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Original article

Synthesis of 2,4-diaryl chromenopyridines and evaluation of their topoisomerase I and II inhibitory activity, cytotoxicity, and structure–activity relationship

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ABSTRACT

Designed and synthesized were a series of 5*H*-chromeno[4,3-*b*]pyridines with substitution at 2- and 4-positions with various 5- or 6-membered heteroaromatics as antitumor agents. They were evaluated for topoisomerase I and II inhibitory activities as well as cytotoxicities against several human cancer cell lines. Structure–activity relationship study showed that 2-furyl or 2-thienyl at 2- or 4-position of central pyridine is crucial in displaying topo I or II inhibitory activity and cytotoxicity.

1. Introduction

DNA is one of the most important pharmacological targets of many drugs. DNA topoisomerases are enzymes that transiently break one or two strands of DNA, which allow to solve various DNA topological problems generated during vital cellular processes [1,2]. Because of the crucial role of topoisomerases for the maintenance and replication of DNA during proliferation, topoisomerase inhibitors are among the most potent anticancer agents to date.

α-Terthiophene has been reported to possess various biological activities such as anti HIV-1 activity, protein kinase C (PKC) inhibitory activity, nematocidal, and phototoxic to the pathogenic yeast *Candida albicans* [3]. Terpyridine, bioisosteres of α-terthiophene, acts as metal-based pseudonuclease (photonuclease/chemical nuclease) [4].

Previously, our research group has reported that terpyridine derivatives showed strong topoisomerase I (topo I) and II (topo II) inhibitory activities as well as a strong cytotoxicity against several human cancer cell lines [5]. From the previous study it was found that number of aryl groups in terpyridine derivatives has important role in determining the antitumor cytotoxicity [5e]. Furthermore, it has been reported by our research group that conformationally constrained rigid analogs of 2,4,6-trisubstituted pyridine containing 5,6-dihydrobenzo[*h*]quinoline moiety showed considerable topo I and II inhibitory activities, and cytotoxicity against several human cancer cell lines [6]. Various compounds possessing chromeno[4,3-*b*]pyridine and chromene moiety were reported as estrogen receptor β-selective ligands [7], TNF-α inhibitors [8], and anti-inflammatory agents [9]. 2,3-Dimethyl-4-chromanol skeleton of natural *Callophyllum* coumarins which consist in 4-chromanone containing pyridine bioisosteres of our synthesized compound is responsible for anti HIV-1 activity [10].

In connection with the previous results, it would be very interesting to observe biological activities by introduction of conformationally constrained rigid molecule in terpyridine skeleton such as 2,4-diaryl chromenopyridines, which possess three fused rings and two non-fused rings. Generally, a drug to its receptor site is influenced by electronic or steric factors and these two functions are considered to be important in the bioactive conformation of

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a drug. Rigid structures are commonly considered to have little conformational entropy compared to flexible structures and can be more efficiently fitted into the active site of a receptor [11]. In here, the rigidification of the flexible ring flattened the molecules so that they could work as DNA intercalators in the topoisomerase I–DNA ternary complex [12]. In this study, twenty-three rigid analogs of 2,4-diaryl chromenopyridines were designed and synthesized as shown in Fig. 1 and Scheme 1. They were evaluated for topo I and II inhibitory activities, cytotoxicity, and studied for structure–activity relationships.

2. Results and discussion

2.1. Synthetic chemistry

Synthetic method for the preparation of 4-chromanone containing pyridine bioisosteres are summarized in Scheme 1. We prepared 23 final compounds of 4-chromanone containing pyridine bioisosteres. 4-Chromanone (**1**) was condensed with aryl aldehydes (**2a**–**g**) to prepare intermediates (**3a**–**g**) in the presence of EtOH and 5% aqueous NaOH with the yield of 46.1–90.5%. Five pyridinium iodide salts **4**–**8** were prepared by reacting aryl acetyls, iodine and pyridine in 72.5–96.2% yield. By treatment of intermediates (**3a**–**g**) with **4**–**8**, using modified Kröhnke synthesis [13], final compounds **9** (R^1 = **a**, **c**, **e**–**g**), **10** (R^1 = **a**–**g**), **11** (R^1 = **a**, **c**, **e**–**g**), **12** (R^1 = **a**–**c**, **e**, **f**), and **13** (R^1 = **d**) were synthesized with the yield of 29.4–53.5%. Structures of the prepared compounds are shown in Fig. 2, and physical properties of the prepared compounds are shown in Table 1.

2.2. Topo I and II inhibitory activities

The conversion of supercoiled plasmid DNA to relaxed DNA by topo I and II was examined in the presence of the prepared 2,4-diaryl chromenopyridines **14**–**36**. Camptothecin and etoposide, well-known topo I and II inhibitors, respectively, were used as positive controls. The effect of the prepared compounds on human DNA topo I was evaluated by the topo I relaxation assays. The reaction products were analyzed by electrophoretic mobility and developed in ethidium bromide in the presence of UV light.

As shown in Fig. 3, compounds **15**, **24**, **27**, and **28** exhibited significant topo I inhibitory activity at 100 μM concentration. Especially, compounds **15**, **24**, and **28** displayed the most significant topo I inhibitory activity as strong as a positive control, camptothecin (Table 2). The compounds (**15**, **24**, **27**, and **28**) having significant topo I inhibitory activity possess 2-furyl or 2-thienyl at 2-position or 4-position of central pyridine, which supports the idea that 2-furyl or 2-thienyl at 2- or 4-position on central pyridine play the crucial role for topo I or II inhibitory activity as reported previously [5b,d,g–j]. Compounds **14**, **15**, **26**, and **36** exhibited moderate topo II inhibitory activity at 100 μM concentration as shown in Fig. 4 and Table 2. In addition, compound **15** showed both considerable topo I and II inhibitory activities. The compounds (**14**, **15**, **26**, and **36**) having moderate topo II inhibitory activity also possess 2-furyl or 2-thienyl at 2-position or 4-position of central pyridine.

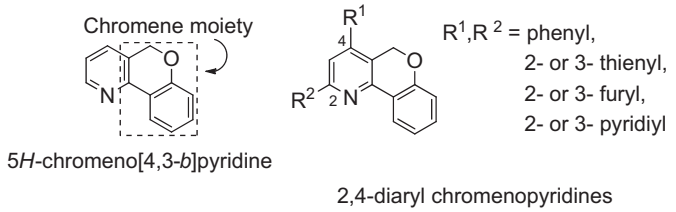


Fig. 1. Structure of 5H-chromeno[4,3-*b*]pyridine and 2,4-diaryl chromenopyridines.

