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Synthesis and antiproliferative evaluation of 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives

Chih-Hua Tseng [a], Cherng-Chyi Tzeng [a], Kuin-Yu Chung [a], Chai-Lin Kao [a], Chih-Yao Hsu [a], Chih-Mei Cheng [b], Keng-Shiang Huang [c], Yeh-Long Chen [a,*]

[a] *Department of Medicinal and Applied Chemistry, College of Life Science, and Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung City 807, Taiwan*
[b] *Department of Biomedical Science and Environmental Biology, College of Life Science, and Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[c] *The School of Chinese Medicine for Post-Baccalaureate, I-Shou University, Kaohsiung, Taiwan*

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ABSTRACT

A number of 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives were synthesized and evaluated for their antiproliferative activities. Among them, (*E*)-6-{4-[3-(dimethylamino)propoxy]phenyl}-2-fluoro-9-hydroxy-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (**23a**) was the most active, exhibited GI_{50} values of 0.64, 0.39, 0.55, 0.67, and 0.65 μM against the growth of Hep G2, Hep 3B, A549, H1299, and MDA-MB-231, respectively. Compound **23a** inhibited the growth of hepatoma cell lines in a dose- and time-dependent manner. The proportion of cells was decreased in the G1 and accumulated in G2/M phase after 12 h treatment of **23a**, while the hypodiploid (sub-G0/G1 phase) cells increased. Further investigations have shown that **23a** induced cell cycle arrest at G2/M phase and induce apoptosis via activation of p53, Bax, and caspase-8 which consequently cause cell death.

1. Introduction

Quinoline moiety constitutes a number of potential anticancer agents. For examples, camptothecin (CPT) which bears a quinoline skeletone, is an anticancer alkaloid isolated from *Camptotheca acuminate*.[1,2] Camptothecin is a prototypical topoisomerase I (Topo I) inhibitor with poor water solubility. Subsequent introduction of hydrophilic side chains led to the discovery of topotecan which are currently used as an anticancer drug. TAS-103 (**1**) (Fig. 1), one of the indeno[2,1-*c*]quinoline derivatives, has been proved to be a novel Topo I and Topo II targeting agent that stabilizes cleavable complexes of Topo-DNA at the cellular level.[3–5] A number of furo[2,3-*b*]quinoline derivatives, such as CIL-102 (**2**), was synthesized and demonstrated to possess significant anticancer activity.[6–10] We have also prepared certain indeno[1,2-*c*]quinoline and 6-arylindeno[1,2-*c*]quinoline derivatives which have shown good antiproliferative activity, DNA binding affinity, and topo I and topo II inhibitory activities.[11–13] For examples, 2,9-bis(3-(dimethylamino)propoxy)-6-(4-(3-(dimethylamino)propoxy)phenyl)-11*H*-indeno[1,2-*c*]-quinolin-11-one (**3**), which exhibited GI_{50} values 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.[12] (*E*)-6-Hydroxy-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (**4**), which exhibited GI_{50} values of 0.84, 0.89, and 0.79 μM against SAS, A549, and BT483, respectively, was more active than CPT.[13] In continuation of our study to explore more potent anticancer drug candidates, we described herein the preparation of certain 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives and their evaluation in vitro against a panel of six cancer cell lines including two human hepatocelluar 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). These cancers are common malignancies in the world, and especially are the leading cause of cancer deaths in Asian countries including Taiwan.[14–17]

2. Chemistry

Treatment of the known 9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**5**)[12] with NH_2OH gave exclusively (*E*)-9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]-quinolin-11-one oxime (**8**) as a sole product. Alkylation of **8** with 3-chloro-*N*,*N*-dimethylpropanamine afforded (*E*)-9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]-quinolin-11-one *O*-3-(dimethylamino)propyl oxime (**11a**) as described in Scheme 1. Compounds

* Corresponding author. Tel.: +886 7 3121101x2684; fax: +886 7 3125339.
E-mail address: yeloch@kmu.edu.tw (Y.-L. Chen).

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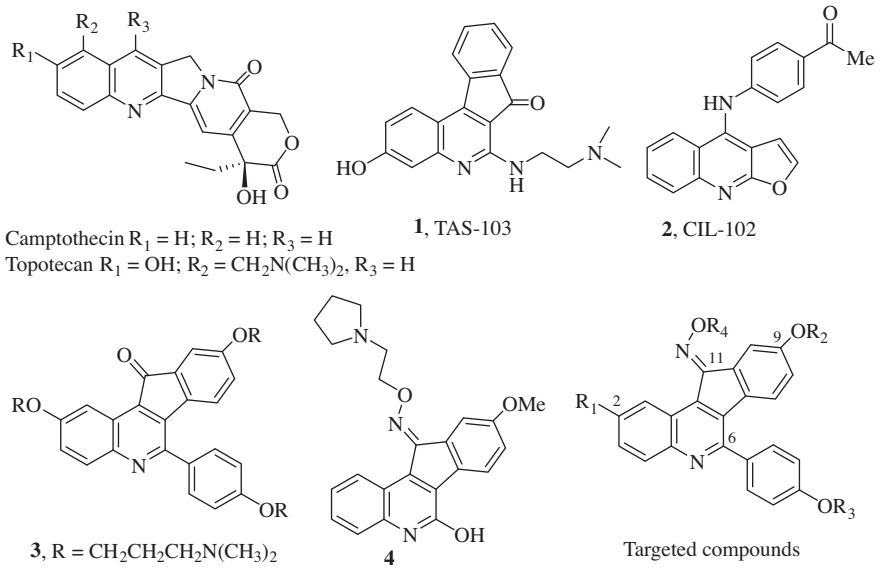
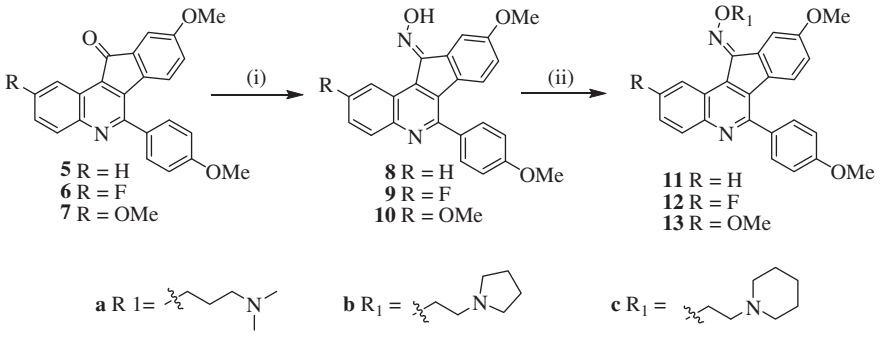


Figure 1. Structures of Camptothecin, Topotecan, 6-[2-(dimethylamino)ethylamino]-3-hydroxy-7*H*-indeno[2,1-*c*]quinolin-7-one (**1**, TAS-103), 1-[4-(Furo[2,3-*b*]quinolin-4-ylamino)phenyl]-ethanone (**2**, CIL-102), Aminoalkyl substituted indenoquinolines **3** and **4**, and targeted compounds.



Scheme 1. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, 2-EtOEtOH, 150 °C, 3 h; (ii) NaH, alkyl halides, DMF, 80 °C, 1 h.

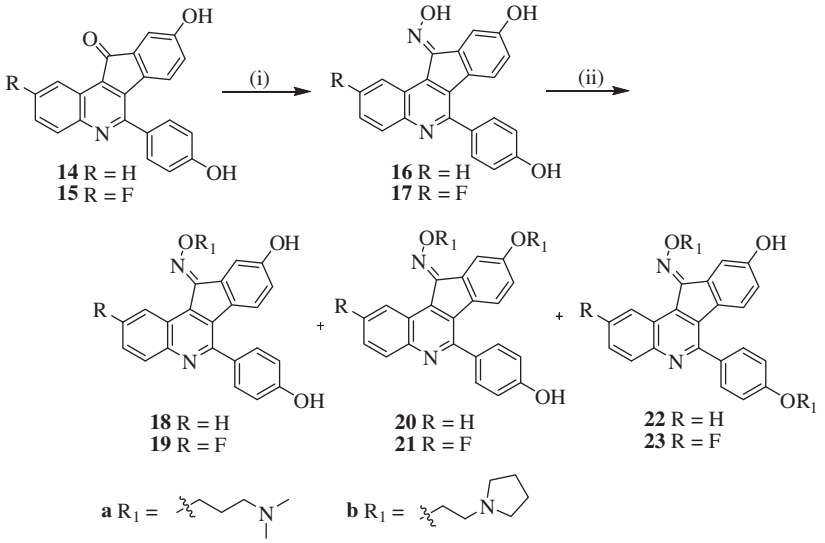
11b and **11c** were obtained by alkylation of **8** with *N*-(2-chloroethyl)pyrrolidine and *N*-(2-chloroethyl)piperidine, respectively, under the same reaction conditions. Compounds **12a**–**12c** were prepared from their oxime precursor **9** which in turn was obtained by the reaction of **6**[12] with NH_2OH. Accordingly, oximization of compound **7**[12] gave its oxime derivative **10** which was then alkylated with various aminoalkyl halides to afford **13a** and **13b**.

Reaction of **14**[12] with NH_2OH afforded 9-hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one oxime (**16**) which was then alkylated with 3-chloro-*N*,*N*-dimethylpropanamine to give a mixture of (*E*)-9-hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (**18a**), (*E*)-9-[3-(dimethylamino)propoxy]-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (**20a**) and (*E*)-6-{4-[3-(dimethylamino)propoxy]phenyl}-9-hydroxy-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (**22a**) in a yield of 27%, 26%, and 21%, respectively as described in Scheme 2. Compounds **18b**, **20b**, and **22b** were obtained by alkylation of their oxime precursor **16** which in turn was prepared from **14** and NH_2OH. Accordingly, oximization of compound **15**[12] gave its oxime derivative **17** which was then alkylated with 3-chloro-*N*,*N*-dimethylpropanamine to give a mixture of **19a**, **21a**, and **23a**. Alkylation compound **17** with *N*-(2-chloroethyl)pyrrolidine afforded a mixture of **19b**, **21b**, and **23b**.

