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ORIGINAL ARTICLE

Design, synthesis and biological evaluation of tetracyclic azafluorenone derivatives with topoisomerase I inhibitory properties as potential anticancer agents

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Abstract Several 9-chloro-11*H*-indeno[1,2-*c*]quinolin-11-one derivatives have been designed which is replacing side chains with different groups containing oxygen, nitrogen or sulfur atoms. Substitution of C-6 on the starting structure, 6,9-dichloro-11*H*-indeno[1,2-*c*]quinolin-11-one, using apposite nucleophilic group with a suitable base or acid could be obtained 28 novel tetracyclic azafluorenone derivatives. The cytotoxic activity of these analogues was examined in cancer cell lines by MTT assay and compounds **4**, **5**, **13**, and **26** were selected to evaluate in topoisomerase I drug screening assay, respectively. At the same time, 17 compounds were selected for NCI-60 anticancer drug screen to prevent the narrower concept of an *in vitro* screening model. Its worth to find

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that 9-chloro-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**12**) showed greater cytotoxicity than another azafluorenone derivatives with an average GI_{50} of 10.498 μM over 60 cell lines. We also found that another analogue, 9-chloro-6-(2-methylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**13**), exhibited preferential growth inhibition effect toward cancer cell lines and showed a significant inhibitory effect on topoisomerase I.

1. Introduction

Camptothecin (CPT) is a cytotoxic quinoline alkaloid, as well as TAS-103 is a quinoline derivative which has been reported to be potent topoisomerase (topo) I and/or topo II poison (Aoyagi et al., 2000; Fujimoto, 2007; Yoshida et al., 2008; Shah et al., 2010). Two clinically anticancer drugs, irinotecan and topotecan, are derivatives of the CPT family that are used in colorectal cancer, either as single agents or in combination with radiotherapy and/or other chemotherapy drugs (Kehrer et al., 2001; Alagoz et al., 2012). Despite clinical success, they are the only Topo I-targeted anticancer drugs which still have several problems with CPT-derived anticancer agents (Teicher, 2008). Ongoing research aims to the lactone and A-D planar ring in CPT and its analogues and derivatives which suffer from major limitation and application drawback of non-mechanism related toxicity and poor solubility profile (Ulukan and Swaan, 2002; Kiselev et al., 2012; Perzyna et al., 2003). Because the direct target of above small molecules remained unclear, we tried structural hybridization of some preclinical and clinical anticancer drugs to design tetracyclic azafluorenone-derived small molecules as potential anticancer drugs (Fig. 1).

The anthracycline antibiotics (e.g. doxorubicin, daunorubicin, mitoxantrone and ametantrone) have been shown to provide significant antiproliferative (or cytostatic) properties (Chen et al., 2013, 2015) and inhibit topoisomerase II (Suzuki et al., 1995) as well as intercalate into the minor groove of double-strand DNA base pairs via the core planar pharmacophore group (Wang et al., 1994). In addition, DMMA (6,8-dihydroxy-7-methoxy-1-methyl-3-azafluorenone), an active compound of the azafluorenone, was isolated from *Polyalthia cerasoides* in 2010 (Pumsalid et al., 2010) which exhibits cytotoxic to various cancer cell lines (Banjerdpongchai et al., 2013). Moreover, recent studies illustrated related indenoquinoline skeleton incorporate ring system and various substituents had been found as potential dual topo I/II inhibitors and anticancer candidates (Tseng et al., 2013, 2010, 2009, 2008). There are also several studies have found that azafluorenone-derived compounds had potent anticancer (Coothankandaswamy et al., 2010; McLaughlin, 2008; Pan et al., 2011), cytotoxic (Pumsalid et al., 2010), antimicrobial (Koyama et al., 2005; Addla et al., 2012), antitubercular (Yempala et al., 2012), anti-inflammatory (Chuang et al., 2008; Rojano et al., 2007) and antiprotozoal activities (Waechter et al., 1999).

Based on above considerations, azafluorenones can be considered as an attractive scaffold for the development of anticancer reagents. In the past, several studies had found that DNA top IB can regulate DNA topological structures by sequential breakage and is involved in DNA transcription, replication, and recombination (Shen et al., 2010; Stewart et al., 1998). Toward supporting the aforementioned hypothesis, we recently disclosed evidence as well as an in-depth continuous investigation toward synthesis of fluorenone analogs (Lee et al., 2013) led to discovery of azafluorenone-derived scaffold small molecules for the polypharmacology. With this motivation, we herein also describe efficient synthetic procedures for the preparation of a series of non-nucleoside fused tetracyclic compounds comprising benzene moiety and an azafluorenone backbone. We attempted to identify additional potent enzyme inhibitors and work toward some azafluorenone-derived structural modifications in order to develop some potential anticancer structures leads which could be compared to parent molecules in cancer chemotherapy.

2. Chemistry

2.1. Materials and instruments

The Pfitzinger synthetic reaction of isatin and 4-chlorophenylacetic acid was stirred at 200 °C with sodium acetate (NaOAc) as a basic catalyst for 3 h to obtain compound **1** (52%) as a key intermediate. An isatin was reacted with NaOAc as base by the amide bond hydrolysis to obtain 2-(2-aminophenyl)-2-oxoacetic acid. A reactant with a ketone group reacted with the aniline to give the imine and the enamine. The enamine cyclized and dehydrated to give the desired quinoline-4-carboxylic acids (**1**). Treatment of **1** with phosphoryl trichloride ($POCl_3$) afforded 6,9-dichloro-11*H*-indeno[1,2-*c*]quinolin-11-one (**2**). A series of 6-substituted 9-chloro-11*H*-indeno[1,2-*c*]quinolin-11-one homologues can be synthesized and the preparation involved various synthetic routes with approximate yields (overall 17–95%) in all steps: (i) reaction of isatin with 4-chlorophenylacetic acid and NaOAc; (ii) reaction of **1** with $POCl_3$; and (iii) reaction of compound **2** with a series of apposite primary or secondary amines, 2-mercaptoethanol, conc. hydrochloride (HCl), or sodium methoxide yielded the corresponding side chain compounds **3**–**29**, respectively (Scheme 1). The quantity of the isolated products was dependent on substrate and reaction condition. All the crude mixtures were purified through tedious recrystallization from ethyl acetate/n-hexane and/or ethanol. The molecular weight of all synthetic compounds was determined by HRMS. The protons and carbons from tetracyclic azafluorenone structures were also obvious from the ^{1}H NMR and ^{13}C NMR spectra.

2.2. Synthesis of target compounds *1*–*29*

The synthetic methods and physical data of compounds **1**–**29** were described and all compounds were tested and compared for their growth inhibition, cytotoxicity and topoisomerase activities. In this study, we synthesized and dedicated on the role of our systematic, tetracyclic, and heterocyclic pharmacophore by introducing a series of side chains linked to the 9-chloro-11*H*-indeno[1,2-*c*]quinolin-11-one moiety.

All reactions were monitored through a TLC (silica gel 60 F_{254}) plate with a 254-nm UV lamp. ^{1}H NMR and ^{13}C NMR were measured on Varian GEMINI-300 (300 MHz) or Agilent 400 MR DD2 (400 MHz); δ values are in ppm relative

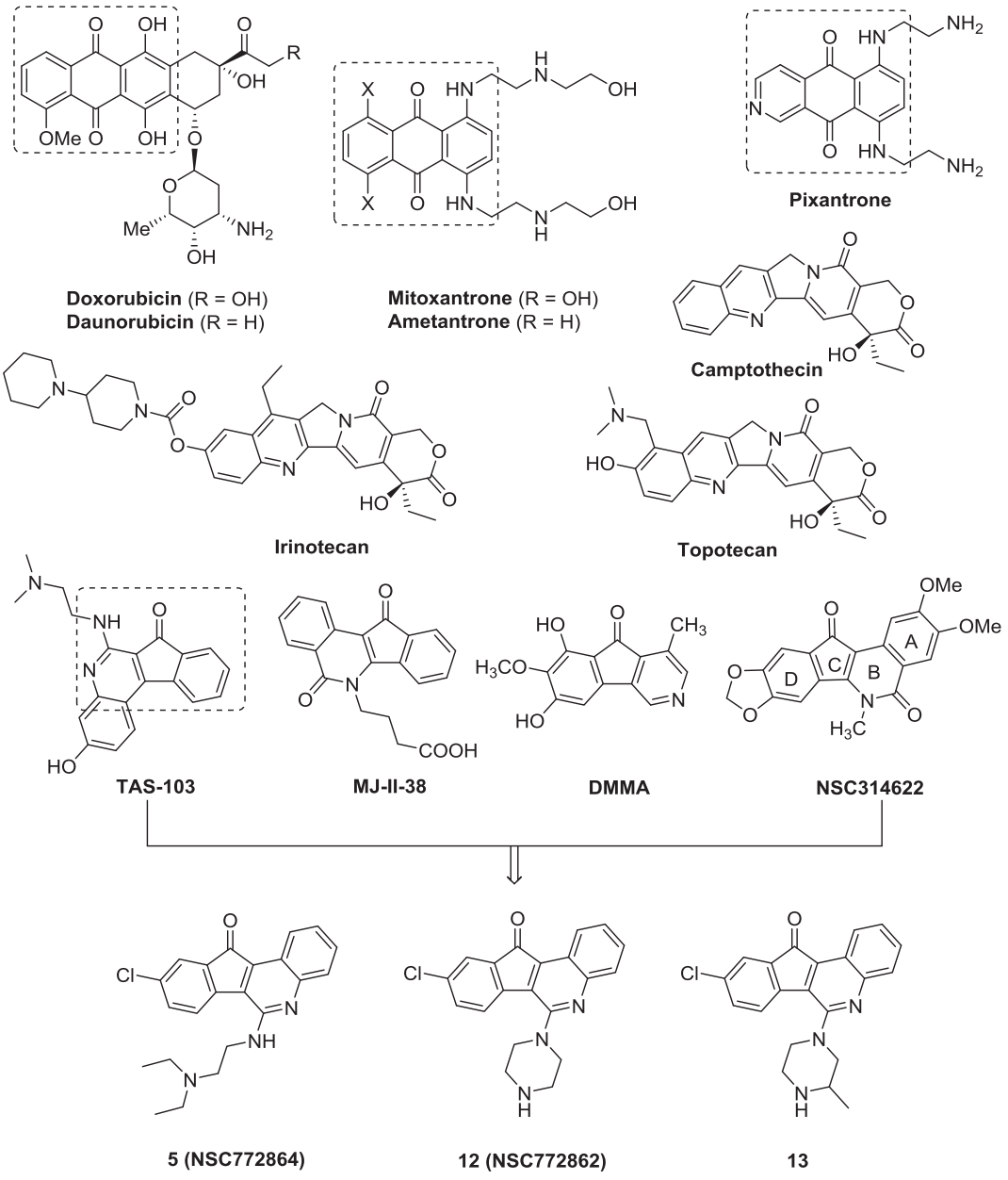


Figure 1 Structural hybridization design of tetracyclic quinoline derivatives.

