MedChemComm

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Synthesis and Antiproliferative Evaluation of 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinoline-11-one Derivatives. Part 4.

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Abstract

We have synthesized certain indeno[1,2-*c*]quinolin-11-ones derivatives for antiproliferative evaluation. Among them, 9-methoxy-6-{4-[(oxiran-2-yl)methyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-(oxiran-2-yl)methyl oxime (**12**) exhibited strong antiproliferative activities aginst the growth of Hela, SKHep, MDA-MB-231, and H1299 cells with an IC_{50} value of 0.54, 0.99, 0.79, and 1.18 μM respectively. Compound **12** was also found to be a potent DNA intercalating agent. Mechanism studies indicated that DNA damage was strongly induced by **12**-treated cells even at a low concentration of 5.0 μM. Our results demonstrated that **12** significantly increased Bax expression, activation of caspase-3, -7, enhanced the cleavage of PARP, and decreased Bcl-2 expression in H1299 cells. Compound **12** also significantly decreased the HDAC6, SIRT1, p-FOXO3a, p-Akt, and p-PTEN expression in H1299 cells.

Key words: Indeno[1,2-*c*]quinoline derivatives; Antiproliferative activity; DNA Intercalating agent; Apoptosis.

The nov

Introduction

Quinoline skeleton constitutes a large number of biologically active compounds and is frequently condensed with various heterocycles.[1-7] For examples, camptothecin (CPT) is an anticancer alkaloid isolated from *Camptotheca acuminate* that bears a condensed quinoline skeletone.[1,2] Amsacrine (*m*-AMSA), a 9-anilinoacridine derivative, has been clinically used for the treatment of leukemia and lymphoma due to its capability of intercalating DNA leading to the inhibition of mammalian topoisomerase II.[3,4] A number of furo[2,3-*b*]quinoline derivatives, such as CIL-102, have been synthesized and demonstrated to possess significant anticancer activity.[5-7] TAS-103, which possesses the tetracyclic indeno[2,1-*c*]quinoline pharmacophore, was initially reported as a dual topoisomerases I and II targeting agent.[8-10] However, a study using yeast suggested that topo II is a primary cellular target of TAS-103 while other mechanisms of action have also been proposed.[11-13] Recently, we have synthesized certain isomeric indeno[1,2-*c*]quinoline derivatives (compounds **1** – **4**, *Figure* 1) of TAS-103 for anticancer evaluation.[14-18] Among them, 9-methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*] quinolin-11-one *O*-3-aminopropyl oxime (**3**) and *(E)*-6-hydroxy-9-methoxy-11*H*-indeno[1,2-*c*] quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyl oxime (**4**)[16] exhibited IC_{50} value of 0.64 and 0.89 μM respectively against the growth of A549, which was more active than CPT. The present report intends to establish the antiproliferative structure-activity relationships (SAR) of indenoquinoline derivatives by the introduction of various substituents at C-6 position and to identify more potential anticancer drug candidates.

< **Insert Figure 1 here** >

Results and Discussion

Chemistry

Treatment of 9-methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one (**1**)[14] with chloroacetyl chloride in the presence of triethylamine gave 6-[4-(2-chloroacetyl)piperazin-1-yl]-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**5**)

which was then reacted with the respective alkylamines to afford aminoalkyl derivatives, **7a - 7g**, respectively in a fairly good overall yield. Acylation of **1** with chloropropionyl chloride afforded 6-[4-(3-chloropropanoyl)piperazin-1-yl]-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**6**) which was then reacted with the respective alkylamines to give aminoalkyl derivatives, **8a - 8g**, as described in *Scheme* 1.

The preparation of hydroxyaminoalkyl derivatives are described in *Scheme 2*. Reaction of **1** with epichlorohydrin gave the epoxide derivative **9** which was then treated with methylamine or dimethylamine to afford 6-{4-[2-hydroxy-3-(methylamino)propyl]piperazin-1-yl}-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**10**) and 6-{4-[3-(dimethylamino)-2-hydroxypropyl]piperazin-1-yl}-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**11**) respectively. Accordingly, 6-{4-[2-hydroxy-3-(dimethylamino)propyl]piperazin-1-yl}-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one-[2-hydroxy-3-(dimethylamino)propyl]oxime (**13**) was prepared by the reaction of dimethylamine and 9-methoxy-6-{4-[(oxiran-2-yl)methyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-(oxiran-2-yl)methyl oxime (**12**) which was obtained by the treatment of **2** [14] with epichlorohydrin.

< **Insert Scheme 1 and 2 here** >

Biological evaluation

Antiproliferative activities

All the synthesized indeno[1,2-*c*]quinoline derivatives were evaluated *in vitro* against a panel of seven cancer cell lines (Hela, SAS, SKHep, AGS, RCC786-O, MB-231, and H1299) using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl- 2*H*-tetrazolium bromide) assay. The normal fibroblast cell line (Detroit 551) was also evaluated since a potential anticancer drug candidate should selectively affect only tumor cells and not somatic cells. The concentration that inhibited the growth of 50% of cells (IC_{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 IC_{50} results of indeno[1,2-*c*]quinoline derivatives are summarized in *Table 1*. 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*] quinolin-11-one (**1**) was inactive against all the cancer cells tested. Introduction of amide side chain enhanced antiproliferative activities with exceptions of the terminal morpholinyl derivatives, **7g** and **8g**, which are inactive. The length of side chains play an important role in which an extra methylene group enhances cytotoxicity, *i.e.* **8a** – **8e** (n = 2) are more active than their respective **7a** – **7e** (n = 1) counterparts. However, in the case of **8f**, the introduction of an extra methylene group has only improved antiproliferative activity in two cancer cell lines (Hela, RCC 786-O), with an increase in IC_{50} for cell lines (SAS, SKHep, AGS, MDA-MB-231, H1299 and Detroit 551). Introduction of the terminal epoxymethyl derivative *i.e.* **9** increased antiproliferative activity againt Hela, MB-231, and H1299 cells while the aminoalcohol counterparts, **10** and **11**, enhanced antiproliferative activities aginst all the cancer cells tested. For the 6,11-disubstituted derivatives, 9-methoxy-6-{4-[(oxiran-2-yl)methyl]piperazin- 1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-(oxiran-2-yl)methyl oxime (**12**) exhibited strong antiproliferative activities aginst the growth of Hela, SKHep, MDA-MB-231, and H1299 cells with an IC_{50} value of 0.54, 0.99, 0.79, and 1.18 μM respectively. 6-{4-[2-Hydroxy-3-(dimethylamino)propyl]piperazin-1-yl}-9-methoxy-11*H*-indeno[1,2-*c*]-quinolin-11-one-[2-hydroxy-3-(dimethylamino)propyl]oxime (**13**) was also active aginst the growth of Hela, SKHep, MDA-MB-231, and H1299 cells with an IC_{50} value of 0.98, 2.46, 0.70, and 0.68 μM respectively. However, compound **12** exhibited a lower cytotoxicity to the normal Detroit 551 cell than that of compound **13** (IC_{50} = 7.95 μM for **12** *v.s.* 2.05 μM for **13**).

DNA intercalation

Previous studies[14-18] indicated that most of indeno[1,2-*c*]quinolin-11-one derivatives interact with DNA and therefore, DNA unwinding assay of these newly synthesized

indeno[1,2-*c*]quinoline-11-one derivatives, including compound **4**[16] as a reference compound, was carried out and the results are presented in *Figure* 2. Compound **12** was found to be more potent than compound **4** in the DNA intercalating activity.

Determination of apoptosis in H1299 cells

Further evaluation of compounds **9** and **12** induced DNA damage in H1299 cells was detected by the comet imaging. *Figure* 3A to 3C represent photomicrographs of DNA lesions induced by **9** (10 μM) and **12** (5 μM). To better understand the antiproliferative mechanisms, apoptotic cell death was measured using an Annexin V binding assay, a DAPI staining assay, and Western blot analysis. The percentage of early and late apoptotic cells treated with **9** for 24h (10 μM) was only 10.9% (early and late, Q2 + Q4; *Figure* 4A), compared to 19.5% (*Figure* 4B) of compound **12** at the same concentration. These findings prompted us to further investigate the apoptotic process of H1299 cells after treatment with compound **12**.

Induction of caspase-dependent apoptosis in H1299 cells

To delineate the possible signaling pathways by which **12** induced H1299 cell apoptosis, we examined the changes in the expression levels of various apoptosis-regulating proteins by Western blot analysis. Treatment with **12** (1, 5 and 10 μM) significantly increased the cleavage of PARP, Bax, activation of caspase-3, -7 and decreased Bcl-2 expression in H1299 cells (*Figure* 5).

Compound 14-induced apoptosis is dependent of PTEN/Akt pathways

The PTEN/Akt pathways are well-characterized cell survival signaling pathways that block apoptosis in a variety of cell types.[19] Akt has a wide range of downstream targets that regulate tumor-associated cell processes such as cell growth, cell cycle progression, survival, migration, epithelial–mesenchymal transition, and angiogenesis. The Akt dysregulation was shown to promote cellular survival and proliferation in a variety of cancers such as, colorectal cancer,[20] breast cancer[21] and lung cancer.[22] The constitutively activated Akt was reported to result in chemoresistant cancer cells. Blockade of Akt signaling resulted in apoptosis and

growth inhibition of tumor cells.[23] Therefore, Akt targeting therapy might be promising in lung cancer treatment.[24] Two deacetylases HDAC6 and SIRT1 have been shown to down-regulate genes that mediate potent apoptotic signals following different stimuli.[25-27] Previous study showed that knockdown of HDAC6 decreases the proliferation of lung cancer cell A549.[28] Additionally, downregulation of SIRT1 was reported to induce apoptosis and enhance radio-sensitization of A549 cells.[29] Consistently, our results showed that treatment with **12** (1, 5 and 10 μM) significantly decreased the HDAC6, SIRT1, FOXO3a, p-Akt, and PTEN expression in H1299 cells (*Figure* 6).

< **Insert Table 1 and Figure 2-6 here** >

Conclusion

We have synthesized certain indeno[1,2-*c*]quinolin-11-ones derivatives for antiproliferative evaluation. Among these derivatives, compounds **12** and **13** are two of the most active agents. However, compound **12** exhibited a lower cytotoxicity to the normal Detroit 551 cell than that of compound **13**. Compound **12** was also found to be a potent DNA intercalating agent, more active than compound **4** (the reference compound). Mechanism studies indicated that **12** significantly increase in Bax/Bcl2 ratio, activate caspase-3 and caspase-7 proteins, induce the cleavage of PARP, and consequently cause the cell death (*Figure* 7). These results were associated with regulation of HDAC6, SIRT1, FOXO3a, Akt and PTEN in **12**-treated cells. Further study on the structural optimization of **12** is ongoing.

