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Design, synthesis and docking study of 5-amino substituted indeno[1,2-*c*]isoquinolines as novel topoisomerase I inhibitors

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ABSTRACT

Various 5-amino group-substituted indeno[1,2-*c*]isoquinolines **7a–f** were synthesized based on the previous QSAR study as rigid structures of 3-arylisoquinolines. Amino group-substituted compounds, especially 5-piperazinyl indeno[1,2-*c*]isoquinoline **7f**, displayed potent topoisomerase I inhibitory activity as well as cytotoxicities against five different tumor cell lines. A Surflex-Dock docking model of **7f** was also studied.

1. Introduction

Camptothecin derivatives, which were approved as anticancer drugs, have been investigated over the last decade as selective DNA topoisomerase I (topo I) inhibitors.[1–3] However, several limitations,[4] such as their chemically unstable structure and rapid efflux from the cell by membrane pumps, prompted us to search for new topo I inhibitors and we have therefore studied 3-arylisoquinolines as topo I inhibitors.[5–8]

In 1999, we synthesized 6,11-dimethyl-6,11-dihydro-5*H*-indeno[1,2-*c*]isoquinolin-5-one via radical cyclization of a 3-arylisoquinoline analog.[9] Surprisingly, indenoisoquinoline **1** (Fig. 1) showed potent cytotoxicities against tumor cell lines (less than 0.001 mg/mL against SK-MEL-2 cells). With this compound as a seed, we designed and synthesized more than 40 indeno[1,2-*c*]isoquinoline analogs and found some of them were potent topo I poisons and had strong cytotoxicities against various tumor cell lines.[8] Through a docking study of these indenoisoquinoline derivatives in the topo I–DNA ternary complex, we realized the flatness of the compounds favored their function as DNA intercalators. Moreover, a carbonyl group at the C-11 position also contributed to strong interaction between the ligand and the topo I–DNA complex through hydrogen bonding with Arg 364 of topo I.[7] Among the synthesized analogs, ethylenediamino-substituted compound **3** exhibited strong cytotoxicities and showed a potent topo I poison.

Cushman's group has also investigated indeno[1,2-*c*]isoquinoline derivatives[10–13] and identified an indeno[1,2-*c*]isoquinoline analog, NSC 314622, as a clinical drug that could serve as a novel compound to overcome some of the limitations of camptothecin-like drugs.[14]

So far, no structural modification of indeno[1,2-*c*]isoquinoline at the C-5 position has been reported except our preparation of compound **3**. In this paper, we introduced various amino groups at the C-5 position because the docking model indicated that the amino group could hydrogen bond with topo I–DNA ternary complex via the carbonyl group.

2. Chemistry

3-Chloroindenoisoquinoline **6**[8] was treated with various amines such as *N*-methylpiperazine, morpholine, *N*-ethylpiperazine, *N*-methyldiazepane, *n*-butylamine and piperazine in the presence of K_2CO_3 in DMF to afford the corresponding amines **7** in good yields. Reaction of compounds **7a–d** with BH_3 gave the corresponding reduced amines **8a–d** in moderate yields. Compounds **7a–c** were reacted with $NaBH_4$ in MeOH to give the alcohols **10a–c**. Alcohol **10b** was acetylated with Ac_2O and 4-dimethylaminopyridine in methylene chloride to afford the acetylated **11** in 87% yield. Compound **7a** was treated with MeMgBr or *n*-hexylMgBr to afford