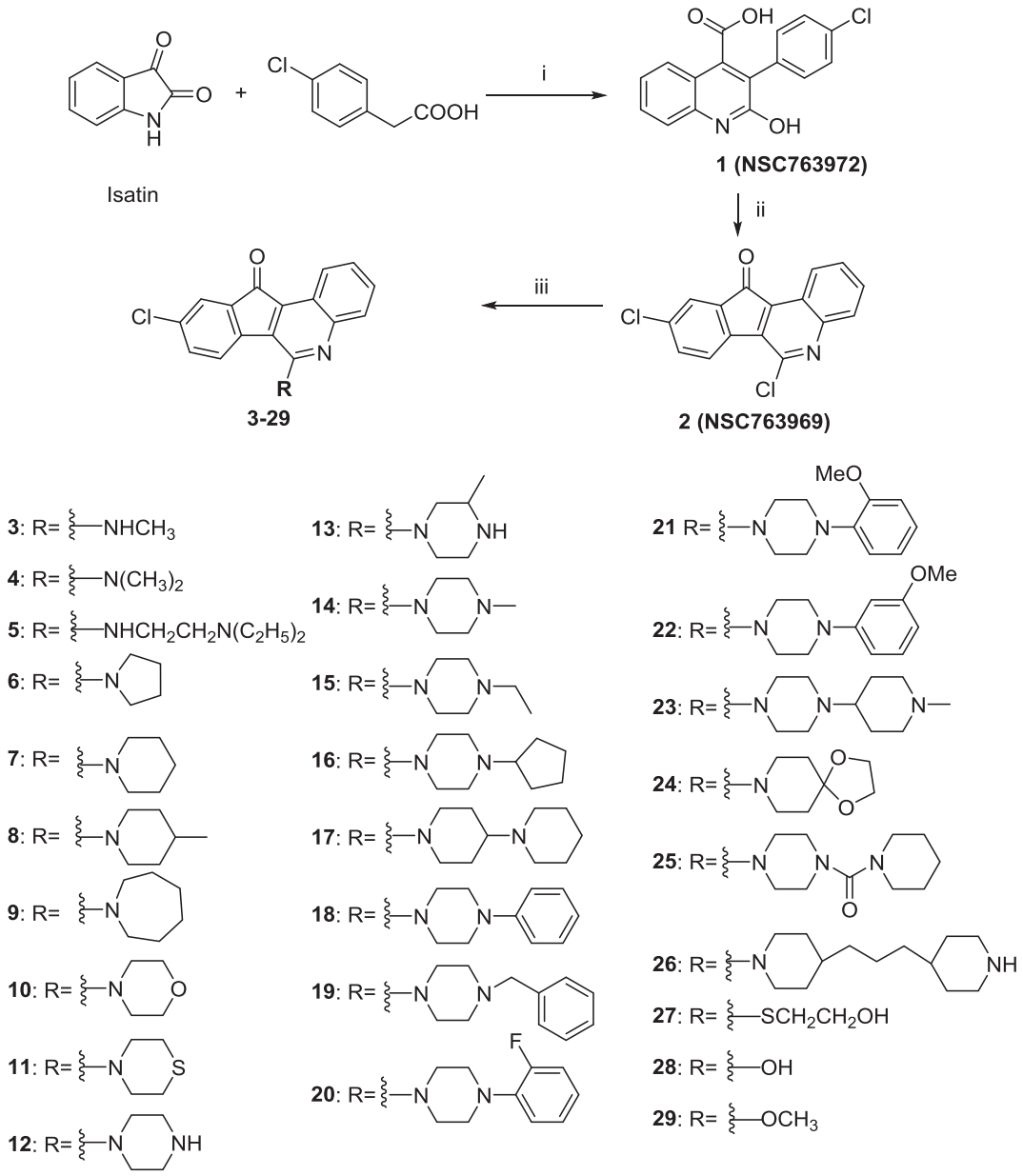
to TMS (tetramethylsilane) as an internal standard. Multiplicities are recorded as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintuplet), dd (doublet of doublets), dt (triplet of doublets), td (doublet of triplets), m (multiplet), and br (broadened). Mass spectra: High resolution electrospray ionization (HRESI): Finnigan MAT 95S (Instrumentation Center, National Taiwan University, Taipei, Taiwan) and High resolution electron impact ionization (HREI): Finnigan MAT MAT-95XL (Instrumentation Center, National Tsing Hua University, Hsinchu, Taiwan). Melting points of synthetic compounds were determined with a Büchi B-545 melting point apparatus. Typical experiments illustrating the synthetic procedures for the preparation of the tetracyclic small molecules are described below. These compounds were synthesized, starting from isatin and 4-chloro-phenylacetic acid. All the reagents and solvents required for synthesis were purchased from either Merck Chemical Company or Sigma–Aldrich Chemical Company without further purification.

*2.2.1. Synthetic procedure i: preparation of compound **1***

A mixture of isatin (3.14 g, 21 mmol), 4-chloro-phenylacetic acid (3.41 g, 20 mmol), and sodium acetate (1.00 g) was heated at 200 °C for 2 h (TLC monitoring). After cooling, 100 mL acetic acid was added to the mixture. The precipitate was filtrated and washed with acetic acid and n-hexane, and then collected the obtained orange compound.

*2.2.2. Synthetic procedure ii: preparation of compound **2***

A suspension of compound **1** (3.03 g, 10.1 mmol) and $POCl_3$ (20 mL) was stirred and heated at 150 °C for 48 h. After cooling, the mixture was poured into ice-water (300 mL) at 0 °C cautiously. The resulting precipitate that separated was col-



Scheme 1 Overall synthetic routes of the tetracyclic azafluorenone derivatives. Reagents and conditions: (i) **1**: NaOAc, 200 °C, 2 h; (ii) **2**: $POCl_3$, 150 °C, reflux, 48 h; (iii) **3**–**26**: appropriate amines, DMF, pyridine, miniclave, 150 °C, 2 h; **27**: mercaptoethanol, K_2CO_3; **28**: DMF, conc. HCl, reflux, 24 h; **29**: NaOMe, MeOH, reflux, 16 h.

lected by filtration. The filtered cake was suspended in 10% $NaHCO_3$ solution (300 mL) with vigorous stirring for 1 h. The resulting precipitate was collected and washed with H_2O. The crude was recrystallized from dichloromethane to give an orange product.

*2.2.3. General procedure iii: preparation of compounds **3**–**26***

Compound **2**, primary or secondary amine (10 mmol) and *N*, *N*-diisopropylethylamine (DIPEA) (2 mmol) were dissolved in miniclave containing DMF (10 mL) and stirred at 150 °C for 4 h. The reaction was poured into ice-water (100 mL). The resulting precipitate was collected by filtration and purified by crystallization from ethanol to afford desired compound.

*2.2.4. Synthetic procedure iv: preparation of compound **27***

Compound **2**, 2-mercaptoethanol (10 mmol) and *N*,*N*-diisopropylethylamine (DIPEA) (2 mmol) were dissolved in miniclave containing DMF (10 mL) and stirred at room 150 °C for 4 h. The reaction was poured into ice-water (100 mL). The resulting precipitate was collected by filtration and purified by crystallization from ethanol to afford desired compounds.

*2.2.5. Synthetic procedure v: preparation of compound **28***

A mixture of compound **2**, conc. HCl (2 mL) and DMF (10 mL) was refluxed at 120 °C. After 4 h, the conc. HCl was added into the reaction again (TLC monitored). The mixture

was evaporated in vacuum or dean-stark trap, treated with H_2O (20 mL), and filtered. The crude solid was washed with EtOH to give **28** as a red solid.

*2.2.6. Synthetic procedure vi: preparation of compound **29***

A methanol solution (20 mL) containing sodium methoxide (1.08 g, 20 mmol) was slowly added into the suspension of compound **2** in MeOH (10 mL) for 10 min. The reaction was refluxed at 100 °C for 16 h (TLC monitored). After cooled, the solvent was removed by rotary evaporator vacuum, filtrated and washed with ethanol and n-hexane to collect an orange solid.

2.3. Physical data

*2.3.1. 3-(4-Chlorophenyl)-2-hydroxyquinoline-4-carboxylic acid (**1**)*

The pure compound was obtained as an orange solid (yield 80%). Mp 310–311 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 3234 (NH), 1637 (CO). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.60 (s, 1H, —OH), 6.91 (td, J = 7.2 Hz, 0.6 Hz, 1H, Ar—H), 7.12 (d, J = 8.4 Hz, 1H, Ar—H), 7.51 (d, J = 8.7 Hz, 2H, Ar—H), 7.53–7.59 (m, 2H, Ar—H), 7.74 (d, J = 8.7 Hz, 2H, Ar—H), 9.83 (s, 1H, —COOH). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 117.12, 120.66, 121.83, 122.20, 126.31, 128.86, 129.15, 132.67, 134.22, 135.21, 136.23, 139.59, 182.29 (CO). HRMS (ESI) m/z calcd for $C_{16}H_{10}NO_3Cl^+$ $[M]^+$ 299.0349, found $[M+H]^+$: 300.0424, $[M-H]^-$: 298.0238.

*2.3.2. 6,9-Dichloro-11H-indeno[1,2-c]quinolin-11-one (**2**)*

Product **2** was cyclized from compound **1** using $POCl_3$ at 150 °C for 48 h. The red solid material was isolated in 30% yield. (R_f = 0.70 at CH_2Cl_2). Mp 241–243 °C. FT-IR (KBr; ν cm^{-1}): 1719 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.52 (dd, J = 8.25, 1.8 Hz, 1H, Ar—H), 7.62–7.68 (m, 2H, Ar—H), 7.70–7.76 (m, 1H, Ar—H), 7.97–8.01 (dt, J = 7.5, 0.6 Hz, 1H, Ar—H), 8.10 (d, J = 7.8 Hz, 1H, Ar—H), 8.77–8.80 (m, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 122.97, 124.59, 125.30, 125.69, 129.13, 130.32, 131.70, 135.06, 135.14, 136.19, 136.74, 136.80, 140.15, 145.25, 150.48, 192.80 (CO). HRMS (ESI) m/z calcd for $C_{16}H_7NOCl_2^+$, $[M]^+$: 298.9905, found $[M+H]^+$: 299.9965 (100), 301.9947 (65), 303.9917 (10).

*2.3.3. 9-Chloro-6-(methylamino)-11H-indeno[1,2-c]quinolin-11-one (**3**)*

Product **3** was prepared from compound **2** and methylamine. The red solid material was isolated in 75% yield (R_f = 0.51 at CH_2Cl_2: n-hexane = 2:1). Mp 189–191 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1716 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.01 (s, 3H, N—CH$_3$), 7.41–7.47 (m, 2H, Ar—H), 7.57–7.62 (m, 3H, Ar—H), 7.84 (d, J = 8.4 Hz, 1H, Ar—H), 8.68 (d, J = 8.1 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 42.22, 120.81, 124.29, 124.93, 124.99, 127.02, 128.13, 130.44, 131.60, 134.33, 135.16, 135.24, 136.59, 141.85, 149.69, 158.14, 194.48 (CO). HRMS (ESI) m/z calcd for $C_{17}H_{11}N_2OCl^+$ $[M]^+$: 294.0560, found $[M+H]^+$: 295.0634.

*2.3.4. 9-Chloro-6-(dimethylamino)-11H-indeno[1,2-c]quinolin-11-one (**4**)*

Product **4** was prepared from compound **2** and dimethylamine. The red solid material was isolated in 74.5% yield (R_f = 0.51 at CH_2Cl_2: n-hexane = 1:1). Mp 193–195 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 3407 (NH), 1718 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.04 (s, 6H, —N—$(CH_3)_2$), 7.41–7.48 (m, 2H, Ar—H), 7.57–7.63 (m, 3H, Ar—H), 7.86 (d, J = 8.7 Hz, 1H, Ar—H), 8.68–8.71 (m, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 42.19, 120.80, 124.27, 124.91, 124.96, 126.99, 128.13, 130.40, 131.57, 134.30, 135.14, 135.22, 136.54, 141.84, 149.70, 158.13, 194.45 (CO). HRMS (EI) m/z calcd for $C_{18}H_{13}N_2OCl^+$ $[M]^+$: 308.0716, found 308.0708.

*2.3.5. 6-(2-(Diethylamino)ethylamino)-9-chloro-11H-indeno[1,2-c]quinolin-11-one (**5**)*

Product **5** was prepared from compound **2** and N^1,N^1-diethylethane-1,2-diamine. The red solid material was isolated in 17.5% yield (R_f = 0.46 at CH_2Cl_2: n-hexane = 2:1). Mp 160–161 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 3317 (NH), 1712 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.11 (t, J = 7.2 Hz, 6H, —CH$_3$), 2.66 (q, J = 7.1 Hz, 4H, —NCH$_2$—), 2.84 (t, J = 5.7 Hz, 2H, —CH$_2$N—), 3.71–3.73 (m, 2H, —NHCH$_2$—), 6.14 (br, 1H, NH), 7.28–7.33 (m, 1H, Ar—H), 7.41–7.55 (m, 3H, Ar—H), 7.60 (d, J = 1.5 Hz, 1H, Ar—H), 7.70 (d, J = 8.7 Hz, 1H, Ar—H), 8.60 (m, J = 8.1 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 12.23, 38.81, 46.92, 51.64, 119.00, 122.48, 124.42, 124.95, 125.40, 126.94, 127.86, 130.53, 134.07, 134.90, 135.08, 135.45, 141.24, 150.68, 152.96, 194.65 (CO). HRMS (ESI) m/z calcd for $C_{22}H_{22}N_3OCl^+$ $[M]^+$: 379.1451, found$[M+H]^+$: 380.1510.