< **Insert Figure 7 here** >

Experimental

Chemistry

TLC: precoated (0.2 mm) silica gel 60 F_{254} plates from EM Laboratories, Inc.; detection by UV light (254 nm). All chromatographic separations were performed using silica gel (Merck 60 230–400 mesh). M.p.: Yamato MP-21 melting-point apparatus; uncorrected. ^{1}H and ^{13}C

NMR spectra: Varian-Unity-400 spectrometer at 400 and 100 MHz, chemical shifts in ppm with $SiMe_4$ as an internal standard (= 0 ppm), coupling constants *J* in Hz. Mass spectra (LRESIMS) were recorded on Finnigan/Thermo Quest MAT 95XL. Elemental analyses were carried out on a Heraeus CHN-O-Rapid elemental analyzer, and results were within ± 0.4% of calculated values.

6-[4-(2-Chloroacetyl)piperazin-1-yl]-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (5). A mixture of **1** (0.42 g, 1 mmol), triethylamine (0.3 g, 3 mmol) and chloroacetyl chloride (0.3 g, 3 mmol) in acetone (20 mL) was stirded at room temperature for 1 h (TLC monitoring). The mixture was then evaporated *in vacuo* to give a residue which was treated with H_2O (50 mL). The resulting precipitate was collected and crystallized from MeOH to give 0.34 g (81%) of **5**. mp: 143-144 °C (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.40 (m, 2H, piperazinyl-H), 3.53 (m, 2H, piperazinyl-H), 3.82 (m, 4H, piperazinyl-H), 3.88 (s, 3H, 9-OMe), 4.16 (s, 2H, CH_2Cl), 6.95 (dd, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.23 (d, 1H, *J* = 2.4 Hz, 10-H), 7.46 (m, 1H, 2-H), 7.50 (d, 1H, *J* = 8.0 Hz, 7-H), 7.58 (m, 1H, 3-H), 7.82 (d, 1H, *J* = 8.4 Hz, 4-H), 8.70 (d, 1H, *J* = 7.6 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 40.81, 41.86, 46.04, 49.42, 49.63, 55.81, 111.10, 118.92, 121.17, 123.76, 123.97, 127.31, 128.00, 129.68, 132.50, 134.75, 135.13, 136.21, 148.45, 156.12, 160.86, 165.41, 194.90. Anal. calc. for $C_{23}H_{20}ClN_3O_3$: C 65.48, H 4.78, N 9.96; found: C 65.40, H 4.80, N 9.91.

6-[4-(3-Chloropropanoyl)piperazin-1-yl]-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (6). Compound **6** was obtained from **1** and chloropropionyl chloride as described for the preparation of **5** in 78% yield. mp: 162-163 °C (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.91 (t, 2H, *J* = 7.2 Hz, CH_2Cl), 3.36 (m, 2H, piperazinyl-H), 3.49 (m, 2H, piperazinyl-H), 3.76 (m, 2H, piperazinyl-H), 3.87-3.90 (m, 7H, piperazinyl-H, $COCH_2$, and 9-OMe), 6.95 (dd, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.24 (d, 1H, *J* = 2.4 Hz, 10-H), 7.46 (m, 1H, 2-H), 7.51 (d, 1H, *J* = 8.0 Hz, 7-H), 7.58 (m, 1H, 3-H), 7.82 (d, 1H, *J* = 8.4 Hz, 4-H), 8.70 (d, 1H, *J* = 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 36.08, 39.92, 41.52, 45.28, 49.49, 49.91, 55.84, 111.11,

118.95, 121.21, 123.80, 124.03, 127.31, 128.03, 129.68, 132.58, 134.83, 135.18, 136.22, 148.50, 156.28, 160.89, 168.49, 194.96. Anal. calc. for $C_{24}H_{22}ClN_3O_3$: C 66.13, H 5.09, N 9.64; found: C 66.02, H 5.09, N 9.56.

General procedure for coupling of substituted-amines derivatives 7a-8g.

A mixture of **5** or **6** (1.0 mmol), substituted amine (3.0 mmol), and K_2CO_3 (0.62 g, 4.5 mmol) in ethanol (50 mL) was refluxed for 30 min (TLC monitoring). The reaction mixture was cooled and the solvent removed *in vacuo* to yield a gum.

9-Methoxy-6-{4-[2-(methylamino)acetyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one (7a). The resulting residue was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and recrystallized from MeOH to give 7a (45%). Mp: 125-126 ^oC (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H, NMe), 3.36 (m, 2H, piperazinyl-H), 3.47 (m, 2H, piperazinyl-H), 3.49 (s, 2H, C(=O)CH_2NH), 3.71 (m, 2H, piperazinyl-H), 3.84-3.89 (m, 5H, piperazinyl-H and 9-OMe), 6.95 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.23 (d, 1H, *J* = 2.4 Hz, 10-H), 7.46 (m, 1H, 2-H), 7.51 (d, 1H, *J* = 8.4 Hz, 7-H), 7.57 (m, 1H, 3-H), 7.81 (d, 1H, *J* = 8.0 Hz, 4-H), 8.70 (dd, 1H, *J* = 0.8, 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 36.59, 41.59, 44.26, 49.44, 49.90, 52.28, 55.81, 111.07, 118.90, 121.16, 123.76, 124.01, 127.26, 128.00, 129.63, 132.56, 134.81, 135.14, 136.18, 148.48, 156.31, 160.85, 169.68, 194.93. Anal. calc. for $C_{24}H_{24}N_4O_3 \cdot 0.2$ HCl: C 68.01, H 5.78, N 13.22; found: C 67.94, H 5.80, N 13.07.

9-Methoxy-6-{4-[2-(dimethylamino)acetyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one (7b). The resulting residue was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and recrystallized from MeOH to give **7b** (68%). Mp: 128-129 ^oC (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 6H, NMe_2), 3.20 (s, 2H, C(=O)CH_2NH), 3.37 (m, 2H, piperazinyl-H), 3.45 (m, 2H, piperazinyl-H), 3.88 (m, 7H, piperazinyl-H and 9-OMe), 6.95 (dd, 1H, *J* = 2.0, 8.4 Hz, 8-H), 7.23 (d, 1H, *J* = 2.0 Hz, 10-H), 7.46 (m, 1H, 2-H), 7.52 (d, 1H, *J* = 8.4 Hz, 7-H), 7.57 (m, 1H, 3-H), 7.82 (d, 1H, *J* = 8.4 Hz, 4-H), 8.69 (d, 1H, *J* = 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 41.56, 45.35, 45.56 (2C), 49.95 (2C), 55.84, 62.80, 111.06, 118.93, 121.15,

123.79, 124.11, 127.20, 128.01, 129.63, 132.65, 134.94, 135.16, 136.16, 148.54, 156.52, 160.84, 168.83, 195.04. Anal. calc. for $C_{25}H_{26}N_4O_3$: C 69.75, H 6.09, N 13.02; found: C 69.60, H 6.05, N 12.96.

6-{4-[2-(2-Hydroxyethylamino)acetyl]piperazin-1-yl}-9-methoxy-11*H*-indeno[1,2-*c*] quinolin-11-one (7c). The resulting residue was collected and chromatographed on a column of silica gel using (CH_2Cl_2–MeOH 1/10) as an eluent to give **7c** (68%) as a red liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.85 (t, 2H, *J* = 5.2 Hz, NCH_2), 3.37 (m, 2H, piperazinyl-H), 3.47 (m, 2H, piperazinyl-H), 3.56 (s, 2H, C(=O)CH_2NH), 3.64 (t, 2H, *J* = 5.2 Hz, OCH_2), 3.70 (m, 2H, piperazinyl-H), 3.88-3.91 (m, 5H, piperazinyl-H and 9-OMe), 6.95 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.23 (d, 1H, *J* = 2.4 Hz, 10-H), 7.47 (m, 1H, 2-H), 7.51 (d, 1H, *J* = 8.4 Hz, 7-H), 7.58 (m, 1H, 3-H), 7.81 (d, 1H, *J* = 8.4 Hz, 4-H), 8.70 (d, 1H, *J* = 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 41.73, 44.17, 49.35, 49.87, 49.96, 51.67, 55.81, 60.91, 111.07, 118.92, 121.17, 123.71, 123.97, 127.31, 127.98, 129.67, 132.53, 134.76, 135.12, 136.18, 148.44, 156.23, 160.84, 170.36, 195.09. Anal. calc. for $C_{25}H_{26}N_4O_4$: C 67.25, H 5.87, N 12.53; found: C 67.16, H 5.92, N 12.52.

6-{4-{2-[2-(Dimethylamino)ethylamino]acetyl}piperazin-1-yl}-9-methoxy-11*H*-indeno [1,2-*c*]quinolin-11-one (7d). This was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and stirred with 1N HCl (1 mL) in MeOH (2 mL) for 0.5 h, then the solvent removed in vacuo to yield **7d** (69%) as a red liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.26 (s, 6H, $N(CH_3)_2$), 2.45, 2.73 (A_2B_2, 4H, NCH_2CH_2N), 3.36 (m, 2H, piperazinyl-H), 3.46 (m, 2H, piperazinyl-H), 3.54 (s, 2H, C(=O)CH_2NH), 3.72 (m, 2H, piperazinyl-H), 3.84-3.91 (m, 5H, piperazinyl-H and 9-OMe), 6.95 (dd, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.23 (d, 1H, *J* = 2.4 Hz, 10-H), 7.46 (m, 1H, 2-H), 7.52 (*d*, 1H, *J* = 8.0 Hz, 7-H), 7.57 (m, 1H, 3-H), 7.82 (d, 1H, *J* = 8.0 Hz, 4-H), 8.70 (dd, 1H, *J* = 1.6, 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 41.60, 44.34, 45.62 (2C), 47.45, 49.49, 49.95, 50.61, 55.84, 59.38, 111.10, 118.94, 121.20, 123.80, 124.06, 127.28, 128.04, 129.66, 132.61, 134.87, 135.19, 136.21, 148.53, 156.39, 160.88, 169.99, 194.98. Anal. calc. for

$C_{27}H_{31}N_5O_3 \cdot 1.25\ H_2O \cdot 0.75$ HCl: C 61.94, H 6.61, N 13.38; found: C 62.20, H 6.87, N 12.98.

6-{4-{2-[3-(Dimethylamino)propylamino]acetyl}piperazin-1-yl}-9-methoxy-11*H*-indeno [1,2-*c*]quinolin-11-one (7e). This was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and stirred with 1N HCl (1 mL) in MeOH (2 mL) for 0.5 h, then the solvent removed in vacuo to yield **7e** (58%) as a red liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.73 (quin, 2H, *J* = 7.2 Hz, $NCH_2C\underline{H_2}CH_2N(CH_3)_2$), 2.25 (s, 6H, $N(CH_3)_2$), 2.38 (t, 2H, *J* = 7.2 Hz, $NCH_2CH_2C\underline{H_2}N(CH_3)_2$), 2.70 (t, 2H, *J* = 7.2 Hz, $NC\underline{H_2}CH_2CH_2N(CH_3)_2$), 3.36 (m, 2H, piperazinyl-H), 3.45 (m, 2H, piperazinyl-H), 3.53 (s, 2H, $C(=O)CH_2NH$), 3.70 (m, 2H, piperazinyl-H), 3.85-3.91 (m, 5H, piperazinyl-H and 9-OMe), 6.95 (dd, 1H, *J* = 2.8, 8.4 Hz, 8-H), 7.22 (d, 1H, *J* = 2.8 Hz, 10-H), 7.46 (m, 1H, 2-H), 7.50 (d, 1H, *J* = 8.4 Hz, 7-H), 7.57 (m, 1H, 3-H), 7.81 (dd, 1H, *J* = 1.2, 8.8 Hz, 4-H), 8.69 (dd, 1H, *J* = 1.2, 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 27.94, 41.57, 44.23, 45.44 (2C), 48.42, 49.40, 49.85, 50.50, 55.79, 57.78, 111.05, 118.87, 121.13, 123.73, 123.99, 127.23, 127.98, 129.61, 132.53, 134.77, 135.10, 136.13, 148.44, 156.28, 160.80, 169.75, 194.92. Anal. calc. for $C_{28}H_{33}N_5O_3 \cdot 1.5\ H_2O \cdot 2.0$ HCl: C 57.22, H 6.53, N 11.92; found: C 56.98, H 6.83, N 11.61.