Structure of **18b** was assigned based on the downfield protons of OCH_2 at 4.68 ppm and two broad signals of OH groups at 9.88 and 9.86 ppm. Structures of dialkylated products **20b** and **22b** were distinguished by the NOESY spectra. Compound **20b** was assigned as 9,11-dialkylated derivative based on the correlation of OCH_2 (δ_H = 4.15 ppm)/8-H (δ_H = 6.61 ppm) and OCH_2 (δ_H = 4.15 ppm)/10-H (δ_H = 7.93 ppm). Compound **22b** was assigned as 6-aryl,11-dialkylated isomer based on the correlations between OCH_2 (δ_H = 3.93 ppm) and meta-H (δ_H = 6.23 ppm). The same assignments were applied for the mixtures of **18a**, **20a**, and **22a**; **19a**, **21a**, and **23a**; and **19b**, **21b**, and **23b** (Fig. 2).

3. Biological results and discussion

All the synthesized 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives were evaluated in vitro against a panel of six cancer cell lines including two human hepatocelluar 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 (2,3-bis[2-methyloxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide) assay.[18] 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



Scheme 2. Reagents and conditions: (i) Hydroxyamine, 2-EtOEtOH, 150 °C, 3 h; (ii) NaH, alkyl halides, DMF, 80 °C, 25 min.

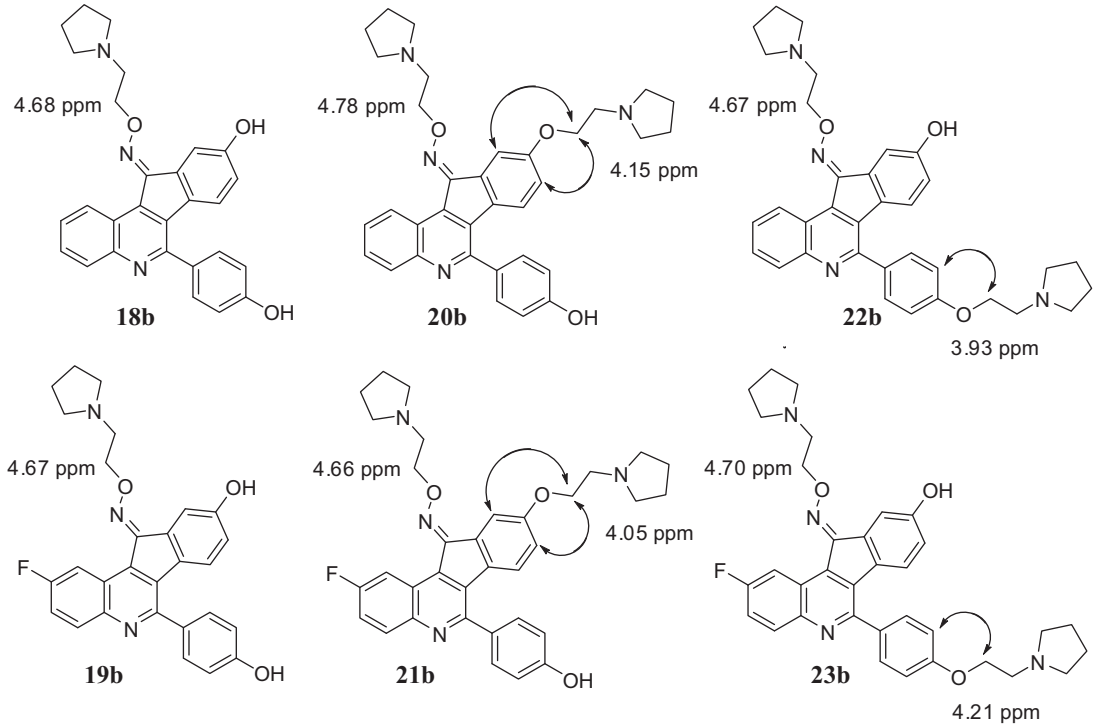
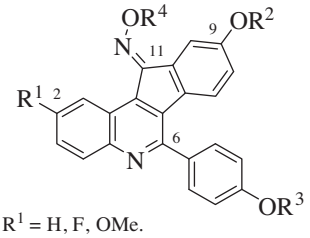


Figure 2. NOESY correlations of compounds **20b**, **21b**, **22b** and **23b**.

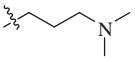
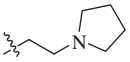
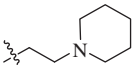
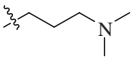
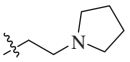
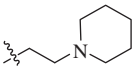
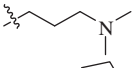
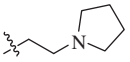
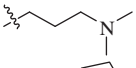
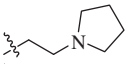
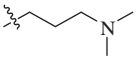
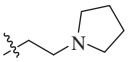
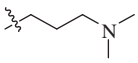
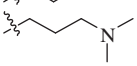
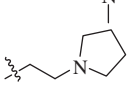
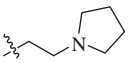
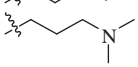
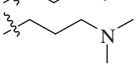
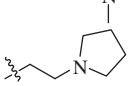
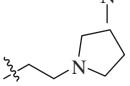
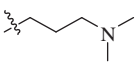
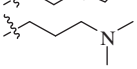
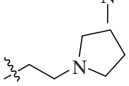
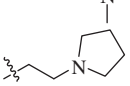
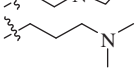
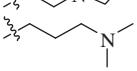
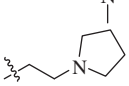
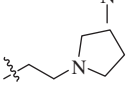
50%. The GI_{50} results of 6-arylindeno[1,2-*c*]quinoline-11-one derivatives are summarized in Table 1. Three oxime precursors **8**–**10** were inactive against all the cancer cells tested with GI_{50} of >10 μM in each case. For the C-2 unsubstituted 6-arylindeno[1,2-*c*]quinoline derivatives, introduction of an acyclic dimethylaminopropyl group or a pyrrolidin-1-yl ethyl side chain at the C-11 oxime moiety enhanced antiproliferative activity in which each of compounds **11a** and **11b** was more active than compound **8**. However, with an exception of Hep 3B, the antiproliferative activity did not improve by the introduction of piperidin-1-yl ethyl on the same position indicated a bulky substituent at C-11 oxime moiety is unfavorable. The same trend was observed for C-2 fluoro derivatives **12a**–**12c** in which each of compounds **12a** and **12b** was more active than their C-11 oxime precursor **9** while **12c** was inactive. With the same aminoalkyl side chain on C-11 oxime moiety, the antiproliferative activity of C-2 methoxy derivative **13a** and its C-2 unsubstituted counterpart **11a** are comparable while C-2 fluoro derivative **12a** was the most active. Compound **12a** exhibited GI_{50} values of 0.66, 0.91, 0.61, and 0.70 μM against the growth of Hep 3B, A549, H1299, and MDA-MB-231, respectively, was more active than the positive Irinotecan.

With an exception of the MDA-MB-231, the oxime precursor **16** was inactive. Introduction of C-2 fluoro group improved antiproliferative activity in which compound **17** was marginally active against the growth of Hep 3B, MCF-7, and MDA-MB-231. However, antiproliferative activity was significantly enhanced by the introduction of

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



R^1 = H, F, OMe.
R^2, R^3, R^4 = H, Me, or aminoalkyl groups.

Compound	R_1	R_2	R_3	R4	Cell line (GI_{50}, μM)					
					Hep G2	Hep 3B	A549	H1299	MCF-7	MDA-MB-231
8	H	Me	Me	H	>10	>10	>10	>10	>10	>10
9	F	Me	Me	H	>10	>10	>10	>10	>10	>10
10	OMe	Me	Me	H	>10	>10	>10	>10	>10	>10
11a	H	Me	Me		6.54 ± 0.11	3.14 ± 0.04	6.69 ± 0.07	6.61 ± 0.06	6.57 ± 0.11	7.01 ± 0.02
11b	H	Me	Me		5.48 ± 0.01	4.68 ± 0.03	5.27 ± 0.03	6.31 ± 0.04	6.71 ± 0.02	5.33 ± 0.03
11c	H	Me	Me		>10	8.29 ± 2.02	>10	>10	>10	>10
12a	F	Me	Me		5.75 ± 0.03	0.66 ± 0.02	0.91 ± 0.24	0.61 ± 0.01	6.18 ± 0.02	0.70 ± 0.03
12b	F	Me	Me		9.46 ± 1.42	3.11 ± 0.02	9.24 ± 2.55	3.15 ± 0.02	6.36 ± 0.03	5.99 ± 0.04
12c	F	Me	Me		>10	>10	>10	>10	>10	>10
13a	OMe	Me	Me		5.95 ± 0.03	6.49 ± 0.03	6.62 ± 0.03	5.70 ± 0.02	6.30 ± 0.07	6.13 ± 0.06
13b	OMe	Me	Me		6.03 ± 0.02	6.17 ± 0.03	6.42 ± 0.02	6.54 ± 0.72	6.25 ± 0.09	5.15 ± 0.11
16	H	H	H	H	>10	>10	>10	>10	>10	8.47 ± 0.82
17	F	H	H	H	>10	7.60 ± 0.87	>10	>10	8.77 ± 0.75	8.41 ± 0.62
18a	H	H	H		1.55 ± 0.01	0.67 ± 0.10	0.50 ± 0.01	6.94 ± 0.06	6.06 ± 0.03	0.93 ± 0.03
18b	H	H	H		5.74 ± 0.04	0.52 ± 0.03	0.88 ± 0.02	6.06 ± 0.06	7.83 ± 0.24	0.58 ± 0.05
19a	F	H	H		6.24 ± 0.04	0.69 ± 0.06	6.46 ± 0.04	6.30 ± 0.02	6.28 ± 0.05	4.74 ± 0.09
19b	F	H	H		5.04 ± 0.05	0.64 ± 0.04	0.84 ± 0.04	6.48 ± 0.02	6.26 ± 0.04	0.68 ± 0.07
20a	H	H			4.96 ± 0.02	5.41 ± 0.02	2.10 ± 0.04	5.30 ± 0.05	>10	3.07 ± 0.01
20b	H	H			4.09 ± 0.05	0.78 ± 0.13	0.79 ± 0.10	6.51 ± 0.17	6.42 ± 0.07	0.68 ± 0.12
21a	F	H			0.66 ± 0.03	0.56 ± 0.01	0.42 ± 0.02	0.84 ± 0.08	6.25 ± 0.01	0.67 ± 0.03
21b	F	H			5.71 ± 0.05	0.23 ± 0.01	0.53 ± 0.01	7.09 ± 0.04	6.20 ± 0.08	0.33 ± 0.02
22a	H		H		3.77 ± 0.04	0.61 ± 0.42	2.03 ± 0.19	0.69 ± 0.12	6.65 ± 0.09	0.63 ± 0.03
22b	H		H		4.11 ± 0.01	0.92 ± 0.39	0.54 ± 0.01	0.57 ± 0.02	5.92 ± 0.02	0.51 ± 0.05
23a	F		H		0.64 ± 0.01	0.39 ± 0.02	0.55 ± 0.05	0.67 ± 0.02	6.46 ± 0.05	0.65 ± 0.01
23b	F		H		1.08 ± 0.01	0.47 ± 0.01	0.31 ± 0.01	0.70 ± 0.06	6.04 ± 0.01	0.48 ± 0.01
Topotecan[a]					3.83 ± 0.15	0.22 ± 0.01	5.98 ± 0.26	>10	>10	0.078 ± 0.002
Irinotecan[a]					5.94 ± 0.75	4.73 ± 0.21	>10	>10	>10	9.17 ± 0.02

