 (m, 4H, piperidinyl-H), 2.59 (m, 4H, piperidinyl-H), 2.83 (m, 2H, CH_2N), 4.23 (t, 2H, J = 6.4 Hz, OCH_2), 6.68–6.74 (m, 2H, 7- and 8-H), 6.97 (d, 1H, J = 2.0 Hz, 10-H), 7.16 (*m*, 2H, Ar-H), 7.56–7.61 (m, 3H, 3- and Ar-H), 8.00 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.19 (dd, 1H, J = 9.6, 2.8 Hz, 1-H), 10.25 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 23.58, 25.19, 30.67, 54.25 (2C), 57.07, 65.37, 106.05 (J = 23.5 Hz), 112.27, 114.51 (2C), 120.05 (J = 25.8 Hz), 120.28, 122.21 (J = 12.1 Hz), 124.64, 130.11 (2C), 131.23, 132.30 (J = 9.1 Hz), 132.62, 132.87, 135.09, 137.29, 145.63, 153.90, 159.21, 159.33, 161.81 (J = 247.9 Hz), 194.30. Anal. Calcd for $C_{29}H_{25}FN_2O_3\cdot0.4HCl$: C, 72.08; H, 5.30; N, 5.79. Found: C, 72.29; H, 5.48; N, 5.49.

Compound **14b** was obtained in 53% yield (0.31 g) as a red solid; Mp 165–166 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.47–1.54 (m, 4H, piperidinyl-H), 1.63–1.75 (m, 8H, piperidinyl-H), 2.57–2.67 (m, 8H, piperidinyl-H), 2.82 (t, 2H, J = 5.6 Hz, CH_2N), 2.95 (t, 2H, J = 5.6 Hz, CH_2N), 4.19 (t, 2H, J = 5.6 Hz, OCH_2), 4.31 (t, 2H, J = 5.6 Hz, OCH_2), 6.73 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.87 (d, 1H, J = 8.4 Hz, 7-H), 7.09 (*m*, 2H, Ar-H), 7.21 (d, 1H, J = 2.4 Hz, 10-H), 7.38–7.43 (ddd, 1H, J = 9.2, 8.4, 2.8 Hz, 3-H), 7.62 (m, 2H, Ar-H), 8.03 (m, 1H, 4-H), 8.43 (dd, 1H, J = 9.6, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.82, 23.91, 25.44 (2C), 25.58 (2C), 54.95 (2C), 55.00, 57.34, 57.55, 57.69, 65.70, 66.28, 107.18 (J = 23.5 Hz), 111.62, 114.87 (2C), 119.66, 120.36 (J = 26.5 Hz), 123.17 (J = 11.4 Hz), 124.74, 129.12, 130.13 (2C), 131.91, 132.09 (J = 9.8 Hz), 133.78, 135.18, 135.54, 137.35, 146.51, 159.52, 160.19, 162.57 (J = 250.2 Hz), 194.67. Anal. Calcd for $C_{36}H_{38}FN_3O_3\cdot0.1HCl$: C, 74.10; H, 6.59; N, 7.20. Found: C, 73.88; H, 6.63; N, 7.15.

5.1.15. 6-{4-[3-(Dimethylamino)propoxy]phenyl}-2-fluoro-9-hydroxy-11*H*-indeno[1,2-*c*] quinolin-11-one (12c) and 9-[3-(dimethylamino)propoxy]-6-{4-[3-(dimethylamino) propoxy]-phenyl}-2-fluoro-11*H*-indeno[1,2-*c*]quinolin-11-one (14c)