9-Methoxy-6-{4-[2-(piperazin-1-yl)acetyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one (7f). The resulting residue was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and recrystallized from MeOH to give **7f** (68%). Mp: 125-126 ^{o}C (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.51 (m, 4H, piperazinyl-H), 2.92 (m, 4H, piperazinyl-H), 2.25 (s, 2H, $C(=O)CH_2NH$), 3.37 (m, 2H, piperazinyl-H), 3.46 (m, 2H, piperazinyl-H), 3.70-4.02 (m, 7H, piperazinyl-H and 9-OMe), 6.96 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.23 (*d*, 1H, *J* = 2.4 Hz, 10-H), 7.46 (*m*, 1H, 2-H), 7.52-7.60 (*m*, 2H, 3-, 7-H), 7.83 (*d*, 1H, *J* = 8.4 Hz, 4-H), 8.70 (*d*, 1H, *J* = 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$): 41.56, 45.39, 46.02 (2C), 49.91, 49.99, 54.42 (2C), 55.83, 62.25, 111.04, 118.92, 121.14, 123.78, 124.07, 127.20, 128.00, 129.63, 132.62, 134.93, 135.17, 136.18, 148.52, 156.47, 160.84, 168.32, 195.01. Anal. calc. for $C_{27}H_{29}N_5O_3 \cdot 1.75\ H_2O \cdot 0.5$ HCl: C 62.19, H 6.39, N 13.44; found: C 62.13, H 6.66, N 13.17.

9-Methoxy-6-[4-(2-morpholinoacetyl)piperazin-1-yl]-11*H*-indeno[1,2-*c*]quinolin-11-one (7g). The resulting residue was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and recrystallized from MeOH to give **7g** (72%). Mp: 132-133 ^oC (MeOH). ^1H-NMR (400 MHz, $CDCl_3$) δ 2.56 (m, 4H, morpholinyl-H), 2.27 (s, 2H, C(=O)CH_2NH), 3.37 (m, 2H, piperazinyl-H), 3.46 (m, 2H, piperazinyl-H), 3.75 (m, 4H, morpholinyl-H), 3.78-4.09 (m, 7H, piperazinyl-H and 9-OMe), 6.96 (dd, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.24 (d, 1H, *J* = 2.4 Hz, 10-H), 7.47 (m, 1H, 2-H), 7.53-7.60 (m, 2H, 3-, 7-H), 7.83 (d, 1H, *J* = 8.4 Hz, 4-H), 8.71 (dd, 1H, *J* = 0.8, 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 41.56, 45.37, 49.93 (2C), 53.54 (2C), 55.83, 61.79, 66.89 (2C), 111.05, 118.95, 121.16, 123.79, 124.05, 127.25, 128.00, 129.66, 132.61, 134.90, 135.18, 136.20, 148.52, 156.42, 160.86, 167.94, 194.98. Anal. calc. for $C_{27}H_{28}N_4O_4 \cdot 0.1\ H_2O$: C 68.37, H 5.99, N 11.81; found: C 68.19, H 6.09, N 11.79.

9-Methoxy-6-{4-[3-(methylamino)propanoyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one (8a). This was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and stirred with 1N HCl (1 mL) in MeOH (2 mL) for 0.5 h, then the solvent removed in vacuo and recrystallized from MeOH to give **8a** (52%). Mp: 121-122 ^oC (MeOH). ^1H-NMR (400 MHz, DMSO-d_6) δ2.57 (s, 3H, NMe), 2.89, 3.13 (A_2B_2, 4H, C(=O)CH_2CH_2N), 3.27 (m, 2H, piperazinyl-H), 3.36 (m, 4H, piperazinyl-H), 3.76 (m, 2H, piperazinyl-H), 3.86 (s, 3H, 9-OMe), 7.12 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.18 (d, 1H, *J* = 2.4 Hz, 10-H), 7.52 (m, 1H, 2-H), 7.57 (d, 1H, *J* = 8.4 Hz, 7-H), 7.64 (m, 1H, 3-H), 7.78 (d, 1H, *J* = 8.4 Hz, 4-H), 8.55 (dd, 1H, *J* = 0.8, 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ 28.62, 32.63, 40.79, 44.36, 44.49, 49.29, 49.41, 55.83, 110.98, 119.15, 120.30, 122.98, 124.69, 127.23, 127.70, 129.76, 132.38, 133.93, 134.44, 135.26, 147.68, 156.29, 160.50, 168.31, 194.35. Anal. calc. for $C_{25}H_{26}N_4O_3 \cdot 1.0\ H_2O \cdot 1.0$ HCl: C 61.92, H 6.03, N 11.55; found: C 61.81, H 6.11, N 11.29.

9-Methoxy-6-{4-[3-(dimethylamino)propanoyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one (8b). The resulting residue was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and recrystallized from MeOH to give 8b (62%). Mp: 133-134 ^oC (MeOH). ^1H-NMR

(400 MHz, $CDCl_3$) δ 2.33 (s, 6H, NMe_2), 2.63, 2.74 (A_2B_2, 4H, $C(=O)CH_2CH_2N$), 3.35 (m, 2H, piperazinyl-H), 3.47 (m, 2H, piperazinyl-H), 3.78 (m, 2H, piperazinyl-H), 3.87-3.89 (m, 5H, piperazinyl-H and 9-OMe), 6.96 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.24 (d, 1H, *J* = 2.4 Hz, 10-H), 7.47 (m, 1H, 2-H), 7.53 (d, 1H, *J* = 8.4 Hz, 7-H), 7.58 (m, 1H, 3-H), 7.82 (d, 1H, *J* = 8.4 Hz, 4-H), 8.71 (dd, 1H, *J* = 0.8, 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 31.58, 41.37, 45.32, 45.42 (2C), 49.57, 49.94, 53.42, 55.17, 55.83, 111.09, 118.92, 121.17, 123.78, 124.06, 127.24, 128.01, 129.63, 132.62, 134.87, 135.17, 136.18, 148.51, 156.40, 160.86, 170.29, 194.98. Anal. calc. for $C_{26}H_{28}N_4O_3$: C 70.25, H 6.35, N 12.60; found: C 69.98, H 6.33, N 12.29.

6-{4-[3-(2-Hydroxyethylamino)propanoyl]piperazin-1-yl}-9-methoxy-11*H*-indeno[1,2-*c*] quinolin-11-one (8c). The resulting residue was collected and chromatographed on a column of silica gel using (CH_2Cl_2–MeOH 1/10) as an eluent to give **8c** (61%) as a red liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.64, 2.98 (A_2B_2, 4H, $C(=O)CH_2CH_2N$), 2.84, 3.69 (A_2B_2, 4H, NCH_2CH_2O), 2.98 (t, 2H, *J* = 6.0 Hz, NCH_2), 3.34 (m, 2H, piperazinyl-H), 3.46 (m, 2H, piperazinyl-H), 3.76 (m, 2H, piperazinyl-H), 3.85-3.90 (m, 5H, piperazinyl-H and 9-OMe), 6.94 (dd, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.22 (d, 1H, *J* = 2.4 Hz, 10-H), 7.46 (m, 1H, 2-H), 7.50 (d, 1H, *J* = 8.0 Hz, 7-H), 7.57 (m, 1H, 3-H), 7.81 (d, 1H, *J* = 8.4 Hz, 4-H), 8.69 (dd, 1H, *J* = 0.8, 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 33.05, 41.33, 44.40, 45.10, 49.43, 49.88, 50.92, 55.80, 60.51, 111.07, 118.88, 121.15, 123.75, 124.01, 127.24, 127.98, 129.61, 132.56, 134.78, 135.12, 136.15, 148.46, 156.30, 160.83, 170.57, 194.91. LRESIMS $[M+H]^+$: 461.

6-{4-{3-[2-(Dimethylamino)ethylamino]propanoyl}piperazin-1-yl}-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (8d). The resulting residue was collected and chromatographed on a column of silica gel using (CH_2Cl_2–MeOH 1/10) as an eluent to give **8d** (72%) as a red liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.24 (s, 6H, NMe_2), 2.43, 2.75 (A_2B_2, 4H, NCH_2CH_2N), 2.64, 2.97 (A_2B_2, 4H, $C(=O)CH_2CH_2N$), 3.35 (m, 2H, piperazinyl-H), 3.46 (m, 2H, piperazinyl-H), 3.76 (m, 2H, piperazinyl-H), 3.88-3.91 (m, 5H, piperazinyl-H and

9-OMe), 6.96 (dd, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.24 (d, 1H, *J* = 2.4 Hz, 10-H), 7.47 (m, 1H, 2-H), 7.53 (d, 1H, *J* = 8.0 Hz, 7-H), 7.58 (m, 1H, 3-H), 7.83 (dd, 1H, *J* = 1.2, 8.4 Hz, 4-H), 8.71 (dd, 1H, *J* = 1.2, 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 33.59, 41.31, 45.18, 45.48, 45.62 (2C), 47.63, 49.53, 49.56, 55.84, 59.22, 111.12, 118.95, 121.19, 123.80, 124.09, 127.26, 128.04, 129.65, 132.65, 134.91, 135.20, 136.21, 148.55, 156.43, 160.89, 170.65, 195.02. LRESIMS $[M+H]^+$: 488.

6-{4-{3-[3-(Dimethylamino)propylamino]propanoyl}piperazin-1-yl}-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (8e). The resulting residue was collected and chromatographed on a column of silica gel using (CH_2Cl_2–MeOH 1/10) as an eluent to give **8e** (61%) as a red liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.69 (quin, 2H, *J* = 7.2 Hz, $NCH_2C\underline{H_2}CH_2NMe_2$), 2.22 (s, 6H, NMe_2), 2.33 (t, 2H, *J* = 7.2 Hz, $NCH_2CH_2C\underline{H_2}NMe_2$), 2.63, 2.93 ($A_2B_2$, 4H, $C(=O)CH_2CH_2N$), 2.69 (t, 2H, *J* = 7.2 Hz, $NC\underline{H_2}CH_2CH_2NMe_2$), 3.33 (m, 2H, piperazinyl-H), 3.43 (m, 2H, piperazinyl-H), 3.75 (m, 2H, piperazinyl-H), 3.81-3.89 (m, 5H, piperazinyl-H and 9-OMe), 6.92 (dd, 1H, *J* = 2.4, 8.0 Hz, 8-H), 7.20 (d, 1H, *J* = 2.4 Hz, 10-H), 7.45 (m, 1H, 2-H), 7.48 (d, 1H, *J* = 8.0 Hz, 7-H), 7.56 (m, 1H, 3-H), 7.80 (dd, 1H, *J* = 0.8, 8.4 Hz, 4-H), 8.67 (dd, 1H, *J* = 1.2, 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 27.87, 33.17, 41.20, 45.05, 45.27, 45.41 (2C), 49.41, 49.81, 55.74, 57.78, 111.99, 118.77, 121.04, 123.66, 123.96, 127.15, 127.92, 129.54, 132.49, 134.71, 135.01, 136.02, 148.38, 156.27, 160.71, 170.62, 194.86. LRESIMS $[M+H]^+$: 502.