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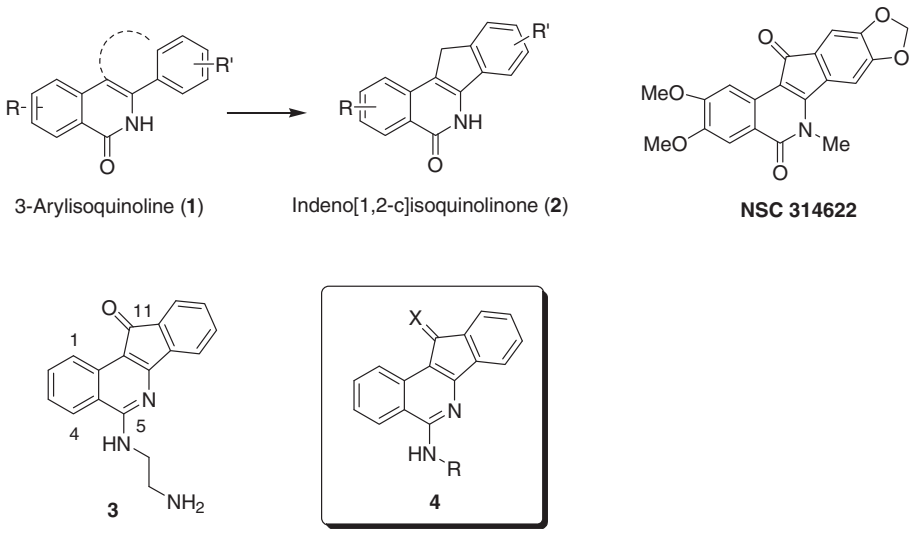
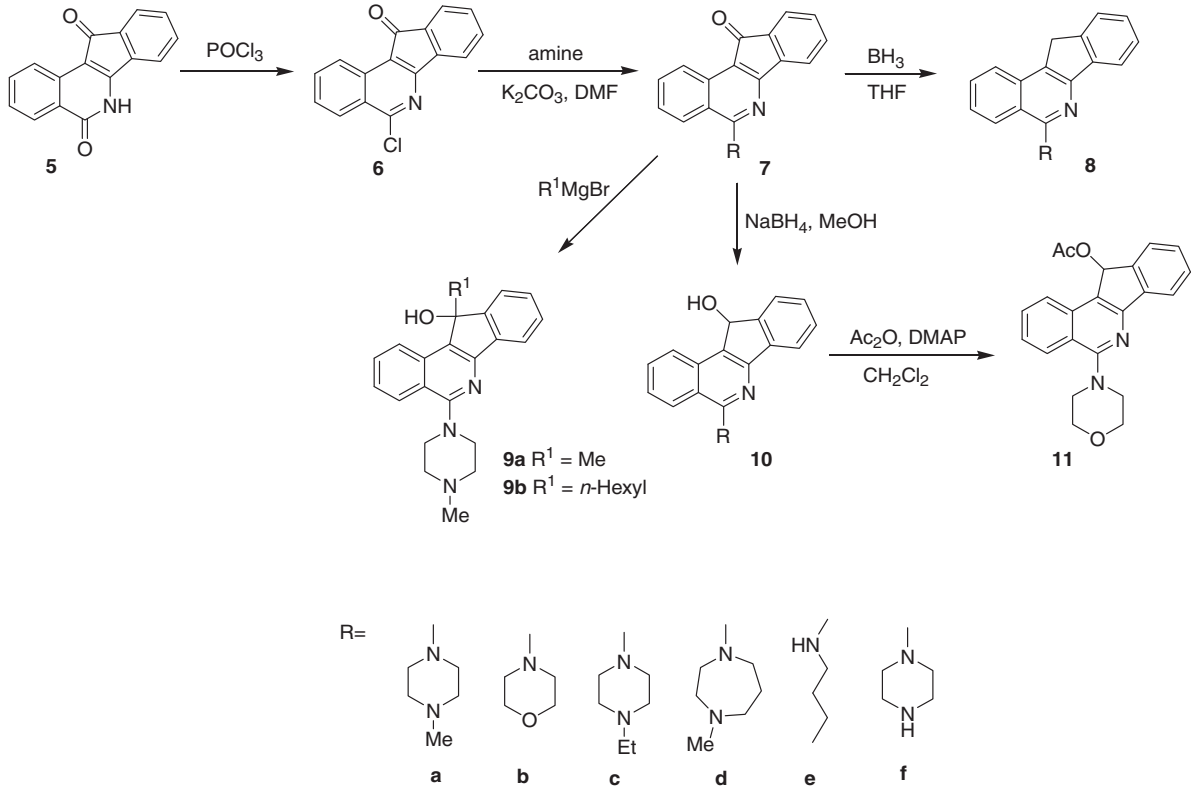


Figure 1. Structures of NSC 314622 and indeno[1,2-*c*]isoquinolines.



Scheme 1. The synthesis of indeno[1,2-*c*]isoquinolines.

the corresponding alcohols **9a** or **9b** in 76% and 65% yield, respectively, as shown in Scheme 1.

3. Results and discussion

3.1. Biological evaluation

The in vitro cytotoxicity experiments with the synthesized compounds were performed by sulforhodamine B (SRB) for A549 (lung), Col2 (colon), SNU-638 (stomach), HT1080 (fibro sarcoma) cell lines and MTT assay for HL-60 (myeloid leukemic) cells.[15]

To determine topo I catalytic activity, assays were carried out with supercoiled pBR322 DNA as the substrate according to protocol. 500 ng of supercoiled pBR322 DNA was incubated with 1 unit topo I in the absence or presence of camptothecin or the synthesized compounds for 30 min at 37 °C. The reaction mixtures were analyzed on 1% agarose gel with ethidium bromide staining.[16]

Semi-quantitative comparisons of the inhibitory activities are shown in Table 1. The IC_{50} cytotoxicity values obtained with cell

lines and the relative potencies of the compounds are expressed semi-quantitatively as follows: +, weak activity; +++, lower activity than 0.1 μM camptothecin; ++++, similar or greater activity than 0.1 μM camptothecin.