From **9** and 3-chloro-*N*,*N*-dimethylpropanamine·HCl as described for **11a** and **13a**. Compound **12c** was obtained in 35% yield (0.15 g). Mp 279–280 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 2.10 (m, 2H, $OCH_2CH_2CH_2$), 2.60 (s, 6H, NMe_2), 2.97 (t, 2H, J = 7.2 Hz, CH_2N), 4.16 (t, 2H, J = 6.4 Hz, OCH_2), 6.72 (m, 2H, 7- and 8-H), 7.01 (*m*, 1H, 10-H), 7.15 (m, 2H, Ar-H), 7.59–7.63 (m, 3H, 3- and Ar-H), 8.02 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.25 (dd, 1H, J = 9.6, 2.8 Hz, 1-H). ^{13}C NMR(100 MHz, DMSO-d_6): δ 24.98, 43.28, 54.70, 54.94, 65.36, 106.12 (J = 23.5 Hz), 112.35, 114.49 (2C), 120.20 (J = 33.4 Hz), 120.36, 122.29 (J = 11.4 Hz), 124.67, 130.20 (2C), 131.35, 132.37 (J = 9.8 Hz), 132.66, 133.02, 135.15, 137.38, 145.69, 153.99, 159.21, 159.41, 161.88 (J = 247.8 Hz), 194.40. Anal. Calcd for $C_{27}H_{23}FN_2O_3\cdot1.5H_2O\cdot2.5HCl$: C, 57.82; H, 5.13; N, 5.00. Found: C, 58.04; H, 5.12; N, 5.17.

Compound **14c** was obtained in 41% yield (0.22 g) as a red solid. Mp 164–165 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.92–2.07 (m, 4H,

$OCH_2CH_2CH_2$), 2.26 (s, 6H, NMe_2), 2.30 (s, 6H, NMe_2), 2.44 (*t*, 2H, *J* = 7.2 Hz, OCH_2N), 2.53 (t, 2H, *J* = 6.8 Hz, OCH_2N), 4.04(t, 2H, *J* = 6.4 Hz, OCH_2), 4.15 (t, 2H, *J* = 6.4 Hz, OCH_2), 6.71 (dd, 1H, *J* = 8.4, 2.4 Hz, 8-H), 6.86 (d, 1H, *J* = 8.4 Hz, 7-H), 7.08 (m, 2H, Ar-H), 7.21 (d, 1H, *J* = 2.4 Hz, 10-H), 7.39 (ddd, 1H, *J* = 9.2, 8.4, 2.8 Hz, 3-H), 7.61 (m, 2H, Ar-H), 8.02 (dd, 1H, *J* = 9.2, 5.6 Hz, 4-H), 8.45 (dd, 1H, *J* = 9.2, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 27.31, 27.49, 45.44 (2C), 45.81 (2C), 56.09, 56.33, 66.42, 66.78, 107.17 (*J* = 23.5 Hz), 111.38, 114.77 (2C), 119.69, 120.27 (*J* = 26.6 Hz), 123.16 (*J* = 12.2 Hz), 124.74, 130.09 (2C), 131.65, 132.10 (*J* = 9.9 Hz), 133.93 (*J* = 6.8 Hz), 135.03, 135.56, 137.45, 146.51, 154.27, 159.97, 160.54, 162.56 (*J* = 250.2 Hz), 194.78. Anal. Calcd for $C_{32}H_{34}FN_3O_3 \cdot 1.0H_2O$: C, 70.70; H, 6.66; N, 7.70. Found: C, 70.49; H, 6.56; N, 7.62.

5.1.16. 2,9-Bis[2-(pyrrolidin-1-yl)ethoxy]-6-{4-[2-(pyrrolidin-1-yl)ethoxy]phenyl}-11*H*-indeno [1,2-*c*]quinolin-11-one (15)