9-Methoxy-6-{4-[3-(piperazin-1-yl)propanoyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin-11-one (8f). This was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and stirred with 1N HCl (1 mL) in MeOH (2 mL) for 0.5 h, then the solvent removed *in vacuo* to give **8f** (63%). Mp: 135-136 °C (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.50 (m, 4H, piperazinyl-H), 2.64, 2.77 (A_2B_2, 4H, $C(=O)CH_2CH_2N$), 2.90 (m, 4H, piperazinyl-H), 3.33 (m, 2H, piperazinyl-H), 3.46 (m, 2H, piperazinyl-H), 3.77 (m, 2H, piperazinyl-H), 3.84-3.89 (m, 5H, piperazinyl-H and 9-OMe), 6.94 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.22 (d,

1H, *J* = 2.4 Hz, 10-H), 7.44-7.59 (m, 3H, 2-, 3-, 7-H), 7.81 (d, 1H, *J* = 8.4 Hz, 4-H), 8.69 (d, 1H, *J* = 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 30.81, 41.34, 45.31, 45.91 (2C), 49.55, 49.93, 54.58 (2C), 54.72, 55.78, 111.03, 118.87, 121.12, 123.73, 124.01, 127.20, 127.96, 129.59, 132.56, 134.81, 135.11, 136.12, 148.46, 156.35, 160.81, 170.52, 194.91. Anal. calc. for $C_{28}H_{31}N_5O_3 \cdot 1.8\ H_2O \cdot 0.6$ HCl: C 62.29, H 6.57, N 12.97; found: C 62.32, H 6.58, N 12.84.

9-Methoxy-6-[4-(3-morpholinopropanoyl)piperazin-1-yl]-11*H*-indeno [1,2-*c*]quinolin-11-one (8g). The resulting residue was purified by column chromatography (MeOH–CH_2Cl_2 1/10) and recrystallized from MeOH to give **8g** (73%). Mp: 158-159 °C (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.53 (m, 4H, morpholinyl-H), 2.63, 2.78 (A_2B_2, 4H, C(=O)CH_2CH_2N), 3.34 (m, 2H, piperazinyl-H), 3.47 (m, 2H, piperazinyl-H), 3.68-3.77 (m, 6H, morpholinyl-H, piperazinyl-H), 3.88 (m, 5H, piperazinyl-H and 9-OMe), 6.95 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.24 (d, 1H, *J* = 2.4 Hz, 10-H), 7.47 (m, 1H, 2-H), 7.53 (d, 1H, *J* = 8.4 Hz, 7-H), 7.58 (m, 1H, 3-H), 7.82 (d, 1H, *J* = 8.4 Hz, 4-H), 8.70 (dd, 1H, *J* = 0.8, 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 30.77, 41.38, 45.31, 49.54, 49.97, 53.74 (2C), 54.51, 55.81, 66.90 (2C), 111.04, 118.92, 121.16, 123.77, 124.02, 127.26, 127.98, 129.64, 132.59, 134.83, 135.15, 136.17, 148.48, 156.36, 160.84, 170.31, 194.95. Anal. calc. for $C_{28}H_{30}N_4O_4 \cdot 0.5\ H_2O$: C 67.86, H 6.31, N 11.31; found: C 68.07, H 6.11, N 10.91.

9-Methoxy-6-{4-[(oxiran-2-yl)methyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*] quinolin-11-one (9). A mixture of **1** (0.36 g, 1 mmol), K_2CO_3 (0.28 g, 2 mol) and epichlorohydrin (0.3 g, 3 mol) in MeCN (30 mL) was refluxed for 4 h (TLC monitoring). The mixture was then cooled and evaporated *in vacuo* to give a residue which was treated with H_2O (50 mL). The resulting residue was purified by column chromatography (MeOH–CH_2Cl_2 1/50) and recrystallized from MeOH to give **9** (0.33 g, 84%). Mp: 146-147 °C (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.38 (dd, 1H, *J* = 7.8, 13.2 Hz, OCH_2), 2.56 (dd, 1H, *J* = 2.8, 5.2 Hz, oxiranyl-H), 2.81 (m, 4H, piperazinyl-H), 2.84 (dd, 1H, *J* = 4.4, 5.2 Hz, oxiranyl-H), 2.94 (dd, 1H, *J* = 2.8, 13.2 Hz,

OCH_2), 3.20 (m, 1H, oxiranyl-H), 3.45 (m, 4H, piperazinyl-H), 3.87 (s, 3H, OMe), 6.94 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.20 (d, 1H, *J* = 2.4 Hz, 10-H), 7.43 (m, 1H, 2-H), 7.54 (m, 2H, 3, 7-H), 7.83 (d, 1H, *J* = 7.6 Hz, 4-H), 8.68 (dd, 1H, *J* = 1.6, 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 44.78 (2C), 49.51 (2C), 50.36, 53.51, 55.75, 61.14, 110.84, 118.78, 120.91, 123.69, 124.34, 126.79, 127.95, 129.39, 132.71, 135.09, 135.17, 148.59, 156.84, 160.63, 195.22. Anal. calc. for $C_{24}H_{23}N_3O_3 \cdot 0.25\ H_2O$: C 70.99, H 5.85, N 10.35; found: C 70.69, H 5.74, N 10.23.

6-{4-[2-Hydroxy-3-(methylamino)propyl]piperazin-1-yl}-9-methoxy-11*H*-indeno [1,2-*c*]quinolin-11-one (10). A mixture of **9** (0.40 g, 1.0 mmol) and 40% methylamine (5 mL) in 2-ethoxyethanol (30 mL) was refluxed for 1 h (by TLC monitoring). The mixture was then cooled and evaporated *in vacuo* to give a residue which was treated with H_2O (50 mL). The resulting residue was collected and chromatographed on a column of silica gel using (CH_2Cl_2–MeOH 1/10) as an eluent to give 0.29 g (67%) of **10** as a red liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.47 (s, 3H, NMe), 2.53-2.71 (m, 4H, 2 CH_2), 2.88 (m, 4H, piperazinyl-H), 3.37 (m, 4H, piperazinyl-H), 3.82 (s, 3H, 9-OMe), 3.93 (m, 1H, CH), 6.86 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.12 (d, 1H, *J* = 2.4 Hz,, 10-H), 7.37-7.53 (m, 3H, 2, 3, 7-H), 7.77 (d, 1H, *J* = 8.4 Hz, 4-H), 8.61 (d, 1H, *J* = 8.4 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 36.38, 49.44, 49.56 (2C), 53.18, 55.62 (2C), 62.11, 65.63, 110.70, 118.59, 120.81, 123.58, 124.16, 126.71, 127.80, 129.27, 132.56, 134.91, 135.73, 148.42, 156.68, 160.50, 194.93. LRESIMS $[M+H]^+$: 433.

6-{4-[3-(Dimethylamino)-2-hydroxypropyl]piperazin-1-yl}-9-methoxy-11*H*-indeno [1,2-*c*]quinolin-11-one (11). Compound **11** was obtained from **9** and 40% dimethylamine as described for **10** in 43% yield (as a red liquid). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.39 (s, 6H, NMe_2), 2.41-2.51 (m, 4H, 2 CH_2), 2.78 (m, 4H, piperazinyl-H), 3.40 (m, 4H, piperazinyl-H), 3.84 (s, 3H, 9-OMe), 3.99 (m, 1H, CH), 6.89 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.15 (d, 1H, *J* = 6.4 Hz,, 10-H), 7.40 (m, 1H, 2-H), 7.52 (m, 2H, 3, 7-H), 7.78 (m, 1H, 4-H), 8.64 (m, 1H, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 45.60 (2C), 49.55 (2C), 53.40, 55.67 (2C), 62.58, 63.58,

64.58, 110.76, 118.65, 120.84, 123.62, 124.26, 126.73, 127.86, 129.30, 132.64, 134.97, 135.00, 135.78, 148.48, 156.76, 160.54, 195.05. LRESIMS $[M+H]^+$: 447.

9-Methoxy-6-{4-[(oxiran-2-yl)methyl]piperazin-1-yl}-11*H*-indeno[1,2-*c*]quinolin- 11-one *O*-(oxiran-2-yl)methyl oxime (12). Compound **12** was obtained from **2** as described for **9**. The resulting residue was purified by column chromatography (MeOH–CH_2Cl_2 1/50) to give **12** in 65% yield (as a red liquid). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.38 (dd, 1H, *J* = 7.8, 13.2 Hz, OCH_2), 2.56 (dd, 1H, *J* = 2.8, 5.2 Hz, oxiranyl-H), 2.81 (m, 4H, piperazinyl-H), 2.84 (dd, 1H, *J* = 4.4, 5.2 Hz, oxiranyl-H), 2.94 (dd, 1H, *J* = 2.8, 13.2 Hz, OCH_2), 3.20 (m, 1H, oxiranyl-H), 3.45 (m, 4H, piperazinyl-H), 3.87 (s, 3H, OMe), 6.87 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.34 (s,1H, 10-H), 7.49 (m, 1H, *J* = 2.4 Hz, 2-H), 7.64 (d, 1H, *J* = 7.6 Hz, 3-H), 7.84 (m, 2H, 4,7-H), 8.69 (dd, 1H, *J* = 1.6, 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 44.55 (2C), 44.66 (2C), 49.40, 49.91, 50.23, 53.46, 55.39, 60.98, 114.98, 116.05, 121.37, 123.29, 125.19, 125.44, 127.36, 128.25, 128.34, 130.91, 131.94, 138.64, 147.00, 154.88, 156.59, 159.54. Anal. calc. for $C_{27}H_{28}N_4O_4 \cdot 0.75\ H_2O$: C 66.71, H 6.13, N 11.53; found: C 66.72, H 6.04, N 11.28.