[a] Topotecan and irinotecan were obtained from ScinoPharm Taiwan Ltd. (Tainan, Taiwan).

at least one aminoalkyl side chain in which each of compounds **18a**, **20a**, and **22a** was much more active than **16**. The same trend was observed in which in which each of compounds **19a**, **21a**, and **23a** was much more active than its oxime precursor **17**. In general, H-bond donating OH group was more favorable than H-bond accepting OMe group at both C-6 aryl and C-9 positions in which compound **18a** was more active than **11a** while **18b** was more active than **11b**, and **19b** was more active than **12b**. For the dialkylated 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives, aminoalkyl side chain substituted at both C-6 aryl and C-11 positions is more favorable than that of substitution at both C-9 and C-11 positions in which compound **22a** was more active than **20a**.

These 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives demonstrated selectivity on subtype of cancer cells. For examples, Hep 3B was more susceptible than Hep G2, A549 was more susceptible than H1299, and MDA-MB-231 was more susceptible than MCF-7. Among six cancer cells tested, MDA-MB-231 and Hep 3B were two of the most sensitive while MCF-7 was the most resistant. Among these 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives evaluated, compound **23a** was the most active, exhibited GI_{50} values of 0.64, 0.39, 0.55, 0.67, and 0.65 μM against the growth of Hep G2, Hep 3B, A549, H1299, and MDA-MB-231, respectively, was more active than the positive Irinotecan.

To gain further into the effects, hepatoma cell lines (Hep G2 & Hep 3B) were treated with 0.1–5.0 μM of compound **23a** for 12, 24, 36 and 48 h and the number of viable cells was counted by XTT method. Compound **23a** inhibited the growth of hepatoma cell lines (Hep G2 & Hep 3B) in a dose- and time-dependent manner as shown in Figure 3. These data indicated that **23a** almost suppressed cell viability at high concentration (5 μM) after 12 h. Compound **23a** was also selected for further evaluation on its effects of hepatoma cell cycle distribution by flow cytometric analysis. Compound **23a** induced cell cycle arrest in a concentration dependent manner as shown in Table 2. The proportion of cells was decreased in the G1 and accumulated in G2/M phase after 12 h treatment of **23a**, while the hypodiploid (sub-G0/G1 phase) cells increased. Apoptosis can be characterized by morphological and biochemical changes in the cell nucleus, including chromatin condensation and nuclear shrinking. Morphological changes of cells treated with **23a** can be visually observed with light microscopy (Fig. 4). We found that the hepatoma cell lines (Hep G2 & Hep 3B) treated with **23a** at 5 μM for 12 h became shrinked. Such morphological changes were not apparent in the control cells. There are many regulators involved in apoptosis induction. The Bcl-2 family proteins are known to modulate apoptosis and the tumor suppressor protein p53 responds to cellular damage and induces apoptosis through caspase family or death receptor apoptosis pathways.[19–21] Our results have shown that **23a** promotes apoptosis by an increase in p53, Bax and activate caspase-8 proteins expression was observed in **23a**-treated Hep G2 cells (Fig. 5). However, Bcl-2 protein was not detected in Hep G2 cells, including control and **23a** treated cells. Previous studies by Kuo et al. have also reported that Bcl-2 protein was not detected in hepatoma cell lines (Hep G2 & Hep 3B).[22] These findings indicate that apoptosis induction may be a mechanism by which **23a** kills the cancer cells. Thus, compound **23a** induces cell cycle arrest at G2/M phase which led to apoptosis.

Table 2
Effects of **23a** on hepatoma cell cycle progression.

Cell lines	Concentration (μM)	Cell cycle distribution (%)			
		Sub G1	G1	S	G2/M
Hep G2 cell	DMSO	3.6	67.5	13.8	18.7
	1.0	4.9	56.5	12.4	31.1
	5.0	19.8	38.1	18.5	23.6
	10.0	29.1	31.3	21.7	17.9
Hep 3B cell	DMSO	2.1	71.6	12.2	16.2
	1	5.3	58.9	10.4	30.7
	5.0	21.8	42.7	17.4	18.1
	10.0	33.5	37.2	15.2	14.1

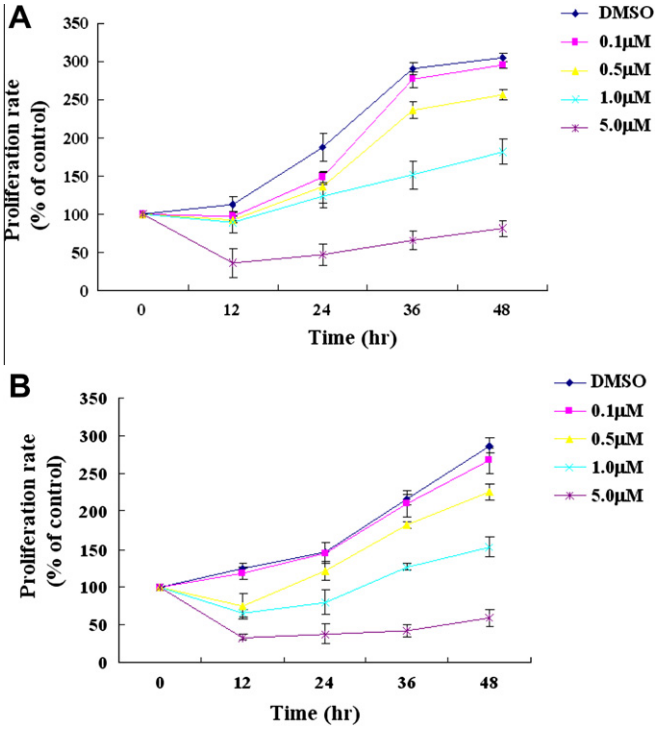


Figure 3. Compound **23a** suppresses the viability of Hep G2 (A) and Hep 3B (B) cells. Hepatoma cells were treated with DMSO, 0.1, 0.5, 1.0 and 5.0 μM of **23a**. At 12, 24, 36 and 48 h after treatment, 50 μL XTT reaction solution was added to each well for an additional 4 h of incubation. The absorbance at 492 nm was measured on a microtiter plate reader. The results represent the mean ± SD ($n = 4$).

4. Conclusion

A number of 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives were synthesized and evaluated for antiproliferative activities against the growth of six cancer cell lines including two human hepatocelluar 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). Among these cancer cells tested, MDA-MB-231 and Hep 3B were two of the most sensitive while MCF-7 was the most resistant. Among these 6-aryl-11-iminoindeno[1,2-*c*]quinoline derivatives evaluated, (*E*)-6-{4-[3-(dimethylamino)propoxy]phenyl}-2-fluoro-9-hydroxy-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (**23a**) was the most active, exhibited GI_{50} values of 0.64, 0.39, 0.55, 0.67, and 0.65 μM against the growth of Hep G2, Hep 3B, A549, H1299, and MDA-MB-231, respectively, was more active than the positive Irinotecan. Flow cytometric analysis indicated **23a** effectively induced G2/M arrest and progress to apoptosis in hepatoma cells after staining with propidium iodide (PI). This suggested that **23a** might inhibit the growth of hepatoma cell lines by the induction of apoptosis. The present work demonstrated that incorporating the flexible aminoalkyl side chains on the pharmacophore of 6-aryl-11-imino-indeno [1,2-*c*]quinoline led to the discovery of novel compounds with

