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Synthesis and antiproliferative evaluation of certain indeno[1,2-*c*]quinoline derivatives

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Abstract—Although the quinoline ring is found in a wide variety of biologically active compounds and is frequently condensed with various heterocycles, synthesis and biological evaluation of the indenoquinoline skeleton attracts only very limited attention. We report herein the synthesis and antiproliferative evaluation of certain indeno[1,2-*c*]quinoline derivatives against the growth of six cancer cell lines including human cervical epithelioid carcinoma (HeLa), oral squamous cell carcinoma (SAS), hepatocellular carcinoma (SKHep), human stomach adenocarcinoma (AGS), prostate cancer (PC-3), and non-small cell lung cancer (A549). The results indicated that 9-methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**17b**) is more active than its C_6-amino derivative **17a**, C_6-morpholine and C_6-piperidine isomers, **17c** and **17d**, respectively. Treatment of **17b** with NH_2OH afforded its hydroxyimino derivative **20** which is more active than the carbonyl precursor **17b**. More potent agents were obtained by further derivatization of **20**. Thus, antiproliferative activities decreased in an order of aminoalkoxyimino **22a–d** > hydroxyimino **20** > alkoxyimino **21**, **22e** > carbonyl **17b**. Both AGS and A549 were resistant to camptothecin with GI_{50} values of 23.76 and 2.80 μM, respectively, while GI_{50} values for **22a–d** were in the range of 5.93–7.11 μM and 0.38–0.87 μM, respectively. Among them, **22b** was the most potent with GI_{50} values of 0.52, 0.74, 6.76, and 0.64 μM against the growth of HeLa, SKHep, AGS, and A549 cells, respectively. Flowcytometric analysis indicated **22c** can induce cell cycle arrest in S phase, and DNA polyploidy (>4*n*) followed by apoptosis.

1. Introduction

Camptothecin (**1**), an alkaloid isolated from *Camptotheca acuminata*,[1,2] and its derivatives such as topotecan (**2**, Hycamptin) and irinotecan (**3**, Camptosar) are prototypical topoisomerase I (Top I) inhibitors which are currently used as anticancer drugs. However, several drawbacks, such as easy opening of the lactone ring to a hydroxycarboxylate which has a high affinity to human serum albumin, and the rapid reversibility of ternary DNA-enzyme-camptothecin complex after the removal of drug, limited their clinical utility.[3,4] Furthermore, the fact that some cancer cells develop resistance to camptothecins has caused an urgent need to search for alternative Top I inhibitors which are chemically more stable. Indenoisoquinoline **4** (NSC 314622) was first identified as a novel Top I inhibitor with better pharmacokinetic features than camptothecin.[5,6] Since then, a number of indenoisoquinolines especially indeno[1,2-*c*]isoquinoline derivatives have been synthesized and proved to possess DNA Top I inhibitory activity.[7–10] These compounds bind to a transient Top I-DNA covalent complex and inhibit the resealing of a single-strand nick that the enzyme creates to relieve superhelical tension in duplex DNA.[11–13]

Since the discovery of indenoisoquinolines as a novel class of potential anticancer drug candidates, extensive structural modifications have been explored by altering the substituent of the tetracyclic pharmacophore. However, synthesis and biological evaluation of the isomeric

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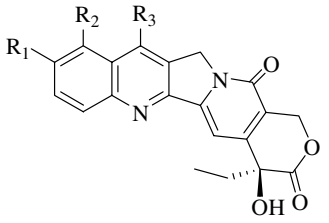
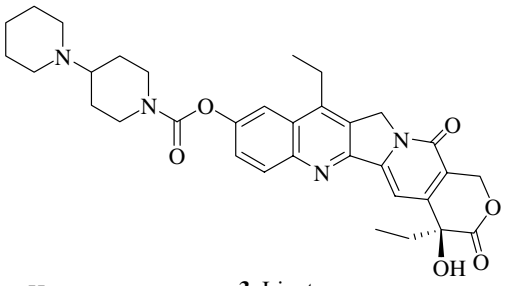
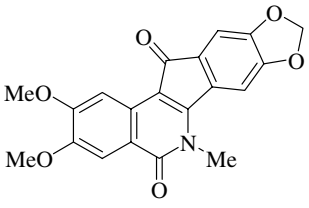


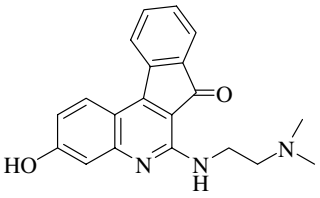
1, Camptothecin $R_1 = H$; $R_2 = H$; $R_3 = H$
2, Topotecan $R_1 = OH$; $R_2 = CH_2N(CH_3)_2$, $R_3 = H$



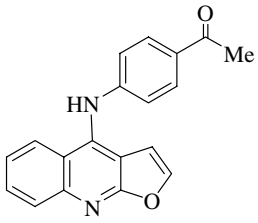
3, Irinotecan



4, NSC 314622



5, TAS-103



6, CIL-102

indenoquinoline skeleton attracts only very limited attention.[14–19] Heterocycles containing the quinoline ring constitute a wide variety of biologically active compounds. For example, certain indeno[1,2-*c*]quinolin-11-one scaffolds have been shown to possess antitumor activity.[15] TAS-103 (**5**), one of the indeno[2,1-*c*]quinoline derivatives, has been proved to be a novel Top I and Top II targeting agent that stabilizes cleavable complexes of Top-DNA at the cellular level.[17–19] A number of furo[2,3-*b*]quinoline derivatives, such as CIL-102 (**6**), have also been synthesized and demonstrated to possess significant anticancer activity.[20–24] We have also synthesized and evaluated antiproliferative activities of certain indolo[2,3-*b*]quinoline derivatives[25] on the ground that these tetracyclic heterocycles may intercalate into the DNA double helix resulting in the inhibition of DNA replication and transcription. In continuation of our study to explore more potent anticancer drug candidates, we describe herein the preparation and antiproliferative evaluation of certain indeno[1,2-*c*]quinoline derivatives.