*2.3.6. 9-Chloro-6-(pyrrolidin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (**6**)*

Product **6** was prepared from compound **2** and pyrrolidine. The red solid material was isolated in 43.6% yield (R_f = 0.51 at CH_2Cl_2: n-hexane = 2:1). Mp 149–150 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1718 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.98 (quin, J = 3.6 Hz, 4H, pyrrolidine-H), 3.56 (t, J = 6.6 Hz, 4H, pyrrolidine-H), 7.33–7.42 (m, 3H, Ar—H), 7.54 (td, J = 7.5, 1.5 Hz, 2H, Ar—H), 7.76 (d, J = 8.4 Hz, 1H, Ar—H), 8.63 (dd, J = 8.4, 0.9 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 25.07, 50.37, 120.39, 124.25, 124.80, 124.96, 126.29, 127.78, 130.33, 130.40, 134.10, 134.83, 135.27, 136.46, 142.50, 149.78, 155.73, 194.65 (CO). HRMS (ESI) m/z calcd for $C_{20}H_{15}N_2OCl^+$ $[M]^+$: 334.0873, found $[M+H]^+$: 335.0952.

*2.3.7. 9-Chloro-6-(piperidin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (**7**)*

Product **7** was prepared from compound **2** and piperidine. The red solid material was isolated in 43% yield (R_f = 0.63 at CH_2Cl_2: n-hexane = 2:1). Mp 191–192 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1717 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.71 (br, 2H, piperidine-H), 1.83–1.86 (m, 4H, piperidine-H), 3.33 (br, 4H, piperidine-H), 7.43–7.48 (m, 2H, Ar—H), 7.58–7.66 (m, 3H, Ar—H), 7.90 (d, J = 8.1 Hz, 1H, Ar—H), 8.70 (d, J = 8.7 Hz, 1H, Ar—H). ^{13}C NMR

(75 MHz, $CDCl_3$): δ (ppm) 224.37, 26.01, 51.30, 120.81, 124.22, 124.41, 124.83, 127.09, 128.29, 130.24, 131.88, 134.24, 135.01, 135.17, 136.35, 142.05, 149.79, 158.35, 194.38 (CO). HRMS (ESI) *m/z* calcd for $C_{21}H_{17}N_2OCl^+$ $[M]^+$: 348.1029, found $[M+H]^+$: 349.1106.

*2.3.8. 9-Chloro-6-(4-methylpiperidin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (**8**)*

Product **8** was prepared from compound **2** and 4-methylpiperidine. The brown solid material was isolated in 25.4% yield (R_f = 0.61 at CH_2Cl_2: n-hexane = 2:1). Mp 190–192 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1718 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.07 (d, *J* = 6 Hz, 3H, —CH$_3$), 1.46–1.60 (m, 3H, —CH$_2$—, —CH—), 1.84–1.87 (m, 2H, —CH$_2$—), 2.96 (t, *J* = 11.6 Hz, 2H, —NCH$_2$—), 3.67–3.71 (m, 2H, —NCH$_2$—), 7.44–7.46 (m, 2H, Ar—H), 7.58 (m, 3H, Ar—H), 7.82–7.85 (m, 1H, Ar—H), 8.66–8.69 (m, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 22.07, 30.87, 34.43, 50.69, 120.88, 124.27, 124.46, 124.91, 127.15, 128.31, 130.32, 131.98, 134.33, 135.07, 135.23, 136.44, 142.13, 149.83, 158.25, 194.52 (CO). HRMS (ESI) *m/z* calcd for $C_{22}H_{19}N_2OCl^+$ $[M]^+$: 362.1186, found $[M+H]^+$: 363.1260.

*2.3.9. 6-(Azepan-1-yl)-9-chloro-11H-indeno[1,2-c]quinolin-11-one (**9**)*

Product **9** was prepared from compound **2** and azepane. The red solid material was isolated in 36% yield (R_f = 0.69 at CH_2Cl_2: n-hexane = 2:1). Mp 146–147 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1712 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.72–1.75 (m, 4H, —CH$_2$—), 1.86 (br, 4H, —CH$_2$—), 3.64 (t, *J* = 5.6 Hz, 4H, —NCH$_2$—), 7.40–7.46 (m, 2H, Ar—H), 7.54–7.60 (m, 3H, Ar—H), 7.78–7.80 (m, 1H, Ar—H), 8.68 (dd, *J* = 8.4, 0.6 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 27.96, 28.40, 52.91, 120.48, 124.24, 124.76, 124.89, 126.60, 127.98, 130.30, 131.09, 134.13, 134.97, 135.15, 136.84, 142.54, 149.74, 157.83, 194.62 (CO). HRMS (ESI) *m/z* calcd for $C_{22}H_{19}N_2OCl^+$ $[M]^+$: 362.1186, found $[M+H]^+$: 363.2000.

*2.3.10. 9-Chloro-6-morpholino-11H-indeno[1,2-c]quinolin-11-one (**10**)*

Product **10** was prepared from compound **2** and morpholine. The red solid material was isolated in 47% yield (R_f = 0.54 at CH_2Cl_2: n-hexane = 2:1). Mp 207–208 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1712 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.41 (t, *J* = 4.5 Hz, 4H, —CH$_2$—), 3.98 (t, *J* = 4.5 Hz, 4H, —CH$_2$—), 7.48 (td, *J* = 8.1, 2.1 Hz, 2H, Ar—H), 7.59–7.65 (m, 3H, Ar—H), 7.87 (d, *J* = 8.7 Hz, 1H, Ar—H), 8.69–8.72 (dt, *J* = 8.1, 0.9 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 50.56, 66.98, 121.18, 124.35, 124.43, 125.23, 127.65, 128.51, 130.59, 131.46, 134.42, 135.21, 135.55, 136.80, 141.66, 149.81, 157.31, 194.14 (CO). HRMS (ESI) *m/z* calcd for $C_{20}H_{15}N_2O_2Cl^+$ $[M]^+$: 350.0822, found $[M+H]^+$: 351.0898.

*2.3.11. 9-Chloro-6-thiomorpholino-11H-indeno[1,2-c]quinolin-11-one (**11**)*

Product **11** was prepared from compound **2** and thiomorpholine. The orange solid material was isolated in 74% yield (R_f = 0.33 at CH_2Cl_2: n-hexane = 2:1). Mp 228–230 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1711 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.91 (t, *J* = 5.1 Hz, 4H, —CH$_2$—), 2.69–2.73 (br, 4H, —CH$_2$—), 7.45–7.50 (td, *J* = 7.8, 1.8 Hz, 2H, Ar—H), 7.57–7.64 (m, 3H, Ar—H), 7.85 (d, *J* = 9.0 Hz, 1H, Ar—H), 8.68–8.71 (dd, *J* = 8.25, 1.2 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 27.38, 52.36, 121.07, 124.29, 125.19, 127.66, 128.48, 129.44, 130.57, 131.59, 134.40, 135.10, 135.54, 136.87, 141.70, 149.70, 157.66, 194.17 (CO). HRMS (ESI) *m/z* calcd for $C_{20}H_{15}N_2OSCl^+$ $[M]^+$: 366.0594, found $[M+H]^+$: 367.0664.

*2.3.12. 9-Chloro-6-(piperazin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (**12**)*

Product **12** was prepared from compound **2** and piperazine. The red solid material was isolated in 48% yield (R_f = 0.43 at CH_2Cl_2: n-hexane = 2:1). Mp 180–181 °C. FT-IR (KBr; *v* cm^{-1}): 3341 (NH), 1718 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.16 (t, *J* = 4.8 Hz, 4H, —CH$_2$—), 3.36 (br, 4H, —CH$_2$—), 7.46–7.49 (m, 2H, Ar—H), 7.62–7.66 (m, 3H, Ar—H), 7.87 (d, *J* = 8.7 Hz, 1H, Ar—H), 8.71 (d, *J* = 8.7 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 46.17, 51.51, 121.05, 124.31, 124.54, 125.11, 127.45, 128.46, 130.48, 131.69, 134.39, 135.16, 135.40, 136.68, 141.88, 149.84, 157.83, 194.37 (CO). HRMS (ESI) *m/z* calcd for $C_{20}H_{16}N_3OCl^+$ $[M]^+$: 349.0982, found $[M+H]^+$: 350.1063.

*2.3.13. 9-Chloro-6-(2-methylpiperazin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (**13**)*

Product **13** was prepared from compound **2** and 3-methylpiperazine. The red solid material was isolated in 18% yield (R_f = 0.49 at CH_2Cl_2: n-hexane = 4:1). Mp 199–200 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 3222 (NH), 1719 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.17 (d, *J* = 6.3 Hz, 3H, —CH$_3$), 2.70 (t, 1H, —CH$_2$—), 3.03–3.07 (m, 1H, —NCH—), 3.15–3.19 (m, 3H, —CH$_2$—, —CH$_2$NH—), 3.60–3.65 (d, *J* = 12.6 Hz, 2H, —NHCH$_2$—), 7.44–7.48 (m, 2H, Ar—H), 7.58–7.62 (m, 3H, Ar—H), 7.86 (d, *J* = 8.4 Hz, 1H, Ar—H), 8.69 (d, *J* = 7.8 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 19.96, 45.98, 50.63, 50.75, 57.67, 120.98, 124.29, 124.47, 125.09, 127.39, 128.41, 130.47, 131.63, 134.37, 135.15, 135.36, 136.65, 141.89, 149.82, 157.56, 194.36 (CO). HRMS (ESI) *m/z* calcd for $C_{21}H_{18}N_3OCl^+$ $[M]^+$: 363.1138, found $[M+H]^+$: 364.1201.

2.3.14. 9-Chloro-6-(4-methylpiperazin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (14)

Product **14** was prepared from compound **2** and 1-methylpiperazine. The red solid material was isolated in 51% yield (R_f = 0.4 at CH_2Cl_2: n-hexane = 2:1). Mp 205–207 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 3462 (NH), 1720 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.42 (s, 3H, N-CH$_3$), 2.70 (br, 4H, —CH$_2$—), 3.43 (br, 4H, N—CH$_2$—), 7.44–7.47 (m, 2H, Ar—H), 7.58–7.61 (m, 3H, Ar—H), 7.84 (d, *J* = 8.4 Hz, 1H, Ar—H), 8.67 (d, *J* = 8.1 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 46.38, 49.97, 55.17, 121.00, 124.28, 124.54, 125.07, 127.38, 128.46, 130.45, 131.51, 134.35, 135.13, 135.38, 136.63, 141.84, 149.80, 157.37, 194.29 (CO). HRMS (ESI) *m/z* calcd for $C_{21}H_{18}N_3OCl^+$ $[M]^+$: 363.1138, found $[M+H]^+$: 364.1222.