As expected, indenoisoquinolines **7a**, **7c**, **7d** and **7f** exhibited more potent topo I inhibitory activities than **8a–e** because the C11 carbonyl group could have hydrogen bond with Arg 364 of topo I as shown in Figure 2. This result is consistent with our previous reports.[8] Overall, the C5 amino groups did not influence the biological activities. In case of morpholine substituted compounds, **7b** and **10b** showed poor cytotoxicities. On the other hand, **9b** exhibited high cytotoxicity (2.57–12.36 μM) as well as strong topo I poison as shown in Table 1. *N*-Methypiperazine substituted compounds such as **7a**, **8a** and **9a** did not show any correlation between C5 amino group and biological activities. However, topo I potencies were highly affected by C11 carbonyl group of the compounds. Compound **7d** showed cytotoxicities ranging from 0.27 to 1.36 μM against the tumor cells and the compounds **8a–e** with reduced carbonyl group also displayed potent cytotoxicities. In particular, compound **8d** exhibited 0.25–8.24 μM potencies against five different cell lines. This discrepancy indicates that topo I may not be the sole biological target for these compounds. Similar results were found in other reports and were explained by the ability of the compounds to penetrate the cell membrane and reach the target, as well as by distribution differences within the cell. The most potent topo I inhibitor, compound **7f**, also exhibited relatively strong cytotoxicities, ranging from 2.01 to 8.55 μM against tumor cells. 11-Hydroxy analogs **10a–b** exhibited weak or moderate cytotoxicities against the tumor cell lines and displayed poor topo I poison. These results were not unexpected due to the fact that the hydroxyl group does not work well as a hydrogen bonding donor with Arg 364 of topo I. On the other hand, compound **9b** showed relatively strong (2.57–12.36 μM) cytotoxicities and topo I activity. 11-Acetoxy compound **11** exhibited weak cytotoxicity (6.99–58.32 μM) or even worse activity than **9b**. Furthermore, this compound did not have any topo I–DNA inhibitory activity.

Table 1
IC_{50} cytotoxicity (μM and topo I activity of the compounds

No.	Compound	A549	Col2	SNU-638	HL-60	HT1080	Topo I [a]
1	**7a**	8.19	7.77	7.98	6.35	5.36	+++
2	**7b**	>100	96.88	>100	>100	69.25	–
3	**7c**	7.10	6.45	5.85	4.21	1.23	+++
4	**7d**	1.05	0.27	1.36	1.28	0.54	+++
5	**7e**	>100	>100	>100	>100	48.24	–
6	**7f**	8.52	7.99	8.25	4.68	2.01	+++++
7	**8a**	11.36	10.12	19.75	5.87	3.85	–
8	**8b**	5.69	4.42	2.58	8.65	1.24	–
9	**8c**	8.19	7.74	3.25	9.98	18.98	–
10	**8d**	5.46	2.13	8.24	3.33	0.25	–
11	**8e**	27.69	75.21	22.21	19.57	4.39	–
12	**9a**	92.49	57.98	58.24	79.87	22.22	–
13	**9b**	7.21	6.24	12.36	8.55	2.57	+++
14	**10a**	18.12	28.26	9.01	13.36	4.87	–
15	**10b**	93.33	>100	>100	99.25	>100	–
16	**10c**	20.94	19.25	18.88	29.37	8.66	–
17	**11**	8.30	7.87	6.99	15.88	58.32	–
18	**3**	0.85	1.43	1.68	10.40	9.60	+++++
19	CPT	0.069	0.045	0.098	0.018	0.080	+++
20	Ellipticine	1.9	2.3	2.2	5.8	4.3	nt[b]

[a] Activity is expressed semi-quantitatively as follows: – very weak activity, + weak activity, +++similar activity to camptothecin, +++++ stronger activity than camptothecin.
[b] nt means not tested.