2.3. Cytotoxicity

For the evaluation of cytotoxicity, 5 different human cancer cell lines were utilized: MDA-MB231 (human breast tumor cell line), HeLa (human cervix tumor cell line), DU145 (human prostate tumor cell line), HCT15 (human colorectal adenocarcinoma cell line), and HL60 (human myeloid leukemic tumor cell line). Cytotoxic evaluation was performed for the selected compounds having significant topo I or II inhibitory activity. The IC_{50} values of 4-chromanone containing pyridine bioisosteres (**14**–**36**) against several human cancer cell lines are as shown in Table 2. Most of the compounds showed moderate cytotoxicity, generally IC_{50} values of 0.1–25 μM. Among them, Compounds **24** and **28** which exhibited strong topo I inhibitory activity, and compound **26** which exhibited strong topo II inhibitory activity showed significant cytotoxicities, as strong as positive controls, camptothecin, etoposide, and adriamycin.

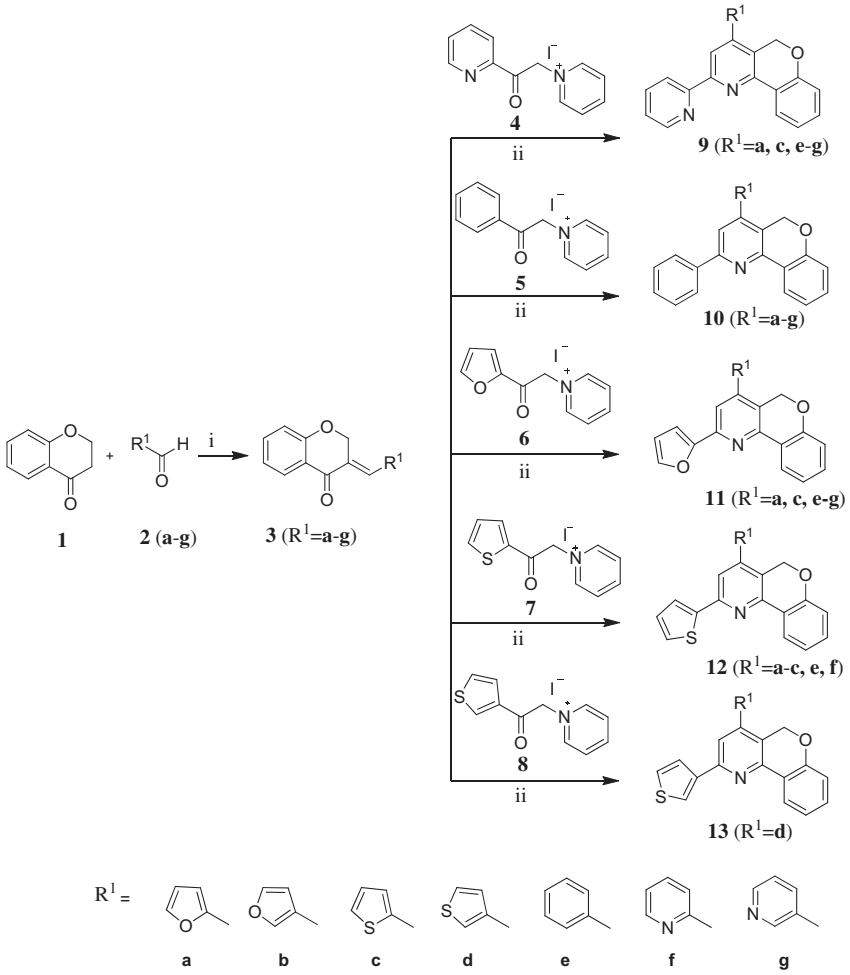
3. Conclusions

We have designed and synthesized 23 compounds by efficient synthetic route and evaluated for topo I and II inhibitory activities along with cytotoxicity against several human cancer cell lines. Among them, **15**, **24**, **27**, and **28** displayed significant topo I inhibitory activity, and **14**, **15**, **26**, and **36** exhibited moderate topo II inhibitory activity. Compounds **24**, **26**, and **28** exhibited strong cytotoxicity. A structure–activity relationship study of 2,4-diaryl chromenopyridines for topo I and II inhibitory activities and cytotoxicity indicates that 2-furyl or 2-thienyl at 2- or 4-position of central pyridine is crucial in displaying topo I or II inhibitory activity and cytotoxicity. In this study, we investigated conformationally constrained rigid molecules such as 2,4-diaryl chromenopyridines for the development of potent antitumor agents, and obtained positive results. This study may provide valuable information to researchers working on the development of antitumor agents.

4. Experimental

Compounds used as starting materials and reagents were obtained from Aldrich Chemical Co., Junsei or other chemical companies, and utilized without further purification. HPLC grade acetonitrile (ACN) and methanol were purchased from Burdick and Jackson, USA. Thin-layer chromatography (TLC) and column chromatography (CC) were performed with Kieselgel 60 F_{254} (Merck) and silica gel (Kieselgel 60, 230–400 mesh, Merck) respectively. Since all the compounds prepared contain aromatic ring, they were visualized and detected on TLC plates with UV light (short wave, long wave or both). NMR spectra were recorded on a Bruker AMX 250 (250 MHz, FT) for ^{1}H NMR and 62.5 MHz for ^{13}C NMR, and chemical shifts were calibrated according to TMS. Chemical shifts (δ) were recorded in ppm and coupling constants (J) in hertz (Hz). Melting points were determined in open capillary tubes on electrothermal 1A 9100 digital melting point apparatus and were uncorrected.

HPLC analyses were performed using two Shimadzu LC-10AT pumps gradient-controlled HPLC system equipped with Shimadzu system controller (SCL-10A VP) and photo diode array detector (SPD-M10A VP) utilizing Shimadzu Class VP program. Sample volume of 10 μL was injected in Waters X-Terra® 5 μM reverse-phase C_{18} column (4.6 × 250 mm) with a gradient elution of



Scheme 1. General synthetic scheme of 2,4-diaryl chromenopyridines Reagents and conditions: i) aq. NaOH (0.5 g in 10 ml), EtOH, room temperature, 46.1–90.5% yield ii) 4–8 (1.0 eq.), NH_4OAc (10.0 eq.), glacial AcOH, 12–24 h, 100–110 °C, 29.4–53.5% yield.

i) 40–100% of B in A for 10 min followed by 100–40% of B in A for 20 min ii) 75–100% of B in A for 10 min followed by 100–75% of B in A for 20 min and iii)85–100% of B in A for 10 min followed by 100–85% of B in A for 20 min at a flow rate of 1.0 mL/min at 254 nm UV detection, where mobile phase A was doubly distilled water with 50 mM ammonium formate (AF) and B was 90% ACN in water with 20 mM AF. Purity of compound is described as percent (%).

ESI LC/MS analyses were performed with a Finnigan LCQ Advantage® LC/MS/MS spectrometry utilizing Xcalibur® program. For ESI LC/MS, LC was performed with 8 μL injection volume on a Waters X-Terra® 3.5 μm reverse-phase C_{18} column (2.1 × 100 mm) with a gradient elution from 10 to 95% of B in A for 10 min followed by 95–10% of B in A for 10 min at a flow rate of 200 μL/min, where mobile phase A was 100% distilled water with 20 mM AF and mobile phase B was 100% ACN. MS ionization conditions were: Sheath gas flow rate: 40 arb, aux gas flow rate: 0 arb, I spray voltage: 5.3 kV, capillary temp.: 275 °C, capillary voltage: 27 V, tube lens offset: 45 V. Retention time is given in minutes.