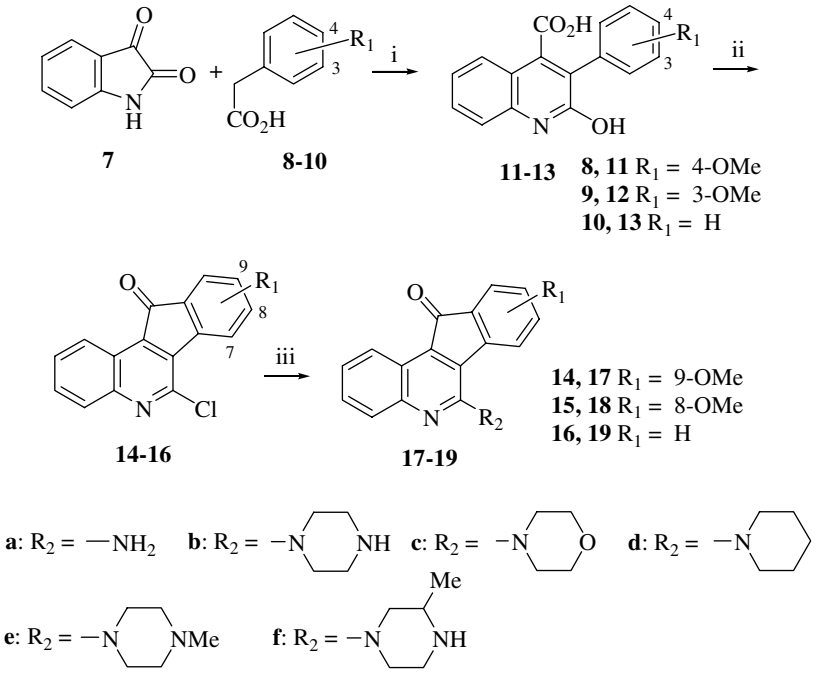
2. Chemistry

Reaction of isatin (**7**) and 4–methoxyphenylacetic acid (**8**) gave 2-hydroxy-3-(4-methoxyphenyl)quinoline-4-carboxylic acid (**11**)[15] as described in Scheme 1. Compounds **12** and **13** were prepared under the same reaction conditions from 3–methoxyphenylacetic acid (**9**) and phenylacetic acid (**10**), respectively, with isatin. Treatment of **11** with $POCl_3$ afforded 6-chloro-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**14**), which was reacted with NH_4OH or cyclic amines to give 6-amino-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**17a**) or its cyclic aminoalkyl derivatives **17b**–**f**. Compound **14** had also been prepared previously from thermal cyclization of **11** followed by chlorination with $POCl_3$.[15] Accordingly, **18b** and **19b** were obtained from **15** and **16**, which in turn were prepared from **12** and **13**, respectively, in a fairly good overall yield.

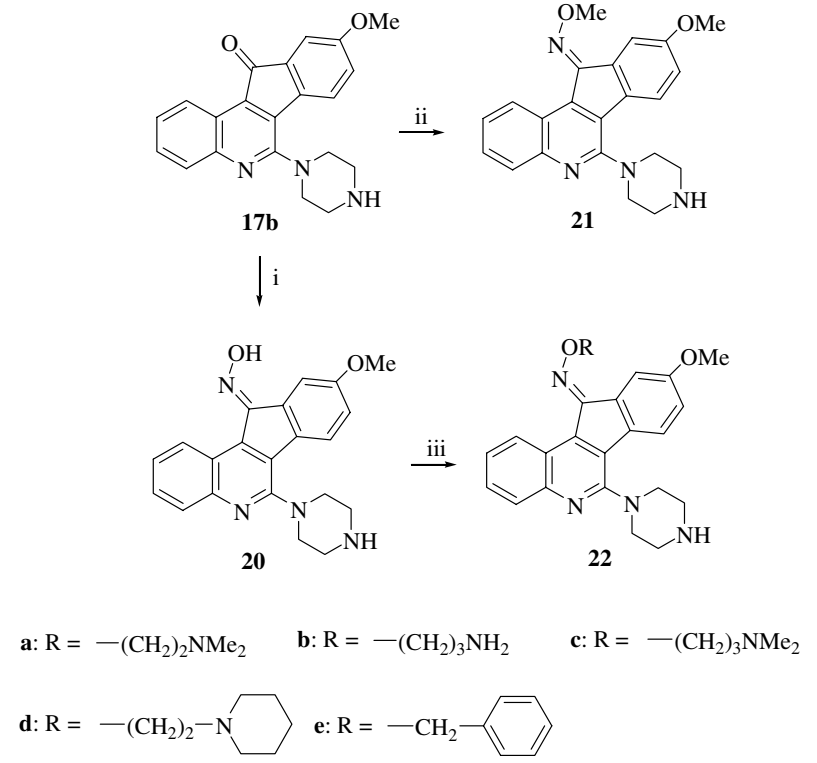
The preparation of 9-methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one oxime (**20**) and its alkylated derivatives **21** and **22a**–**f** is described in Scheme 2. Reaction of **17b** with NH_2OH or NH_2OMe gave **20** or **21**, respectively. Alkylation of **20** with various aminoalkyl chlorides afforded their respective aminoalkoxyimino derivatives **22a**–**e**.

3. Pharmacological results and discussion

All the synthesized indeno[1,2-*c*]quinoline derivatives were evaluated in vitro against a panel of six cancer cell lines including human cervical epithelioid carcinoma (HeLa), oral squamous cell carcinoma (SAS), hepatocellular carcinoma (SKHep), human stomach adenocarcinoma (AGS), prostate cancer (PC-3), and non-small cell lung cancer (A549) using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. 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 11*H*-indeno[1,2-*c*]quinoline-11-one derivatives are summarized in Table 1. For the 9-methoxy derivatives, the C_6-piperazine substituted derivative **17b** is more active than its amino derivative **17a** with exceptions of HeLa and SKHep, in which **17a** is fairly active against the growth of HeLa cell with a GI_{50} value of 9.48 μM. Inactiveness of **17c** and **17d** indicated that the replacement of C_6-piperazine with its isosteric moieties such as morpholine or piperidine is unfavorable. Comparable activities were observed by the replacement of C_6-piperazine with *N*-4 methyl piperazine (**17b** vs **17e**) while the C-3 methyl piperazine counterpart **17f** exhibited more potent antiproliferative activities than **17b**. Comparison of positional isomers indicated that the 8-OMe derivative **18b** is more active than its 9-OMe isomer **17b**. The fact that **17b** is less active than its unsubstituted parent **19b** implies that the methoxy group is not essential for antiproliferative



Scheme 1. Reagents and conditions: (i) NaOAc, 200 °C, 3h; (ii) $POCl_3$, 150 °C, 48 h; (iii) Nh_4OH in sealed tube, 200 °C, 48 h or cyclic amines in ethoxyethanol.

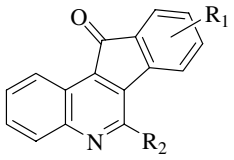


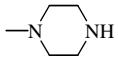
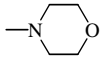
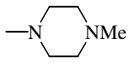
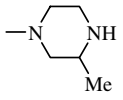
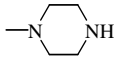
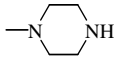
Scheme 2. Reagents and conditions: (i) NH_2OH HCl, MW (100 W), 30 min; (ii) NH_2OMe HCl, MW (100 W), 30 min; (iii) NaH, RCl.

activities. On the contrary, the C-6 piperazine or substituted piperazine is crucial because morpholine counterpart **17c** and piperidine counterpart **17d** are inactive. Although camptothecin exhibited more active antiproliferative activities than 11*H*-indeno[1,2-*c*]quinoline-11-one derivatives against the growth of most cancer cell lines tested, it was inactive against the growth of AGS with a GI_{50} value of 23.76 μM, while **18b** and **19b** were relatively active with GI_{50} values of 4.11 and 6.54 μM, respectively.