3.2. Docking study

In order to determine the binding mode of action of the most potent topo I inhibitor **7f**, we performed a docking study. The resulting docking model revealed a different binding mode than the former indeno[1,2-*c*]isoquinoline model. In this model, the isoquinoline ring intercalated between the −1 and +1 bases, parallel to the plane of the base pairs, and the carbonyl group of C-11

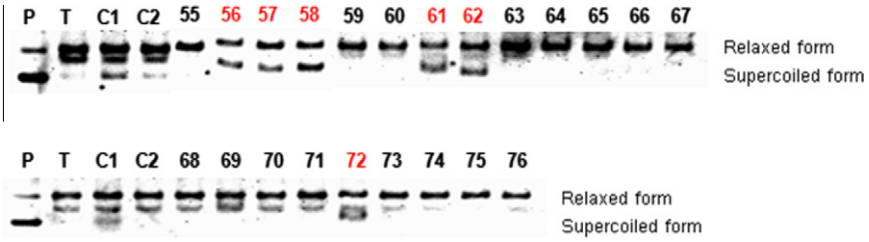


Figure 2. Topo I inhibitory activities of the compounds. Lane P: pBR322; lane T: pBR322 + topo I; lane C: pBR322 + topo I + camptothecin (0.01 mg/mL); lane 55–76 (prepared compound number, 0.1 mg/mL): 55 (**11**), 56 (**7d**), 57 (**7c**), 58 (**7f**), 59 (**7e**), 60 (**7d**), 61 (**7a**), 62 (**9b**), 63 (**9a**), 64 (**10a**), 65 (**8e**), 69 (**8a**), 70 (**10b**), 71 (**8b**), 72 (**8d**), 73 (8c), 75 (**10a**), 76 (**10c**).

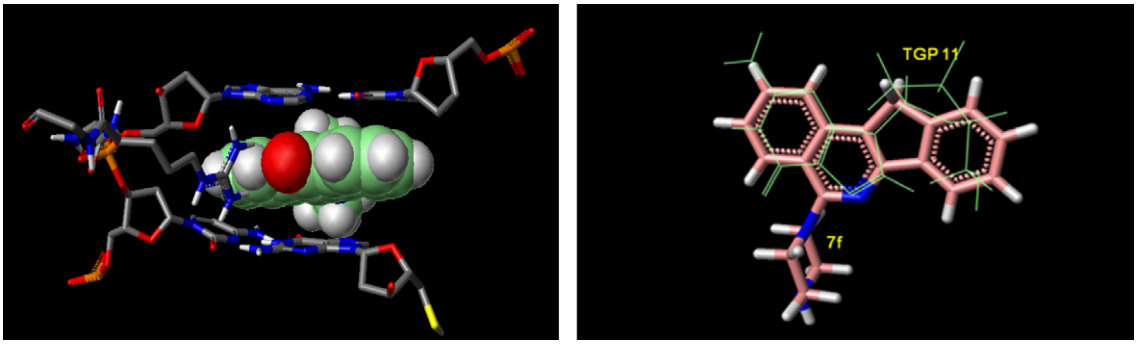


Figure 3. Space filling (left) and top view (right) models of compound **7f**.

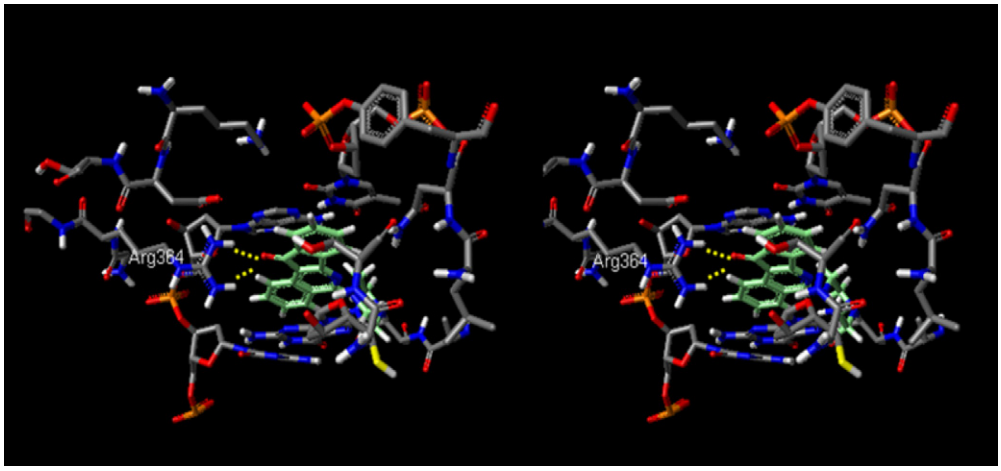


Figure 4. Wall-eyed viewing docked model of compound **7f**.

position had a H-bond to Asp 533, which is considered an important amino acid that interacts with the ligand in the DNA-topo I active site. In this model, the indenoisoquinoline ring functioned as a DNA intercalator as a blocker of the rejoining process of the phosphoester. In the previous study, the binding geometry of camptothecin in the DNA–topo I complex was studied with an ab initio quantum mechanics calculation and indicated that the π–π stacking interactions contributed much more than the hydrogen bonding effect of the ligand.[17] From this molecular docking study, the importance of the DNA intercalation of **7f** and the hydrogen bond was clarified through the molecular docking study. Space filling model of **7f** and the stereoview picture are shown in Figures 3 and 4.