4.1. General method for preparation of **3**

4-Chromanone (**1**) was dissolved in ethanol followed by the addition of equivalent amount of aryl aldehydes (**2a**–**g**). The 5% aqueous solution of NaOH was added dropwise to the mixture at room temperature. On addition of NaOH, precipitation was observed, cooled for 30 min, filtered, and washed with water and cold methanol. The precipitate was dried to give compound **3** (R^1 = **a**–**g**) in the yield of 46.1–90.5%.

4.2. General method for the preparation of ***4***–***8***

Aryl acetyl was mixed with pyridine followed by addition of equivalent amount of iodine. The mixture was then refluxed at 140 °C for 3 h. Precipitate occurred during reaction was cooled to room temperature. Then it was filtered and washed with cold pyridine followed by drying overnight to yield 72.5–96.2% of **4**–**8**.

4.3. General method for the preparation of ***14***–***36***

Anhydrous ammonium acetate (10.0 equiv) was mixed with glacial acetic acid followed by addition of the rigid chalcones of 4-chromanone (1.0 equiv), **3** (R^1 = **a**–**g**) and pyridinium iodide salts **4**–**8** (1.0 equiv). The mixture was then refluxed at 100–110 °C for 12–24 h. The reaction mixture was then extracted with ethyl acetate, washed with water and brine solution. The organic layer was dried with magnesium sulfate and filtered. The filtrate was evaporated at reduced pressure, which was then purified with silica gel column chromatography with the gradient elution of ethyl acetate/n-hexane to afford solid compounds **14**–**36** in 29.4–53.5% yield.

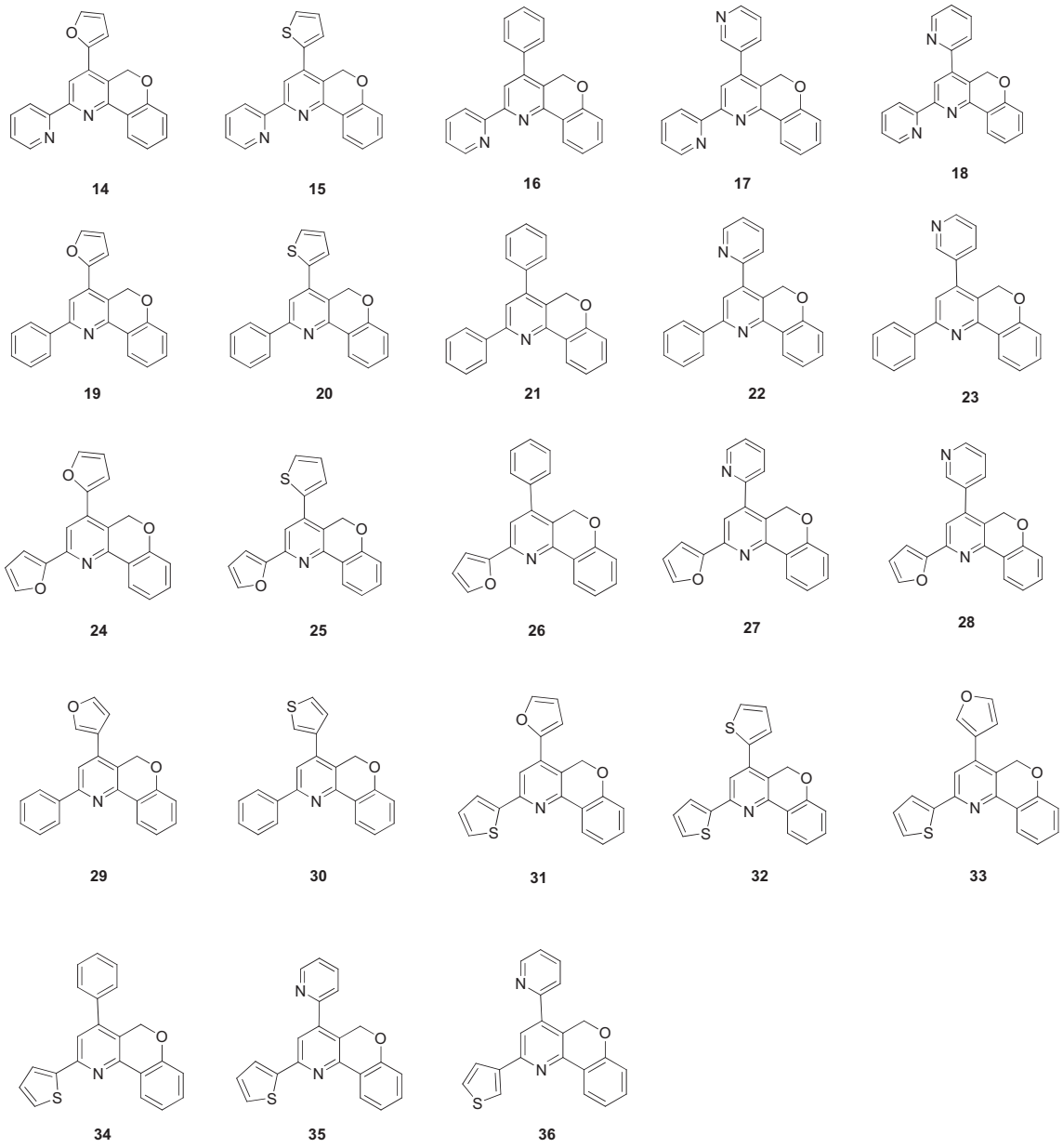


Fig. 2. Structure of the prepared compounds.

*4.3.1. 4-(furan-2-yl)-2-(pyridin-2-yl)-5H-chromeno[4,3-b]pyridine (**14**)*

A white solid, Yield: 52.0%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 7.62 min, $[MH]^+$: 327.27.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.70 (dd, J = 4.7, 2.2 Hz, 1H, 2-pyridine H-6), 8.64 (d, J = 7.6 Hz, 1H, 2-pyridine H-3), 8.59 (s, 1H, pyridine H-3), 8.42 (d, J = 7.7, 1.5 Hz, 1H, chromeno H-5), 7.86 (td, J = 7.7, 1.6 Hz, 1H, 2-pyridine H-4), 7.63 (dd, J = 1.4, 0.5 Hz, 1H, furan H-5), 7.37–7.31 (m, 2H, chromeno H-7 and 2-pyridine H-5), 7.16 (t, J = 7.2 Hz, 1H, chromeno H-6), 6.99 (d, J = 8.0, 1H, chromeno H-8), 6.84 (d, J = 3.4, 1H, furan H-3), 6.59 (dd, J = 3.3, 1.7 Hz, 1H, furan H-4), 5.62 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.22, 156.87, 154.94, 150.81, 149.06, 148.72, 143.94, 136.89, 134.87, 131.21, 125.12, 123.86, 123.39, 122.19, 121.97, 121.22, 116.73, 116.63, 112.03, 111.60, 66.32.