The fact that both **20** and **21** are more active than **17b** implied that a hydroxyimino or methoxyimino derivative is more favorable than the carbonyl precursor as

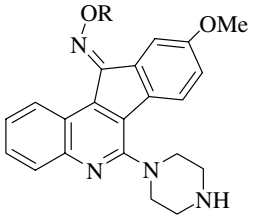
Table 1. Antiproliferative activity of 11*H*-indeno[1,2-*c*]quinoline-11-one derivatives (GI_{50}, μM)

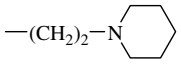
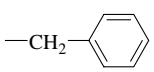


	R_1	R_2	HeLa	SAS	SKHep	AGS	PC-3	A549
17a	9-OMe	NH_2	9.48 ± 0.59	>30	21.69 ± 5.93	>30	>30	>30
17b	9-OMe		15.18 ± 1.50	>30	25.34 ± 15.46	10.13 ± 1.19	20.38 ± 11.02	16.78 ± 2.18
17c	9-OMe		>30	>30	>30	>30	>30	>30
17d	9-OMe		>30	>30	>30	>30	>30	>30
17e	9-OMe		7.29 ± 1.94	25.13 ± 11.23	20.24 ± 7.86	7.40 ± 0.30	>30	16.00 ± 6.50
17f	9-OMe		3.44 ± 1.01	13.40 ± 2.60	2.90 ± 0.50	7.93 ± 1.16	16.25 ± 5.00	7.83 ± 1.31
18b	8-OMe		0.36 ± 0.11	5.10 ± 0.82	0.73 ± 0.31	4.11 ± 1.20	10.63 ± 2.08	8.60 ± 4.72
19b	H		1.07 ± 0.61	10.70 ± 0.92	1.75 ± 1.08	6.54 ± 2.59	11.91 ± 3.96	3.60 ± 1.25
Camptothecin			0.18 ± 0.10	6.00 ± 2.70	0.22 ± 0.13	23.76 ± 0.73	0.09 ± 0.02	2.80 ± 0.70

summarized in Table 2. The antiproliferative activity decreased by substitution of **20** with methyl or benzyl group, **21** and **22e**, respectively, but was significantly enhanced by the introduction of aminoalkyl side chains **22a**–**d**. Among these aminoalkoxyimino derivatives **22a**–**d**, **22b** was the most potent with GI_{50} values of 0.52, 0.74, 6.76, and 0.64 μM against the growth of HeLa, SKHep, AGS, and A549 cells, respectively. Among these cancer cells tested, AGS was the most resistant to camptothecin with a GI_{50} value of

Table 2. Antiproliferative activity of 9-methoxy 11*H*-indeno[1,2-*c*]quinoline-11-one derivatives (GI_{50}, μM)



	R	HeLa	SAS	SKHep	AGS	PC-3	A549
20	H	2.35 ± 0.25	6.10 ± 0.90	3.75 ± 1.28	1.74 ± 0.47	4.86 ± 2.03	3.80 ± 0.85
21	Me	10.63 ± 4.38	10.30 ± 0.51	9.31 ± 2.90	5.18 ± 0.48	13.82 ± 4.30	6.16 ± 0.12
22a	$-(CH_2)_2NMe_2$	0.92 ± 0.38	6.33 ± 0.86	3.01 ± 2.18	5.93 ± 1.24	4.61 ± 1.18	0.71 ± 0.12
22b	$-(CH_2)_3NH_2$	0.52 ± 0.17	2.41 ± 1.16	0.74 ± 0.21	6.76 ± 2.02	2.98 ± 2.16	0.64 ± 0.01
22c	$-(CH_2)_3NMe_2$	0.37 ± 0.06	4.93 ± 0.57	2.95 ± 2.10	6.84 ± 1.75	5.28 ± 0.67	0.38 ± 0.21
22d		0.78 ± 0.22	5.81 ± 0.48	1.57 ± 0.42	7.11 ± 0.84	5.62 ± 0.48	0.87 ± 0.12
22e		6.14 ± 0.34	7.62 ± 0.63	4.15 ± 1.82	6.12 ± 1.40	7.81 ± 1.07	5.40 ± 0.22
Camptothecin		0.18 ± 0.10	6.00 ± 2.70	0.22 ± 0.13	23.76 ± 0.73	0.09 ± 0.02	2.80 ± 0.70

23.76 μM while GI_{50} values for **22a–d** were in the range of 5.93–7.11 μM. Results have also shown that **22b** and **22c** exhibited more active antiproliferative activities than camptothecin against the growth of SAS, AGS, and A549. Compound **22c** was the most active against the growth of HeLa with a GI_{50} value of 0.37 μM and, therefore, was selected along with its hydroxyimino precursor **20** for further investigation of their effects on the growth curves of HeLa cells. Total 48 h period of growth time was monitored and the results showed that both compounds can effectively suppress the cell proliferation in dosage dependent manners (Fig. 1A and B). Compound **22c** inhibited about 50% and almost 100% of the cell proliferation at 0.3 and 1.0 μM, respectively, which was more effective than **20**. These results were also consistent with GI_{50} values obtained from MTT viability assay (Table 2).

The indeno[1,2-*c*]quinoline treated cells with higher DNA content indicated that the cells may be arrested in S phase or in G2 phase of cell cycle. To examine the association between indeno[1,2-*c*]quinoline-induced growth inhibition and cell cycle arrest, the cell cycle distribution was analyzed by flow cytometry. The results in Figure 2 and Table 3 shows that **22c** can induce cell accumulation in S phase in a dose dependent manner after treatment of **22c** for 24 h. Figure 2D showed that, at the concentration of 1.0 μM, 22**c** can not only induce

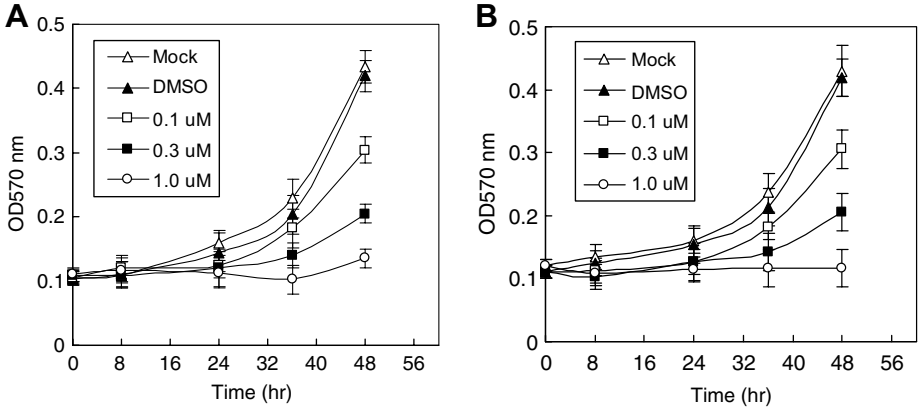


Figure 1. Antiproliferative effects of **20** (A) and **22c** (B) on HeLa cells. Adherent cells proliferated in 96-well plates (10^4 cells/well) were incubated with different concentrations of **20** (1.0, 3.0, and 10 μM) and **22c** (0.1, 0.3, and 1.0 μM), respectively, and determined by MTT assay at various time intervals.