*2.3.15. 9-Chloro-6-(4-ethylpiperazin-1-yl)-11H-indeno[1,2-c] quinolin-11-one (**15**)*

Product **15** was prepared from compound **2** and 1-ethylpiperazine. The red solid material was isolated in 20% yield (R_f = 0.43 at CH_2Cl_2: n-hexane: MeOH = 2:1: 0.5). Mp 182–184 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1710 (C═O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.19 (t, 3H, J = 7.2 Hz, —C<u>H</u>$_3$), 2.57 (q, 2H, J = 7.4 Hz, —NC<u>H</u>$_2$—), 3.03 (br, 4H, —C<u>H</u>$_2$—), 3.46 (br, 4H, —C<u>H</u>$_2$—), 7.43–7.48 (m, 2H, Ar—H), 7.57–7.60 (m, 3H, Ar—H), 7.85 (d, J = 8.4 Hz, 1H, Ar—H), 8.69 (dd, J = 8.25, 0.9 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 12.06, 49.99, 52.69, 52.85, 121.02, 124.30, 124.59, 125.07, 127.36, 128.49, 130.43, 131.53, 134.36, 135.19, 135.38, 136.64, 141.89, 149.84, 15,740, 194.32 (<u>C</u>O). HRMS (ESI) m/z calcd for $C_{22}H_{20}N_3OCl^+$ $[M]^+$: 377.1295, found $[M+H]^+$: 378.1380.

*2.3.16. 9-Chloro-6-(4-cyclopentylpiperazin-1-yl)-11H-indeno [1,2-c]quinolin-11-one (**16**)*

Product **16** was prepared from compound **2** and 1-cyclopentylpiperazine. The red solid material was isolated in 37% yield (R_f = 0.46 at CH_2Cl_2: n-hexane: MeOH = 2:1: 0.5). Mp 183–184 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1716 (C═O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.19 (t, 3H, J = 7.2 Hz, —C<u>H</u>$_3$), 2.57 (q, 2H, J = 7.4 Hz, —NC<u>H</u>$_2$—), 3.03 (br, 4H, —C<u>H</u>$_2$—), 3.46 (br, 4H, —C<u>H</u>$_2$—), 7.43–7.48 (m, 2H, Ar—H), 7.57–7.63 (m, 3H, Ar—H), 7.85 (d, J = 8.7 Hz, 1H, Ar—H), 8.69 (d, J = 8.1 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 24.32, 30.67, 50.12, 52.36, 67.87, 120.99, 124.28, 124.61, 125.05, 127.31, 128.50, 130.40, 131.56, 134.36, 135.19, 135.32, 136.58, 141.94, 149.85, 157.48, 194.40 (<u>C</u>O). HRMS (ESI) m/z calcd for $C_{25}H_{24}N_3OCl^+$ $[M]^+$: 417.1608, found $[M+H]^+$: 418.1689.

*2.3.17. 9-Chloro-6-(4-(piperidin-1-yl)piperidin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (**17**)*

Product 1**7** was prepared from compound **2** and 1-(piperidin-4-yl)piperidine. The red solid material was isolated in 57% yield. (R_f = 0.51 at CH_2Cl_2: n-hexane: MeOH = 2:1: 0.5). Mp 174–175 °C. FT-IR (KBr; ν cm^{-1}): 1718 (C═O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.48–1.50 (m, 2H, —C<u>H</u>$_2$—), 1.63–1.65 (m, 2H, —C<u>H</u>$_2$—), 1.72–1.85 (m, 4H, —C<u>H</u>$_2$—), 2.08 (d, J = 11.4 Hz, 2H, —C<u>H</u>$_2$—), 2.38–2.46 (m, 1H, —C<u>H</u>$_2$-—), 2.60 (s, 4H, —C<u>H</u>—), 2.91–3.02 (m, 2H, —C<u>H</u>$_2$—), 3.76 (d, J = 12.3 Hz, 2H, —C<u>H</u>$_2$—), 7.41–7.45 (m, 2H, Ar—H), 7.55–7.61 (m, 3H, Ar—H), 7.82 (d, J = 8.4 Hz, 1H, Ar—H), 8.66 (d, J = 7.8 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 24.99, 26.61, 28.77, 50.17, 50.85, 62.51, 120.98, 124.27, 124.42, 124.96, 127.26, 128.36, 130.34, 131.86, 134.37, 135.08, 135.32, 136.45, 141.99, 149.80, 157.82, 194.39 (<u>C</u>O). HRMS (ESI) m/z calcd for $C_{26}H_{26}ClN_3O^+$ $[M]^+$: 431.1764, found $[M+H]^+$: 432.1822.

*2.3.18. 9-Chloro-6-(4-phenylpiperazin-1-yl)-11H-indeno[1,2-c] quinolin-11-one (**18**)*

Product 1**8** was prepared from compound **2** and 1-phenylpiperazine. The red solid material was isolated in 52% yield (R_f = 0.91 at CH_2Cl_2: n-hexane: MeOH = 3:1:0.5). Mp 193–194 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1714 (C═O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.47 (br, 4H, —C<u>H</u>$_2$—), 3.57 (br, 4H, —C<u>H</u>$_2$—), 6.93 (t, J = 7.2 Hz, 1H, Ar—H), 7.04 (d, J = 7.8 Hz, 2H, Ar—H), 7.30–7.36 (m, 2H, Ar—H), 7.45–7.51 (m, 2H, Ar—H), 7.58–7.70 (m, 3H, Ar—H), 7.88 (d, J = 7.8 Hz, 1H, Ar—H), 8.72 (d, J = 8.1 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 49.44, 50.16, 116.66, 120.53, 121.14, 124.32, 124.52, 125.21, 127.60, 128.49, 129.57, 130.56, 131.60, 134.43, 135.16, 135.49, 136.68, 141.74, 149.78, 151.65, 157.41, 194.30 (<u>C</u>O). HRMS (ESI) m/z calcd for $C_{26}H_{20}N_3OCl^+$ $[M]^+$: 425.1295, found $[M+H]^+$: 426.1370.

*2.3.19. 6-(4-Benzylpiperazin-1-yl)-9-chloro-11H-indeno[1,2-c] quinolin-11-one (**19**)*

Product **19** was prepared from compound **2** and 1-benzylpiperazine. The red solid material was isolated in 41% yield (R_f = 0.37 at CH_2Cl_2: n-hexane = 2:1). Mp 178–180 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1718 (C═O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.73 (br, 4H, —C<u>H</u>$_2$—), 3.41 (br, 4H, —C<u>H</u>$_2$—), 3.65 (s, 2H, —C<u>H</u>$_2$—) 7.28–7.47 (m, 7H, Ar—$H_{8,10}$, Ar—H), 7.57–7.63 (m, 3H, Ar—$H_{2,3,4}$), 7.84 (d, J = 8.7 Hz, 1H, Ar—H_7), 8.68 (d, J = 7.05 Hz,1H, Ar—H_1). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 50.11, 53.21, 63.34, 120.97, 124.26, 124.60, 125.04, 127.34, 127.50, 128.42, 128.64, 129.40, 130.42, 131.61, 134.35, 135.11, 135.30, 136.53, 138.46, 141.83, 149.78, 157.55, 194.38 (<u>C</u>O). HRMS (ESI) m/z calcd for $C_{27}H_{22}N_3OCl^+$ $[M]^+$: 439.1451, found $[M+H]^+$: 440.1503.

*2.3.20. 9-Chloro-6-(4-(2-fluorophenyl)piperazin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (**20**)*

Product **20** was prepared from compound **2** and 1-(2-fluorophenyl)piperazine. The red solid material was isolated in 41% yield (R_f = 0.46 at CH_2Cl_2: n-hexane = 2:1). Mp 182–183 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1715 (C═O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.37 (br, 4H, —C<u>H</u>$_2$—), 3.59 (br, 4H, —C<u>H</u>$_2$—), 7.00–7.12 (m, 4H, Ar—H), 7.44–7.50 (m, 2H, Ar—H), 7.59–7.68 (m, 3H, Ar—H), 7.88 (d, J = 8.1 Hz, 1H, Ar—H), 8.71 (d, J = 8.1 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 50.27, 50.63, 116.47, 116.75, 119.48, 121.09, 123.06, 123.17, 124.31, 124.53, 124.80, 124.86, 125.17, 127.54, 128.49, 130.54, 131.55, 134.40, 135.16, 135.45, 141.76, 149.78, 157.37, 194.29 (<u>C</u>O). HRMS (ESI) m/z calcd for $C_{26}H_{19}ClN_3O^+$ $[M]^+$: 443.1201, found $[M+H]^+$: 444.1269.

*2.3.21. 9-Chloro-6-(4-(2-methoxyphenyl)piperazin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (**21**)*

Product **21** was prepared from compound **2** and 1-(2-methoxyphenyl)piperazine. The red solid material was isolated in 37% yield (R_f = 0.38 at CH_2Cl_2: n-hexane = 2:1). Mp 129–131 °C (EtOH). FT-IR (KBr; ν cm^{-1}): 1714 (C═O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.36 (br, 4H, —C<u>H</u>$_2$—), 3.60 (br, 4H, —C<u>H</u>$_2$—), 3.90 (s, 3H, —OC<u>H</u>$_3$), 6.91–7.07 (m, 4H, Ar—H), 7.47 (t, J = 7.5 Hz, 2H, Ar—H), 7.59–7.63 (m, 2H, Ar—H), 7.68 (d, J = 7.8 Hz, 1H, Ar—H), 7.87 (d, J = 8.1 Hz, 1H, Ar—H), 8.70 (d, J = 8.4 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 50.39, 50.75, 55.75, 112.15, 118.68, 121.03, 121.49, 123.55, 124.28, 124.61, 125.09, 127.40, 128.47, 130.46, 131.58, 134.36, 135.15, 135.36, 136.61,

141.66, 141.85, 149.81, 152.86, 157.53, 194.38 (CO). HRMS (ESI) *m/z* calcd for $C_{27}H_{22}ClN_3O_2^+$ $[M]^+$: 455.1401, found $[M+H]^+$: 456.1473.

2.3.22. 9-Chloro-6-(4-(3-methoxyphenyl)piperazin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (22)

Product **22** was prepared from compound **2** and 1-(3-methoxyphenyl)piperazine. The red solid material was isolated in 86% yield (R_f = 0.43 at CH_2Cl_2: n-hexane = 2:1). Mp 189–191 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1723 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.46 (br, 4H, —CH$_2$—), 3.55 (br, 4H, —CH$_2$—), 3.83 (s, 3H, —OCH$_3$), 6.50 (m, *J* = 8.1 Hz, 1H, Ar—H), 6.57 (s, 1H, Ar—H), 6.65 (d, *J* = 8.4 Hz, 1H, Ar—H), 7.22 (d, *J* = 8.1 Hz, 1H, Ar—H), 7.44–7.51 (m, 2H, Ar—H), 7.60–7.68 (m, 3H, Ar—H), 7.88 (d, *J* = 8.1 Hz, 1H, Ar—H), 8.71 (d, *J* = 7.2 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 49.36, 50.08, 55.46, 103.25, 105.29, 109.41, 121.14, 124.31, 124.50, 125.18, 127.60, 128.49, 130.25, 130.56, 131.56, 134.42, 135.15, 135.49, 136.68, 141.70, 149.77, 153.00, 157.35, 161.17, 194.23 (CO). HRMS (ESI) *m/z* calcd for $C_{27}H_{22}ClN_3O_2^+$ $[M]^+$: 455.1401, found $[M+H]^+$: 456.1464.

2.3.23. 9-Chloro-6-(4-(1-methylpiperidin-4-yl)piperazin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (23)

Product **23** was prepared from compound **2** and 1-(1-methylpiperidin-4-yl)piperazine. The red solid material was isolated in 31% yield (R_f = 0.90 at CH_2Cl_2: n-hexane: MeOH = 2:1:0.5). Mp 208–209 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1710 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.64–1.72 (m, 2H, —CH$_2$—), 1.88 (d, *J* = 10.5 Hz, 1H, —CH$_2$—), 1.95–2.03 (m, 2H, —CH$_2$—), 2.29 (s, 4H, —CH—, —CH$_3$), 2.82 (br, 4H, —CH$_2$—), 2.95 (d, *J* = 9.6 Hz, 1H, Ar—H), 3.40 (br, 4H, —CH$_2$—), 6.47–6.50 (m, 1H, Ar—H), 6.57 (s, 1H, Ar—H), 6.65 (d, *J* = 8.4 Hz, 1H, Ar—H), 7.22 (d, *J* = 8.1 Hz, 1H, Ar—H), 7.42–7.47 (m, 2H, Ar—H), 7.57–7.61 (m, 3H, Ar—H), 7.84 (d, *J* = 8.4 Hz, 1H, Ar—H), 8.68 (d, *J* = 7.8 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 28.54, 46.34, 49.24, 50.49, 55.62, 61.83, 120.96, 124.27, 124.61, 125.04, 127.31, 128.45, 130.40, 131.54, 134.33, 135.15, 135.31, 136.55, 141.89, 149.81, 157.49, 194.38 (CO). HRMS (ESI) *m/z* calcd for $C_{26}H_{27}ClN_4O^+$ $[M]^+$: 446.1873, found, $[M+H]^+$: 447.1944.