*4.3.2. 2-(pyridin-2-yl)-4-(thiophen-2-yl)-5H-chromeno[4,3-b] pyridine (**15**)*

A light yellow solid, Yield: 50.3%.

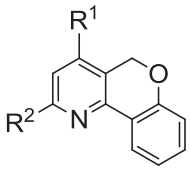
R_f (ethyl acetate/n-hexane 1:5 v/v): 0.36.

LC/MS/MS: retention time: 6.30 min, $[MH]^+$: 343.27.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.68 (ddd, J = 3.9, 1.7, 0.8 Hz, 1H, 2-pyridine H-6), 8.64 (dd, J = 7.9, 0.6 Hz, 1H, 2-pyridine H-3), 8.44 (s, 1H, pyridine H-3), 8.42 (dd, J = 7.6, 1.5 Hz, 1H, chromeno H-5), 7.82 (td, J = 7.7, 1.7 Hz, 1H, 2-pyridine H-4), 7.50 (dd, J = 5.0, 1.2 Hz, 1H, 4-thiophene H-5), 7.36–7.30 (m, 2H, 4-thiophene H-3 and chromeno H-7), 7.19–7.12 (m, 3H, 4-thiophene H-4, 2-pyridine H-5 and chromeno H-6), 7.01 (dd, J = 8.0, 0.8 Hz, 1H, chromeno H-8), 5.47 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.28, 155.80, 155.01, 149.08, 148.69, 139.92, 138.52, 136.89, 131.30, 127.80, 127.51, 125.19, 123.89, 123.55, 12.53, 122.31, 121.29, 120.43, 116.80, 66.04.

Table 1
Prepared compounds with yield, purity by HPLC, and melting point.



Entry	R^1	R^2	Yield (%)	Purity (%)	mp (°C)
14	2-furyl	2-pyridyl	52.0	100.0	153.3–155.2
15	2-thienyl	2-pyridyl	50.3	100.0	167.6–168.2
16	phenyl	2-pyridyl	36.5	100.0	147.1–148.9
17	3-pyridyl	2-pyridyl	29.4	100.0	177.5–180.6
18	2-pyridyl	2-pyridyl	41.4	100.0	178.6–182.4
19	2-furyl	phenyl	38.2	100.0	164.9–165.7
20	2-thienyl	phenyl	31.7	96.1	172.2–174.6
21	phenyl	phenyl	30.9	96.4	116.9–117.6
22	2-pyridyl	phenyl	32.7	97.8	165.3–167.1
23	3-pyridyl	phenyl	29.4	99.3	146.2–147.8
24	2-furyl	2-furyl	43.9	97.8	140.4–142.4
25	2-thienyl	2-furyl	35.3	97.6	130.4–131.3
26	phenyl	2-furyl	41.6	98.8	107.8–109.9
27	2-pyridyl	2-furyl	43.0	100.0	170.6–171.5
28	3-pyridyl	2-furyl	48.8	97.5	128.3–130.2
29	3-furyl	phenyl	43.0	95.5	152.8–161.1
30	3-thienyl	phenyl	34.5	96.2	142.6–144.6
31	2-furyl	2-thienyl	33.9	99.3	184.3–185.7
32	2-thienyl	2-thienyl	33.9	98.9	147.1–148.7
33	3-furyl	2-thienyl	32.4	96.6	149.5–150.7
34	phenyl	2-thienyl	53.5	96.3	127.9–128.2
35	2-pyridyl	2-thienyl	35.3	99.1	164.6–165.8
36	2-pyridyl	3-thienyl	52.5	99.0	181.6–182.5

*4.3.3. 4-phenyl-2-(pyridin-2-yl)-5H-chromeno[4,3-b]pyridine (**16**)*

A white solid, Yield: 36.5%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.35.

LC/MS/MS: retention time: 8.37 min, $[MH]^+$: 337.32.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.69–8.65 (m, 2H, 2-pyridine H-3, H-6), 8.47 (dd, J = 7.7, 1.63 Hz, 1H, chromeno H-5), 8.34 (s, 1H, pyridine H-3), 7.86 (td, J = 7.8, 1.5 Hz, 1H, 2-pyridine H-4), 7.49–7.45 (m, 3H, 4-phenyl H-3, H-4, H-5), 7.40–7.30 (m, 4H, 4-phenyl H-2, H-6, 2-pyridine H-5 and chromeno H-8), 7.16 (t, J = 7.7 Hz, 1H, chromeno H-7), 6.97 (d, J = 7.6 Hz, 1H, chromeno H-6), 5.05 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.42, 156.01, 154.89, 149.05, 147.59, 137.36, 136.89, 131.21, 128.62, 128.54, 125.15, 123.81, 123.75, 123.69, 122.28, 121.30, 120.58, 116.83, 66.03.

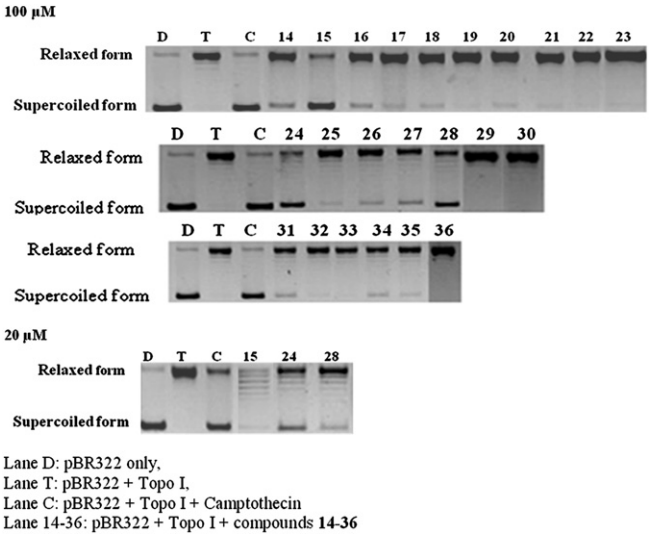


Fig. 3. Topoisomerase I inhibitory activity of the prepared compounds **14**–**36**.

*4.3.4. 2-(pyridin-2-yl)-4-(pyridin-3-yl)-5H-chromeno[4,3-b] pyridine (**17**)*

A light yellow solid, Yield: 29.4%.

R_f (ethyl acetate/n-hexane 1:2 v/v): 0.36.

LC/MS/MS: retention time: 3.73 min, $[MH]^+$: 338.34.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.95 (d, J = 2.2 Hz, 1H, 4-pyridine H-2), 8.75 (d, J = 5.1 Hz, 1H, 2-pyridine H-6), 8.73–8.67 (m, 2H, 2-pyridine H-3 and 4-pyridine H-6), 8.42 (d, J = 7.9 Hz, 1H, 4-pyridine H-4), 8.20 (d, J = 7.7, 1.6 Hz 1H, chromeno H-5), 7.89–7.82 (m, 2H, 2-pyridine H-4 and pyridine H-3), 7.50 (dd, J = 5.1, 1.8 Hz, 1H, 2-pyridine H-5), 7.39–7.32 (m, 2H, 4-pyridine H-5 and chromeno H-7), 7.14 (dt, J = 7.6, 1.12 Hz, 1H, chromeno H-6), 6.90 (dd, J = 8.1, 0.8 Hz, 1H, chromeno H-8), 5.31(s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.95, 156.52, 155.69, 149.94, 149.27, 149.18, 147.75, 145.78 137.07, 132.58, 131.79, 130.50, 126.18, 124.85, 124.05, 122.62, 122.55, 121.32, 121.00, 118.57, 117.10, 67.79.