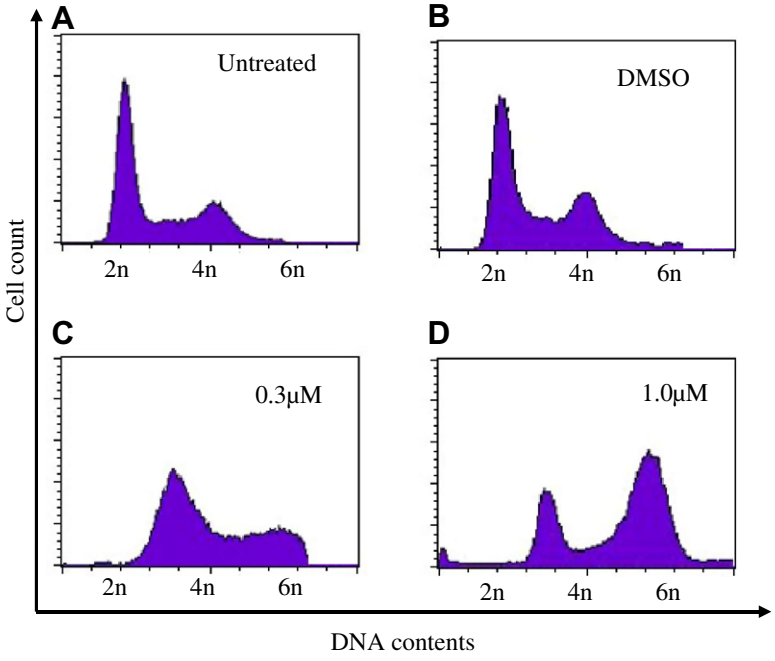


Figure 2. Flow cytometric analysis of HeLa cells. Untreated HeLa cells were taken as control (A). Cells were treated DMSO (B), 0.3 μM (C), or 1.0 μM (D) of **22c**; 24 h later the cells were harvested, fixed, and stained with propidium iodide as described in Experimental Section prior to analysis by flow cytometry. The percentage of cells in each cell cycle phase was quantified and is given in Table 3.

Table 3. Effects of **22c** on HeLa cell cycle progression

PI staining	Cell cycle distribution (%)				
	SubG1	G1	S	G2/M (4*n*)	>4*n* (6*n*+8*n*)
Untreated	0.4 ± 0.1	58.3 ± 2.9	13.8 ± 1.8	25.2 ± 1.9	1.3 ± 0.4
DMSO	0.4 ± 0.2	57.6 ± 3.9	12.9 ± 3.4	29.3 ± 1.8	1.8 ± 0.6
22c (0.3 μM)	1.5 ± 0.2	10.8 ± 1.4	50.8 ± 4.4	19.8 ± 1.3	17.2 ± 3.7
22c (1.0 μM)	5.9 ± 0.5	3.9 ± 0.8	21.8 ± 2.8	15.3 ± 2.4	53.2 ± 4.1

the cell accumulation in S phase (between 2*n* and 4*n*) but also increase DNA polyploidy (>4*n*) and the subG1 phase after 24 h drug treatment. The sub G0/G1 phase increased after 24 h drug treatment indicated that these cells may cause the DNA fragmentation and apoptosis.

4. Conclusion

Based on the structure of indeno[1,2-*c*]quinolines, we found that the features for optimum antiproliferative activities are: (1) substituent at C-6 should be piperazine or alkylated piperazine; (2) substituent at C-11 is crucial in which the potency decreased in an order of aminoalkoxyimino **22a**–**d** > hydroxyimino **20** > alkoxyimino **21**, **22e** > carbonyl **17b**.

Among these aminoalkoxyimino derivatives **22a**–**d**, **22b** exhibited GI_{50} values of 0.52, 0.74, and 0.64 μM against the growth of HeLa, SKHep, and A549 cells, respectively, which are approximately 30-fold increase in potency compared to the parent core **17b**. Compound **22b** was more active than camptothecin against the growth of SAS, AGS, and A549 cells, with GI_{50} values of 2.41, 6.76, and 0.64 μM, respectively. Results have also shown that **22b** and **22c** exhibited approximately 4-fold increase in potency compared to camptothecin against the growth of AGS and A549. Flowcytometric analysis indicated that **22c** can induce cell cycle arrest in S phase, DNA polyploidy (>4*n*) followed by apoptosis. Further studies on the structural optimization and the antiproliferative mechanism 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 Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts are 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 Chung-Hsing University using Heraeus CHN-O Rapid EA, and all values are within ±0.4% of the theoretical compositions.

5.1.1. 2-Hydroxy-3-(4-methoxyphenyl)quinoline-4-carboxylic acid (11). A mixture of isatin (2.21 g, 15 mmol), 4-methoxyphenylacetic acid (4.36 g, 26.25 mmol), and sodium acetate (0.3 g) was heated at 200 °C for 3 h (TLC monitoring). After cooling, the mixture was added AcOH (100 mL), and the precipitate was collected, washed with H_2O, and then crystallized from EtOH to give **11** (3.14 g, 71%). mp 338–339 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (*s*, 3H, OMe), 7.03 (*m*, 2H, Ar-H), 7.33 (*m*, 2H, Ar-H), 7.70 (*m*, 1H, 6-H), 7.84 (*m*, 2H, 7, 8-H), 8.14 (*m*, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.31, 114.12 (2C), 119.50, 120.75, 123.96, 123.99, 128.80, 129.10, 129.32, 131.26, 131.49 (2C), 146.81, 150.73, 160.42, 167.44. Anal. calcd for $C_{17}H_{13}NO_4$ 0.1 H_2O: C 68.73, H 4.48, N 4.71; found: C 68.65, H 4.49, N 4.69.