6-{4-[2-Hydroxy-3-(dimethylamino)propyl]piperazin-1-yl}-9-methoxy-11*H*-indeno [1,2-*c*]quinolin-11-one-[2-hydroxy-3-(dimethyl-amino)propyl] oxime (13). Compound **13** was obtained from **12** and 40% dimethylamine as described for **10** in 41% yield (as a red liquid). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 6H, NMe_2), 2.37 (s, 6H, NMe_2), 2.38-2.51 (m, 4H, 2 CH_2), 2.57 (t, 2H, *J* = 7.6 Hz, CH_2), 2.92 (m, 4H, piperazinyl-H), 3.42 (m, 4H, piperazinyl-H), 3.88 (s, 3H, 9-OMe), 3.97 (m, 1H, CH), 4.22 (m, 1H, CH), 4.57 (t, 1H, *J* = 4.4 Hz, CH_2), 6.95 (dd, 1H, *J* = 2.4, 8.4 Hz, 8-H), 7.38 (m, 1H, 10-H), 7.54 (m, 1H, 2-H), 7.75 (d, 1H, *J* = 8.4 Hz, 3-H), 7.86 (d, 1H, *J* = 8.4 Hz, 7-H), 7.98 (d, 1H, *J* = 2.4 Hz, 4-H), 8.75 (d, 1H, *J* = 8.0 Hz, 1-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 45.56 (2C), 45.71 (2C), 49.70, 53.52, 55.63, 61.96, 62.66, 63.72, 64.66, 65.83, 66.38, 115.36, 116.08, 116.25, 121.64, 123.50, 125.40, 125.71, 127.55, 128.42, 128.57, 131.27, 132.18, 139.01, 147.21, 154.91, 156.89, 159.84. LRESIMS $[M+H]^+$: 563.

Pharmacology

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 lg/mL amphotericin. Culture was maintained at 37 ^{o}C with 5% CO2 in a humidified atmosphere.

Antiproliferative Assay.

Cancer cells were treated as indicated for 72 h in medium containing 10% FBS. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium 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 ($Ab_{treated}$/ $Ab_{control}$) x 100, whereAb represents the mean absorbance (n = 3). The concentration that killed 50% of cells (IC_{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%.

DNA Mobility assay (DNA unwinding).[16]

Negative supercolied pBR322 (400 ng) was incubated in TE buffer, pH = 8.0, with 10 μM of different indenoquinoline derivatives for 12 h at room temperature. Following the addition of 2 μL of loading buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol), the samples was loaded onto a 1% agarose gel. The gel was run at 6 V/cm for 2.5 h in TAE buffer (40mM Tris, 20 mM sodium acetate, 1 mM EDTA-Na2, pH = 8.5) and stained with ethidium bromide and photographed under UV illumination using Polaroid instant film.

Alkaline comet assay

To determine whether compounds **9** and **12** induce the alkaline sensitive lesions, the single cell electrophoresis-based alkaline comet assay was performed as described previously with minor modifications.[30] Briefly, 70 μl low melting point agarose (0.5%, w/v) (Gibco) was mixed with 2 × 10^4 cells; the mixture was then layered onto the slides, and overlaid with a

coverslip. After agarose solidification, the coverslip was removed and the slide was immersed at 4 °C in fresh lysing solution (2.5 M NaCl; 100 mM Na_2EDTA, 10 mM Tris, pH 10 containing 1% Triton X-100). The slide was equilibrated with an alkaline solution (1 mM Na_2EDTA, 300 mM NaOH) and then replaced with alkaline electrophoresis solution (200 mM NaOH, 1 mM EDTA). Afterwards, slides were neutralized by Tris buffer (0.4 M, pH 7.5) and rinsed with deionized water. 5 μg/ml PI was added. The slide was viewed under a fluorescence microscopy (TE2000-U; Nikon, Tokyo, Japan) at 460 nm for visual scoring. The migration of DNA from the nucleus of each cell was measured using the computer program Cometscore™ (TriTek Corp, Sumerduck, VA).

Assessment of apoptosis

Apoptotic cells were quantified using annexin V and PI double staining described previously.[31] Annexin V-FITC detection kit was purchased from Strong Biotech (Taipei, Taiwan). In brief, 1×10^6 cells were seeded onto a 100-mm petri-dish and exposed to indicated concentrations of compound 11 and 14 for 24 h respectively. Afterwards, cells were harvested by trypsinization and labeled with annexin V-fluorescein isothiocyanate (10 μg/ml) and PI (20 μg/ml). Cells were washed, resuspended with a binding buffer then analyzed using a flow cytometry (FACS Calibur; Becton Dickinson, Mountain View, CA) and WinMDI 2.8 software (written by Joseph Trotter, Scripps Research Institute, La Jolla, CA)..

Immunoblot analysis.

After treatment of compound **12**, cells were harvested and lysed. Lysates were centrifuged and the protein concentration was determined. A total of 30 μg protein lysates were resolved by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred to 0.22 μm pore-size nitrocellulose membranes (Pall Life Sciences, Ann Arbor, MI). Membranes were blocked with 5% non-fat milk. Afterwards, the membranes were incubated with primary antibodies against Caspase-3 (IMGENEX, #IMG-144A), Caspase-7 (IMGENEX, #IMG-277), Bcl-2 (Epitomics, #1017), Bax (Epitomics, #1063), Histone Deacetylase 6 (HDAC6) (Epitomics, #3781), SIRT1 (Epitomics, #1104), FOXO3a (Epitomics, #2071), Cleaved PARP (Cell Signaling, #9541), PTEN (GeneTex, #GTX101025), β-actin (GeneTex, #GTX109639)

and phosphorylated-Akt (Ser^{473}, Santa Cruz Biotech, sc-7985-R), and their corresponding secondary antibodies respectively. The signals were detected using a chemiluminescence detection kit ECL^{TM} (Amersham Piscataway, NJ, USA).

Acknowledgements

Financial support of this work by the *National Science Council of the Republic of China* (NSC101-2313-B-037-001, NSC 102-2320-B-037-001) is gratefully acknowledged. We also thank the *National Center for High-Performance Computing* for providing computer resources and chemical database services.

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Figure captions:

Figure 1. Structures of Camptothecin (CPT), CIL-102, TAS-103, indeno[1,2-*c*]quinoline derivatives **1** – **4**, and target compounds.

Figure 2. Indeno[1,2-*c*]quinoline derivatives unwind plasmid DNA. Compounds (10 μM) were incubated with negatively supercolied pBR322 (0.4 μg) for 12 h in TE buffer and then run for 2.5 h on a 1 % gel.

Figure 3. Compound **9** (A) and **12** (B) induced DNA damage in H1299 cells detected by the comet images (200X). Cells were treated with DMSO (a), compound at 1.0 μM (b), 5.0 μM (c) or 10.0 μM (d) for 24 h and then the cells were suspended in agarose to make a gel, were mounted on a microscopic slide, lysed, electrophoresed, stained with a dye that binds DNA, and viewed with a fluorescence microscope. DNA containing breaks moved from the brightly fluorescent core (the head) towards the anode, forming the image described as a comet. (C) Average of % tail DNA for compound **9** and **12**-treated H1299 cells respectively.

Figure 4. Compound **9** (A) and **12** (B) possesses apoptosis-inducing effects on H1299 cells. Cells were treated with DMSO (a), compound at 1.0 μM (b), 5.0 μM (c) or 10.0 μM (d) for 24 h and then the cells were harvested for flow cytometry analysis of PI and Annexin-V staining. The experimental details were described in Section 5.6.5. Representative cell distribution by PI and Annexin-V staining were shown (n = 3).

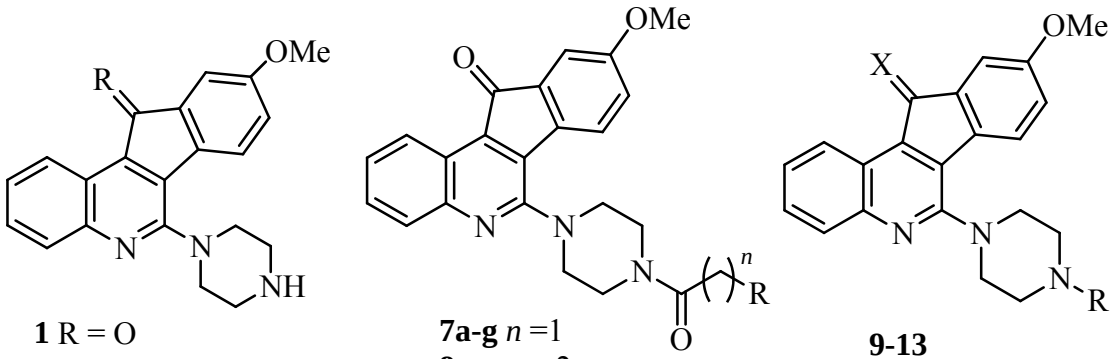
Figure 5. The effect of the **12** on apoptosis-related protein expression in H1299 cells. Western blot analysis of proteins extracted from H1299 cells treated with 0, 1, 5, and 10 μM of **12**, respectively, for 24 h. Total protein lysates were separated by electrophoresis, transferred to nitrocellulose membranes, and analyzed with antibodies against procaspase-3, and -7; Bcl-2; Bax; and cleaved PARP. β-actin was used as an internal control.

Figure 6. Expression of HDAC6, SIRT1, p-FOXO3a, p-Akt, and p-PTEN in H1299 cells by treatment with compound **12**. Exponentially growing cells were treated with **12** for 24 h. Equal amounts of cell lysate were resolved using SDS–PAGE and analyzed by Western blot using

anti-HDAC6, anti-SIRT1, anti-FOXO3a, anti-p-Akt, anti-p-PTEN, and β-actin antibody to confirm equal protein loading.

Figure 7. Proposed mechanism of compound **12**-induced apoptosis of lung cancer cells. Compound **12** may intercalate DNA, inactivate the Akt signaling, increase the Bax/Bcl-2 ratio and activate caspase-3 and caspase-7 proteins, and consequently to cause the cell death of lung cancer. Simultaneously, compound **12** dramatically down-regulates the protein level of pro-survival deacetylases HDAC6 and SIRT1.

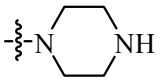
Table 1. Antiproliferative activity of 9-methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinoline derivatives (IC_{50}, μM)

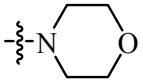
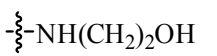
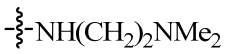
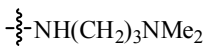
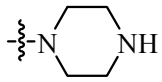
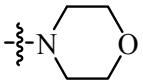
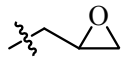
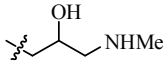
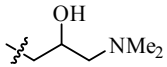
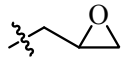
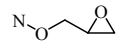
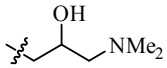
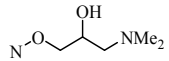