2.3.24. 9-Chloro-6-(4-(1,4-dioxa-8-azaspiro[4,5]dec-8-yl)-11H-indeno[1,2-c]quinolin-11-one (24)

Product **24** was prepared from compound **2** and 1,4-dioxa-8-azaspiro[4,5]dec-8-yl. The red solid material was isolated in 56% yield (R_f = 0.34 at CH_2Cl_2: n-hexane = 2:1). Mp 218–219 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1718 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.98 (t, *J* = 5.7 Hz, 4H, —CH$_2$—), 3.51 (br, 4H, —NCH$_2$—), 4.04 (s, 4H, —OCH$_2$—), 7.46 (td, *J* = 8.7, 2.1 Hz, 2H, Ar—H), 7.57–7.62 (m, 3H, Ar—H), 7.83 (d, *J* = 8.7 Hz, 1H, Ar—H), 8.69 (dd, *J* = 8.4, 0.9 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 35.00, 48.35, 64.66, 107.31, 120.98, 124.25, 125.06, 127.32, 128.41, 130.42, 131.65, 134.38, 135.09, 135.36, 136.57, 141.97, 149.74, 157.34, 194.43 (CO). HRMS (ESI) *m/z* calcd for $C_{23}H_{19}ClN_2O_3^+$ $[M]^+$: 406.1084, found $[M+H]^+$: 407.1154.

2.3.25. 9-Chloro-6-(4-((piperazin-1-yl)(piperidin-1-yl)methanone)-11H-indeno[1,2-c]quinolin-11-one (25)

Product **25** was prepared from compound **2** and (piperazin-1-yl)(piperidin-1-yl)methanone. The red solid material was isolated in 47% yield (R_f = 0.17 at CH_2Cl_2: n-hexane = 2:1). Mp 266–267 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1647, 1718 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.63 (s, 2H, —CH$_2$—), 3.28–3.47 (m, 16H, —CH$_2$—), 7.42–7.49 (m, 2H, Ar—H), 7.57–7.61 (m, 3H, Ar—H), 7.82 (d, *J* = 8.4 Hz, 1H, Ar—H), 8.69 (d, *J* = 7.8, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 24.86, 25.97, 47.12, 47.99, 49.99, 121.16, 124.32, 124.47, 125.20, 127.65, 128.39, 130.58, 131.62, 134.49, 135.09, 135.54, 136.68, 141.56, 149.69, 157.36, 164.84 (CO), 194.20 (CO). HRMS (ESI) *m/z* calcd for $C_{23}H_{19}ClN_2O_3^+$ $[M]^+$: 460.1666, found $[M+H]^+$: 461.1739.

2.3.26. 9-Chloro-6-(4-(3-(piperidin-4-yl)propyl)piperidin-1-yl)-11H-indeno[1,2-c]quinolin-11-one (26)

Compound **26** was prepared from compound **2** and 4-(3-(piperidin-4-yl)propyl)piperidine. The pure product was obtained as red powder (yield 8.4%) (R_f = 0.41 at CH_2Cl_2: n-hexane = 2:1). Mp 149–151 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1718 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.07–1.46 (m, 12H, —CH$_2$—, —CH—), 1.66–1.91 (m, 4H, —CH$_2$—), 2.54–2.62 (m, 2H, —NCH$_{2(axial)}$—), 2.90–2.94 (m, 2H, —NCH$_{2(axial)}$—), 3.06 (d, *J* = 12 Hz, 2H, —NCH$_{2(equatorial)}$—), 3.70 (d, *J* = 12.3 Hz, 2H, —NCH$_{2(equatorial)}$—), 7.41–7.47 (m, 2H, Ar—H), 7.56–7.61 (m, 3H, Ar—H), 7.84 (d, *J* = 8.4 Hz, 1H, Ar—H), 8.68 (d, *J* = 8.1, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 23.81, 32.58, 33.95, 35.88, 36.49, 37.04, 37.65, 47.07, 50.75, 120.89, 124.27, 124.44, 124.92, 127.15, 128.31, 130.32, 131.96, 134.33, 135.08, 135.23, 136.43, 142.12, 149.83, 158.22, 194.52 (CO). HRMS (ESI) *m/z* calcd for $C_{29}H_{32}ClN_3O^+$ $[M]^+$: 473.2234, found $[M+H]^+$: 474.2318.

2.3.27. 6-(2-Hydroxyethylthio)-9-chloro-11H-indeno[1,2-c]quinolin-11-one (27)

Compound **27** was prepared from compound **2** and 2-mercaptoethanol. The pure product was obtained as red powder (yield 95%) (R_f = 0.23 at CH_2Cl_2: n-hexane = 2:1). Mp 169–170 °C (EtOH). FT-IR (KBr; *v* cm^{-1}): 1718 (C=O). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.66 (t, *J* = 5.4 Hz, 2H, —SCH$_2$—), 4.11 (t, *J* = 5.25 Hz, 2H, —CH$_2$OH), 4.34 (br, 1H, —OH), 7.47 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar—H), 7.53 (td, *J* = 8.4, 1.5 Hz, 1H, Ar—H), 7.61 (d, *J* = 2.1 Hz, 1H, Ar—H), 7.65 (td, *J* = 8.4, 1.5 Hz, 1H, Ar—H), 7.87 (d, *J* = 8.1 Hz, 1H, Ar—H), 7.92 (d, *J* = 8.1 Hz, 1H, Ar—H), 8.72 (d, *J* = 8.4 Hz, 1H, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 34.05, 63.23, 121.54, 124.64, 125.36, 125.83, 127.90, 128.79, 131.14, 134.11, 134.61, 135.02, 136.08, 136.70, 140.76, 149.91, 155.11, 193.79 (CO). HRMS (EI) *m/z* calcd for $C_{18}H_{12}ClNO_2S^+$ $[M]^+$: 341.0277, found 341.0287.

2.3.28. 6-Hydroxy-9-chloro-11H-indeno[1,2-c]quinolin-11-one (28)

The pure product was obtained as red solid (yield 57%) (R_f = 0.24 at ethyl acetate: n-hexane = 3:2). Mp 384 °C (dec.). ^1H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.30 (t,

J = 7.6 Hz, 1H, Ar—H), 7.40 (d, J = 8.0 Hz, 1H, Ar—H), 7.56 (t, J = 7.6 Hz, 1H, Ar—H), 7.61 (s, 1H, Ar—H), 7.63 (d, J = 6.8 Hz, 1H, Ar—H), 7.93 (d, J = 7.6 Hz, 1H, Ar—H), 7.37 (d, J = 8.4 Hz, 1H, Ar—H), 12.42 (br, 1H, —OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 115.12, 116.39, 123.91, 124.41, 125.02, 131.62, 133.69, 134.33, 134.82, 135.68, 136.68, 140.78, 141.10, 159.20, 194.44 (C═O). HRMS (ESI) calcd for $C_{16}H_8NO_2Cl$ $[M]^+$ 281.0244; found $[M+H]^+$ 282.0322, $[M-H]^-$ 280.0178.

2.3.29. 6-Methoxy-9-chloro-11H-indeno[1,2-c]quinolin-11-one (29)

The pure product was obtained as orange powder (yield 60%) (R_f = 0.52 at CH_2Cl_2: n-hexane = 1:1). Mp 259–261 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.24 (3H, s, —OCH_3), 7.44 (1H, dd, J = 8.0 Hz, 2.0 Hz, Ar—H), 7.47 (1H, td, J = 7.6 Hz, 1.2 Hz, Ar—H), 7.59 (1H, d, J = 2.0 Hz, Ar—H), 7.62 (1H, td, J = 8.0 Hz, 1.6 Hz, Ar—H), 7.75 (1H, d, J = 7.6 Hz, Ar—H), 7.85 (1H, d, J = 8.4 Hz, Ar—H), 8.67 (1H, dd, J = 8.0 Hz, 1.2 Hz, Ar—H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 53.92, 120.66, 124.09, 124.86, 125.03, 126.68, 127.48, 129.08, 130.25, 134.31, 134.51, 135.16, 136.14, 140.25, 148.79, 158.13, 193.88 (C═O). HRMS (ESI) calcd for $C_{17}H_{10}NO_2Cl$ $[M]^+$ 295.0400; found $[M+H]^+$ 296.0482.

2.4. Cell culture and MTT assay

All of the synthesized compounds (**1**–**29**) were tested against renal CAKI-1 cell line by MTT assay. Moreover, we selected four compounds **4**, **5**, **13**, and **26** to evaluate the topo I inhibitory activity. Simultaneously, seventeen of our structures (**1**, **2**, **3**, **4**, **5**, **8**, **10**, **11**, **12**, **14**, **16**, **18**, **22**, **25**, **27**, **28** and **29**) were selected by NCI and were investigated against a panel of 60 human tumor cell lines. According to the primary screening, compounds **1**, **5** and **12** were chosen for further cell growth inhibition screening for the GI_{50} (50% growth inhibitory concentration), TGI (the total growth inhibition), and LC_{50} (the 50% lethal concentration) by NCI.

A 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) (Sigma, USA) assay was performed to determine the cell viability and IC_{50} values of our synthetic compounds against the MCF-7 (Mosmann, 1983; Denizot and Lang, 1986) and CAKI-1 cell lines. MCF-7 (3000 cells/well) and CAKI-1 (5000 cells/well) cells were seeded in 96-well microplates with RPMI-1641 supplemented with 10% FBS and treated with various concentrations of compounds for 72 h. After treatment, each well was washed with phosphate-buffered saline (PBS) three times, 100 μL of the MTT solution (0.5 mg/mL final concentration in the medium) was added to each well, and cells were incubated at 37 °C for 1 h. MTT is converted to blue formazan crystals by mitochondrial succinate dehydrogenase. The plates were then washed with PBS and solubilized in 100 μL of dimethyl sulfoxide (DMSO) per well. The absorbances at 540 nm were determined using an enzyme-linked immunosorbent assay (ELISA) microplate reader. Effects of our synthetic compounds on cell viability were demonstrated as the relative activity (relative to the DMSO control group).

2.5. DNA topoisomerase I assay

The topoisomerase I assay kit (TopoGEN, Inc., USA) was performed as described previously with modifications (Shchekotikhin et al., 2011). The activity of DNA topoisomerase I was determined by evaluating the relaxation of supercoiled DNA pHOT. The selected synthetic compounds and camptothecin were dissolved in DMSO at 10 mM as stock solution. A mixture containing 0.25 μg of the plasmid PHOT DNA and 1–2 units of recombinant human DNA topoisomerase I (TopoGEN INC., USA) was incubated with the prepared 0.5% DMSO (negative control), camptothecin 100 μM (positive control) or compounds in the buffer (10 mM Tris-HCl, pH 7.9; 1 mM EDTA; 0.15 M NaCl; 0.1% BSA;

Table 1 Effect of synthetic compounds on cell viability against renal CAKI-1 cells and breast MCF-7 cells.