5.1.2. 2-Hydroxy-3-(3-methoxyphenyl)quinoline-4-carboxylic acid (12). From isatin and 3-methoxyphenylacetic acid as described for **11**: 63% yield. mp 327–329 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.77 (*s*, 3H, 3′-OMe), 6.93–6.98 (*m*, 3H, Ar-H), 7.26 (*m*, 1H, 6-H), 7.34 (*m*, 1H, Ar-H), 7.39 (*d*, 1H, J = 8.0 Hz, 8-H), 7.48 (*dd*, 1H, J = 0.8, 8.0 Hz, 5-H), 7.58 (*m*, 1H, 7-H), 12.15 (*br s*, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.04, 113.39, 115.46, 115.50, 115.58, 122.04, 122.47, 125.46, 128.54, 128.88, 130.89, 135.71, 138.51, 142.24, 158.62, 160.64, 167.48. Anal. calcd for $C_{17}H_{13}NO_4$: C 69.15, H 4.44, N 4.74; found: C 69.10, H 4.36, N 4.74.

5.1.3. 2-Hydroxy-3-phenylquinoline-4-carboxylic acid (13). From isatin and phenylacetic acid as described for **11**: 78% yield. mp 332–333 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 7.26 (*m*, 1H, 6-H), 7.33–7.45 (*m*, 6H, Ar-H), 7.49 (*dd*, 1H, J = 1.2, 8.4 Hz, 5-H), 7.58 (*m*, 1H, 7-H), 12.17 (*br s*, 1H, COOH), 13.84 (*br s*, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 115.44, 115.61, 122.43, 125.43, 127.73 (2C), 128.01, 128.74, 129.64 (2C), 130.82, 134.44, 138.50, 142.17, 160.74, 167.45. Anal. calcd for $C_{16}H_{11}NO_3$: C 72.45, H 4.14, N 5.27; found: C 72.42, H 4.18, N 5.28.

5.1.4. 6-Chloro-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (14). A solution of **11** (2.95 g, 10 mmol) in $POCl_3$ (30 mL) was heated at 150 °C for 48 h (TLC monitoring). After cooling, the mixture was poured into ice-water (150 mL). The resulting precipitate that separated was collected by filtration. The filtered cake was suspended in 5% $NaHCO_3$ solution (200 mL) with vigorous stirring for 1 h. The resulting precipitate was collected, washed with H_2O, and crystallized from EtOH to give **14** (2.69 g, 91%). mp 227–228 °C ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (*s*, 3H, 9-OMe), 6.99 (*dd*, 1H, J = 2.4,

8.4 Hz, 8-H), 7.24 (*d*, 1H, *J* = 2.4 Hz, 10-H), 7.61 (*m*, 1H, 2-H), 7.67 (*m*, 1H, 3-H), 7.95 (*dd*, 1H, *J* = 0.4, 8.8 Hz, 4-H), 8.02 (*d*, 1H, *J* = 8.4 Hz, 7-H), 8.76 (*dd*, 1H, *J* = 0.8, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.81, 111.21, 119.48, 122.80, 123.97, 125.12, 128.59, 129.64, 130.61, 133.55, 135.14, 136.27, 137.20, 144.68, 149.30, 161.45, 193.63. Anal. calcd for $C_{17}H_{10}ClNO_2$: C 69.05, H 3.41, N 4.74; found: C 68.66, H 3.57, N 4.64.

5.1.5. 6-Chloro-11*H*-indeno[1,2-*c*]quinolin-11-one (16).[26] Chlorination of **13** as described for **14**: 88% yield. mp 149–150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (*s*, 3H, 9-OMe), 7.35 (*m*, 1H, 9-H), 7.54 (*m*, 1H, 8-H), 7.61 (*m*, 1H, 2-H), 7.67–7.72 (*m*, 2H, 3, 7-H), 7.96 (*d*, 1H, *J* = 8.8 Hz, 4-H), 8.14 (*d*, 1H, *J* = 7.6 Hz, 10-H), 8.79 (*dd*, 1H, *J* = 0.4, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 122.63, 123.98, 124.26, 124.94, 128.60, 129.69, 129.95, 131.11, 133.08, 135.36, 136.30, 136.55, 141.52, 145.03, 149.83, 193.65. Anal. calcd for $C_{16}H_8ClNO\cdot 0.1\ H_2O$: C 71.35, H 3.03, N 5.20; found: C 71.64, H 3.18, N 5.23.

5.1.6. 6-Amino-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (17a). A mixture of **14** (0.3 g, 1 mmol), ammonia water (5 mL), and 2-ethoxyethanol (20 mL) was heated in the sealed tube at 200 °C for 48 h. The solvent was removed in vacuo and the residue was suspended in H_2O (50 mL). The resulting precipitate that separated was collected, washed with H_2O, and dried to give a crude solid, which was crystallized from MeOH to give **17a** (0.17 g, 62%). mp 148–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.85 (*s*, 3H, 9-OMe), 6.70 (*br s*, 2H, NH_2), 7.04 (*dd*, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.16 (*d*, 1H, *J* = 2.4 Hz, 10-H), 7.29 (*m*, 1H, 2-H), 7.48–7.55 (*m*, 2H, 3, 4-H), 7.87 (*d*, 1H, *J* = 8.4 Hz, 7-H), 8.45 (*d*, 1H, *J* = 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 56.24, 111.70, 118.89, 123.42, 124.38, 124.53, 124.71, 126.12, 128.37, 129.89, 134.16, 134.27, 134.92, 149.38, 153.79, 160.63, 195.55. Anal. calcd for $C_{17}H_{12}N_2O_2$: C 73.91, H 4.38, N 10.14; found: C 73.82, H 4.42, N 9.76.

5.1.7. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one (17b). From **14** and piperazine as described for **17a**: 83% yield. mp 150–151 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.29 (*m*, 4H, piperazinyl-H), 3.45 (*m*, 4H, piperazinyl-H), 3.85 (*s*, 3H, 9-OMe), 7.12 (*dd*, 1H, *J* = 2.0, 8.0 Hz, 8-H), 7.17 (*d*, 1H, *J* = 2.0 Hz, 10-H), 7.50-7.56 (*m*, 2H, 2-, 7-H), 7.64 (*m*, 1H, 3-H), 7.79 (*d*, 1H, *J* = 8.4 Hz, 4-H), 8.54 (*d*, 1H, *J* = 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 42.80 (2C), 47.10 (2C), 55.84, 111.01, 119.27, 120.41, 122.98, 124.77, 127.38, 127.79, 129.83, 132.34, 133.81, 134.43, 135.37, 147.62, 155.93, 160.55, 194.35. Anal. calcd for $C_{21}H_{19}N_3O_2\cdot 0.7\ H_2O\cdot 0.8$ HCl: C 65.15, H 5.52, N 10.85; found: C 65.38, H 5.45, N 10.59.