4. Conclusion

In conclusion, based on the previous QSAR investigation of the indeno[1,2-*c*]isoquinolines, we designed and synthesized various amino group-substituted analogs at the C-5 position. The newly synthesized indeno[1,2-*c*]isoquinolines were also structurally modified. As expected, molecules containing the 11-carbonyl group exhibited potent topo I inhibitory activities with relatively strong cytotoxicities against five different tumor cell lines. On the other hand, 11-hydrogenated compounds showed no topo I poison even though they exhibited strong cytotoxicities. Alkyl group introduction at the C11 position did not enhance the biological activities, except for the compound containing the *n*-hexyl group, which showed moderate topo I activity. For an explanation of the topo I activity of **7f**, molecular docking studies were performed with the Surflex-Dock program to provide the reasonable binding mode of the compound into the binding sites of the DNA–topo I complex.

In further studies of the other constrained structures of 3-arylisoquinolines, diverse structural modifications such as 4-arylation or heterocyclization of 3-arylisoquinolines are currently being carried out and these will be reported in due course.

5. Experimental section

5.1. Chemistry

Melting points were determined by the capillary method on Electrothermal IA9200 digital melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) data for ^{1}H NMR were collected on Varian 300 FT spectrometer of Korea Basic Science Institute and were reported in ppm, downfield from the peak of the internal standard, tetramethylsilane. The data are reported as follows: chemical shift, number of protons, multiplicity (s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, b: broadened). IR spectra were recorded on JASCO-FT IR spectrometer using $CHCl_3$ or KBr pellets. Mass spectra were obtained on JEOL JNS-DX 303 using the electron-impact (EI) method. Column chromatography was performed on Merck silica gel 60 (70–230 mesh). TLC was performed using plates coated with silica gel 60 F254 that were purchased from Merck.

5.1.1. 5-(4-Methylpiperazin-1-yl)indeno[1,2-*c*]isoquinolin-11-one (7a)

The mixture of compound **6** (100 mg, 0.38 mmol), *N*-methylpiperazine (57 mg, 0.56 mmol) and K_2CO_3 (156 mg, 1.13 mmol) in 10 mL of DMF was refluxed for 2 h. After the reaction was completed, the reaction mixture was diluted with 40 mL of water, and the aqueous phase was extracted with diethyl ether. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent in vacuo gave the residue which was purified by column chromatography on silica gel with CH_2Cl_2/methanol (10:1) to afford the desired amine **7a** (80 mg, 64%) as a yellow solid. mp: 176–177 °C. IR cm^{-1}: 1678. ^{1}H NMR ($CDCl_3$): δ 8.68–8.65 (m, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.62–7.28 (m, 6H), 3.83 (t, J = 4.8 Hz, 4H), 2.66 (t, J = 4.9 Hz, 4H), 2.40 (s, 3H). EIMS: *m/z* 329 (M^+, 100).

5.1.2. 5-Morpholin-4-yl-indeno[1,2-*c*]isoquinolin-11-one (7b)

The procedure described for the preparation of **7a** gave **7b** (92%) as a yellow solid. mp: 204–206 °C. ^{1}H NMR ($CDCl_3$): δ 8.67–8.64 (m, 1H), 7.90–7.30 (m, 7H), 3.95 (t, J = 4.6 Hz, 4H), 3.80 (t, J = 4.7 Hz, 4H). EIMS: *m/z* 316 (M^+, 86).

5.1.3. 5-(4-Ethylpiperazin-1-yl)indeno[1,2-*c*]isoquinolin-11-one (7c)

The procedure described for the synthesis of **7a** gave **7c** (93%) as a yellow solid. mp: 134–135 °C. ^{1}H NMR ($CDCl_3$): δ 8.67–8.64 (m, 1H), 7.93–7.90 (m, 1H), 7.66–7.32 (m, 6H), 3.88 (t, J = 4.9 Hz, 4H), 2.71 (t, J = 4.9 Hz, 4H), 2.54 (m, 2H), 1.16 (t, J = 7.2 Hz, 3H). EIMS: *m/z* 343 (M^+, 54).

5.1.4. 5-(4-Methyl[1,4]diazepan-1-yl)indeno[1,2-*c*]isoquinolin-11-one (7d)

The procedure described for the preparation of **7a** provided **7d** (83%) as a yellow solid. mp: 107–108 °C.^{1}H NMR ($CDCl_3$): δ 8.66–

8.63 (m, 1H), 7.96–7.93 (m, 1H), 7.60–7.30 (m, 6H), 4.17–4.14 (m, 2H), 4.11–4.07 (m, 2H), 2.94–2.91 (m, 4H), 2.67–2.64 (m, 2H), 2.41 (s, 3H). EIMS: *m/z* 343 (M^+, 100).