*4.3.5. 2,4-di(pyridin-2-yl)-5H-chromeno[4,3-b]pyridine (**18**)*

A white solid, Yield: 41.4%.

R_f (ethyl acetate/n-hexane 1:2 v/v): 0.36.

LC/MS/MS: retention time: 5.76 min, $[MH]^+$: 338.32.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.74–8.67 (m, 3H, 2-pyridine H-3, H-6 and 4-pyridine H-6), 8.53 (s, 1H, pyridine H-3), 8.43 (dd, J = 7.7, 1.6 Hz, 1H, chromeno H-5), 7.88–7.83(m, 2H, 4-pyridine H-3 and 2-pyridine H-4), 7.74 (d, J = 8.0, 1.5 Hz, 1H, 4-pyrridine H-4), 7.38–7.31 (m, 3H, 2-pyridine H-5, 4-pyridine H-5 and chromeno H-7), 7.10 (dt, J = 7.5, 1.0 Hz, 1H, chromeno H-6), 6.90 (d, J = 8.0 Hz, 1H, chromeno H-8), 5.53 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.62, 156.23, 155.88, 154.93, 149.17, 149.01, 148.96, 145.40, 136.95, 136.93, 131.20, 125.12, 124.92, 123.86, 123.71, 123.60, 123.07, 122.11, 121.26, 119.64, 116.81, 66.50.

*4.3.6. 4-(furan-2-yl)-2-phenyl-5H-chromeno[4,3-b]pyridine (**19**)*

A light yellow solid, Yield: 38.2%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 9.06 min, $[MH]^+$: 326.28.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.40 (dd, J = 7.7, 1.6 Hz, 1H, chromeno H-5), 8.15–8.19 (m, 2H, 2-phenyl H-2,6), 7.89 (s, 1H, pyridine H-3), 7.60 (d, J = 1.9 Hz, 1H, 4-furan H-5), 7.51–7.44 (m, 3H, 2-phenyl H-3, H-4, H-5), 7.34 (dt, J = 8.0, 1.8 Hz, 1H, chromeno H-7), 7.10 (dt, J = 7.6, 1.1 Hz, 1H, chromeno H-6), 6.90 (dd. J = 8.0, 0.6 Hz, 1H, chromeno H-8), 6.68 (d, J = 3.4 Hz, 1H, 4-furan H-3), 6.60 (dd, J = 3.4, 1.8 Hz, 1H, 4-furan H-4), 5.57 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.22, 156.17, 150.50, 148.96, 145.09, 143.88, 139.03, 134.67, 131.17, 129.12, 128.69, 126.86, 125.28, 123.47, 122.22, 120.41, 116.63, 125.98, 112.07, 111.42, 66.13.

*4.3.7. 2-phenyl-4-(thiophen-2-yl)-5H-chromeno[4,3-b]pyridine (**20**)*

A white solid, Yield: 31.7%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.51.

LC/MS/MS: retention time: 9.84 min, $[MH]^+$: 342.27.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.46 (dd, J = 7.6, 1.5 Hz, 1H, chromeno H-5), 8.17–8.14 (m, 2H, 2-phenyl H-2, H-6), 7.71 (s, 1H, pyridine H-3), 7.54–4.43 (m, 4H, 2-phenyl H-3, H-4, H-5 and 4-thiophene H-5), 7.34 (dt, J = 7.7, 1.6 Hz, 1H, chromeno H-6), 7.20–7.14 (m, 2H, 4-thiophene H-3 and chromeno H-6), 7.09 (dt, J = 4.2, 0.7 Hz, 1H, 4-thiophene H-4) 6.98 (d, J = 8.1 Hz, 1H, chromeno H-8), 5.43 (s, 2H, chromeno H-2).

Table 2
Topo I and II inhibitory activities, and cytotoxicity of the prepared compounds.

Compounds	Topo I (% Inhibition)		Topo II (% Inhibition)		IC_{50} (μM)				
	100 μM	20 μM	100 μM	20 μM	MDA-MB231[a]	HeLa[b]	DU145[c]	HCT15[d]	HL60[e]
Adriamycin[f]					0.4 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.0	0.7 ± 0.0
Etoposide[g]			40.5	22.3	0.7 ± 0.1	1.4 ± 0.2	0.5 ± 0.0	1.1 ± 0.0	1.0 ± 0.0
Camptothecin[h]	58.0	20.9			0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.1 ± 0.0
14	4.4	NA	23.4	NA	3.3 ± 0.2	>50	22.9 ± 0.3	>50	18.6 ± 0.5
15	48.2	4.1	19.4	NA	2.4 ± 0.1	6.2 ± 0.2	13.1 ± 1.0	14.0 ± 0.8	13.7 ± 0.5
16	3.1	NA	7.2	NA	11.1 ± 0.2	5.0 ± 0.1	7.6 ± 0.8	15.6 ± 1.1	14.5 ± 0.6
17	2.6	NA	8.7	NA	7.2 ± 0.7	1.3 ± 0.0	4.4 ± 0.4	3.3 ± 0.3	>50
18	0.6	NA	10.7	NA	4.7 ± 0.3	>50	>50	23.1 ± 0.3	0.2 ± 0.0
19	0.0	NA	11.5	NA	11.6 ± 0.7	1.4 ± 0.0	18.6 ± 3.1	12.3 ± 2.0	7.3 ± 0.3
20	0.0	NA	10.8	NA	3.9 ± 0.1	16.0 ± 0.2	2.6 ± 0.3	1.2 ± 0.4	>50
21	0.0	NA	15.4	NA	1.8 ± 0.6	14.2 ± 0.3	6.9 ± 2.1	5.1 ± 1.4	18.8 ± 0.3
22	0.0	NA	8.7	NA	1.9 ± 0.0	2.8 ± 0.0	1.9 ± 0.1	12.1 ± 2.9	>50
23	0.0	NA	14.0	NA	NA	NA	NA	NA	NA
24	46.4	22.9	11.6	NA	0.6 ± 0.2	1.3 ± 0.0	0.9 ± 0.2	0.6 ± 0.2	3.4 ± 0.1
25	0.0	NA	6.8	NA	NA	NA	NA	NA	NA
26	4.9	NA	28.4	NA	0.2 ± 0.1	2.3 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	1.4 ± 0.0
27	11.5	NA	2.0	NA	NA	NA	NA	NA	NA
28	41.6	15.5	5.6	NA	1.1 ± 0.1	2.2 ± 0.1	4.6 ± 0.3	0.6 ± 0.2	1.8 ± 0.1
29	1.9	NA	10.5	NA	10.0 ± 0.1	39.6 ± 5.7	>50	31.1 ± 2.6	6.6 ± 1.0
30	3.7	NA	17.6	NA	11.9 ± 0.2	>50	>50	>50	>50
31	9.2	NA	14.1	NA	NA	NA	NA	NA	NA
32	0.7	NA	7.9	NA	NA	NA	NA	NA	NA
33	0.0	NA	8.4	NA	NA	NA	NA	NA	NA
34	5.5	NA	7.5	NA	NA	NA	NA	NA	NA
35	1.8	NA	8.5	NA	NA	NA	NA	NA	NA
36	3.3	NA	25.3	3.2	25.8 ± 0.2	>50	5.0 ± 1.9	8.3 ± 0.8	>50