5.1.8. 9-Methoxy-6-morpholino-11*H*-indeno[1,2-*c*]quinolin-11-one (17c). From **14** and morpholine as described for **17a**: 81% yield. mp 170–171 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.40 (*m*, 4H, morpholinyl-H), 3.87 (*s*, 3H, 9-OMe), 3.98 (*m*, 4H, morpholinyl-H), 6.95 (*dd*, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.21 (*d*, 1H, *J* = 2.4 Hz, 10-H), 7.45 (*m*, 1H, 2-H), 7.55 (*m*, 2H, 3-, 7-H), 7.83 (*d*, 1H, *J* = 8.4 Hz, 4-H), 8.69 (*dd*, 1H, *J* = 1.2, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 50.13 (2C), 55.81, 66.85 (2C), 110.99, 118.87, 121.07, 123.78, 124.25, 127.04, 128.01, 129.55, 132.65, 135.03, 135.19, 136.11, 148.62, 156.79, 160.76, 195.11. Anal. calcd for $C_{21}H_{18}N_2O_3$: C 72.82, H 5.24, N 8.09; found: C 72.77, H 5.26, N 8.03.

5.1.9. 9-Methoxy-6-(piperidin-1-yl)-11*H*-indeno[1,2-*c*]-quinolin-11-one (17d). From **14** and piperidine as described for **17a**: 76% yield. mp 147–148 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.70 (*m*, 2H, piperidinyl-H), 1.84 (*m*, 4H, piperidinyl-H), 3.32 (*m*, 4H, piperidinyl-H), 3.87 (*s*, 3H, 9-OMe), 6.95 (*dd*, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.21 (*d*, 1H, *J* = 2.4 Hz, 10-H), 7.42 (*m*, 2H, 2-H), 7.54 (*m*, 1H, 3-H), 7.59 (*d*, 1H, *J* = 8.0 Hz, 7-H), 7.82 (*d*, 1H, *J* = 8.4 Hz, 4-H), 8.68 (*dd*, 1H, *J* = 1.6, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 24.27, 25.92 (2C), 50.98 (2C), 55.79, 110.68, 118.84, 120.86, 123.74, 124.29, 126.60, 127.87, 129.31, 133.28, 135.11, 135.63, 135.83, 148.72, 158.03, 160.64, 195.50. Anal. calcd for $C_{22}H_{20}N_2O_2\cdot 0.1\ H_2O$: C 76.33, H 5.88, N 8.09; found: C 76.14, H 5.90, N 7.99.

5.1.10. 9-Methoxy-6-(4-methylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one (17e). From **14** and 4-methylpiperazine as described for **17a**: 74% yield. mp 147–148 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 2.42 (*s*, 3H, NMe), 2.70 (*m*, 4H, piperazinyl-H), 3.43 (*m*, 4H, piperazinyl-H), 3.87 (*s*, 3H, 9-OMe), 6.95 (*dd*, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.21 (*d*, 1H, *J* = 2.4 Hz, 10-H), 7.43 (*m*, 1H, 2-H), 7.53–7.57 (*m*, 2H, 3-, 7-H), 7.83 (*d*, 1H, *J* = 8.4 Hz, 4-H), 8.68 (*d*, 1H, *J* = 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 46.28, 49.56 (2C), 55.03 (2C), 55.77, 110.78, 118.80, 120.88, 123.69, 124.33, 126.76, 127.95, 129.39, 132.70, 135.11, 135.26, 135.92, 148.59, 156.85, 160.62, 195.27. Anal. calcd for $C_{22}H_{21}N_3O_2\cdot 0.3\ H_2O$: C 72.43, H 5.97, N 11.52; found: C 72.19, H 6.02, N 11.70.

5.1.11. 9-Methoxy-6-(3-methylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one (17f). From **14** and 3-methylpiperazine as described for **17a**: 55% yield as a gum. ^{1}H NMR (400 MHz, $CDCl_3$): 1.17 (*d*, 3H, *J* = 6.4 Hz, Me), 1.82 (*br s*, 1H, piperidinyl-NH), 1.68 (*m*, 1H, piperazinyl-H), 3.01 (*m*, 1H, piperazinyl-H), 3.14–3.25 (*m*, 3H, piperazinyl-H), 3.64 (*m*, 2H, piperazinyl-H), 3.88 (*s*, 3H, 9-OMe), 6.95 (*dd*, 1H, *J* = 2.8, 8.4 Hz, 8-H), 7.21 (*d*, 1H, *J* = 2.4 Hz, 10-H), 7.43 (*m*, 1H, 2-H), 7.53–7.58 (*m*, 2H, 3-, 7-H), 7.83 (*d*, 1H, *J* = 8.4 Hz, 4-H), 8.69 (*dd*, 1H, *J* = 1.2, 8.0 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 19.79, 45.74, 50.17, 50.53, 55.78, 57.19, 110.85, 118.81, 120.90, 123.72, 124.26, 126.79, 127.92, 129.41, 132.81, 135.14, 135.28, 136.00, 148.64, 157.02, 160.68, 195.24. Anal. calcd for $C_{22}H_{21}N_3O_2\cdot 0.75.H_2O$: C 70.84, H 6.09, N 11.27; found: C 70.50, H 6.12, N 11.20.

5.1.12. 8-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]-quinolin-11-one (18b). A solution of **12** (1.48 g, 5 mmol) in $POCl_3$ (15 mL) was heated at 150 °C for 36 h (TLC monitoring). After cooling, the mixture was poured into ice-water (80 mL). The resulting precipitate that

separated was collected by filtration. The filtered cake was suspended in 5% $NaHCO_3$ solution (100 mL) with vigorous stirring for 1 h. The resulting precipitate was collected, washed with H_2O, and dried to give **15** as the crude intermediate which was used for the next step without further purification.

The mixture of above crude intermediate **15**, 2-ethoxyethanol (15 mL), and piperazine (2.15 g, 25 mmol) was heated in the sealed tube at 200 °C for 48 h. The solvent was removed in vacuo and the residue was suspended in H_2O (50 mL). The resulting precipitate that separated was collected, washed with H_2O, and dried to give a crude solid, which was purified by column chromatography (MeOH:CH_2Cl_2 1/50) to give **18b** (0.59 g, 34%). mp 193–194 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.17 (*m*, 4H, piperazinyl-H), 3.36 (*m*, 4H, piperazinyl-H), 3.93 (*s*, 3H, 8-OMe), 6.70 (*dd*, 1H, *J* = 2.4, 8.4 Hz, 9-H), 7.30 (*d*, 1H, *J* = 2.4 Hz, 7-H), 7.45 (*m*, 1H, 2-H), 7.57–7.62 (*m*, 2H, 3-, 10-H), 7.85 (*d*, 1H, *J* = 8.4 Hz, 4-H), 8.76 (*dd*, 1H, *J* = 0.8, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 46.03 (2C), 51.33 (2C), 55.85, 111.32, 111.54, 113.47, 120.94, 124.12, 125.95, 126.70, 127.93, 129.94, 136.88, 137.85, 145.81, 149.38, 157.49, 165.31, 193.84. Anal. calcd for $C_{21}H_{19}N_3O$·0.1 HCl: C 72.27, H 5.52, N 12.04; found: C 72.07, H 5.64, N 12.03.