5.1.5. 5-Butylaminoindeno[1,2-*c*]isoquinolin-11-one (7e)

The procedure described for the preparation of **7a** afforded **7e** (98%) as a yellow solid. mp: 215–218 °C. ^{1}H NMR ($CDCl_3$): δ 8.72–8.69 (m, 1H), 7.68–7.26 (m, 7H), 5.99 (s, 1H), 3.88–3.82 (m, 2H), 1.83–1.73 (m, 2H), 1.58–1.48 (m, 2H), 1.03 (t, J = 7.3 Hz, 3H). EIMS: *m/z* 302 (M^+, 55).

5.1.6. 5-Piperazin-1-ylindeno[1,2-*c*]isoquinolin-11-one (7f)

The procedure described for the preparation of **7a** gave **7f** (73%) as a yellow solid. mp: 188–189 °C. ^{1}H NMR ($CDCl_3$): δ 8.73–8.70 (m, 1H), 7.89–7.28 (m, 7H), 3.80 (t, J = 4.9 Hz, 4H), 3.14 (t, J = 4.9 Hz, 4H), 1.98 (s, 1H). EIMS: *m/z* 315 (M^+, 66).

5.1.7. 5-(4-Methylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]isoquinolin (8a)

A reaction mixture of compound **7a** (475 mg, 1.79 mmol) and 1 M borane–tetrahydrofuran complex (4 mL) in dry THF (10 mL) was refluxed for 6 h. After cooling to room temperature, the mixture was concentrated in vacuo and the residue was diluted with EtOAc (100 mL). Glacial acetic acid was added dropwise to the reaction mixture until pH 5. The organic layer was washed with saturated sodium bicarbonate (100 mL) and brine, dried over Na_2SO_4 and concentrated to give the residue which was purified by short silica gel column with CH_2Cl_2/methanol (15:1) to yield the dehydrogenated compound **8a** (315 mg, 56%) as a yellow solid. mp: 178–180 °C. ^{1}H NMR ($CDCl_3$): δ 8.01 (d, J = 8.4 Hz, 1H), 8.02–7.99 (m, 1H), 7.91 (d, J = 8.3 Hz, 1H), 7.68–7.34 (m, 5H), 4.03 (s, 2H), 3.86–3.83 (m, 2H), 3.68–3.65 (m, 2H), 3.36–3.33 (m, 2H), 3.12–3.05 (m, 2H), 2.77 (s, 3H). EIMS: *m/z* 315 (M^+, 92).

5.1.8. 5-Morpholin-4-yl-11*H*-indeno[1,2-*c*]isoquinolin (8b)

The procedure described for the preparation of **8a** yielded **8b** (91%) as a yellow solid. mp: 159–160 °C. ^{1}H NMR ($CDCl_3$): δ 8.17–8.14 (m, 1H), 8.03–8.00 (m, 1H), 7.87–7.84 (m, 1H), 7.64–7.31 (m, 5H), 4.04–3.99 (m, 4H), 3.97 (s, 2H), 3.52–3.48 (m, 4H). EIMS: *m/z* 302 (M^+, 100).

5.1.9. 5-(4-Ethylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]isoquinolin (8c)

The procedure described for the preparation of **8a** gave **8c** (69%) as a yellow solid. mp: 176–179 °C. ^{1}H NMR ($CDCl_3$): δ 8.11–8.08 (m, 1H), 8.02–7.99 (m, 1H), 7.92–7.89 (m, 1H), 7.68–7.34 (m, 5H), 4.02 (s, 2H), 3.91–3.87 (m, 2H), 3.69–3.67 (m, 2H), 3.29–3.27 (m, 2H), 3.14–3.09 (m, 2H), 3.07–3.00 (m, 2H), 1.36 (t, J = 7.3 Hz, 3H). EIMS: *m/z* 329 (M^+, 87).

5.1.10. 5-(4-Methyl-[1,4]diazepan-1-yl)-11*H*-indeno[1,2-*c*]isoquinolin (8d)

The procedure described for the preparation of **8a** gave **8d** (82%) as a yellow solid. mp: 147–149 °C. ^{1}H NMR ($CDCl_3$): δ 8.07 (d, J = 8.5 Hz, 1H), 7.99–7.96 (m, 1H), 7.90–7.87 (m, 1H), 7.66–7.35 (m, 5H), 4.00 (s, 2H), 3.99–3.92 (m, 2H), 3.81–3.76 (m, 2H), 3.50–3.35 (m, 3H), 3.15–3.07 (m, 1H), 3.12 (s, 3H), 2.34–2.32 (m, 1H), 2.21–2.13 (m, 1H). EIMS: *m/z* 329 (M^+, 54).