Each data represents mean ± S.D. from three different experiments performed in triplicate.
NA: not applicable.
[a] MDA-MB231: human breast adenocarcinoma.
[b] HeLa: human cervix tumor.
[c] DU145: human prostate tumor.
[d] HCT15 human colorectal adenocarcinoma.
[e] HL60: chronic myelogenous leukemia.
[f] Adriamycin; positive control for cytotoxicity.
[g] Etoposide: positive control for topo II and cytotoxicity.
[h] Camptothecin: positive control for topo I and cytotoxicity.

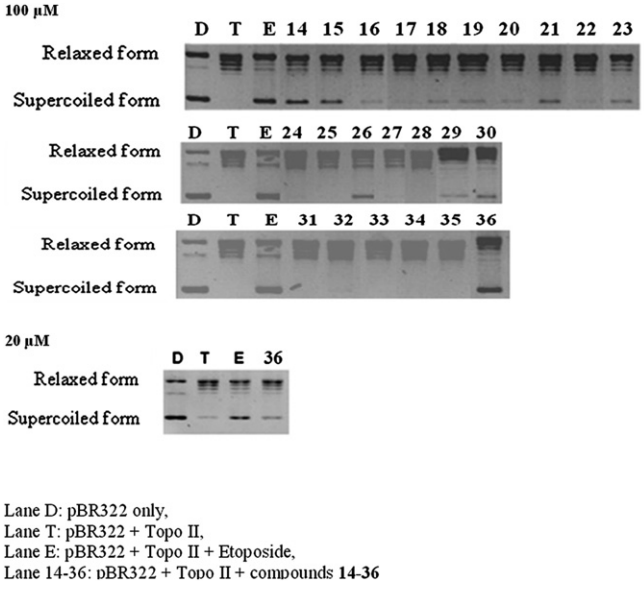


Fig. 4. Topoisomerase II inhibitory activity of the prepared compounds **14**–**36**.

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.26, 149.04, 139.60, 138.88, 138.54, 131.26, 129.18, 128.71, 128.00, 127.92, 127.39, 126.91, 125.36, 125.11, 123.61, 122.31, 121.98, 119.82, 116.71, 111.42, 65.89.

*4.3.8. 2,4-diphenyl-5H-chromeno[4,3-b]pyridine (**21**)*

A white solid, Yield: 30.9%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 9.84 min, $[MH]^+$: 336.31.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.48 (dd, J = 7.7, 1.6 Hz, 1H, chromeno H-5), 8.18–8.14 (m, 2H, 2-phenyl H-2 and H-6), 7.63 (s, 1H, pyridine H-3), 7.54–7.43 (m, 6H, 2-phenyl H-3, H-4, H-5 and 4-phenyl H-3, H-4, H-5), 7.37–7.34 (m, 3H, 4-phenyl H-2, H-6 and chromeno H-7), 7.15 (t, J = 7.5, 1.0 Hz, 1H, chromeno H-6), 6.96 (dd, J = 8.0, 0.9 Hz, 1H, chromeno H-8) 5.25 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.40, 156.12, 148.71, 147.27, 139.08, 137.49, 131.17, 129.08, 128.75, 128.69, 128.59, 128.43, 127.10, 126.90, 125.32, 123.77, 122.28, 122.12, 119.94, 116.73, 65.90.

*4.3.9. 2-phenyl-4-(pyridin-2-yl)-5H-chromeno[4,3-b]pyridine (**22**)*

A yellow solid, Yield: 32.7%.

R_f (ethyl acetate/n-hexane 1:2 v/v): 0.50.

LC/MS/MS: retention time: 7.45 min, $[MH]^+$: 337.30.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.75 (dd, J = 4.0, 0.8 Hz, 1H, 4-pyridine H-6), 8.47 (dd, J = 7.7, 1.6 Hz, 1H, 4-pyridine H-5), 8.20–8.17 (m, 2H, 2-phenyl H-2, H-6), 7.87 (dt, J = 7.6, 1.6 Hz, 1H, 4-pyridine H-4), 7.80 (s, 1H, pyridine H-3), 7.62 (d, J = 7.8 Hz, 1H, chromeno H-8), 7.54–7.44 (m, 3H, 2-phenyl H-3, H-4, H-5), 7.40–7.31 (m, 2H, 4-pyridine H-3, H-5), 7.15 (d, J = 7.5 Hz, 1H, chromeno H-7), 6.98 (d, J = 8.06 Hz, 1H, chromeno H-6), 5.05 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.57, 156.23, 156.17, 149.46, 149.23, 145.24, 139.03, 136.97, 131.18, 129.11, 128.69, 126.91, 125.32, 123.69, 123.45, 123.14, 123.02, 122.15, 119.12, 116.72, 66.26.

4.3.10. 2-phenyl-4-(pyridin-3-yl)-5H-chromeno[4,3-b]pyridine (**23**)

A white solid, Yield: 29.4%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 5.22 min, $[MH]^+$: 337.36.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.90 (d, J = 2.1 Hz, 1H, 4-pyridine H-2), 8.80 (d, J = 5.1 Hz, 1H, 4-pyridine, H-6), 8.20 (dd, J = 7.7, 1.6 Hz, 1H, chromeno H-5), 8.00 (dd, J = 9.4, 1.2 Hz, 2H, 2-phenyl H-2, H-6), 7.93 (s, 1H, pyridine H-3), 7.70 (d, J = 2.0 Hz, 1H, 4-pyridine H-4), 7.56–7.45 (m, 4H, 4-pyridine H-5 and 2-phenyl H-3, H-4, H-5), 7.30 (dt, J = 7.7, 1.6 Hz, 1H, chromeno H-7), 7.10 (dt, J = 8.0, 1.0 Hz, 1H, chromeno H-6), 6.90 (dt, J = 8.0 Hz, 1H, chromeno H-8), 5.32 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 158.50, 156.17, 156.53, 150.41, 147.75, 145.78, 139.06, 132.81, 131.88, 130.44, 129.32, 128.87, 127.05, 126.26, 124.85, 122.62, 119.73, 118.31, 117.15, 111.42, 67.82.