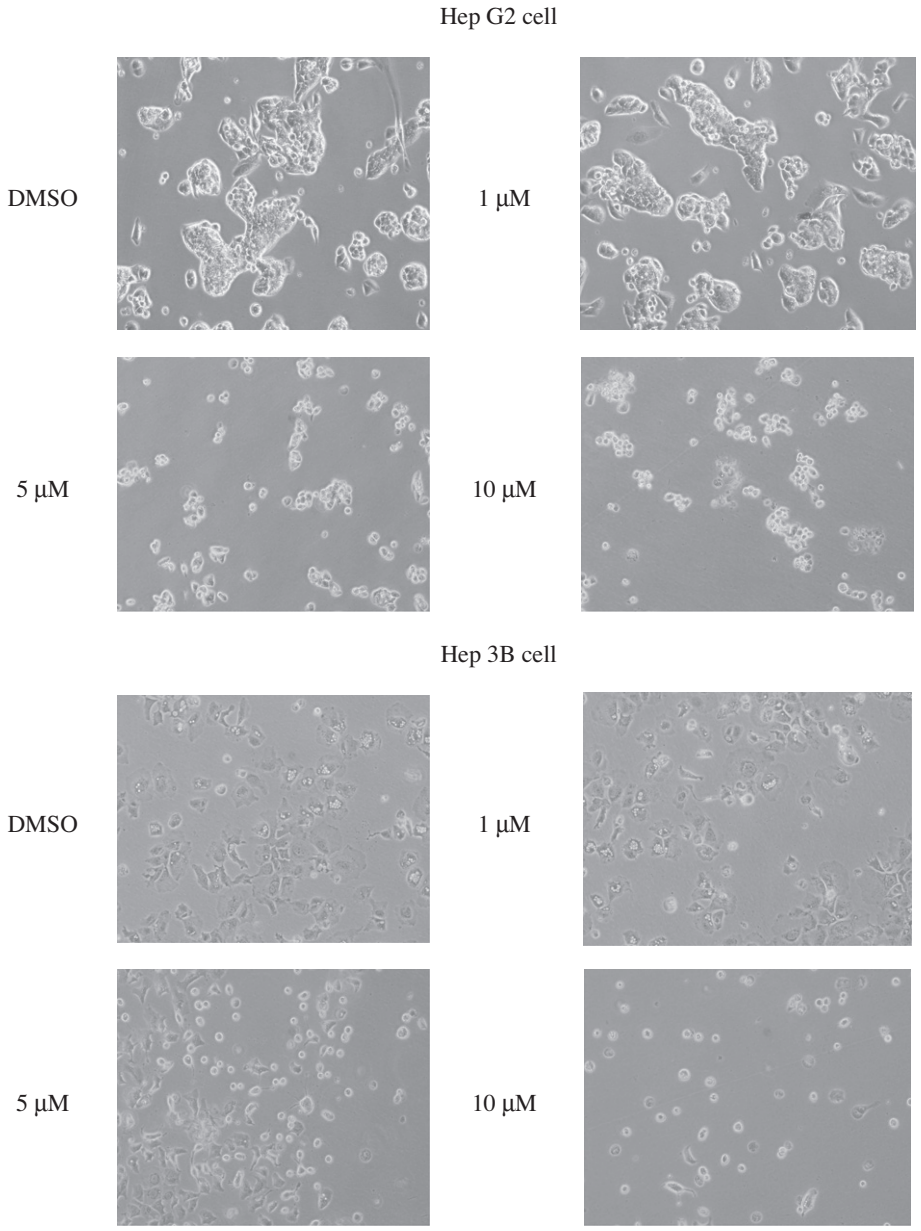


Figure 4. Induction of morphological change in Hep G2 and Hep 3B cells. Cells were treated with DMSO or compound **23a** (1–10 μM) for 12 h at 37 °C and photographed under a microscope.

potential apoptosis-inducing and anticancer activities. Further structural optimization and detailed biological studies on the molecular mechanism of action are ongoing.

5. Experimental

5.1. General

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

5.2. General procedure for the preparation of oxime compounds 8–10, 16 and 17

A mixture of 2-substituted 6-aryllindeno[1,2-*c*]quinolin-11-one **5–7** (1.0 mmol), hydroxylamine·HCl (3.0 mmol), and 2-ethoxyethanol (30 mL) was heated at reflux for 3–6 h (TLC monitoring). The solvent was removed in vacuo and the residue was suspended in H_2O (20 mL). The resulting precipitate that separated was collected, washed with H_2O, and dried to give a crude solid. The crude product was purified by crystallization from EtOH to afford compounds **8–10**. Accordingly, compounds **16** and **17** were prepared from their carbonyl precursors **14** and **15**, respectively.

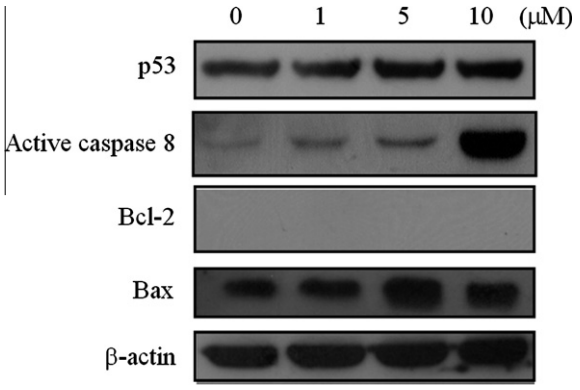


Figure 5. Immunoblot analysis of the expression of p53, caspase-8, Bcl-2, and Bax in Hep G2 cells. Cells were incubated with compound **23a** (0–10 μM) and the expression of the apoptosis-related proteins p53, caspase-8, Bcl-2, and Bax was determined at 12 h by western blot. Protein loading was normalized to the expression of β-actin. A representative experiment is shown of three performed.

5.2.1. (*E*)-9-Methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one oxime (8)

Yield 72% as a yellow solid. Mp 270 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 3.79 and 3.89 (two s, 6H, 4′- and 9-OMe), 6.86–6.91 (m, 2H, Ar-H), 7.13–7.17 (m, 2H, Ar-H), 7.59–7.74 (m, 4H, Ar-H), 8.00–8.03 (m, 2H, Ar-H), 8.90 (dd, 1H, J = 8.4, 1.2 Hz, 1-H), 13.56 (br s, 1H, NOH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.31, 55.51, 113.93 (2C), 115.09, 115.26, 122.25, 123.09, 125.21, 128.01, 129.14, 129.52, 130.15 (2C), 130.97, 131.04, 131.08, 132.01, 137.56, 146.71, 153.52, 154.33, 159.84 (2C). Anal. Calcd for $C_{24}H_{18}N_2O_3$·0.3HCl: C, 73.28; H, 4.69; N, 7.12. Found: C, 73.05; H, 4.79; N, 6.87.

5.2.2. 2-Fluoro-9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one oxime (9)

Yield 70% as a yellow solid. Mp: 299–300 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 3.76 and 3.88 (two s, 6H, 4′- and 9-OMe), 6.85 (s, 2H), 7.12–7.14 (m, 2H, Ar-H), 7.56–7.61 (m, 3H, Ar-H), 7.95 (s, 1H, 10-H), 8.06 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.49 (dd, 1H, J = 10.4, 2.8 Hz, 1-H), 13.61 (s, 1H, NOH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.39, 55.60, 108.51 (J = 24.3 Hz), 114.04 (2C), 114.98, 115.50, 119.42 (J = 23.1 Hz), 122.70 (J = 9.1 Hz), 123.43, 130.25 (2C), 130.46, 131.11, 131.74, 132.08, 132.56 (J = 9.9 Hz), 135.81 (J = 5.4 Hz), 145.43, 153.08, 153.68, 160.06, 160.18, 161.12 (J = 239.3 Hz). Anal. Calcd for $C_{24}H_{17}FN_2O_3$·0.1HCl: C, 71.34; H, 4.27; N, 6.93. Found: C, 71.56; H, 4.29; N, 6.83.

5.2.3. 2,9-Dimethoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one oxime (10)

Yield 60% as a yellow solid. Mp: 273–274 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 3.77, 3.87 and 3.92 (three s, 9H, 4′-, 2-, and 9-OMe), 6.84–6.89 (m, 2H, Ar-H), 7.10–7.14 (m, 2H, Ar-H), 7.35 (ddd, 1H, J = 9.2, 2.8, 0.8 Hz, 3-H), 7.54–7.58 (m, 2H), 7.91 (d, 1H, J = 9.2 Hz, 4-H), 8.02 (d, 1H, J = 2.4 Hz, 10-H), 8.25 (d, 1H, J = 2.8 Hz, 1-H), 13.44 (s, 1H, NOH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.28, 55.44, 55.50, 103.24, 113.87 (2C), 115.05, 115.25, 121.46, 123.10, 123.28, 130.19 (2C), 131.05 (2C), 131.21, 131.25, 132.30, 135.98, 143.22, 151.72, 153.66, 158.66, 159.62, 159.80. Anal. Calcd for $C_{25}H_{20}N_2O_4$·1.7HCl: C, 63.29; H, 4.61; N, 5.90. Found: C, 63.04; H, 4.69; N, 5.51.

5.3. General procedure for preparation of aminoalkylated oxime derivatives: 11a–c, 12a–c, and 13a–b

To a stirred solution of oxime derivatives **8**–**10** (1.0 mmol) in dry DMF (20 mL) was added NaH (60% in oil, 0.50 g) at 0 °C for 1 h. Appropriate tertiary-aminoalkyl halide derivatives (3 mmol) was added and heated at 80 °C for 1–2 h (TLC monitoring). The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuo. Crude product was purified by flash chromatography on silica gel (MeOH/CH_2Cl_2 1:10) and crystallized from EtOH.

5.3.1. (*E*)-9-Methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethyl-amino)propyl oxime (11a)

Yield 80% a yellow solid. Mp 122–123 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.02–2.08 (m, 2H, $OCH_2CH_2CH_2$), 2.25 (s, 6H, NMe_2), 2.52–2.55 (m, 2H, CH_2N), 3.78 and 3.89 (two s, 6H, 4′- and 9-OMe), 4.62 (t, 2H, J = 6.4 Hz, OCH_2), 6.85–6.91 (m, 2H, 7- and 8-H), 7.12–7.16 (m, 2H, Ar-H), 7.57–7.73 (m, 4H, 2-, 3- and Ar-H), 7.84–7.85 (m, 1H, 10-H), 8.01 (dd, 1H, J = 8.4, 0.8 Hz, 4-H), 8.83–8.86 (m, 1H, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 26.64, 44.87(2C), 55.29, 55.46, 55.50, 74.86, 113.90(2C), 115.47, 115.61, 122.10, 123.29, 125.06, 128.22, 129.19, 129.69, 130.10(2C), 130.83, 131.30, 131.46, 132.01, 136.62, 147.00, 153.41, 154.35, 159.82 (2C). Anal. Calcd for $C_{29}H_{29}N_3O_3$·0.2 H_2O: C, 73.93; H, 6.29; N, 8.92. Found: C, 73.74; H, 6.33; N, 8.74.