5.1.11. *n*-Butyl(11*H*-indeno[1,2-*c*]isoquinolin-5-yl)amine(8e)

The procedure described for the preparation of **8a** gave **8e** (55%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.00 (d, J = 7.4 Hz, 1H), 7.81–7.77 (m, 2H), 7.62–7.55 (m, 2H), 7.42–7.31 (m, 3H), 5.27 (s, 1H), 3.93 (s, 2H), 3.78 (q, J = 6.5 Hz, 2H), 1.77 (m, 2H), 1.53 (m, 2H), 1.03 (t, J = 7.3 Hz, 3H). EIMS: *m/z* 288 (M^+, 100).

5.1.12. 11-Methyl-5-(4-methylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]isoquinolin-11-ol (9a)

To a stirred solution of **7a** (100 mg, 0.27 mmol) in THF (10 mL), 1 M CH_3MgBr in *n*-butyl ether (0.6 mL, 0.54 mmol) was added at 0 °C and the reaction mixture was continuously stirred while the temperature increased to room temperature. After 12 h, the reaction was quenched with water and the mixture was filtered through celite. The filtrate was washed with CH_2Cl_2 and brine, dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (2:1) to yield **9a** (82 mg, 79%) as a yellow solid. mp: 201–202 °C. IR cm^{-1}: 3421. ^{1}H NMR ($CDCl_3$): δ 8.28 (d, J = 8.0 Hz, 1H), 7.96–7.35 (m, 7H), 3.13 (m, 4H), 2.46 (m, 4H), 2.21 (s, 3H), 1.87 (s, 3H). EIMS: *m/z* 345 (M^+, 85).

5.1.13. 11-Hexyl-5-(4-methylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]isoquinolin-11-ol (9b)

The procedure described for the preparation of **9a** gave **9b** (70%) as a yellow solid. mp: 83–84 °C. ^{1}H NMR ($CDCl_3$): δ 8.27 (d, J = 8.1 Hz, 1H), 8.00–7.27 (m, 7H), 4.50 (s, 1H), 3.22 (s, 4H), 2.53 (s, 4H), 2.48–2.30 (m, 2H), 2.24 (s, 3H), 1.08–0.96 (m, 6H), 0.71 (t, J = 6.9 Hz, 3H), 0.66–0.61 (m, 2H). EIMS: *m/z* 415 (M^+, 100).

5.1.145-(4-Methylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]isoquinolin-11-ol (10a)

To a stirred solution of **7a** (100 mg, 0.27 mmol) in 10 mL MeOH was added $NaBH_4$ (31 mg, 0.82 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 4 h. The reaction was quenched with water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (2:1) to yield the alcohol **10a** (68 mg, 77%) as a white solid. mp: 195–197 °C. ^{1}H NMR ($CDCl_3$): δ 8.21–8.18 (m, 1H), 7.91 (t, J = 8.2 Hz, 2H), 7.68–7.60 (m, 2H), 7.45–7.27 (m, 3H), 5.75 (s, 1H), 3.16 (m, 4H), 2.53–2.38 (m, 4H), 2.31 (s, 3H). EIMS: *m/z* 331 (M^+, 78).

5.1.15. 5-Morpholin-4-yl-11*H*-indeno[1,2-*c*]isoquinolin-11-ol (10b)

The procedure described for the preparation of **10a** afforded **10b** (78%) as a white solid. mp: 149–151 °C. IR cm^{-1}: 3382. ^{1}H NMR ($CDCl_3$): δ 8.23–7.34 (m, 8H), 5.82 (d, J = 5.8 Hz, 1H), 3.95 (t, J = 4.7 Hz, 4H), 3.50–3.45 (m, 4H). EIMS: *m/z* 318 (M^+, 67).

5.1.16. 5-(4-Ethylpiperazin-1-yl)-11*H*-indeno[1,2-*c*]isoquinolin-11-ol (10c)

The procedure described for the preparation of **9a** gave **9c** (87%) as a white solid. mp: 203–207 °C. ^{1}H NMR ($CDCl_3$): δ 8.22–8.19 (m, 1H), 7.99–7.35 (m, 7H), 5.79 (s, 1H), 3.32 (m, 2H), 3.21 (m, 2H), 2.68–2.62 (m, 4H), 2.52–2.45 (m, 2H), 1.59 (t, J = 7.2 Hz, 3H). EIMS: m/*z* 345 (M^+, 100).