4.3.11. 2,4-di(furan-2-yl)-5H-chromeno[4,3-b]pyridine (**24**)

A yellow solid, Yield: 43.9%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 13.14 min, $[MH]^+$: 316.22.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.30 (dd, J = 7.7, 1.4 Hz, 1H, chromeno H-5), 7.84 (s, 1H, pyridine H-3), 7.63 (t, J = 0.8 Hz 1H, 2-furan H-5), 7.55 (t, J = 0.8 Hz, 1H, 4-furan H-5), 7.33 (tt, J = 7.0, 0.8 Hz, 1H, chromeno H-7), 7.20 (d, J = 3.3 Hz, 1H, 2-furan H-3), 7.10 (t, J = 7.4 Hz, 1H, chromeno H-6), 6.92 (d, J = 8.0 Hz, 1H, chromeno H-8), 6.70 (d, J = 3.4 Hz, 1H, 4-furan H-3), 6.59–6.56 (m, 2H, 2-furan H-4 and 4-furan H-4), 5.54 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.19, 153.73, 150.45, 149.08, 148.50 143.94, 143.23, 134.52, 131.25, 125.23, 123.19, 122.22, 120.23, 116.63, 113.99, 112.09, 112.06, 111.55, 108.90, 35.73.

4.3.12. 2-(furan-2-yl)-4-(thiophen-2-yl)-5H-chromeno[4,3-b] pyridine (**25**)

A white solid, Yield: 35.3%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 5.85 min, $[MH]^+$: 332.22.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.30 (dd, J = 7.7, 1.5 Hz, 1H, chromeno H-5), 7.68 (s, 1H, pyridine H-3), 7.50 (d, J = 0.8 Hz, 1H, 2-furan H-5), 7.49 (dd, J = 5.1, 1.0 Hz, 1H, 4-thiophene H-5), 7.30 (dt, J = 7.7, 1.6 Hz, 1H, chromeno H-6), 7.22–7.13 (m, 3H, 4-thiophene H-4, 2-furan H-3 and chromeno H-7), 7.00 (dd, J = 3.5, 1 Hz, 1H, 4-thiophene H-3), 6.90 (dd, J = 8.0, 0.7 Hz, 1H, chromeno H-8), 6.50 (dd, J = 3.3, 1.7 Hz, 1H, 2-furan H-4), 5.41 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.29, 153.60, 149.13, 148.51 143.34, 139.52, 138.36, 131.34, 128.07, 127.89, 127.49, 125.30, 123.35, 122.33, 121.80, 117.75, 116.71, 112.10, 109.10, 65.92.

4.3.13. 2-(furan-2-yl)-4-phenyl-5H-chromeno[4,3-b]pyridine (**26**)

A white solid, Yield: 41.6%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 8.57 min, $[MH]^+$: 326.25.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.32 (dd, J = 7.6, 1.4 Hz, 1H, chromeno H-5), 7.59 (s, 1H, pyridine H-3), 7.53–7.46 (m, 4H, 4-phenyl H-2, H-6, furan H-5 and chromeno H-6), 7.36–7.30 (m, 3H, 4-phenyl, H-3, H-4, H-5), 7.20 (d, J = 4.3 Hz, 1H, furan H-3), 7.10 (t, J = 7.4 Hz, 1H, chromeno H-7), 6.90 (d, J = 8.0 Hz, 1H, chromeno H-8), 6.50 (dd, J = 3.1, 1.7 Hz, 1H, furan H-4), 3.98 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.41, 153.81, 148.82, 148.43 147.20, 143.25, 137.27, 131.26, 128.27, 128.63, 128.42, 125.27, 123.50, 122.32, 121.99, 117.99, 116.74, 112.08, 108.92, 65.93.

4.3.14. 2-(furan-2-yl)-4-(pyridin-2-yl)-5H-chromeno[4,3-b] pyridine (**27**)

A light green soild, Yield: 43.0%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 6.01 min, $[MH]^+$: 327.23.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.70 (dd, J = 3.6, 0.8 Hz, 1H, 4-pyridine H-6), 8.38 (dd, J = 7.7, 1.6 Hz, 1H, chromeno H-5), 7.81 (dt, J = 7.7, 1.7 Hz, 1H, 4-pyridine H-3), 7.76 (s, 1H, pyridine H-3), 7.62 (d, J = 7.8 Hz, 1H, 4-pyridine H-4), 7.51 (d, J = 0.9 Hz, 1H, 2-furan H-5), 7.39–7.23 (m, 2H, 4-pyridine H-5 and chromeno H-7), 7.23 (d, J = 3.3 Hz, 1H, 2-furan H-3), 7.15 (dt, J = 7.5, 1 Hz, 1H, chromeno H-6), 6.90 (dd, J = 8.6, 0.7 Hz, 1H, chromeno H-8), 6.5 (dd, J = 3.3, 1.7 Hz, 1H, 2-furan H-4), 5.44 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.60, 156.02, 153.77, 149.36, 148.52 145.11, 143.24, 136.99, 131.27, 125.26, 123.49, 123.42, 123.17, 123, 122.16, 117.12, 116.73, 112.13, 108.96, 66.32.

4.3.15. 2-(furan-2-yl)-4-(pyridin-3-yl)-5H-chromeno[4,3-b] pyridine (**28**)

A light yellow solid, Yield: 48.8%.

R_f (ethyl acetate/n-hexane 1:1 v/v): 0.24.

LC/MS/MS: retention time: 4.41 min, $[MH]^+$: 327.26.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.74 (td, J = 4.6, 1.3, 1.1 Hz, 1H, 4-pyridine H-6), 8.63 (t, J = 1.1 Hz, 1H, 4-pyridine H-2), 8.37 (dd, J = 7.1, 1.1 Hz, 1H, chromeno H-5), 7.71 (dd, J = 5.4, 2.3 Hz, 1H, 2-furan H-5), 7.57–7.54 (m, 2H, 4-pyridine H-4 and pyridine H-3), 7.46 (dd, J = 7.7, 4.8 Hz, 1H, 4-pyridine H-5), 7.35 (tt, J = 6.6, 1.5, 1.1 Hz, 1H, chromeno H-7), 7.25 (d, J = 4.3 Hz, 1H, 2-furan H-3), 7.15 (tt, J = 7.5, 1.0 Hz, 1H, chromeno H-6), 6.97 (d, J = 8.1 Hz, 1H, chromeno H-8), 6.57 (dd, J = 3.0, 1.4 Hz, 1H, furan H-4), 5.20 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.36, 153.48, 149.93, 149.16 149.04, 148.73, 143.46, 135.81, 133.12, 131.55, 125.32, 123.51, 123.20, 122.45, 121.96, 117.75, 116.81, 112.18, 109.33, 65.59.