5.1.13. 6-(Piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one (19b). From **16** and piperazine as described for **17a**: 81% yield. mp 152–153 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.39 (*m*, 4H, piperazinyl-H), 3.51 (*m*, 4H, piperazinyl-H), 7.34 (*m*, 1H, 9-H), 7.47 (*m*, 1H, 8-H), 7.55–7.63 (*m*, 4H, 2-, 3-, 7-, 10-H), 7.77 (*d*, 1H, *J* = 8.4 Hz, 4-H), 8.54 (*dd*, 1H, *J* = 1.2, 8.0 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 42.70 (2C), 46.88 (2C), 120.58, 123.51, 123.79, 124.68, 127.84, 128.07, 129.82, 130.75, 131.68, 132.55, 136.06, 136.13, 142.15, 148.43, 156.23, 194.89. Anal. calcd for $C_{20}H_{17}N_3O$·1.0 HCl: C 68.28, H 5.16, N 11.94; found: C 68.08, H 5.25, N 11.71.

5.1.14. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]-quinolin-11-one oxime (20). To a suspension of **17b** (0.35 g, 1.0 mmol) in 2-ethoxyethanol (30 mL) was added NH_2OH·HCl (0.20 g, 3.0 mmol). The reaction mixture was heated with stirring under microwave irradiation (100 W) for 30 min (TLC monitoring). The solvent was removed in vacuo and the residue suspended in H_2O (20 mL). The resulting precipitate that separated was collected, washed with H_2O, and crystallized from MeOH to give **20** (0.29 g, 81%). mp 146–147 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 2.06 (*m*, 4H, piperazinyl-H), 3.24 (*m*, 4H, piperazinyl-H), 3.84 (*s*, 3H, OMe), 7.13 (*d*, 1H, *J* = 7.2 Hz, 8-H), 7.46 (*m*, 1H, 2-H), 7.59 (*m*, 1H, 3-H), 7.80 (*m*, 2H, 4-, 7-H), 8.02 (*s*, 1H, 10-H), 8.76 (*d*, 1H, *J* = 8.0 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 44.91 (2C), 50.15 (2C), 55.52, 115.04, 115.32, 120.99, 123.56, 125.03, 125.66, 126.39, 128.14, 128.70, 130.18, 130.52, 138.82, 146.37, 153.66, 156.94, 159.60. ESIMS $[M+H]^+$: 361.

5.1.15. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]-quinolin-11-one *O*-methyl oxime (21). From **17b** and NH_2OMe·HCl as described for **20**: 83% yield. mp 149–150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.35 (*m*, 8H, piperazinyl-H), 3.85 (*s*, 3H, OMe), 4.36 (*s*, 3H, NOMe), 7.15 (*d*, 1H, *J* = 7.2 Hz, 8-H), 7.52 (*m*, 1H, 2-H), 7.65 (*m*, 1H, 3-H), 7.74 (*d*, 1H, *J* = 8.4 Hz, 4-H), 7.86 (*m*, 2H, 7-, 10-H), 8.74 (*d*, 1H, *J* = 8.0 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 42.36 (2C), 46.45 (2C), 55.62, 64.57, 115.79, 115.87, 121.06, 123.87, 124.96, 126.46, 126.69, 128.26, 129.14, 130.34, 130.53, 138.28, 146.22, 153.45, 155.54, 159.80. Anal. calcd for $C_{22}H_{22}N_4O_2$·1.4 HCl: C 62.11, H 5.54, N 13.17; found: C 62.20, H 5.66, N 13.01.

5.1.16. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]-quinolin-11-one O-2-(dimethylamino)ethyl oxime (22a). To a stirred solution of **20** (0.36 g, 1 mmol) in dry DMF (20 mL) was added NaH (60% in oil, 0.50 g) at 0 °C and stirring was continued for 1h. After stirring at rt for 8 h, 2-chloro-*N*,*N*-dimethylethanamine·HCl (0.42 g, 3 mmol) was added and stirring was continued for 1 h. The reaction mixture was partitioned between H_2O (50 mL) and CH_2Cl_2(50 mL). The organic layer was collected, dried over $MgSO_4$, and evaporated in vacuo to give the residue which was purified by column chromatography (MeOH:CH_2Cl_2 1/10) to give **22a** (0.23 g, 53%). mp 84–86 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 2.39 (*s*, 6H, NMe_2), 2.89 (*t*, 2H, *J* = 6.0 Hz, NCH_2), 3.19 (*m*, 4H, piperazinyl-H), 3.47 (*m*, 4H, piperazinyl-H), 3.88 (*s*, 3H, OMe), 4.68 (*t*, 2H, *J* = 6.0 Hz, NCH_2), 6.95 (*dd*, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.41 (*m*, 1H, 2-H), 7.55 (*m*, 1H, 3-H), 7.76 (*d*, 1H, *J* = 8.4 Hz, 7-H), 7.87 (*d*, 1H, *J* = 8.4 Hz, 4-H), 7.98 (*d*, 1H, *J* = 2.4 Hz, 10-H), 8.79 (*dd*, 1H, *J* = 1.2, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 45.73 (2C), 46.01 (2C), 50.78 (2C), 55.58, 58.20, 74.97, 115.23, 115.86, 121.67, 123.34, 125.48, 125.67, 127.40, 128.39, 128.53, 131.26, 132.12, 139.20, 147.18, 154.46, 157.09, 159.78. Anal. calcd for $C_{25}H_{29}N_5O_2 \cdot 0.4\ H_2O$: C 68.44, H 6.85, N 15.96; found: C 68.67, H 6.91, N 15.60.

5.1.17. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]-quinolin-11-one O-3-aminopropyl oxime (22b). From **20** and 3-chloropropylamine · HCl as described for **22a**: 41% yield. mp 89–90 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 2.10 (*quin*, 2H, *J* = 6.4 Hz, $NCH_2C\underline{H_2}CH_2N$), 3.00 (*t*, 2H, *J* = 6.4 Hz, NCH_2), 3.15 (*m*, 4H, piperazinyl-H), 3.32 (*m*, 4H, piperazinyl-H), 3.87 (*s*, 3H, OMe), 4.67 (*t*, 2H, *J* = 6.4 Hz, NCH_2), 7.18 (*dd*, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.51 (*m*, 1H, 2-H), 7.63 (*m*, 1H, 3-H), 7.78 (*d*, 1H, *J* = 8.4 Hz, 7-H), 7.83 (*d*, 1H, *J* = 8.4 Hz, 4-H), 7.87 (*d*, 1H, *J* = 2.4 Hz, 10-H), 8.73 (*dd*, 1H, *J* = 1.2, 7.6 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 27.45, 36.07 (2C), 44.08, 49.14 (2C), 55.68, 73.47, 115.69, 115.90, 120.67, 123.93, 124.99, 126.19, 126.89, 128.24, 129.05, 130.28, 130.91, 138.09, 146.46, 153.92, 156.55, 159.74. ESIMS $[M+H]^+$: 418.