5.3.2. (*E*)-9-Methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (11b)

Yield 86% a yellow solid. Mp: 135–136 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.81–1.84 (m, 4H, pyrrolidinyl-H), 2.69–2.71 (m, 4H, pyrrolidinyl-H), 3.09 (t, 2H, J = 6.0 Hz, CH_2N), 3.82 and 3.92 (two s, 6H, 4′- and 9-OMe), 4.76 (t, 2H, J = 6.0 Hz, OCH_2), 6.71 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.91 (d, 1H, J = 8.4 Hz, 7-H), 7.06–7.09 (m, 2H, Ar-H), 7.53–7.65 (m, 4H, 2-, 3-, and Ar-H), 7.98 (d, 1H, J = 2.4 Hz, 10-H), 8.10 (d, 1H, J = 8.4 Hz, 4-H), 8.94 (dd, 1H, J = 8.8, 1.6 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.52 (2C), 54.84 (2C), 54.93, 55.41, 55.57, 76.03, 114.08 (2C), 115.28, 115.75, 122.98, 123.52, 125.63, 127.78, 128.04, 129.97, 130.11 (2C), 131.71, 132.07, 132.49, 132.76, 137.80, 147.59, 154.32, 154.74, 159.95, 160.14. Anal. Calcd for $C_{30}H_{29}N_3O_3$: C, 75.13; H, 6.10; N, 8.76. Found: C, 74.97; H, 6.13; N, 8.60.

5.3.3. (*E*)-9-Methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(piperidin-1-yl)ethyl oxime (11c)

Yield 78% a yellow solid. Mp 161–162 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43–1.49 (m, 2H, piperidinyl-H), 1.61–1.66 (m, 4H, piperidinyl-H), 2.59 (br s, 4H, piperidinyl-H), 2.95 (t, 2H, J = 6.0 Hz, CH_2N), 3.83, 3.92 (two s, 6H, 4′- and 9-OMe), 4.75 (t, 2H, J = 6.0 Hz, OCH_2), 6.71 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.91 (d, 1H, J = 8.4 Hz, 7-H), 7.06–7.09 (m, 2H, Ar-H), 7.53–7.65 (m, 4H, 2-, 3-, and Ar-H), 7.97 (d, 1H, J = 2.4 Hz, 10-H), 8.08–8.11 (m, 1H, 4-H), 8.92–8.95 (m, 1H, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 24.14, 25.97 (2C), 55.03 (2C), 55.41, 55.56, 57.93, 74.94, 114.08 (2C), 115.28, 115.68, 122.99, 123.50, 125.66, 127.75, 128.82, 129.97, 130.11 (2C), 131.72, 132.03, 132.49, 132.80, 137.83, 147.60, 154.25, 154.74, 159.94, 160.14. Anal. Calcd for $C_{31}H_{31}N_3O_3$: C, 75.43; H, 6.33; N, 8.51. Found: C, 75.04; H, 6.28; N, 8.42.

5.3.4. (*E*)-2-Fluoro-9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quin-olin-11-one *O*-3-(dimethylamino)propyl oxime (12a)

Yield 82% a yellow solid. Mp 164–165 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 2.23–2.30 (m, 2H, $OCH_2CH_2CH_2$), 2.47 (s, 6H, NMe_2), 2.78 (t, 2H, J = 7.2 Hz, CH_2N), 3.84 and 3.92 (two s, 6H, 4′- and 9-OMe), 4.67 (t, 2H, J = 6.4 Hz, OCH_2), 6.70 (dd, 1H, J = 8.4, 2.8 Hz, 8-H), 6.92 (d, 1H, J = 8.8 Hz, 7-H), 7.06–7.10 (m, 2H, Ar-H), 7.38 (ddd, 1H, J = 9.2, 8.0, 2.8 Hz, 3-H), 7.58–7.61 (m, 2H, Ar-H), 7.89 (d, 1H, J = 2.4 Hz, 10-H), 8.07 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.51 (dd, 1H, J = 10.4, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$):

26.68, 44.62 (2C), 55.42, 55.62, 56.03, 74.60, 108.95 (J = 24.2 Hz), 114.13 (2C), 115.13, 115.96, 119.06 (J = 25.7 Hz), 123.42 (J = 11.4 Hz), 123.83, 130.09 (2C), 131.65, 132.05, 132.39 (J = 9.1 Hz), 132.44, 132.64, 137.16, 137.22, 144.77, 154.04 (2C), 160.22 (J = 3.1 Hz), 161.64 (J = 246.4 Hz). Anal. Calcd for $C_{29}H_{28}FN_3O_3$: C, 71.74; H, 5.81; N, 8.65. Found: C, 71.66; H, 5.89; N, 8.61.

5.3.5. (*E*)-2-Fluoro-9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (12b)

Yield 95% a yellow solid. Mp 169–170 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.81–1.86 (m, 4H, pyrrolidinyl-H), 2.69–2.72 (m, 4H, pyrrolidinyl-H), 3.08 (t, 2H, J = 6.4 Hz, CH_2N), 3.83 and 3.92 (two s, 6H, 4′- and 9-OMe), 4.76 (t, 2H, J = 6.0 Hz, OCH_2), 6.72 (dd, 1H, J = 8.8, 2.8 Hz, 8-H), 6.93 (d, 1H, J = 8.8 Hz, 7-H), 7.06–7.09 (m, 2H, Ar-H), 7.38 (ddd, 1H, J = 9.6, 8.4, 2.8 Hz, 3-H), 7.58–7.61 (m, 2H, Ar-H), 7.97 (d, 1H, J = 2.8 Hz, 10-H), 8.07 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.55 (dd, 1H, J = 10.4, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.55 (2C), 54.84 (2C), 54.96, 55.42, 55.56, 76.22, 109.08 (J = 23.5 Hz), 114.13 (2C), 115.45, 115.65, 119.02 (J = 26.5 Hz), 123.48 (J = 11.3 Hz), 123.76, 130.11 (2C), 131.75, 132.06, 132.36 (J = 9.1 Hz), 132.52, 132.59, 137.28, 137.34, 144.78, 153.92, 154.05, 160.20, 161.64 (J = 246.4 Hz). Anal. Calcd for $C_{30}H_{28}FN_3O_3{\cdot}0.2\ H_2O$: C, 71.90; H, 5.71; N, 8.38. Found: C, 71.95; H, 5.71; N, 8.33.

5.3.6. (*E*)-2-Fluoro-9-methoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quin-olin-11-one *O*-2-(piperidin-1-yl)ethyl oxime (12c)

Yield 81% a yellow solid. Mp 180–181 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.44–1.49 (m, 2H, piperidinyl-H), 1.60–1.66 (m, 4H, piperidinyl-H), 2.58 (br s, 4H, piperidinyl-H), 2.93 (t, 2H, J = 6.0 Hz, CH_2N), 3.83 and 3.92 (two s, 6H, 4′- and 9-OMe), 4.74 (t, 2H, J = 6.0 Hz, OCH_2), 6.70 (dd, 1H, J = 8.4, 2.8 Hz, 8-H), 6.91 (d, 1H, J = 8.4 Hz, 7-H), 7.05–7.09 (m, 2H, Ar-H), 7.38 (ddd, 1H, J = 9.2, 8.4, 2.8 Hz, 3-H), 7.58–7.61 (m, 2H, Ar-H), 7.95 (d, 1H, J = 2.8 Hz, 10-H), 8.07 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.54 (dd, 1H, J = 10.4, 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 24.17, 26.00 (2C), 55.03 (2C), 55.42, 55.57, 57.96, 75.08, 109.10 (J = 24.2 Hz), 114.13 (2C), 115.45, 115.60, 119.02 (J = 25.7 Hz), 123.48 (J = 11.3 Hz), 123.74, 130.11 (2C), 131.75, 132.06, 132.35, 132.55, 137.29, 137.35, 144.78, 153.83, 154.02, 160.20, 161.63 (J = 246.4 Hz). Anal. Calcd for $C_{31}H_{30}FN_3O_3$: C, 72.78; H, 5.91; N, 8.21. Found: C, 72.61; H, 5.72; N, 7.97.

5.3.7. (*E*)-2,9-Dimethoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (13a)

Yield 76% a yellow solid. Mp 143–144 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.98–2.05 (m, 2H, $OCH_2CH_2CH_2$), 2.16 (s, 6H, NMe_2), 2.42 (t, 2H, J = 7.2 Hz, CH_2N), 3.76, 3.87 and 3.90 (three s, 9H, 2-, 4′-, and 9-OMe), 4.57 (t, 2H, J = 6.4 Hz, OCH_2), 6.85–6.87 (m, 2H, 7- and 8-H), 7.09–7.12 (m, 2H, Ar-H), 7.32 (dd, 1H, J = 9.2, 2.8 Hz, 3-H), 7.53–7.55 (m, 2H, Ar-H), 7.81–7.88 (m, 2H, 4- and 10-H), 8.17 (d, 1H, J = 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 26.94, 45.21 (2C), 55.27, 55.29, 55.50, 55.72, 74.97, 102.99, 113.86 (2C), 115.47, 121.63, 123.10, 123.32, 130.19 (2C), 130.99, 131.25, 131.54, 132.15, 135.19, 143.35, 151.62, 153.30, 158.77, 159.66, 159.79. Anal. Calcd for $C_{30}H_{31}N_3O_4{\cdot}0.1H_2O$: C, 72.15; H, 6.30; N, 8.41. Found: C, 72.00; H, 6.40; N, 8.33.