Compound	IC_{50} (μM)[a] ± SD[b]		Compound	IC_{50} (μM) ± SD	
	CAKI-1	MCF-7		CAKI-1	MCF-7
1	> 30	> 30	**16**	19.66 ± 4.75	> 30
2	> 30	> 30	**17**	5.83 ± 0.37	3.07 ± 0.36
3	23.26 ± 5.74	3.28 ± 0.88	**18**	3.59 ± 1.40	3.51 ± 1.4
4	13.52 ± 2.79	3.06 ± 0.77	**19**	> 30	> 30
5	3.85 ± 0.56	2.13 ± 0.57	**20**	10.80 ± 2.91	> 30
6	> 30	> 30	**21**	> 30	> 30
7	> 30	> 30	**22**	12.96 ± 3.16	> 30
8	> 30	> 30	**23**	1.81 ± 1.10	2.31 ± 0.35
9	29.64 ± 2.55	> 30	**24**	5.95 ± 0.03	> 30
10	7.19 ± 0.35	> 30	**25**	> 30	> 30
11	9.07 ± 3.86	4.0 ± 0.55	**26**	21.83 ± 2.74	> 30
12	1.52 ± 0.21	2.2 ± 0.33	**27**	1.99 ± 0.03	> 30
13	1.66 ± 0.11	2.4 ± 0.433	**28**	> 30	> 30
14	8.18 ± 0.69	> 30	**29**	> 30	> 30
15	9.66 ± 1.91	3.24 ± 0.66	CPT[c]	–[d]	1.24 ± 0.72

[a] IC_{50} is the concentration of drug (μM) required to inhibit cell growth by 50% of the mean (N = 3).
[b] SD: standard derivation, all experiments were independently performed at least three times.
[c] CPT (camptothecin) as a reference drug.
[d] –: not determined.

0.1 mM Spermidine; 5% glycerol) at 37 °C for 45 min. The reaction was quenched by the addition of sodium dodecyl sulfate (final 1% concentration) and proteinase K (final 50 μg/mL concentration) at 37 °C for 15 min. To the reaction mixtures, the loading buffer containing 0.25% bromophenol blue and 50% glycerol was added 0.1 volume in reactions mixtures. These samples were electrophoresed on 1% agarose gel at 60 V for 1.5 h with TAE (Tris-acetate-EDTA) as the buffer. The gels were stained with ethidium bromide for 10 min and destained with water for 20 min after electrophoresis.

2.6. NCI in vitro 60-cell drug screening experiments

Eight of our synthesized compounds were selected by the NCI, and their anticancer activities at a single dose of 10 μM were determined by a sulforhodamine B (SRB) colorimetric assay according to previous protocols (Sikic, 1991; Monks et al., 1997; Kandeel et al., 2015). Cells (3000–5000 per well) were seeded into 96-well microtiter plates for 24 h at 37 °C, with 5% CO_2, 95% air, and 100% relative humidity. Two plates of each cell line were fixed with trichloroacetic acid (TCA) as

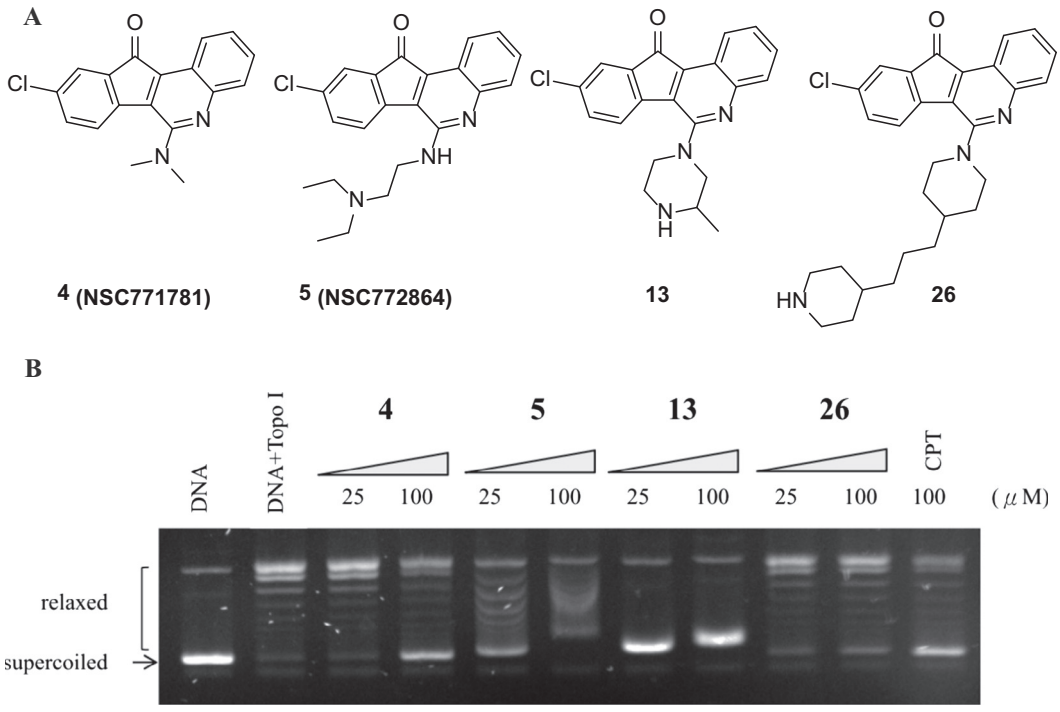


Figure 2 Structures and the inhibition of topoisomerase I relaxation activities of compounds **4**, **5**, **13**, and **26**. (A) Structure of compounds **4**, **5**, **13**, and **26**. (B) Effect of compounds **4**, **5**, **13**, and **26** on topoisomerase I mediated supercoiled pHOT DNA relaxation. Lane 1: untreated supercoiled pHOT DNA. Lane 2: the pHOT DNA treated with topoisomerase I in the absence of drugs. Lanes 3–4 (compound **4)**, 5–6 (compound **5)**, 7–8 (compound **13)** and 9–10 (compound **26)** are the pHOT DNA treated with topoisomerase I in the present of drugs at 25 or 100 μM, respectively. Lane 11 is the pHOT DNA treated with topoisomerase I in the presence of CPT at 100 μM.

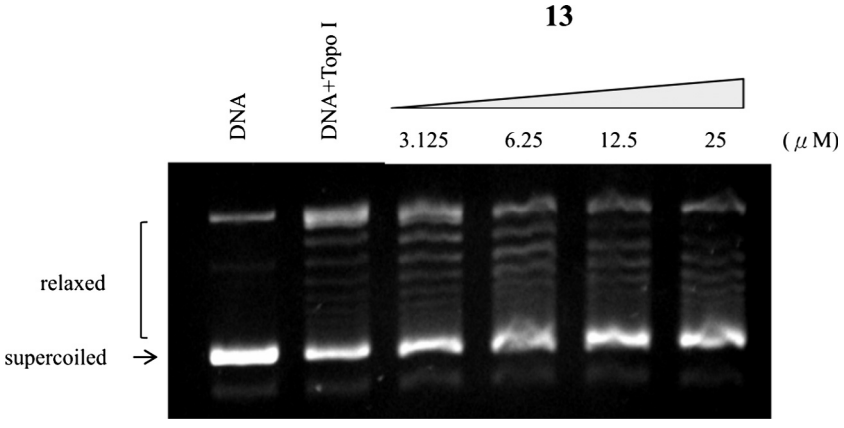


Figure 3 Effect of compound **13** on topoisomerase I mediated supercoiled pHOT DNA relaxation. Lanes 1: untreated supercoiled pHOT DNA. Lanes 2: the pHOT DNA treated with topoisomerase I in the absence of drugs. Lanes 3–6 are the pHOT DNA treated with topoisomerase I in the presence of compound **13** at 3.125, 6.25, 12.5 and 25 μM.

Table 2 Cytotoxicity of selected compounds in the NCI *in vitro* 60-cell drug screen program.

Panel/cell line	Compounds/growth percent[a]																
Compd. No.	**1**	**2**	**3**	**4**	**5**	**8**	**10**	**11**	**12**	**14**	**16**	**18**	**22**	**25**	**27**	**28**	**29**
NCI No.	**NSC763972**	**NSC763969**	**NSC772856**	**NSC771781**	**NSC772864**	**NSC772860**	**NSC771782**	**NSC772857**	**NSC772862**	**NSC771783**	**NSC772859**	**NSC772861**	**NSC772863**	**NSC772858**	**NSC763971**	**NSC763970**	**NSC765596**
Leukemia																	
CCRF-CEM	22.62	64.86	101.50	92.13	42.00	104.33	88.85	105.04	−35.45	92.44	95.17	93.52	93.78	109.87	71.56	105.78	89.25
HL-60 (TB)	3.18	102.00	102.75	95.49	94.33	98.84	97.09	94.50	−38.36	101.15	103.04	105.45	99.09	109.78	91.75	102.31	108.77
K-562	12.16	102.16	90.99	88.81	45.29	103.57	89.01	100.89	−65.71	92.13	99.26	95.17	94.25	95.70	63.81	97.48	108.53
MOLT-4	43.74	70.83	93.26	88.92	34.63	94.41	88.74	93.29	−43.22	92.90	98.39	98.13	95.46	104.77	59.49	95.10	110.33
RPMI-8226	20.68	92.83	91.82	88.05	81.94	97.99	83.40	95.20	−43.57	85.92	94.89	95.33	93.63	104.31	62.28	90.60	90.30
SR	29.86	65.85	91.67	86.12	46.92	98.51	85.94	99.53	−55.19	75.13	97.09	95.93	95.05	105.15	61.70	77.02	N.T.
Non-small cell lung cancer																	
A549/ATCC	11.94	108.98	96.83	98.85	51.79	101.69	101.47	104.35	18.70	96.43	101.56	90.89	101.22	99.12	76.42	104.88	99.70
EKVX	N.T.[b]	79.27	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	30.15	72.14	N.T.
HOP-62	29.61	94.89	87.10	78.02	24.86	64.18	78.71	78.17	−61.80	61.42	108.83	N.T.	90.22	96.39	91.46	91.26	96.01
HOP-92	N.T.	71.79	N.T.	44.26	N.T.	N.T.	66.08	N.T.	N.T.	38.50	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	77.75
NCI-H226	66.09	91.86	92.19	84.79	80.86	94.59	87.21	94.22	49.94	86.17	92.80	79.43	89.19	96.06	80.52	85.66	89.22
NCI-H23	58.72	79.30	86.83	82.62	80.63	90.42	90.40	94.33	49.19	86.27	92.80	83.34	87.22	95.41	66.22	81.56	93.26
NCI-H322M	89.67	95.38	80.85	78.83	69.83	107.08	91.28	92.29	35.89	87.41	98.50	98.63	91.93	94.59	73.47	90.72	99.80
NCI-H460	79.73	98.68	84.38	94.17	25.15	101.21	102.14	93.32	−66.36	91.83	101.46	97.04	101.76	98.85	79.22	83.32	98.27
NCI-H522	1.78	89.37	90.50	88.97	77.39	98.45	91.11	92.76	18.08	96.62	98.72	80.10	79.75	99.28	68.89	92.22	95.89
Colon cancer																	
COLO 205	14.63	99.18	66.47	100.97	45.69	104.79	100.71	96.30	−81.49	93.65	104.54	102.19	106.63	98.52	87.39	98.25	108.91
HCC-2998	88.12	98.52	57.51	81.50	88.65	102.76	107.23	103.16	−55.83	103.21	106.04	106.23	105.79	106.23	96.27	102.24	104.46
HCT-116	35.26	105.87	88.31	98.57	36.96	102.51	93.64	89.08	−83.19	92.78	91.34	78.18	93.02	101.67	77.94	103.43	88.60
HCT-15	21.44	114.88	66.20	80.49	50.38	97.78	80.29	97.88	−78.92	92.83	89.64	80.71	100.39	99.35	80.10	108.54	94.98
HT29	81.42	104.27	99.44	94.89	9.75	104.84	91.34	95.52	−80.89	93.76	93.22	82.40	95.93	108.65	84.03	102.38	111.60
KM12	79.30	111.92	66.82	94.44	53.42	94.80	109.99	97.97	−47.09	99.06	99.33	98.34	105.95	101.58	84.62	102.73	98.35
SW-620	66.13	98.29	96.25	96.35	43.72	88.89	103.71	91.88	−73.67	92.76	93.06	93.14	91.16	92.51	86.99	95.26	98.89
CNS cancer																	
SF-268	84.54	117.27	97.27	100.26	65.88	96.49	103.58	96.13	34.33	92.35	96.39	85.45	83.89	107.51	96.53	106.64	113.64
SF-295	N.T.	92.80	95.71	93.22	N.T.	96.18	102.43	93.33	23.75	90.01	96.87	60.91	90.05	97.83	73.28	92.48	N.T.
SF-539	104.63	110.08	92.08	87.80	85.04	90.22	96.24	92.93	36.91	95.23	101.34	83.17	83.97	92.98	100.05	105.79	91.49
SNB-19	93.45	101.30	95.95	104.19	94.02	103.87	100.01	113.43	67.25	85.41	95.06	88.40	105.27	108.66	89.19	99.28	96.35
SNB-75	74.33	82.91	79.70	69.12	71.52	84.42	81.55	72.13	22.34	70.00	86.36	33.20	37.76	80.84	66.06	80.33	59.75
U251	20.22	100.63	96.03	98.98	N.T.	N.T.	95.72	111.96	N.T.	85.86	N.T.	N.T.	N.T.	98.15	78.04	96.46	97.67
Melanoma																	
LOX IMVI	33.38	84.23	93.40	85.45	N.T.	N.T.	91.20	100.04	N.T.	87.17	N.T.	N.T.	N.T.	103.19	68.41	86.91	116.91