1 R = O
2 R = NOH

7a-g *n* =1
8a-g *n* = 2

9-13

Compd	R	X	Cell line[a] (IC_{50}, μM)							
			Hela	SAS	SKHep	AGS	RCC 786-O	MDA-MB-231	H1299	Detroit 551
CPT	-	-	0.18 ± 0.10	6.00 ± 2.70	0.22 ± 0.13	23.76 ± 0.73	0.015 ± 0.001	2.11 ± 0.61	3.14 ± 0.78	0.99 ± 0.09
1	-	-	15.18 ± 1.50	> 30	25.34 ± 15.46	10.13 ± 1.19	12.47 ± 0.96	23.76 ± 3.69	17.14 ± 2.09	5.71 ± 0.61
2	-	-	2.35 ± 0.25	6.10 ± 0.90	3.75 ± 1.28	1.74 ± 0.47	1.20 ± 0.90	5.87 ± 1.08	5.72 ± 0.71	ND[b]
7a	-NHMe	O	2.04 ± 0.62	7.40 ± 0.90	4.83 ± 2.09	7.15 ± 1.50	6.20 ± 1.90	5.92 ± 0.16	5.14 ± 0.16	4.84 ± 0.46
7b	-NMe_2	O	11.84 ± 2.78	> 30	19.22 ± 5.40	8.70 ± 1.21	14.13 ± 3.36	6.15 ± 0.04	6.28 ± 0.10	31.47 ± 0.47
7c	-$NH(CH_2)_2OH$	O	4.74 ± 2.31	11.30 ± 0.30	6.64 ± 0.94	6.55 ± 0.57	6.70 ± 0.53	5.90 ± 0.08	5.41 ± 0.89	8.78 ± 1.78
7d	-$NH(CH_2)_2NMe_2$	O	8.17 ± 2.19	19.50 ± 3.20	16.03 ± 3.83	14.12 ± 2.28	9.37 ± 0.59	6.15 ± 0.55	6.48 ± 0.07	4.59 ± 0.60
7e	-$NH(CH_2)_3NMe_2$	O	10.76 ± 3.03	> 30	13.10 ± 2.72	5.62 ± 2.18	9.97 ± 2.12	6.00 ± 0.50	5.99 ± 0.19	8.89 ± 0.63
7f		O	> 30	7.40 ± 1.10	2.22 ± 1.33	3.37 ± 0.41	4.00 ± 1.51	5.45 ± 0.14	5.32 ± 0.17	2.55 ± 0.14

7g		O	18.02 ± 8.10	> 30	19.93±10.03	21.40 ± 0.24	> 30	> 30	> 30	ND [b]
8a		O	1.90 ±0.78	6.90 ±0.70	9.10 ±4.82	8.11 ±1.06	6.00 ±1.40	5.89 ± 0.11	5.46 ± 0.08	2.92 ± 0.06
8b		O	11.66 ± 1.75	17.00 ± 2.90	16.61 ± 1.99	8.11 ± 1.12	11.47 ± 1.76	6.61 ± 0.25	5.79 ± 0.12	2.84 ± 0.03
8c		O	3.13 ± 1.32	9.10 ± 1.60	4.51 ± 1.14	3.67 ± 0.46	2.03 ± 1.43	6.08 ± 0.19	5.67 ± 0.15	8.38 ± 1.73
8d		O	4.78 ± 1.53	8.80 ± 1.00	5.08 ± 1.47	4.94 ± 1.02	7.03 ± 1.29	7.44 ± 0.12	5.65 ± 0.28	2.53 ± 0.05
8e		O	5.43 ± 1.27	13.50 ± 2.20	8.60 ± 0.67	2.80 ± 1.32	5.90 ± 0.92	5.70 ± 0.07	5.44 ± 0.12	6.42 ± 1.40
8f		O	4.71 ± 1.37	7.70 ± 0.90	4.93 ± 1.14	5.46 ± 0.52	1.50 ± 1.30	6.26 ± 0.10	5.87 ± 0.18	3.35 ± 0.64
8g		O	14.31 ± 4.38	> 30	15.05 ± 5.87	11.95 ± 1.28	> 30	21.87 ± 3.91	18.71 ± 2.35	ND [b]
9		O	10.66 ± 1.21	> 30	> 30	21.45 ± 10.10	19.10 ± 3.10	7.65 ± 0.66	7.92 ± 0.04	ND [b]
10		O	4.64 ± 0.96	7.40 ± 0.70	3.44 ± 1.23	9.58 ± 3.84	6.40 ± 0.20	6.05 ± 0.13	6.89 ± 0.17	9.40 ± 1.72
11		O	2.47± 0.72	6.90 ± 0.50	2.22 ± 0.76	7.86 ± 3.84	6.80 ± 0.10	6.11 ± 0.09	6.84 ± 0.20	2.58 ± 0.04
12			0.54 ± 0.20	6.20 ± 0.90	0.99 ± 0.44	8.76 ± 4.10	8.80 ± 2.20	0.79 ± 0.05	1.18 ± 0.14	7.95 ± 1.03
13			0.98 ± 0.38	9.00 ± 2.70	2.46 ± 1.72	18.60 ± 6.20	13.90 ± 10.50	0.70 ± 0.14	0.68 ± 0.08	2.05 ± 0.12

[a] Theses cell lines are: cervix carcinoma cell (Hela); tongue cancer cell (SAS); liver cancer cell (SKHep); gastric cancer cell (AGS); renal cancer cell (RCC 786-O); breast cancer cell (MDA-MB-231); non-small cell lung cancer (H1299); and normal skin fibroblast cell (Detroit 551).

[b] ND: Not determined.

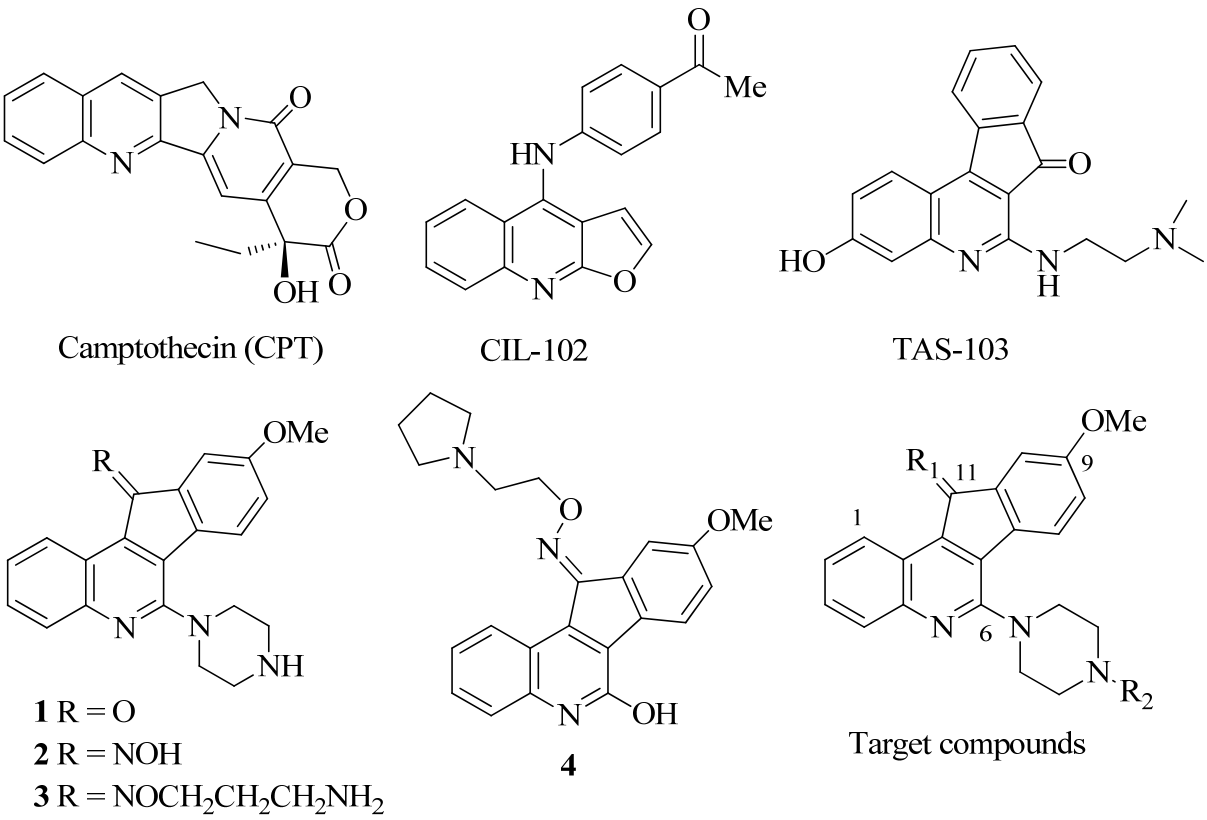


Figure 1.