5.3.8. (*E*)-2,9-Dimethoxy-6-(4-methoxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (13b)

Yield 98% a yellow solid. Mp 202–203 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.77 (br s, 4H, pyrrolidinyl-H), 2.85 (br s, 4H, pyrrolidinyl-H), 3.25 (br s, 2H, CH_2N), 3.78, 3.88 and 3.93 (three s, 9H, 2-, 4′-, and 9-OMe)), 4.75 (br s, 2H, OCH_2), 6.86–6.90 (m, 2H, 7- and 8-H), 7.11–7.13 (m, 2H, Ar-H), 7.35 (dd, 1H, J = 9.2, 2.8 Hz, 3-H), 7.54–7.56 (m, 2H, Ar-H), 7.88–7.90 (m, 2H, 4- and 10-H), 8.18 (d, 1H, J = 2.8 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 22.90 (2C), 53.99 (2C), 55.29 (2C), 55.39, 55.57, 76.23, 103.04, 113.89 (2C), 115.72, 121.75, 123.10, 123.36, 130.16 (2C), 130.96, 131.27, 131.68, 132.06, 135.08, 143.41, 151.64, 153.96, 158.85, 159.69, 159.88. Anal. Calcd for $C_{31}H_{31}N_3O_4{\cdot}0.7\ H_2O$: C, 71.30; H, 6.25; N, 8.05. Found: C, 71.15; H, 6.23; N, 7.82.

5.4. General procedure for 16 and 17

5.4.1. 9-Hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one oxime (16)

Yield 61% as a yellow solid. Mp 340 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 6.75–6.84 (m, 2H, 7- and 8-H), 7.11–7.14 (m, 2H, Ar-H), 7.67–7.69 (m, 1H, Ar-H), 7.86–7.99 (m, 3H, 2-, 3-, and 10-H), 8.28 (d, 1H, J = 8.4 Hz, 4-H), 9.03 (dd, 1H, J = 8.4, 0.8 Hz, 1-H), 10.47 (br s, 2H, OH), 14.34 (br s, 1H, NOH). ^{13}C NMR (100 MHz, DMSO-d_6): 115.86 (2C), 116.07, 117.51, 122.52, 123.66, 125.85, 127.15, 129.94, 131.06, 131.10 (2C), 132.30, 132.58, 139.25, 142.80, 150.99, 153.03, 159.44, 160.32. Anal. Calcd for $C_{22}H_{14}N_2O_3{\cdot}0.5\ H_2O{\cdot}1.0$ HCl: C, 66.09; H, 4.03; N, 7.01. Found: C, 66.27; H, 3.79; N, 6.83.

5.4.2. 2-Fluoro-9-hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one oxime (17)

Yield 65% as a red solid. Mp 320 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 6.71 (dd, 1H, J = 8.8, 2.8 Hz, 8-H), 6.87 (d, 1H, J = 8.4 Hz, 7-H), 7.02–7.04 (m, 2H, Ar-H), 7.53–7.56 (m, 2H, Ar-H), 7.66–7.71 (m, 1H, 3-H), 7.93 (d, 1H, J = 2.4 Hz, 10-H), 8.12 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.55 (dd, 1H, J = 10.4, 2.8 Hz, 1-H), 10.11 (br s, 2H, OH), 13.81 (br s, 1H, NOH). ^{13}C NMR (100 MHz, DMSO-d_6): 108.91 (J = 24.2 Hz), 115.61 (2C), 116.40, 117.13 (J = 9.1 Hz), 120.31 (J = 26.5 Hz), 123.09 (J = 11.3 Hz), 123.91, 128.51, 129.91, 129.99, 130.66 (2C), 131.34, 132.76, 152.86, 152.92, 153.24, 159.05, 161.45 (J = 246.3 Hz). Anal. Calcd for $C_{22}H_{13}FN_2O_3{\cdot}0.6H_2O{\cdot}1.0$HCl: C, 62.97; H, 3.65; N, 6.68. Found: C, 62.71; H, 3.87; N, 6.51.

5.5. General procedure for preparation of aminoalkylated oxime derivatives and disubstituted derivatives: 18–23

To a stirred solution of oxime derivatives 16 or 17 (1.0 mmol) in dry DMF (20 mL) was added NaH (60% in oil, 0.50 g) at 0 °C for 1 h. An appropriate tertiary-aminoalkyl halide (5 mmol) was added and heated at 80 °C for 2–5 h (TLC monitoring). The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuo. Crude product was purified by flash chromatography on silica gel, using a gradient of MeOH/CH_2Cl_2 (1:30 to 1:10) and crystallized from EtOH to give the **18**–**23**.

5.5.1. (*E*)-9-Hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (18a), (*E*)-9-[3-(dimethylamino)propoxy]-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (20a) and (*E*)-6-{4-[3-(dimethylamino)propoxy]phenyl}-9-hydroxy-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (22a)

Compound **18a** was obtained in 27% yield (0.12 g) as a yellow solid. Mp 185–186 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 2.01–2.08 (m, 2H, $OCH_2CH_2CH_2$), 2.23 (s, 6H, NMe_2), 2.50 (t, 2H, J = 7.2 Hz, CH_2N), 4.61 (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), 6.94–6.98 (m, 2H, Ar-H), 7.45–7.49 (m, 2H, Ar-H), 7.60–7.70 (m, 2H, 2- and 3-H), 7.81 (d, 1H, J = 2.4 Hz, 10-H), 7.97 (d, 1H, J = 7.6 Hz, 4-H), 8.84

(dd, 1H, J = 8.4, 1.2 Hz, 1-H), 9.88 (br s, 2H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.83, 45.01 (2C), 48.61, 55.46, 74.78, 115.23 (2C), 116.83, 117.15, 122.13, 123.54, 125.01, 128.02, 128.91, 129.64, 129.98, 130.10 (2C), 130.53, 131.00, 131.79, 136.23, 146.86, 153.70, 154.63, 158.10, 158.28. Anal. Calcd for $C_{27}H_{25}N_3O_3{\cdot}0.3H_2O{\cdot}0.3HCl$: C, 71.14; H, 5.73; N, 9.22. Found: C, 71.12; H, 6.02; N, 8.95.

Compound **20a** was obtained in 26% yield (0.14 g) as a red solid. Mp 187–188 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.98–2.04 (m, 2H, $OCH_2CH_2CH_2$), 2.13–2.20 (m, 2H, $OCH_2CH_2CH_2$), 2.31 and 2.37 (two s, 12H, NMe_2), 2.54 (t, 2H, J = 7.2 Hz, CH_2N), 2.66 (t, 2H, J = 7.2 Hz, CH_2N), 4.06 (t, 2H, J = 6.4 Hz, OCH_2), 4.49 (t, 2H, J = 6.0 Hz, OCH_2), 6.39–6.46 (m, 2H, 7- and 8-H), 6.91–6.94 (m, 2H, Ar-H), 7.36–7.39 (m, 2H, Ar-H), 7.41–7.55 (m, 2H, 2- and 3-H), 7.69 (d, 1H, J = 2.4 Hz, 10-H), 8.02 (dd, 1H, J = 8.4, 0.8 Hz, 4-H), 8.67 (dd, 1H, J = 8.4, 0.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 27.12, 27.19, 44.88 (2C), 45.29 (2C), 55.75, 56.27, 66.12, 73.95, 114.42 (2C), 116.97, 117.36, 122.78, 123.73, 125.72, 127.34, 128.43, 129.61, 130.04 (2C), 130.59, 131.43, 132.13, 132.42, 137.53, 147.11, 154.31, 154.33, 157.91, 159.20. Anal. Calcd for $C_{32}H_{36}N_4O_3{\cdot}1.5H_2O{\cdot}1.0HCl$: C, 65.35; H, 6.86; N, 9.53. Found: C, 65.46; H, 6.82; N, 9.18.

Compound **22a** was obtained in 21% yield (0.11 g) as a red solid. Mp 75–76 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.94–2.03 (m, 2H, $OCH_2CH_2CH_2$), 2.14–2.19 (m, 2H, $OCH_2CH_2CH_2$), 2.31 and 2.33 (two s, 12H, NMe_2), 2.52 (t, 2H, J = 7.6 Hz, CH_2N), 2.60 (t, 2H, J = 7.6 Hz, CH_2N), 3.95 (t, 2H, J = 6.0 Hz, OCH_2), 4.62 (t, 2H, J = 6.4 Hz, OCH_2), 6.58 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.82–6.89 (m, 3H, 7- and Ar-H), 7.42–7.44 (m, 2H, Ar-H), 7.49–7.62 (m, 2H, 2- and 3-H), 7.86 (d, 1H, J = 2.0 Hz, 10-H), 8.08 (d, 1H, J = 8.0 Hz, 4-H), 8.89 (dd, 1H, J = 8.4, 0.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 27.07, 27.46, 45.16 (2C), 45.23 (2C), 45.31, 45.35, 56.18, 56.26, 66.31, 74.93, 115.30, 116.05 (2C), 116.56, 122.98, 123.61, 125.69, 127.69, 128.89, 129.51, 130.18 (2C), 131.31, 131.59, 132.15, 132.33, 137.93, 147.32, 154.13, 155.00, 157.78, 159.22. Anal. Calcd for $C_{32}H_{36}N_4O_3{\cdot}0.7H_2O{\cdot}3.0HCl$: C, 59.44; H, 6.30; N, 8.66. Found: C, 59.48; H, 6.01; N, 8.29.