5.1.18. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]-quinolin-11-one O-3-(dimethylamino)propyl oxime (22c). From **20** and 3-chloro-*N*,*N*-dimethylpropanamine·HCl as described for **22a**: 52% yield. mp 145–146 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 2.14 (*quin*, 2H, *J* = 6.4 Hz, $NCH_2C\underline{H_2}CH_2N$), 2.33 (*s*, 6H, NMe_2), 2.59 (*t*, 2H, *J* = 6.4 Hz, NCH_2), 3.29 (*m*, 4H, piperazinyl-H), 3.44

(*m*, 4H, piperazinyl-H), 3.88 (*s*, 3H, OMe), 4.62 (*t*, 2H, J = 6.4 Hz, NCH_2), 6.95 (*dd*, 1H, J = 2.8, 8.4 Hz, 8-H), 7.41 (*m*, 1H, 2-H), 7.55 (*m*, 1H, 3-H), 7.80 (*d*, 1H, J = 8.4 Hz, 7-H), 7.86 (*d*, 1H, J = 8.0 Hz, 4-H), 7.92 (*d*, 1H, J = 2.8 Hz, 10-H), 8.78 (*dd*, 1H, J = 1.2, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 27.28, 44.68 (2C), 45.49 (2C), 50.41 (2C), 55.58, 56.24, 74.81, 115.01, 115.97, 121.69, 123.15, 125.45, 125.67, 127.09, 128.30, 128.64, 131.16, 131.73, 139.37, 146.96, 154.11, 156.30, 159.82. ESIMS $[M+H]^+$: 446.

5.1.19. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one O-2-(piperidin-1-yl)ethyl oxime (22d). From **20** and *N*-(2-chloroethyl)piperidine·HCl as described for **22a**: 54% yield. mp 95–96 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 1.38 (*m*, 2H, piperidinyl-H), 1.51 (*m*, 4H, piperidinyl-H), 2.51 (*m*, 4H, piperidinyl-H), 2.81 (*t*, 2H, J = 5.6 Hz, NCH_2), 3.28 (*m*, 4H, piperazinyl-H), 3.46 (*m*, 4H, piperazinyl-H), 3.85 (*s*, 3H, OMe), 4.66 (*t*, 2H, J = 5.6 Hz, NCH_2), 7.15 (*dd*, 1H, J = 2.8, 8.4 Hz, 8-H), 7.48 (*m*, 1H, 2-H), 7.61 (*m*, 1H, 3-H), 7.76 (*d*, 1H, J = 8.4 Hz, 7-H), 7.81 (*d*, 1H, J = 8.4 Hz, 4-H), 7.92 (*d*, 1H, J = 2.8 Hz, 10-H), 8.72 (*dd*, 1H, J = 0.8, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 23.92, 25.62 (2C), 44.60 (2C), 49.70 (2C), 54.31 (2C), 55.54, 57.41, 74.44, 115.55, 115.85, 120.87, 123.79, 124.98, 126.03, 126.81, 128.18, 128.95, 130.34, 130.91, 138.10, 146.47, 153.58, 156.70, 159.68. Anal. calcd for $C_{28}H_{33}N_5O_2 \cdot 0.1\ H_2O$: C 71.04, H 7.07, N 14.79; found: C 70.76, H 7.40, N 14.45.

5.1.20. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]-quinolin-11-one O-benzyl oxime (22e). From **20** and benzyl chloride as described for **22a**: 83% yield. mp 211–212 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.16 (*m*, 4H, piperazinyl-H), 3.49 (*m*, 4H, piperazinyl-H), 3.80 (*s*, 3H, OMe), 5.61 (*s*, 3H, OCH_2), 7.13 (*dd*, 1H, J = 2.4, 8.4 Hz, 8-H), 7.36–7.51 (*m*, 4H), 7.58–7.63 (*m*, 3H), 7.74 (*d*, 1H, J = 8.4 Hz, 7-H), 7.81 (*d*, 1H, J = 8.4 Hz, 4-H), 7.84 (*d*, 1H, J = 2.4 Hz, 10-H), 8.70 (*d*, 1H, J = 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 40.13 (2C), 48.81 (2C), 55.49, 78.18, 115.56, 115.90, 120.87, 123.86, 124.97, 126.15, 126.86, 128.20, 128.39, 128.57 (2C), 128.60 (2C), 129.00, 130.28, 130.86, 136.80, 138.10, 146.41, 153.91, 156.36, 159.63. Anal. calcd for $C_{17}H_{15}N_3O \cdot 1.0.H_2O$: C 71.76, H 6.03, N 11.96; found: C 71.53, H 5.81, N 11.70.

5.2. Antiproliferative activity

5.2.1. Cell culture. Cancer cells were purchased from Bioresources Collection and Research Center, Taiwan. Each 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 amphotericin. Culture was maintained at 37 °C with 5% CO_2 in a humidified atmosphere.

5.2.2. Antiproliferative assay. Cancer cells were treated as indicated for 48 h in medium containing 10% FBS. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, (2 mg/mL) (MTT, 20 mL) was added to the cultures and incubated during the final 1.5 h. The resultant tetrazolium salt was then dissolved by the addition of dimethylsulfoxide. Color was measured spectrophotometrically in a microtiter plate reader at 570 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%.

5.2.3. Immunofluorescence analysis.[27,28] HeLa cells grown on cover glasses in 12-well plates with the drug treatment for 24 h were used for DAPI staining. At different time points, the cells were washed twice with 1X HBSS and with 1X PBS three times. Then, the cells were fixed with 0.4% paraformaldehyde for 5 min, followed by three times washing by 1X PBS. In order to permeabilize cells, cells were incubated in 2% FBS and 0.4% Triton X-100 in 1X PBS for 15 min at room temperature followed by three times washing with 0.2% Triton X-100 in PBS. To stain DNA, DAPI (0.06 μg/mL) was added onto cells and incubated at room temperature for 5 min. After washing several times with 0.2% Triton X-100 in PBS, the cells with DAPI staining were examined by fluorescence microscopy.

5.2.4. Flow cytometric analysis.[29] HeLa cells treated with DMSO or **22c** at a concentration of 0.3 or 1.0 μM for 24 h were harvested, rinsed in PBS, resuspended and fixed in 80% ethanol, and stored at −20 °C in fixation buffer until ready for analysis. Then the pellets were suspended in 1 mL of propidium iodide (PI) solution containing 20 μg/μL of PI, 0.2 mg/mL RNase, and 0.1% (v/v) Triton X-100. Cell samples were incubated at room temperature in the dark for at least 30 min and analyzed by a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Data recording was made using CELLQuest software (Becton Dickinson, Mountain View, CA) and cell cycle data were analyzed using ModFitLT software (Veruty Software House, USA).

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