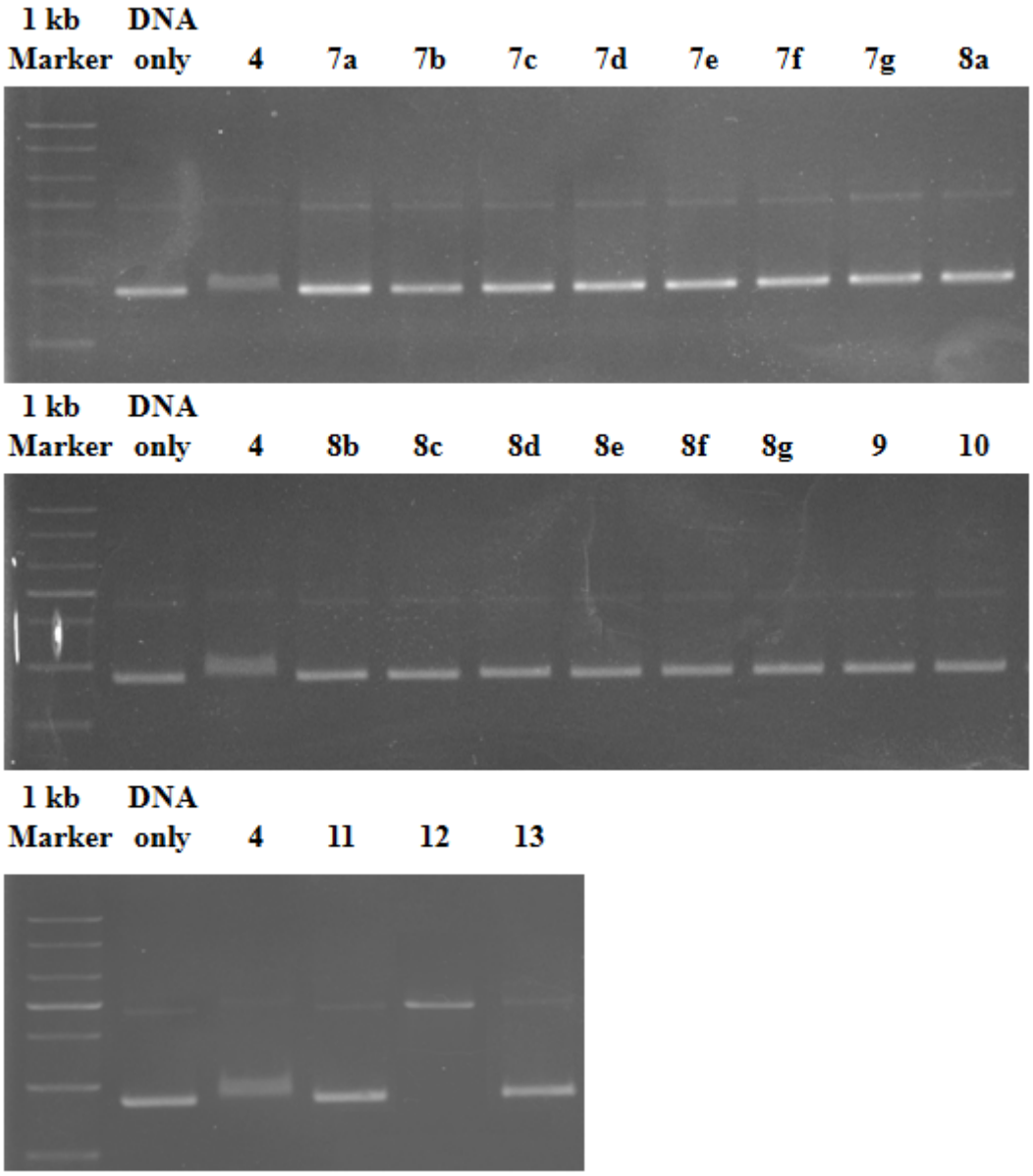
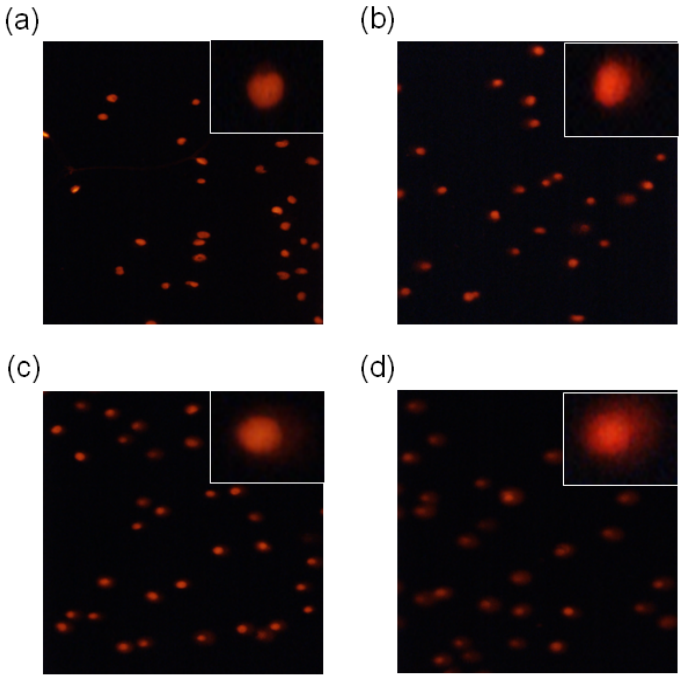
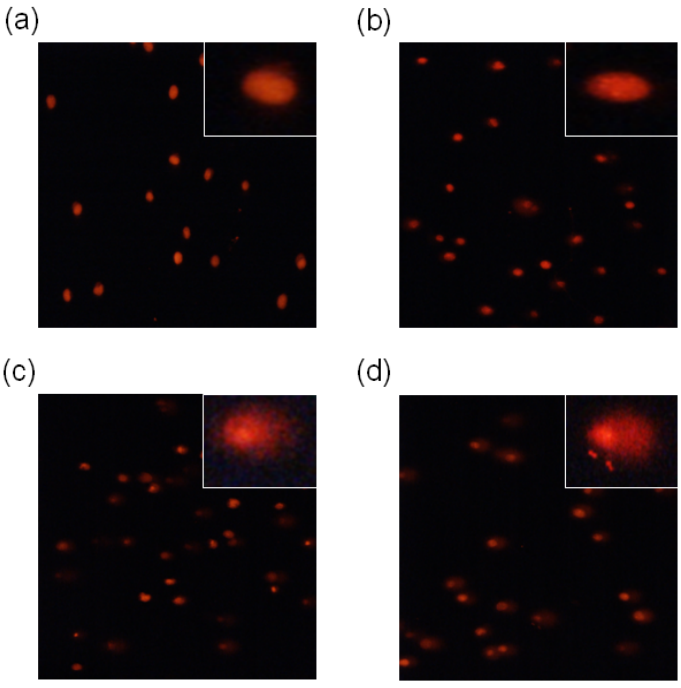


Figure 2.

(A)



(B)



(C)

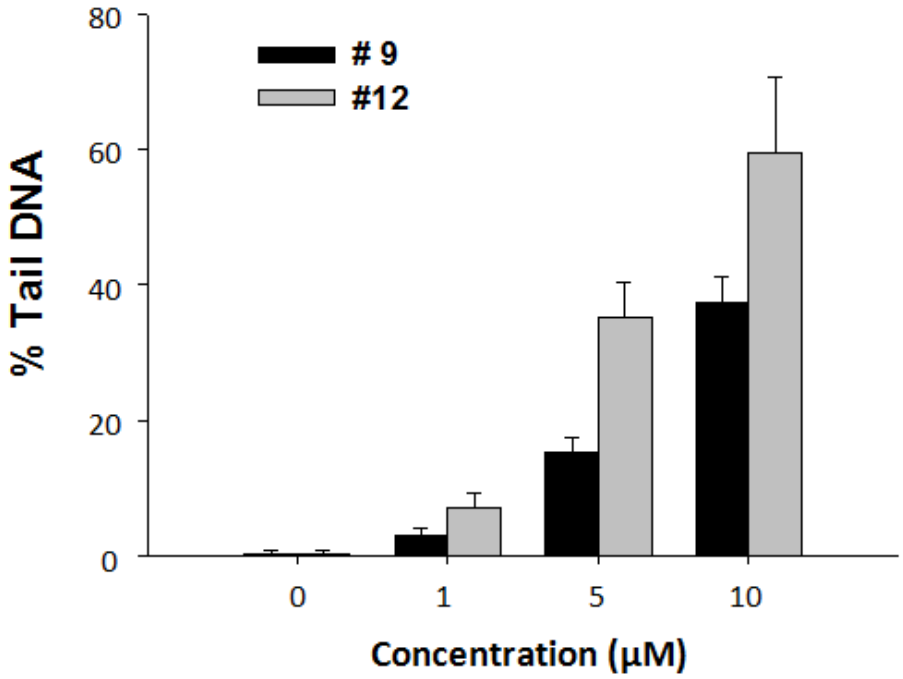
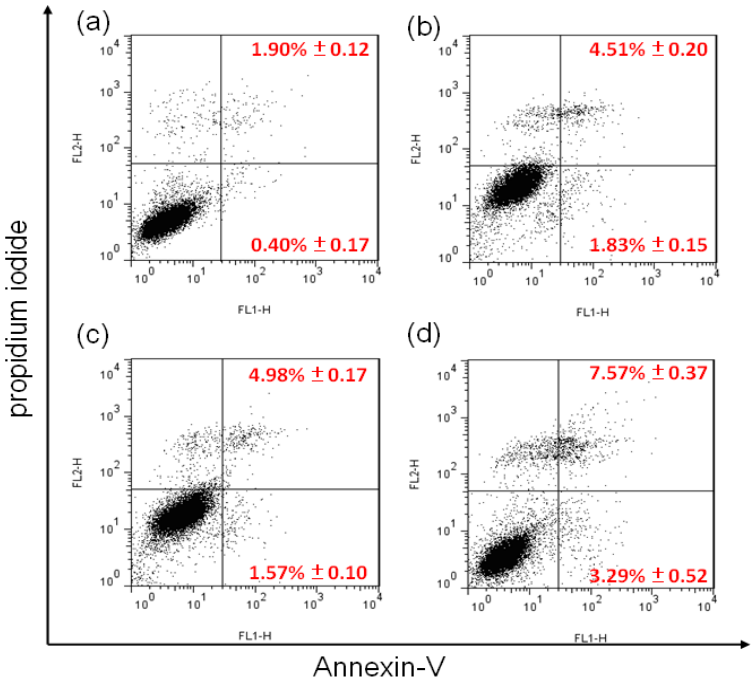


Figure 3.

(A)



(B)

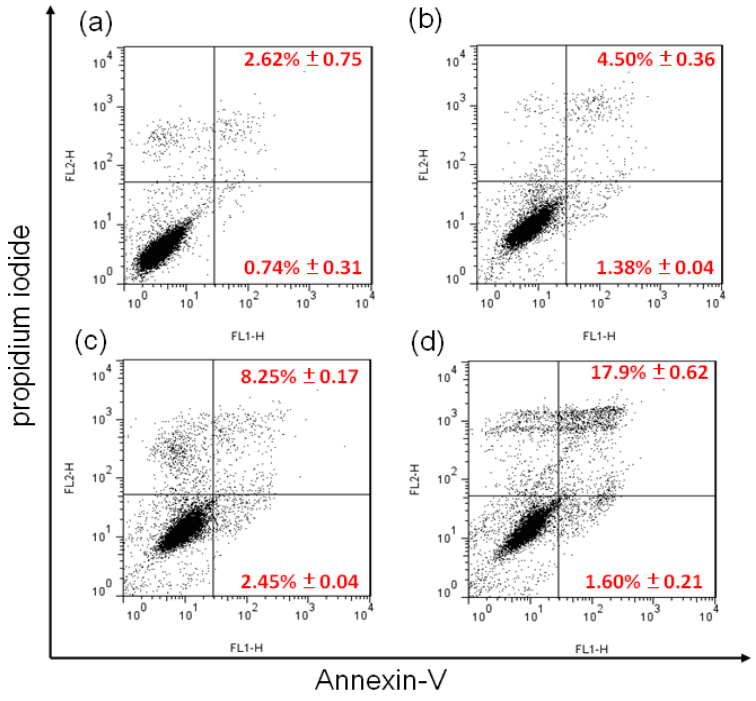


Figure 4.

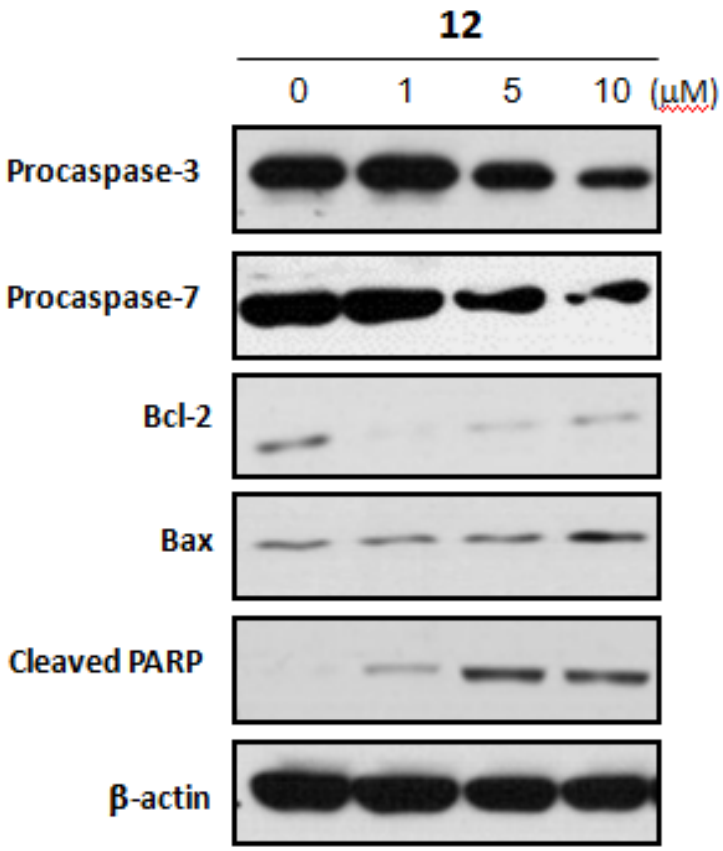


Figure 5.