5.5.2. (*E*)-9-Hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (18b), (*E*)-6-(4-hydroxyphenyl)-9-[2-(pyrrolidin-1-yl)ethoxy]-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (20b) and (*E*)-9-hydroxy-6-{4-[2-(pyrrolidin-1-yl)ethoxy]phenyl}-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (22b)

Compound **18b** was obtained in 24% yield (0.11 g) as a yellow solid. Mp 145–146 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.69–1.72 (m, 4H, pyrrolindinyl-H), 2.62 (br s, 4H, pyrrolidinyl-H), 3.00 (t, 2H, J = 6.0 Hz, CH_2N), 4.68 (t, 2H, J = 5.6 Hz, OCH_2), 6.68 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.84 (d, 1H, J = 8.4 Hz, 7-H), 6.94–6.97 (m, 2H, Ar-H), 7.45–7.48 (m, 2H, Ar-H), 7.61–7.70 (m, 2H, 2- and 3-H), 7.83 (d, 1H, J = 2.0 Hz, 10-H), 7.97 (d, 1H, J = 8.0 Hz, 4-H), 8.84 (d, 1H, J = 8.4 Hz, 1-H), 9.88 (br s, 2H, OH). ^{13}C NMR(100 MHz, DMSO-d_6): 23.21 (2C), 54.11 (2C), 54.36, 75.77, 115.23 (2C), 116.94, 117.18, 122.13, 123.54, 125.04, 128.06, 128.93, 129.65, 130.12 (2C), 130.28, 130.54, 131.02, 131.81, 136.25, 146.87, 153.75, 154.64, 158.11, 158.28. Anal. Calcd for $C_{28}H_{25}N_3O_3{\cdot}0.7H_2O{\cdot}0.4HCl$: C, 70.25; H, 5.64; N, 8.78. Found: C, 70.63; H, 5.98; N, 8.40.

Compound **20b** was obtained in 28% yield (0.15 g) as a red solid. Mp 174–175 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.5–1.91 (m, 8H, pyrrolidinyl-H), 2.76–2.80 (m, 8H, pyrrolidinyl-H), 3.00 (t, 2H, J = 5.6 Hz, CH_2N), 3.17 (t, 2H, J = 5.6 Hz, CH_2N), 4.15 (t, 2H, J = 5.6 Hz, OCH_2), 4.78 (t, 2H, J = 5.6 Hz, OCH_2), 6.61 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.82 (d, 1H, J = 8.4 Hz, 7-H), 6.86–6.90 (m, 2H, Ar-H), 7.38–7.42 (m, 2H, Ar-H), 7.49–7.62 (m, 2H, 2- and 3-H), 7.93 (d, 1H, J = 2.8 Hz, 10-H), 8.08 (d, 1H, J = 8.0 Hz, 4-H), 8.85 (dd, 1H, J = 8.4, 0.8 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.28 (2C), 23.45 (2C), 54.54 (2C), 54.78 (2C), 54.85, 54.90, 66.35, 75.36, 115.94 (2C), 116.09 (2C), 122.91, 123.67, 125.60, 127.73, 128.89, 129.60, 130.09 (2C), 131.52, 132.17, 132.68, 137.77, 147.38, 154.50, 155.10, 158.02, 158.86. Anal. Calcd for $C_{34}H_{36}N_4O_3{\cdot}1.3H_2O{\cdot}1.0HCl$: C, 67.10; H, 6.56; N, 9.21. Found: C, 67.19; H, 6.32; N, 9.11.

Compound **22b** was obtained in 29% yield (0.16 g) as a red solid. Mp 176–177 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): 1.88–1.94 (m, 8H, pyrrolidinyl-H), 2.75 (br s, 4H, pyrrolidinyl-H), 2.88–2.94 (m, 6H, CH_2N and pyrrolidinyl-H), 3.18–3.20 (m, 2H, CH_2N), 3.93 (t, 2H, J = 5.6 Hz, OCH_2), 4.65–4.68 (m, 2H, OCH_2), 5.97 (d, 1H, J = 8.4 Hz, 7-H), 6.12 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.22–6.24 (m, 2H, Ar-H), 6.68–6.70 (m, 2H, Ar-H), 7.53–7.67 (m, 3H, 2-, 3- and 10-H), 8.12 (d, 1H, J = 8.4 Hz, 4-H), 8.84 (d, 1H, J = 8.0 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.08 (2C), 23.47 (2C), 54.02 (2C), 54.90 (2C), 54.99, 55.45, 66.69, 73.98, 114.23 (2C), 116.40, 118.84, 122.31, 123.32, 125.83, 127.45, 128.15, 129.29 (2C), 129.60, 130.22, 130.73, 132.41, 132.97, 136.84, 147.20, 154.82, 154.91, 158.41 (2C). Anal. Calcd for $C_{34}H_{36}N_4O_3$: C, 74.43; H, 6.61; N, 10.21. Found: C, 74.12; H, 6.74; N, 10.05.

5.5.3. (*E*)-2-Fluoro-9-hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]-quinolin-11-one *O*-3-(dimethylamino)propyl oxime (19a), (*E*)-9-[3-(dimethylamino)propoxy]-2-fluoro-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (21a) and (*E*)-6-{4-[3-(dimethylamino)propoxy]phenyl}-2-fluoro-9-hydroxy-11*H*-indeno[1,2-*c*] quinolin-11-one *O*-3-(dimethylamino)propyl oxime (23a)

Compound **19a** was obtained in 30% yield (0.14 g) as a red solid. Mp 262 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 2.01 (quin, 2H, J = 6.8 Hz, $OCH_2CH_2CH_2$), 2.19 (s, 6H, NMe_2), 2.45 (t, 2H, J = 6.8 Hz, CH_2N), 4.59 (t, 2H, J = 6.8 Hz, OCH_2), 6.67 (dd, 1H, J = 8.4, 2.8 Hz, 8-H), 6.82 (d, 1H, J = 8.4 Hz, 7-H), 6.92–6.95 (m, 2H, Ar-H), 7.42–7.45 (m, 2H, Ar-H), 7.58 (ddd, 1H, J = 9.2, 8.4, 2.8 Hz, 3-H), 7.76 (d, 1H, J = 2.4 Hz, 10-H), 8.01 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.43 (dd, 1H, J = 10.4, 2.8 Hz, 1-H), 9.88 (br s, 2H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.94, 45.13 (2C), 55.53, 74.96, 108.19 (J = 23.5 Hz), 115.25 (2C), 116.75, 117.26, 118.93 (J = 25.7 Hz), 122.49 (J = 11.3 Hz), 123.82, 129.65, 130.13 (2C), 130.28, 131.05, 132.40, 132.54 (J = 9.9 Hz), 135.77 (J = 6.1 Hz), 144.10, 153.24, 154.11, 158.15, 158.57, 160.99 (J = 244.8 Hz). Anal. Calcd for $C_{28}H_{23}FN_2O_3{\cdot}0.6HCl$: C, 67.65; H, 5.17; N, 8.77. Found: C, 67.65; H, 5.26; N, 8.63.

Compound **21a** was obtained in 24% yield (0.13 g) as a yellow solid. Mp 193–194 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.80–2.03 (m, 4H, $OCH_2CH_2CH_2$), 2.13 (s, 6H, NMe_2), 2.18 (s, 6H, NMe_2), 2.33 (t, 2H, J = 7.2 Hz, CH_2N), 2.42 (t, 2H, J = 7.2 Hz, CH_2N), 3.98 (t, 2H, J = 6.4 Hz, OCH_2), 4.59 (t, 2H, J = 6.4 Hz, OCH_2), 6.85–6.96 (m, 4H, 7-, 8- and Ar-H), 7.42–7.46 (m, 2H, Ar-H), 7.57 (ddd, 1H, J = 8.8, 8.8, 2.4 Hz, 3-H), 7.78 (d, 1H, J = 2.4 Hz, 10-H), 8.01 (dd, 1H, J = 9.2, 6.0 Hz, 4-H), 8.40 (dd, 1H, J = 10.0, 2.4 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 26.80, 26.97, 45.18 (4C), 55.58, 55.71, 66.19, 75.04, 108.2 (J = 24.2 Hz), 115.23 (2C), 115.84, 116.01, 119.06 (J = 25.8 Hz), 122.36 (J = 11.4 Hz), 123.62, 130.02, 130.06 (2C), 130.80, 131.00, 131.90, 132.48 (J = 11.3 Hz), 136.03 (J = 6.1 Hz), 144.21, 152.95, 154.19, 158.30, 159.37, 160.92 (J = 244.9 Hz). Anal. Calcd for $C_{32}H_{35}FN_4O_3{\cdot}0.3HCl$: C, 69.43; H, 6.43; N, 10.12. Found: C, 69.44; H, 6.34; N, 10.00.

Compound **23a** was obtained in 22% yield (0.12 g) as a yellow solid. Mp 189–190 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.88–2.05 (m, 4H, $OCH_2CH_2CH_2$), 2.18 (s, 6H, NMe_2), 2.19 (s, 6H, NMe_2), 2.39–2.46 (m, 4H, CH_2N), 4.11 (t, 2H, J = 6.4 Hz, OCH_2), 4.61 (t, 2H, J = 6.4 Hz, OCH_2), 6.68 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.81 (d, 1H, J = 8.4 Hz, 7-H), 7.09–7.12 (m, 2H, Ar-H), 7.54–7.61

(m, 3H, 3-H and Ar-H), 7.78 (d, 1H, J = 2.4 Hz, 10-H), 8.04 (dd, 1H, J = 9.2, 6.0 Hz, 4-H), 8.44 (dd, 1H, J = 10.4, 2.8 Hz, 1-H), 10.21 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.92, 26.97, 45.16 (2C), 45.23 (2C), 55.53, 55.70, 65.97, 74.98, 108.16 (J = 23.5 Hz), 114.35 (2C), 116.76, 117.26, 118.95 (J = 25.8 Hz), 122.54 (J = 11.4 Hz), 123.72, 129.44, 130.10 (2C), 131.02, 131.74, 132.34, 132.56 (J = 9.9 Hz), 135.81 (J = 5.4 Hz), 144.07, 153.18, 153.70, 158.65, 159.23, 161.02 (J = 244.8 Hz). Anal. Calcd for $C_{32}H_{35}FN_4O_3$·0.4HCl: C, 68.97; H, 6.40; N, 10.05. Found: C, 69.00; H, 6.33; N, 9.90.