MALME-3M	73.87	102.06.	114.83	111.50	52.55	98.31	136.93	101.05	−32.28	106.59	92.48	100.05	92.79	102.62	107.23	103.37	107.54
M14	72.55	109.79	112.68	113.63	76.44	96.60	106.99	101.69	−56.24	106.31	105.34	97.52	105.07	101.18	98.89	108.50	96.50
MDA-MB-435	N.T.	100.46	107.24	97.33	92.72	104.53	106.62	107.70	−6.57	98.43	99.93	99.55	100.75	102.57	95.15	100.35	100.31
SK-MEL-2	110.64	117.11	N.T.	95.09	N.T.	N.T.	104.29	N.T.	N.T.	104.36	N.T.	N.T.	N.T.	N.T.	95.85	117.12	109.44
SK-MEL-28	73.75	90.08	114.89	113.43	87.40	105.21	113.81	110.84	−91.38	110.81	107.58	104.82	103.93	107.03	86.90	100.02	106.24
SK-MEL-5	66.21	86.30	80.96	94.89	82.51	98.52	99.19	89.52	59.18	97.67	97.48	99.23	96.87	92.50	68.56	84.87	98.51
UACC-257	38.15	127.99	107.69	101.11	92.18	106.47	103.31	107.44	−39.24	95.34	98.37	98.40	93.79	104.23	106.91	106.31	98.20
UACC-62	86.86	94.45	77.41	78.16	99.56	93.36	88.33	94.90	−81.71	96.67	95.06	87.17	86.44	93.92	84.47	95.17	74.08
Ovarian cancer																	
IGROV1	87.42	92.82	54.41	83.38	43.43	94.36	86.47	72.45	3.23	75.03	85.84	65.13	80.87	81.63	69.73	91.28	96.92
OVCAR-3	82.98	119.93	84.91	103.48	73.13	97.59	102.34	96.58	22.86	93.41	99.54	98.07	104.27	103.33	89.42	117.80	111.89
OVCAR-4	40.23	94.13	72.83	84.28	52.50	97.11	96.67	98.19	35.59	94.70	94.20	90.32	87.08	98.31	73.19	80.74	N.T.
OVCAR-5	104.97	137.00	81.54	89.14	89.34	104.92	90.30	94.37	39.14	99.31	106.41	88.56	98.22	102.33	112.54	126.77	96.81
OVCAR-8	16.01	101.08	94.33	84.55	61.36	96.38	95.25	96.11	−46.09	87.51	96.55	75.82	83.98	104.73	90.21	107.63	98.75
NCI/ADR-RES	43.83	96.78	92.22	92.57	69.55	90.28	94.32	102.85	−4.00	88.19	100.39	97.88	92.82	107.13	73.58	97.00	94.29
SK-OV-3	N.T.	N.T.	92.62	99.12	84.20	99.78	107.26	84.60	57.81	87.98	96.85	78.58	86.02	95.59	N.T.	N.T.	119.79
Renal cancer																	
786-0	103.15	112.40	103.34	108.01	74.13	102.73	103.71	99.53	25.31	89.51	97.90	81.41	81.67	105.05	100.58	114.55	103.51
A498	N.T.	N.T.	82.03	96.00	88.98	87.55	93.88	99.15	59.79	72.81	88.64	97.48	104.53	104.44	N.T.	N.T.	93.51
ACHN	51.33	111.67	88.46	87.55	55.86	94.75	81.66	86.42	−83.29	88.10	100.03	68.58	93.51	95.59	88.12	104.75	90.83
CAKI-1	N.T.	75.67	73.84	84.65	64.35	83.89	94.99	82.68	17.84	83.81	93.83	80.23	86.59	90.50	41.86	71.62	90.65
RXF 393	82.62	112.80	97.15	101.32	51.90	97.51	114.61	106.09	20.51	94.00	95.60	72.95	78.38	107.22	79.39	99.93	114.49
SN12C	48.72	88.03	94.82	95.38	61.60	99.08	96.95	93.10	15.89	83.57	99.08	87.90	88.17	101.12	84.58	96.69	89.48
TK-10	94.62	138.45	65.31	91.84	95.55	134.79	101.98	121.79	47.07	99.56	N.T.	115.61	133.34	131.97	99.82	126.63	N.T.
UO-31	15.98	80.62	56.91	48.63	25.86	76.25	45.74	65.33	−40.61	47.60	72.57	65.02	86.64	82.74	56.23	70.50	55.69
Prostate cancer																	
PC-3	32.02	92.73	89.44	82.55	41.01	90.64	79.64	87.77	−58.51	82.93	86.19	79.79	72.83	93.07	58.35	93.44	74.44
DU-145	89.22	113.91	106.88	106.50	65.48	103.47	119.02	106.04	−41.83	102.37	110.73	102.96	98.98	114.04	92.27	111.80	121.06
Breast cancer																	
MCF7	50.00	74.25	5.24	23.99	12.70	64.65	52.31	49.61	−100.00	68.30	75.18	53.31	77.46	97.24	67.82	68.09	71.98
MDA-MB-231/ATCC	41.97	87.66	83.87	76.68	25.16	90.96	79.28	93.08	−10.45	62.53	82.96	73.80	79.81	93.54	72.56	86.98	74.61
HS 578T	84.98	91.01	85.28	72.84	84.70	98.38	88.93	87.68	60.88	70.47	99.40	N.T.	71.75	102.06	58.28	105.98	92.74
BT-549	91.18	119.62	101.88	102.55	87.48	95.52	93.95	92.91	58.35	82.89	102.74	89.83	101.57	105.34	92.88	120.08	92.95
T-47D	−30.98	86.97	23.53	44.88	43.28	67.68	88.81	83.86	−31.54	76.27	92.20	75.93	86.68	95.99	63.89	67.23	78.97
MDA-MB-468	45.23	107.92	−31.61	0.60	33.81	97.12	59.47	97.71	−35.66	95.71	103.99	95.37	101.04	106.42	77.36	80.08	99.51

(*continued on next page*)

Table 2 (*continued*)

Panel/cell line	Compounds/growth percent[a]																				
Compd. No.	**1**	**2**	**3**	**4**	**5**	**8**	**10**	**11**	**12**	**14**	**16**	**18**	**22**	**25**	**27**	**28**	**29**				
NCI No.	NSC763972	NSC763969	NSC772856	NSC771781	NSC772864	NSC772860	NSC771782	NSC772857	NSC772862	NSC771783	NSC772859	NSC772861	NSC772863	NSC772858	NSC763971	NSC763970	NSC765596				
Mean	55.93	98.17	84.15	87.15	62.40	96.20	93.66	94.75	−14.37	88.15	96.53	87.29	92.15	100.53	79.52	96.60	96.23				
Delta	86.91	33.31	115.76	86.55	52.65	32.02	47.92	45.14	85.63	49.65	23.96	54.09	54.39	19.69	49.37	29.37	40.54				
Range	141.62	73.59	146.50	113.03	89.81	70.61	91.19	72.18	167.25	72.31	38.16	82.41	95.58	51.13	82.39	59.54	65.37				

[a] Data obtained from NCI *in vitro* 60-cell drug screen program at 10 μM.
[b] N.T. = no test.

a control of the cell population for each cell line at the time of drug exposure (T_0). After additional incubation with the vehicle (DMSO) or the test compounds for 48 h, cells were fixed with cold 50% (w/v) TCA (final concentration, 10% TCA) and then incubated for 60 min at 4 °C. Plates were then washed with tap water, and cells were treated with 100 μL of the SRB solution at 0.4% (w/v) in 1% acetic acid for 10 min at room temperature. After staining, the plates were washed with 1% acetic acid to remove any unbound dye, and SRB-bound cells were solubilized with 0.01 M Trizma base. The absorbance was measured using a spectrophotometer at a wavelength of 515 nm. Using the absorbance measurements, including time zero (T_0), control growth (C), and test growth in the presence of a drug (T_X), the percentage growth was calculated for each compound as follows: $100 - [(T_X - T_0)/(C - T_0)] \times 100$ for which concentrations in which $T_X \geqslant T_0$.

3. Results and discussion

The synthetic methods of 6-substituted 9-chloro-11*H*-indeno[1,2-*c*]quinolin-11-ones are depicted in Scheme 1. The intermediate 3-(4-Chlorophenyl)-2-hydroxyquinoline-4-carboxylic acid (**1**) was obtained from the reaction of isatin with 4-chlorophenylacetic acid *via* the Pfitzinger reaction (Pfitzinger, 1886, 1888). It is a common reaction to provide quinoline-4-carboxylic acids under basic condition. The following is the starting material compound **2**, 6,9-dichloro-11*H*-indeno[1,2-*c*]quinolin-11-one, and it was produced from intermediate (**1**) in $POCl_3$ and carried out in an open system round-bottle flask with a condenser. The synthetic strategy of compounds **3**–**26** was accomplished *via* the reaction of an appropriate amine with compound **2** in DMF, and then treatment with pyridine to afford the desired compounds, which were purified by recrystallization. It was also worthy to note that compound **2** could react with HCl to obtain **28** or with sodium methoxide (NaOMe) to afford **29**, respectively. All the synthetic methods are illustrated in the procedure Scheme 1.

The effects of the synthesized indenoquinolone derivatives **2**–**29** on cell viability of the MCF-7 breast cancer cell line and CAKI-1 renal cancer cell line were experimentally assessed by performing a MTT assay. Results are summarized in Table 1 and are expressed as IC_{50} (μM) values. From the obtained results, we observed that some indenoquinolones exhibited interesting activities on the tested cancer cell lines. We found that the most potent compounds against CAKI-1 cells were **12, 13, 23** and **27** with IC_{50} values less than 2 μM. In addition, the potent compounds against MCF-7 cells were **5** (2.13 ± 0.57), **12** (2.20 ± 0.33), **13** (2.40 ± 0.43), and **23** (2.31 ± 0.35) which showed low IC_{50} values of cell viability. Of the compounds analyzed, we observed that introduction of substituted piperazinyl groups at the 6-position of the indenoquinolone scaffold could modulate the inhibition of cell viability of MCF-7 and CAKI-1 compared to the indenoquinolone **2** which possess two chloro atoms. However, the IC_{50} values of compounds **14**–**22** containing a N-substituted piperazinyl group decreased potency significantly. Further, the micromolar IC_{50} values of some potent compounds are presented in Table 1.

According to the antiproliferative activities and the similar side chains, compounds **4**, **5**, **13**, and **26** were selected to evaluate the topoisomerase I inhibition. In Fig. 2, the selected com-

Table 3 *In vitro* anticancer activity by selected compounds **1** (NSC763972), **5** (NSC772864), **12** (NSC772862) in the NCI's 60 human cancer cell lines.