To a solution of **10** (0.36 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 (0.51 g, 3 mmol) was added and the mixture was heated at 80 °C for 60 min. The reaction mixture was partitioned between H_2O (50 mL) and CH_2Cl_2 (50 mL). The organic layer was separated, dried over $MgSO_4$, and evaporated. The resulting residue was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/10) to give 15 (0.21 g, 57%) as a orange solid. Mp 157–158 °C. ^1H NMR (400 MHz, $CDCl_3$): 1.81–1.88 (m, 12H, pyrrolidinyl-H), 2.64 (m, 4H, pyrrolidinyl-H), 2.71 (m, 8H, pyrrolidinyl-H), 2.91 (t, 2H, CH_2N), 2.98–3.04 (m, 4H, $CH_2N \times 2$), 4.13 (t, 2H, *J* = 5.6 Hz, OCH_2), 4.24 (t, 2H, *J* = 5.6 Hz, OCH_2), 4.33 (t, 2H, *J* = 5.6 Hz, OCH_2), 6.73 (dd, 1H, *J* = 8.0, 2.8 Hz, 8-H), 6.84 (d, 1H, *J* = 8.0 Hz, 7-H), 7.08 (m, 2H, Ar-H), 7.20 (d, 1H, *J* = 2.8 Hz, 10-H), 7.31 (dd, 1H, *J* = 9.2, 2.8 Hz, 3-H), 7.61 (m, 2H, Ar-H), 7.90 (d, 1H, *J* = 9.2 Hz, 4-H), 8.13 (d, 1H, *J* = 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.47 (2C), 23.51 (4C), 54.69 (2C), 54.73 (2C), 54.78 (2C), 54.85, 54.87, 55.03, 67.15, 67.43, 67.56, 101.2, 111.25, 114.81 (2C), 119.52, 123.74, 123.98, 124.48, 130.16 (2C), 131.03, 132.23, 132.81, 135.64, 135.79, 136.57, 146.13, 152.00, 159.47, 159.60, 160.05, 195.41. Anal. Calcd for $C_{40}H_{46}N_4O_4 \cdot 1.5H_2O \cdot 1.0HCl$: C, 67.62; H, 7.11; N, 7.89. Found: C, 67.45; H, 6.76; N, 7.58.

5.1.17. 2,9-Bis[2-(piperidin-1-yl)ethoxy]-6-{4-[2-(piperidin-1-yl)ethoxy]phenyl}-11*H*-indeno [1,2-*c*]quinolin-11-one (16)

From **10** and *N*-(2-chloroethyl)piperidine·HCl as described for **15**: yield: 53%. Mp 159–160 °C. ^1H NMR (400 MHz, $CDCl_3$): 1.49 (m, 6H, piperidinyl-H), 1.61–1.72 (m, 12H, piperidinyl-H), 2.54 (m, 4H, piperidinyl-H), 2.62 (m, 8H, piperidinyl-H), 2.80 (t, 2H, *J* = 5.6 Hz, CH_2N), 2.88–2.93 (m, 4H, $CH_2N \times 2$), 4.15 (t, 2H, *J* = 5.6 Hz, OCH_2), 4.26 (t, 2H, *J* = 5.6 Hz, OCH_2), 4.33 (t, 2H, *J* = 5.6 Hz, OCH_2), 6.70 (dd, 1H, *J* = 8.4, 2.8 Hz, 8-H), 6.84 (d, 1H, *J* = 8.4 Hz, 7-H), 7.08 (m, 2H, Ar-H), 7.18 (d, 1H, *J* = 2.8 Hz, 10-H), 7.28 (dd, 1H, *J* = 9.2, 2.8 Hz, 3-H), 7.61 (m, 2H, Ar-H), 7.89 (d, 1H, *J* = 9.2 Hz, 4-H), 8.11 (d, 1H, *J* = 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.96, 23.99, 24.07, 25.63 (2C), 25.69 2C), 25.76 (2C), 54.99 (2C), 55.02 (2C), 55.05 (2C), 55.15, 57.64, 57.78, 65.85, 66.12, 66.30, 101.26, 111.29, 114.79 (2C), 119.43, 123.67, 123.94, 124.45, 130.17 (2C), 131.01, 132.23, 132.77, 135.58, 135.74, 136.52, 146.09, 151.94, 159.32, 159.48, 159.94, 195.33. Anal. Calcd for $C_{43}H_{52}N_4O_4 \cdot 0.5H_2O$: C, 73.99l; H, 7.67; N, 8.03. Found: C, 73.74; H, 7.48; N, 7.94.