5.5.4. (*E*)-2-Fluoro-9-hydroxy-6-(4-hydroxyphenyl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (19b), (*E*)-2-fluoro-6-(4-hydroxyphenyl)-9-[2-(pyrrolidin-1-yl) ethoxy]-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (21b) and (*E*)-2-fluoro-9-hydroxy-6-{4-[2-(pyrrolidin-1-yl)ethoxy]phenyl}-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (23b)

Compound **19b** was obtained in 25% yield (0.12 g) as a red solid. Mp 255 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 1.69–1.72 (m, 4H, pyrrolidinyl-H), 2.61 (br s, 4H, pyrrolidinyl-H), 2.99 (t, 2H, J = 5.6 Hz, CH_2N), 4.67 (t, 2H, J = 5.6 Hz, OCH_2), 6.68 (dd, 1H, J = 8.4, 2.4 Hz, 8-H), 6.83 (d, 1H, J = 8.4 Hz, 7-H), 6.93–6.96 (m, 2H, Ar-H), 7.43–7.46 (m, 2H, Ar-H), 7.56 (ddd, 1H, J = 9.2, 8.8, 3.2 Hz, 3-H), 7.79 (d, 1H, J = 2.4 Hz, 10-H), 8.02 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.42 (dd, 1H, J = 10.8, 3.2 Hz, 1-H), 9.86 (br s, 2H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 23.19 (2C), 54.07 (2C), 54.37, 75.83, 108.17 (J = 24.3 Hz), 115.21 (2C), 116.83, 117.24, 118.88 (J = 25.7 Hz), 122.46 (J = 11.4 Hz), 123.76, 129.62, 130.08 (2C), 130.24, 131.02, 132.37, 132.51 (J = 9.1 Hz), 135.73 (J = 6.1 Hz), 144.08, 153.26, 154.09, 158.12, 158.51, 160.96 (J = 244.8 Hz). Anal. Calcd for $C_{28}H_{23}FN_2O_3$·0.4HCl: C, 69.47; H, 5.08; N, 8.68. Found: C, 69.49; H, 5.15; N, 8.52.

Compound **21b** was obtained in 20% yield (0.11 g) as a yellow solid. Mp 159–160 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.66–1.71 (m, 8H, pyrrolidinyl-H), 2.57 (br s, 4H, pyrrolidinyl-H), 2.78 (t, 2H, J = 5.6 Hz, CH_2N), 2.94 (t, 2H, J = 5.6 Hz, CH_2N), 4.05 (t, 2H, J = 5.6 Hz, OCH_2), 4.66 (t, 2H, J = 5.6 Hz, OCH_2), 6.86–6.97 (m, 4H, 7-, 8-, and Ar-H), 7.43–7.46 (m, 2H, Ar-H), 7.55–7.60 (m, 1H, 3-H), 7.85 (d, 1H, J = 2.0 Hz, 10-H), 8.02 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.40 (dd, 1H, J = 10.4, 2.8 Hz, 1-H), 9.86 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 23.15 (2C), 23.19 (2C), 53.95 (2C), 54.05 (2C), 54.15, 54.41, 67.11, 75.94, 108.17 (J = 24.3 Hz), 115.22 (2C), 115.53, 115.70, 116.27, 118.98 (J = 25.7 Hz), 122.32 (J = 11.4 Hz), 123.59, 130.09 (2C), 130.83, 131.07, 131.91, 132.52 (J = 9.1 Hz), 136.03 (J = 6.1 Hz), 144.22, 152.99, 154.16, 158.17, 159.21, 160.94 (J = 244.8 Hz). Anal. Calcd for $C_{28}H_{23}FN_2O_3$·0.7HCl: C, 68.96; H, 6.08; N, 9.46. Found: C, 68.88; H, 6.06; N, 9.36.

Compound **23b** was obtained in 16% yield (0.09 g) as a yellow solid. Mp 192 °C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 1.72–1.74 (m, 8H, pyrrolidinyl-H), 2.64 (br s, 4H, pyrrolidinyl-H), 2.94, 3.02 (m, 4H, $CH_2N \times 2$), 4.21 (t, 2H, J = 5.6 Hz, OCH_2), 4.70 (t, 2H, J = 5.6 Hz, OCH_2), 6.68 (dd, 1H, J = 8.8, 2.4 Hz, 8-H), 6.81 (d, 1H, J = 8.8 Hz, 7-H), 7.12–7.15 (m, 2H, Ar-H), 7.56–7.63 (m, 3H, 3-H and Ar-H), 7.82 (d, 1H, J = 2.0 Hz, 10-H), 8.04 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.46 (dd, 1H, J = 10.4, 2.8 Hz, 1-H), 10.04 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 23.10 (2C), 23.16 (2C), 54.03 (4C), 54.18, 54.30, 66.52, 75.73, 108.18 (J = 23.5 Hz), 114.41 (2C), 116.87, 117.28, 118.99 (J = 25.7 Hz), 122.55 (J = 11.3 Hz), 123.70, 129.49, 130.09 (2C), 131.02, 131.87, 132.35, 132.59 (J = 9.9 Hz), 135.81 (J = 6.1 Hz), 144.08, 153.27, 153.66, 158.59, 158.97, 161.03 (J = 244.9 Hz). Anal. Calcd for $C_{28}H_{23}FN_2O_3$·1.0HCl: C, 67.71; H, 6.02; N, 9.29. Found: C, 67.53; H, 6.28; N, 8.86.

5.6. Pharmacological methods

5.6.1. Antiproliferative assay

Cancer cells (Hep G2, Hep 3B, A549, H1299, MCF-7 and MDA-MB-231) 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%.[18]

5.6.2. Cell cycle analysis

Hepatoma cells were treated with DMSO, **23a** at different concentrations (1.0, 5.0, 10.0 μM) for 12 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).

5.6.3. Immunoblot analysis

After treatment of compound **23a**, 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 12,000g at 4 °C for 20 min and the supernatants were stored at −70 °C. The protein concentration was determined by the Bradford method. 20 μg protein 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. 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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References and notes

1. Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888.
2. Werbovetz, K. A.; Bhattacharjee, A. K.; Brendle, J. J.; Scovill, J. P. *Bioorg. Med. Chem.* **2000**, 8 1741.
3. Utsugi, T.; Aoyagi, K.; Asao, T.; Okazaki, S.; Aoyagi, Y.; Sano, M.; Wierzba, K.; Yamada, Y. *Jpn. J. Cancer Res.* **1997**, *88*, 992.
4. Aoyagi, Y.; Kobunai, T.; Utsugi, T.; Oh-hara, T.; Yamada, Y. *Jpn. J. Cancer Res.* **1999**, *90*, 578.
5. Ishida, K.; Asao, T. *Biochim. Biophys. Acta* **2002**, *1587*, 155.
6. Chen, I. L.; Chen, Y. L.; Tzeng, C. C.; Chen, I. S. *Helv. Chim. Acta* **2002**, *85*, 2214.
7. Chen, I. L.; Chen, Y. L.; Tzeng, C. C. *Chin. Pharm. J.* **2003**, *55*, 49.
8. Zhao, Y. L.; Chen, Y. L.; Tzeng, C. C.; Chen, I. L.; Wang, T. C.; Han, C. H. *Chem. Biodiver.* **2005**, *2*, 205.
9. Chen, Y. L.; Chen, I. L.; Wang, T. C.; Han, C. H.; Tzeng, C. C. *Eur. J. Med. Chem.* **2005**, *40*, 928.
10. Huang, Y. T.; Huang, D. M.; Guh, J. H.; Chen, I. L.; Tzeng, C. C.; Teng, C. M. *J. Biol. Chem.* **2005**, *280*, 2771.
11. Tseng, C. H.; Chen, Y. L.; Lu, P. J.; Yang, C. N.; Tzeng, C. C. *Bioorg. Med. Chem.* **2008**, *16*, 3153.
12. Tseng, C. H.; Chen, Y. L.; Chung, K. Y.; Cheng, C. M.; Wang, C. H.; Tzeng, C. C. *Bioorg. Med. Chem.* **2009**, *17*, 7465.
13. Tseng, C. H.; Tzeng, C. C.; Yang, C. L.; Lu, P. J.; Chen, H. L.; Li, H. Y.; Chuang, Y. C.; Yang, C. N.; Chen, Y. L. *J. Med. Chem.* **2010**, *53*, 6164.
14. Chen, C. H.; Su, W. W.; Yang, S. S.; Chang, T. T.; Cheng, K. S.; Lin, H. H.; Wu, S. S.; Lee, C. M.; Chang, C. S.; Chen, C. J.; Sheu, J. C.; Chen, D. S.; Lu, S. N. *J. Gastroenterol. Hepatol.* **2006**, *21*, 1561.
15. Chen, C. H.; Yang, P. M.; Huang, G. T.; Lee, H. S.; Sung, J. L.; Sheu, J. C. *J. Formosan Med. Assoc.* **2007**, *106*, 148.
16. El-Serag, H. B.; Rudolph, K. L. *Gastroenterology* **2007**, *132*, 2557.
17. Mazzanti, R.; Gramantieri, L.; Bolondi, L. *Mol. Aspects Med.* **2008**, *29*, 130.
18. Roehm, N. W.; Rodgers, G. H.; Hatfield, S. M.; Glasebrook, A. L. *J. Immunol. Methods* **1991**, *142*, 2575.
19. Movassagh, M.; Foo, R. S. *Heart Fail. Rev.* **2008**, *13*, 111.
20. Katiyar, S. K.; Roy, A. M.; Balinga, M. S. *Mol. Cancer Ther.* **2005**, *4*, 207.
21. Hayward, R. L.; Macpherson, J.; Cummings, S. J.; Monia, B. P.; Smyth, J. F.; Jordell, D. L. *Mol. Cancer Ther.* **2004**, *3*, 169.
22. Kuo, P. L.; Lin, T. C.; Lin, C. C. *Life Sci.* **2002**, *71*, 1879.