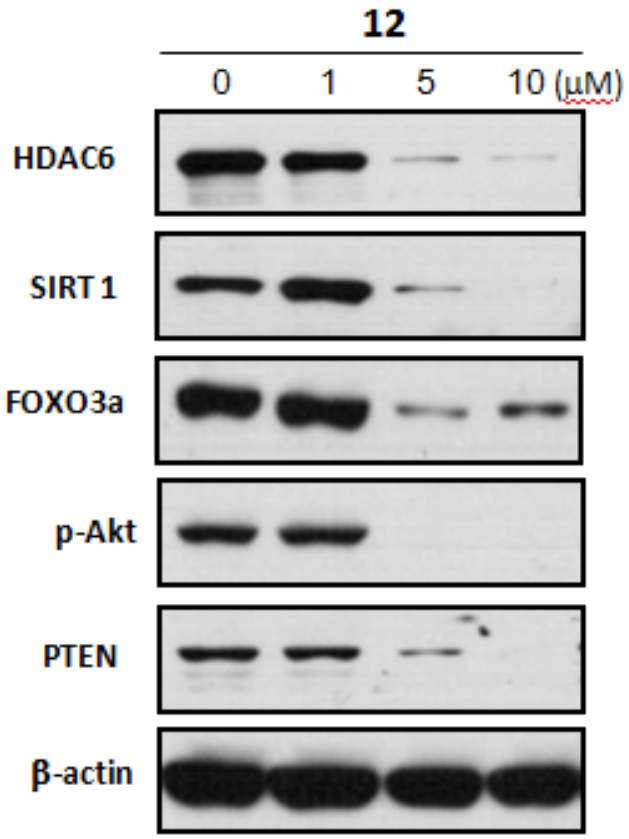


Figure 6.

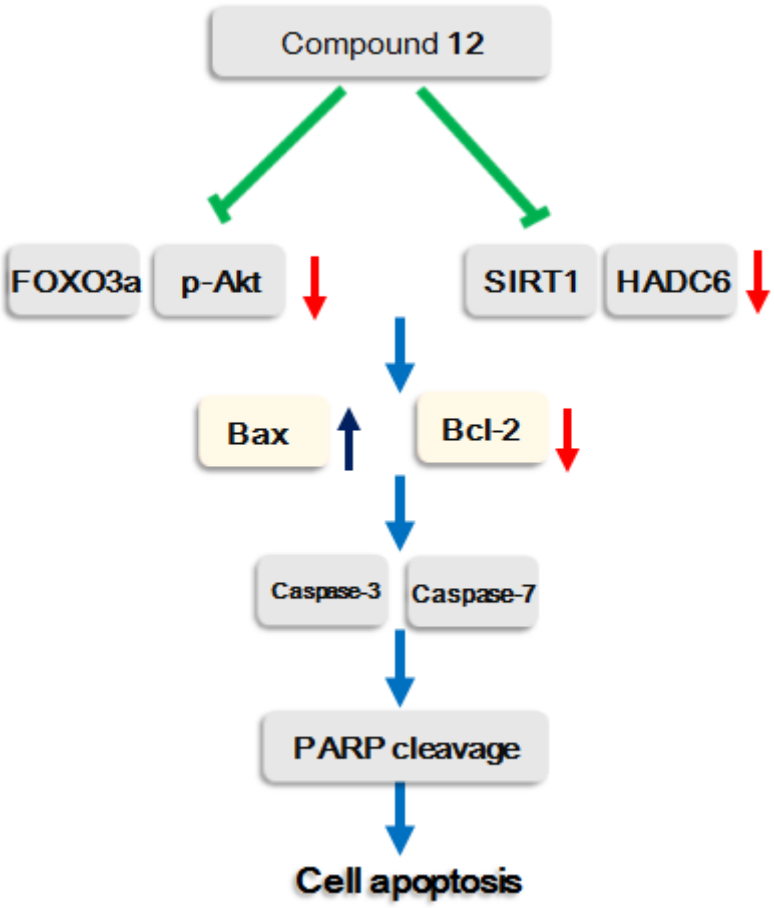
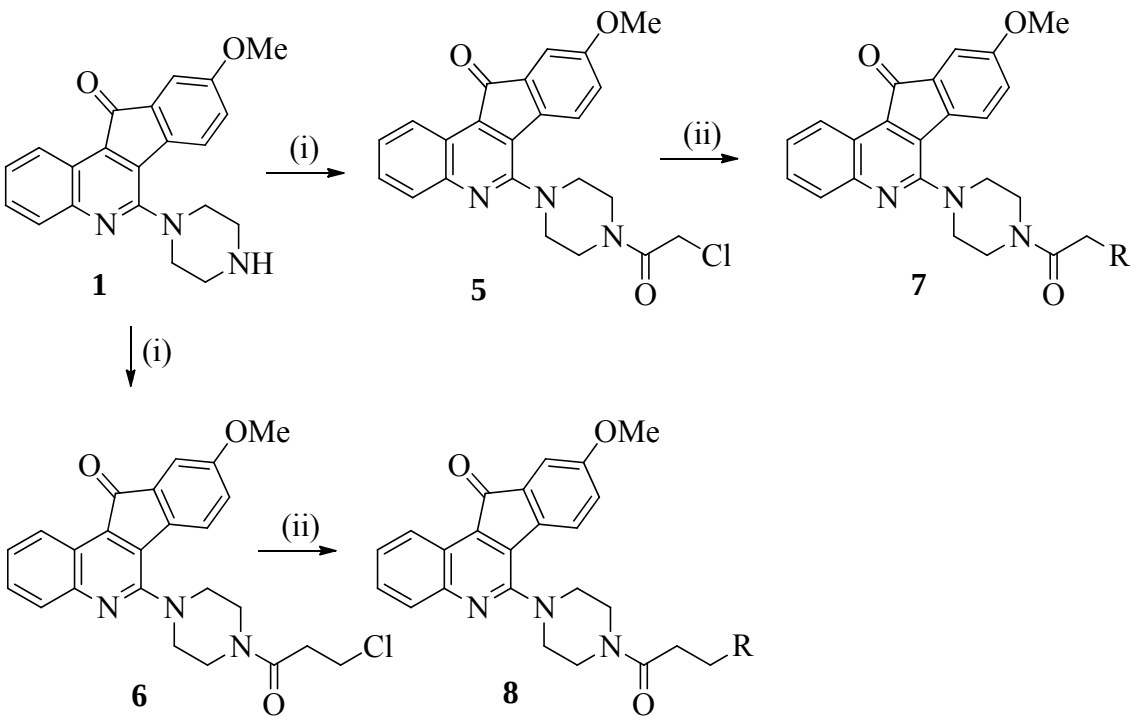
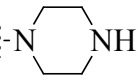
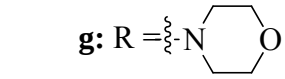


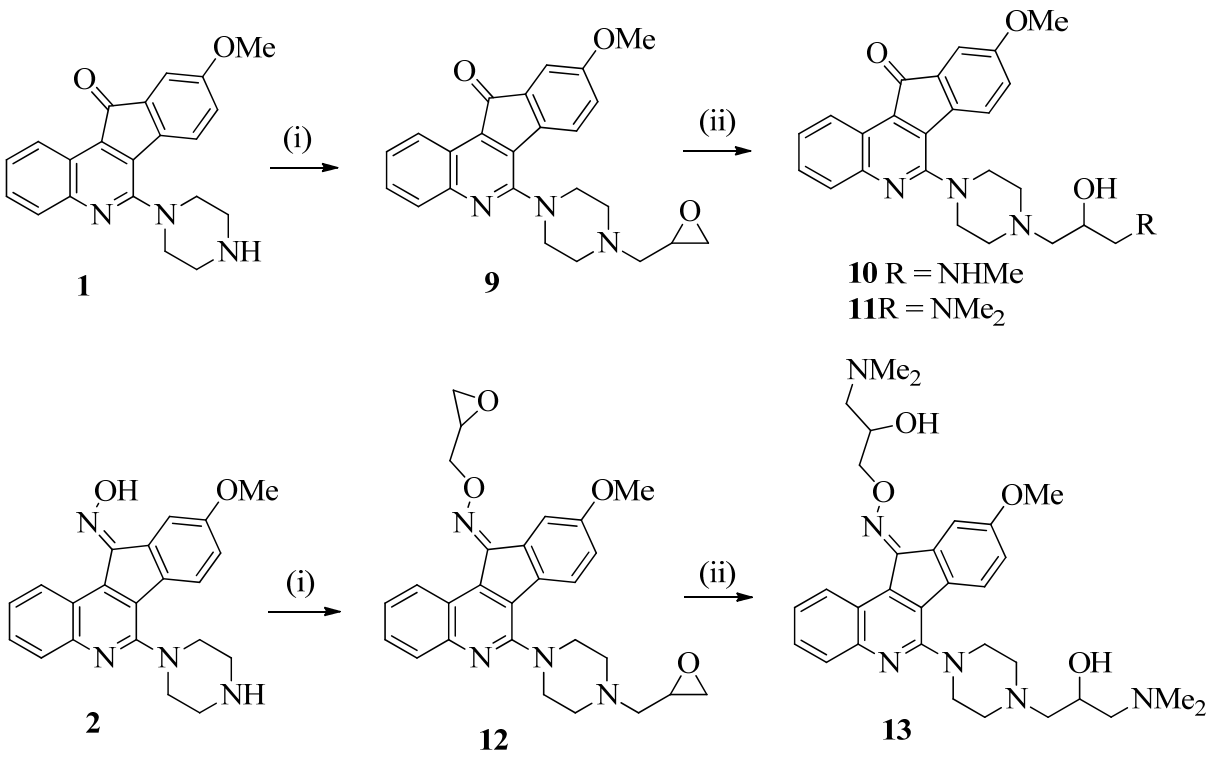
Figure 7.



a: R = NHMe **b:** R = NMe_2 **c:** R = $NH(CH_2)_2OH$ **d:** R = $NH(CH)_2NMe_2$

e: R = $NH(CH_2)_3NMe_2$ **f:** R =  **g:** R = 

Scheme 1: reagents and conditions: (i) chloroacyl chloride, Et_3N, rt, 1 h; (ii) alkyl amine, K_2CO_3, reflux, 30 min.



Scheme 2: reagents and conditions: (i) epichlorohydrin, K_2CO_3, reflux, 4h; (ii) NH_2Me or $NHMe_2$, reflux, 4h.

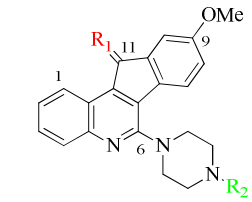
Synthesis and Antiproliferative Evaluation of 9-Methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinoline-11-one Derivatives. Part 4.

Chih-Hua Tseng, Cherng-Chyi Tzeng, Chien-Chih Chiu, Chiao-Li Yang, Pei-Jung Lu[d] Chon-Kit Chou, Chun-Yen Liu, [e]and Yeh-Long Chen *

Text:

A number of 6,11-disubstituted indenoquinolines were synthesized and evaluated for their antiproliferative activities and mechanism studies.

Colour graphic:



6,11-disubstituted-indenoquinolines