5.1.18. 2,9-Bis[3-(dimethylamino)propoxy]-6-{4-[3-(dimethylamino)propoxy]phenyl}-11*H*-indeno[1,2-*c*]quinolin-11-one (17)

From **10** and 3-chloro-*N*,*N*-dimethylpropanamine·HCl as described for **15**: yield: 50%. Mp 140–141 °C. ^1H NMR (400 MHz, $CDCl_3$): 1.93–2.08 (m, 6H, $OCH_2CH_2CH_2$), 2.25, 2.29, and 2.30 (three s, 18H, $NMe_2 \times 3$), 2.45 (t, 2H, *J* = 6.8 Hz, CH_2N), 2.49–2.54 (*m*, 4H, $CH_2N \times 2$), 4.05 (t, 2H, *J* = 6.4 Hz, OCH_2), 4.15 (t, 2H, *J* = 6.4 Hz, OCH_2), 4.24 (t, 2H, *J* = 6.4 Hz, OCH_2), 6.69 (dd, 1H, *J* = 8.4, 2.4 Hz, 8-H), 6.84 (d, 1H, *J* = 8.4 Hz, 7-H), 7.07 (m, 2H, Ar-H), 7.19 (d, 1H, *J* = 2.4 Hz, 10-H), 7.27 (dd, 1H, *J* = 9.2, 2.8 Hz, 3-H), 7.60 (*m*, 2H, Ar-H), 7.90 (d, 1H, *J* = 9.2 Hz, 4-H), 8.12 (d, 1H, *J* = 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 27.40 (2C), 27.56, 45.50 (2C), 45.56 (4C), 56.16, 56.38 (2C), 66.40, 66.70, 66.74, 101.23, 111.12, 114.70 (2C), 119.40, 123.57, 124.01, 124.47, 130.15 (2C), 131.03, 132.08, 132.75, 135.51, 135.81, 136.60, 146.09, 151.93, 159.69, 159.72, 160.24, 165.46. Anal. Calcd for $C_{37}H_{46}N_4O_4 \cdot 1.0H_2O \cdot 0.2HCl$: C, 69.85; H, 7.65; N, 8.81. Found: C, 69.69; H, 7.45; N, 8.65.

5.2. Pharmacological methods

5.2.1. Antiproliferative assay

Cancer cells (Hep G2, Hep 3B, Hep 2.2.1, A549, H1299) and normal cell (MRC-5) were purchased from Bioresources Collection and Research Center, Taiwan. Cell line was 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 that is, 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. (The medium was then changed (100 μL), and the cells were incubated for another day. Fifty microliters of serum-free medium containing XTT (1 mg/mL) and phenazine methosulfate (PMS) (10 μM) was added to the cells which were then 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 $(Ab_{treated}/Ab_{control}) \times 100$, 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%.[20]

5.2.2. Cell cycle analysis

A549 cells were treated with DMSO, **7** or **17** at different concentrations (0.1, 1.0, 5.0, 10.0 μM) for 24 h. Cells were harvested, rinsed in PBS, resuspended, fixed in 70% ethanol, and stored at −20 °C in fixation buffer until ready for analysis. 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

A549 Cells were seeded on cover glasses in 12-well plates with DMSO or compound **17** for 24 h were used for DAPI staining. 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 (Santa Cruze, 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. Samples were mounted before analyzing under a fluorescence microscopy.

5.2.4. DNA fragmentation assay

DNA fragmentation was determined by agarose gel electrophoresis. Cells were treated with various concentrations of compound **17** (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 1× TAE buffer (40 mmol/L of Tris, 2 mmol/L of EDTA, 20 mmol/L of acetic acid). DNA ladder marker (0.2–14.0 kb; GeneMark) was added to gel as a reference for the analysis of internucleosomal DNA fragmentation. The gel was stained with ethidium bromide (20 μg/mL) and photographed under ultraviolet illumination.

5.2.5. Immunoblot analysis

After treatment of compound **17**, cells were collected and washed twice with cold PBS and 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 μg/mL aprotinin, and 25 μg/mL leupeptin) and kept on ice for 30 min. The lysates were centrifuged at 12,000*g* at 4 °C for 20 min and the supernatants were stored at −70 °C. The protein concentration was determined by the Bradford method. Protein (20 μg) were separated by 10% 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 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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