5.1.17. Acetic acid 5-morpholin-4-yl-11*H*-indeno[1,2-*c*]isoquinolin-11-yl ester (11)

To a solution of **10b** (331 mg, 1.04 mmol) in 10 mL CH_2Cl_2, pyridine (107 mg, 1.35 mmol) and 4-dimethylaminopyridine (40 mg) was added anhydride acetic acid (127 mg, 1.25 mmol) at 0 °C under nitrogen. The reaction mixture was stirred overnight and then poured into water followed by extraction with ether and the ether layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography with *n*-hexane/ethyl acetate (6:1) on silica gel to afford **11** (278 mg, 74%) as a yellow solid. mp: 164–166 °C. IR cm^{-1}: 1726. ^{1}H NMR ($CDCl_3$): δ 8.14 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 7.4 Hz, 1H), 7.78–7.75 (m, 1H), 7.68–7.33 (m, 5H), 7.14 (s, 1H), 4.02–3.99 (m, 4H), 3.58–3.55 (m, 4H), 2.23 (s, 3H). EIMS: *m/z* 360 (M^+, 57).

5.2. Molecular modeling

The docking study was performed using Surflex-Dock in Sybyl version 8.1.1 by Tripos Associates, operating under Red Hat Linux 4.0 with an IBM computer (Intel Pentium 4, 2.8 GHz CPU, 1 GB memory). The structure of compound **7f** was sketched in the Sybyl package and minimized using the conjugate gradient method until the gradient was 0.001 kcal/mol with the Tripos force field with Gasteiger-Huckel charge. The 1SC7 (PDB code) structure in Protein Data Bank was taken and the structure was refined as follows. After the phosphoester bond of G12 in 1SC7 was produced, the SH of G11 on the scissile strand was modified to OH. With this DNA–topo I complex, Surflex-Dock was carried out with **7f**. After the protomol generation, which was positioned to scissile bond cavity between the −1 and +1 bases, Surflex-Dock was operated to afford 10 docked conformers. The conformer with the best total score (7.89) was chosen for speculation regarding the detailed binding patterns in the cavity.

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References and notes

1. Takagi, K.; Dexheimer, T. S.; Redon, C.; Sordet, O.; Agama, K.; Lavielle, G.; Pierre, A.; Bates, S. E.; Pommier, Y. *Mol. Cancer Ther.* **2007**, *6*, 3229.
2. Bjornsti, M. A.; Benedetti, P.; Viglianti, G. A.; Wang, J. C. *Cancer Res.* **1989**, *49*, 6318.
3. Hsiang, Y. H.; Liu, L. F. *Cancer Res.* **1988**, *48*, 1722.
4. Gounder, M. K.; Nazar, A. S.; Saleem, A.; Pungaliya, P.; Kulkarni, D.; Versace, R.; Rubin, E. H. *Invest. New Drugs* **2008**, *26*, 205.
5. Van, H. T. M.; Cho, W. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2551.
6. Lee, S. H.; Van, H. T. M.; Yang, S. H.; Lee, K. T.; Kwon, Y.; Cho, W. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2444.
7. Van, H. T. M.; Le, Q. M.; Lee, K. Y.; Lee, E. S.; Kwon, Y.; Kim, T. S.; Le, T. N.; Lee, S. H.; Cho, W. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5763.
8. Cho, W. J.; Le, Q. M.; Van, H. T. M.; Lee, K. Y.; Kang, B. Y.; Lee, E. S.; Lee, S. K.; Kwon, Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3531.
9. Cho, W. J.; Park, M. J.; Imanishi, T.; Chung, B. H. *Chem. Pharm. Bull.* **1999**, *47*, 900.
10. Song, Y.; Shao, Z.; Dexheimer, T. S.; Scher, E. S.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2010**, *53*, 1979.
11. Morrell, A.; Placzek, M. S.; Steffen, J. D.; Antony, S.; Agama, K.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2007**, *50*, 2040.
12. Morrell, A.; Placzek, M.; Parmley, S.; Grella, B.; Antony, S.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2007**, *50*, 4388.
13. Nagarajan, M.; Morrell, A.; Ioanoviciu, A.; Antony, S.; Kohlhagen, G.; Agama, K.; Hollingshead, M.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2006**, *49*, 6283.
14. Kohlhagen, G.; Paull, K. D.; Cushman, M.; Nagafuji, P.; Pommier, Y. *Mol. Pharmacol.* **1998**, *54*, 50.
15. Arnould, R.; Dubois, J.; Abikhalil, F.; Libert, A.; Ghanem, G.; Atassi, G.; Hanocq, M.; Lejeune, F. J. *Anticancer Res.* **1990**, *10*, 145.
16. Cho, W. J.; Min, S. Y.; Le, T. N.; Kim, T. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4451.
17. Xiao, X.; Cushman, M. *J. Am. Chem. Soc.* **2005**, *127*, 9960.