Panel/cell line (μM)	**1 (NSC763972)**					**5 (NSC772864)**					**12 (NSC772862)**				
	GI_{50}			TGI	LC_{50}	GI_{50}			TGI	LC_{50}	GI_{50}			TGI	LC_{50}
		Subpanel MID[b]	Selectivity ratio				Subpanel MID[b]	Selectivity ratio				Subpanel MID[b]	Selectivity ratio		
Leukemia															
CCRF-CEM	1.42	1.114	8.063	15.5	> 100	8.24	9.696	1.475	3.50	7.80	6.32	11.000	0.954	> 100	> 100
HL-60(TB)	1.06			4.28	> 100	N.T.			N.T.	N.T.	36.9			> 100	> 100
K-562	0.315			> 100	> 100	8.75			3.61	7.46	7.80			> 100	> 100
MOLT-4	1.97			15.3	> 100	6.24			3.43	7.91	4.41			> 100	> 100
RPMI-8226	0.549			> 100	> 100	20.20			4.31	9.73	7.48			> 100	> 100
SR	1.37			43.4	> 100	5.05			4.06	11.60	3.09			> 100	> 100
Non-small cell lung cancer															
A549/ATCC	2.95	5.840	1.538	> 100	> 100	15.20	11.819	1.210	3.51	6.72	4.87	6.477	1.621	> 100	> 100
EKVX	N.T.			N.T.	N.T.	N.T.			N.T.	N.T.	N.T.			N.T.	N.T.
HOP-62	3.84			> 100	> 100	4.51			2.83	5.74	12.3			> 100	> 100
HOP-92	1.21			13.4	> 100	3.08			5.80	25.10	N.T.			N.T.	N.T.
NCI-H226	11.6			> 100	> 100	1.88			–	–	7.77			> 100	> 100
NCI-H23	3.44			63.6	> 100	2.63			4.00	8.35	6.65			> 100	> 100
NCI-H322M	16			92.3	> 100	34.50			13.10	36.20	5.62			> 100	> 100
NCI-460	7.44			86.3	> 100	5.15			3.20	6.74	4.15			> 100	> 100
NCI-H522	0.238			0.644	3.57	27.60			3.80	7.23	3.98			> 100	> 100
Colon cancer															
COLO 205	3.77	9.560	0.939	15.8	39.8	2.41	18.400	0.778	–	–	12.2	9.763	1.075	> 100	> 100
HCC-2998	11.0			> 100	> 100	> 100			4.08	7.94	30.9			> 100	> 100
HCT-116	0.807			> 100	> 100	6.19			3.19	6.06	5.42			> 100	> 100
HCT-15	0.716			> 100	> 100	4.37			3.51	7.50	4.34			> 100	> 100
HT29	39.0			> 100	> 100	4.23			3.16	6.28	7.84			> 100	> 100
KM12	4.62			> 100	> 100	6.48			3.11	5.96	2.50			> 100	> 100
SW-620	7.01			> 100	> 100	5.12			3.06	6.67	5.14			> 100	> 100
CNS cancer															
SF-268	4.28	14.364	0.625	> 100	> 100	17.70	14.875	0.962	3.27	6.52	8.71	8.053	1.304	> 100	> 100
SF-295	10.8			87.9	> 100	17.80			4.60	9.85	7.79			> 100	> 100
SF-539	23.1			64.2	> 100	20.90			3.15	5.86	14.2			> 100	> 100
SNB-19	30.9			> 100	> 100	18.20			3.42	–	8.27			> 100	> 100
SNB-75	N.T.			N.T.	> 100	3.25			2.35	4.86	4.53			> 100	> 100
U251	2.74			> 100	> 100	11.40			3.06	5.88	4.82			> 100	> 100
Melanoma															
LOX IMV1	0.64	10.637	0.844	57.00	> 100	6.04	15.654	0.914	3.04	6.02	4.74	19.894	0.528	> 100	> 100
MALME-3M	8.32			42.20	> 100	17.30			3.83	7.46	8.87			> 100	> 100
M14	3.75			> 100	> 100	16.50			5.48	19.30	7.49			> 100	> 100
MDA-MB-435	3.97			> 100	> 100	20.20			3.86	7.62	7.01			> 100	> 100
SK-MEL-2	17.10			50.20	> 100	37.70			4.73	10.40	7.36			> 100	> 100
SK-MEL-28	33.00			> 100	> 100	6.00			3.21	5.83	> 100			> 100	> 100
SK-MEL-5	7.36			22.50	56.5	1.15			2.61	–	4.10			> 100	> 100

(*continued on next page*)

Table 3 (*continued*)

Panel/cell line (μM)	**1 (NSC763972)**					**5 (NSC772864)**					**12 (NSC772862)**				
	GI_{50}			TGI	LC_{50}	GI_{50}			TGI	LC_{50}	GI_{50}			TGI	LC_{50}
		Subpanel MID[b]	Selectivity ratio				Subpanel MID[b]	Selectivity ratio				Subpanel MID[b]	Selectivity ratio		
UACC-257	6.39			65.10	> 100	21.20			3.39	6.37	35.1			> 100	> 100
UACC-62	15.20			88.40	> 100	14.80			3.31	6.68	4.38			> 100	> 100
Ovarian cancer															
IGROV1	14.3	13.649	0.658	> 100	> 100	11.20	17.448	0.820	3.62	7.82	5.94	13.591	0.772	> 100	> 100
OVCAR-3	4.46			95.1	> 100	N.T.			N.T.	N.T.	12.8			> 100	> 100
OVCAR-4	8.83			21.9	49.30	2.99			2.79	5.68	6.39			> 100	> 100
OVCAR-5	16.1			66.1	> 100	21.50			3.32	6.52	34.5			> 100	> 100
OVCAR-8	1.49			> 100	> 100	13.80			3.63	7.76	8.66			> 100	> 100
NCI/ADR-RES	2.26			25.2	> 100	19.30			3.67	7.41	22.5			> 100	> 100
SK-OV-3	48.1			> 100	> 100	35.90			5.57	17.20	4.35			> 100	> 100
Renal cancer															
786-O	23.4	10.988	0.817	> 100	> 100	26.80	12.733	1.124	3.29	6.01	23.5	6.370	1.648	> 100	> 100
A498	6.75			71.7	> 100	8.76			1.78	50.80	2.63			> 100	> 100
ACHN	4.2			> 100	> 100	6.44			3.04	5.89	3.14			> 100	> 100
CAKI-1	7.92			> 100	> 100	14.80			3.26	6.80	2.52			> 100	> 100
RXF 393	17.9			63.6	> 100	N.T.			N.T.	N.T.	4.55			> 100	> 100
SN12C	3.24			> 100	> 100	4.12			2.87	6.16	5.42			> 100	> 100
TK-10	22.7			68.9	> 100	24.60			3.70	6.38	N.T.			N.T.	N.T.
UO-31	1.79			35.3	> 100	3.61			2.88	6.48	2.83			> 100	> 100
Prostate cancer															
PC-3	0.606	2.968	3.026	> 100	> 100	12.50	15.650	0.914	3.82	8.98	5.93	5.360	1.959	> 100	> 100
DU-145	5.33			> 100	> 100	18.80			2.94	5.43	4.79			> 100	> 100
Breast cancer															
MCF7	7.73	7.280	1.234	> 100	> 100	0.44	12.345	1.159	2.60	5.54	5.42	6.813	1.541	> 100	> 100
MDA-MB-231/ATCC	2.44			13.8	75.1	2.32			2.71	5.70	7.23			> 100	> 100
HS 578T	20.3			> 100	> 100	19.30			10.00	46.10	4.77			> 100	> 100
BT-549	7.55			> 100	> 100	32.50			9.50	45.50	10.6			> 100	> 100
T-47D	0.84			2.86	8.96	17.80			3.56	7.19	8.44			> 100	> 100
MDA-MB-468	4.82			29.4	> 100	1.71			3.07	–	4.42			> 100	> 100
MID[a]	8.982					14.306					10.498				

MID[a] = Average sensitivity of all cell lines in μM.
MID[b] = Average sensitivity of all cell lines of a particular subpanel in μM.
Selectivity ratio = MID[a]:MID[b].
N.T. = no test.

pounds showed various inhibitory effects against topoisomerase I at 25 and 100 μM. Among them, the synthetic compound **13** not only exhibited more potent inhibitory activity than compounds **4**, **5**, **26**, and CPT, but also completely blocked topoisomerase I-mediated DNA relaxation at 25 μM. Compound **7** was chosen for further testing against topoisomerase I in a concentration-dependent manner doses at 3.125, 6.25, 12.5, and 25 μM (Fig. 3).

The effects of sixteen compounds **1 (NSC763972)**, **2 (NSC763969)**, **3 (NSC772856)**, **4 (NSC771781)**, **5 (NSC772864)**, **8 (NSC772860)**, **10 (NSC771782)**, **12 (NSC772862)**, **14 (NSC771783)**, **16 (NSC772859)**, **18 (NSC772861)**, **22 (NSC772863)**, **25 (NSC772858)**, **27 (NSC763971)**, **28 (NSC763970)**, and **29 (NSC765596)** on cell viability were evaluated against the NCI-60 human tumor cell lines at 10 μM *in vitro* using the SRB protein-binding dye (Sikic, 1991; Monks et al., 1997). As shown in Table 2, this class of compounds bearing ethanolthiol group (**27**), hydroxyl group (**28**), methoxy group (**29**), and several N-substituted piperazinyl groups revealed no significant activities for all the cancer cell lines which respond to the previous *in vitro* consequences. Three synthetic compounds **1**, **5**, and **12** were selected for an advanced 60-cell panel assay at five logarithm concentrations (10^{-2}, 10^{-1}, 10^{0}, 10^{1} and 10^{2} μM) and the results were indicated with GI_{50}, TGI and LC_{50}, because of their significant cytotoxicity when tested using MTT viability assay.

Compound **1**, the average concentration required to inhibit GI_{50} was 8.982 μM with a range of 0.238 μM (non-small cell lung cancer: NCI-H522) to >39.0 μM (colon cancer: HT-29). With **5**, the average concentration required to inhibit GI_{50} was 14.36 μM with a range of 0.44 μM (breast cancer: MCF-7) to >100 μM (colon cancer: HCC-2998). Furthermore, the average GI_{50} concentration of **12** was 10.498 μM, with a range of 2.52 μM (ovarian cancer: IGROV1) to >100 μM (melanoma: SK-MEL-28). Compounds **1**, **5**, and **12** exhibited dose-dependent inhibition of proliferation in all 60 cancer cell lines. Compound **1** is active against most of the cancer cell lines with GI_{50} values <1 μM for 12% (7/58) of the cell lines. With initial assessment at relative doses, **12** is more potent than **5**. Compound **12** exhibited highly inhibitory effect against most of the cancer cell lines with GI_{50} values <5 mM for 36.8% of the 57 cell lines. The GI_{50}, TGI and LC_{50} values of the active compounds within each series are given in Table 3.

4. Conclusion

Because of the anticancer potential demonstrated by the indenoquinolone scaffold, an approach for synthesizing 6-substituted-9-chloro-indenoquinolones was developed, and the inhibition activities of the synthetic compounds on cell viability were evaluated. Based on our biological results, it was envisioned that introduction of piperazinyl groups with a 4-substituted side chain at the 6-position of the indenoquinolone scaffold decreased the cell viability of the breast cancer cell line MCF-7 and renal cancer cell line CAKI-1. Among the synthesized compounds, **12** (with a piperazinyl group) and **13** (with a 2-methylpiperazinyl group) were the most-active compound exhibiting potent inhibitory activity on the cell viability of MCF-7 and CAKI-1 cells. Through a series of promising *in vitro* experiments, we found that 6-substituted-9-chloro-indenoquinolone derivatives, especially compound **13**, not only exhibited preferential growth inhibition effects toward cancer cell lines but also showed the inhibitory effect on topoisomerase I. Based on our results and structure–activity relationships (SARs) studies, compounds **12** and **13** could be potent antibreast cancer candidates and promising lead compounds that warrants further structure optimization.

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