4.3.16. 4-(Furan-3-yl)-2-phenyl-5H-chromeno[4,3-b]pyridine (**29**)

A white solid, Yield: 43.0%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 8.18 min, $[MH]^+$: 326.27.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.44 (dd, J = 8.0, 1.5 Hz, 1H, chromeno H-5), 8.16–8.12 (m, 2H, 2-phenyl H-2, H-6), 7.63 (s, 1H, pyridine H-3), 7.59–7.56 (m, 2H, 4-furan H-2, H-5), 7.52–7.43 (m, 3H, 2-phenyl H-3, H-4, H-5), 7.30 (ddd, J = 6.8, 1.7, 0.7 Hz, 1H, chromeno H-7), 7.14 (dt, J = 7.5, 1.1 Hz, 1H, chromeno H-6), 6.90 (dd, J = 7.6, 0.7 Hz, 1H, chromeno H-8), 6.60 (dd, J = 1.8, 0.9 Hz, 1H, 4-furan H-4), 5.36 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.32, 156.24, 148.73, 143.84, 140.92, 139.03, 137.80, 131.19, 129.11, 128.70, 126.88, 125.29, 123.57, 122.30, 122.11, 116.69, 110.57, 65.84.

4.3.17. 2-phenyl-4-(thiophen-3-yl)-5H-chromeno[4,3-b]pyridine (**30**)

A white solid, Yield: 34.5%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 9.22 min, $[MH]^+$: 342.27.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.45 (dd, J = 7.6, 1,5 Hz, 1H, chromeno H-5), 8.17–8.13 (m, 2H, 2-phenyl H-2,6) 7.66 (S, 1H, pyridine H-3), 7.53–7.43 (m, 4H, 2-phenyl H-3,4,5 and 4-thiophene H-2), 7.37–7.30 (m, 2H, 4-thiophene H-4 and chromeno H-7), 7.20–7.11 (m, 2H, chromeno H-6,8), 6.97 (dd, J = 8.1, 0.8 Hz, 1H, chromeno H-8), 5.05 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.33, 156.21, 148.79, 141.78, 139.04, 137.95, 131.18, 129.10, 128.70, 127.82, 126.89, 126.70, 125.31, 125.46, 123.71, 122.30, 122.12, 119.56, 116.72,65.93.

*4.3.18. 4-(furan-2-yl)-2-(thiophen-2-yl)-5H-chromeno [4,3-b] pyridine (**31**)*

A white solid, Yield: 33.9%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.40.

LC/MS/MS: retention time: 8.56 min, $[MH]^+$: 332.24.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.30 (dd, J = 7.7, 1.6 Hz, 1H, chromeno H-5), 7.76 (s, 1H, pyridine H-3), 7.68 (dd, J = 3.6, 1.1 Hz, 1H, 2-thiophene H-3), 7.61 (d, J = 1.2 Hz, 1H, 4-furan H-5), 7.40 (dd, J = 5.0, 1.0 Hz, 1H, 2-thiophene H-5), 7.30 (dt, J = 8.0, 1.7 Hz, 1H, chromeno H-7), 7.16–7.10 (m, 2H, 4-furan H-3 and chromeno H-6), 6.90 (dd, J = 8.1, 0.8 Hz, 1H, chromeno H-8), 6.70 (d, J = 3.1 Hz, 1H, 2-thiophene H-4), 6.63 (dd, J = 3.4, 1.8 Hz, 1H, 4-furan H-4), 5.53 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.17, 151.65, 150.25, 148.91, 144.94, 143.91, 134.64, 131.29, 127.94, 127.62, 125.33, 124.63, 123.07, 122.25, 120.18, 116.59, 114.24, 112.07, 111.55, 66.12.

*4.3.19. 2,4-di(thiophen-2-yl)-5H-chromeno[4,3-b]pyridine (**32**)*

A white solid, Yield: 33.9%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 9.35 min, $[MH]^+$: 348.23.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.30 (dd, J = 8.7, 1.7 Hz, 1H, chromeno H-5), 7.64 (dd, J = 4.7, 1.0 Hz, 1H, 2-thiophene H-3), 7.59 (s, 1H, pyridine H-3), 7.48 (dd, J = 5.1, 1.1 Hz, 1H, 4-thiophene H-3), 7.41 (dd, J = 5.0, 1.0 Hz, 1H, 2-thiophene H-5), 7.32 (dt, J = 8.4, 1.7 Hz, 1H, chromeno H-7), 7.18–7.11 (m, 2H, 4-thiophene H-5 and chromeno H-6), 7.10 (dd, J = 3.4, 1.3 Hz, 1H, 2-thiophene H-4), 7.02 (dd, J = 3.6, 1.0 Hz, 1H, 4-thiophene H-4), 6.97 (dd, J = 8.0, 1.8 Hz, 1H, chromeno H-8), 5.38 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.29, 151.65, 149, 144.76, 139.62, 138.26, 131.39, 128.03, 127.97, 127.93, 127.78, 127.44, 125.42, 124.76, 123.22, 122.35, 121.79, 118.05, 116.68, 65.89.

*4.3.20. 4-(furan-3-yl)-2-(thiophen-2-yl)-5H-chromeno[4,3-b] pyridine (**33**)*

A white solid, Yield: 32.4%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 7.78 min, $[MH]^+$: 332.32.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.30 (dd, J = 7.7, 1.6 Hz, 1H, chromeno H-5), 7.64 (dd, J = 3.6, 0.9 Hz, 1H, 2-thiophene H-3), 7.58 (d, J = 2.7 Hz, 1H, 4-furan H-5), 7.57 (d, J = 0.8 Hz, 1H, 4-furan H-2), 7.59 (s, 1H, pyridine H-3), 7.41 (dd, J = 5.0, 0.9 Hz, 1H, thiophene H-5), 7.32 (dt, J = 7.7, 1.6 Hz, 1H, chromeno H-7), 7.17–7.11 (m, 2H, 2-thiophene H-4 and chromeno H-6), 6.97 (dd, J = 7.65, 0.8 Hz, 1H, chromeno H-8), 6.63 (dd, J = 1.5, 0.8 Hz, 1H, 4-furan H-4), 5.32 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.22, 151.67, 148.66, 144.87, 143.86, 140.96, 137.81, 131.30, 127.95, 127.67, 125.31, 124.58, 123.16, 122.34, 122.11, 117.27, 116.65, 110.53, 65.81.

*4.3.21. 4-phenyl-2-(thiophen-2-yl)-5H-chromeno[4,3-b]pyridine (**34**)*

A white solid, Yield: 53.5%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.50.

LC/MS/MS: retention time: 9.50 min, $[MH]^+$: 342.27.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.42 (dd, J = 7.6, 1.5 Hz, 1H, chromeno H-5), 7.64 (d, J = 3.6 Hz, 1H, 2-thiophene H-3), 7.51–7.49 (m, 4H, pyridine H-3, 6-phenyl H-2,4,6), 7.40 (d, J = 5.0 Hz, 1H, 2-thiophene H-5), 7.36–7.30 (m, 3H, chromeno H-7 and 6-phenyl H-3,5), 7.18–7.10 (m, 2H, chromeno H-6 and 2-thiophene H-4), 6.97 (d, J = 8.0 Hz, 1H, chromeno H-8), 5.21 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 160.94, 156.56, 155.51, 148.98 143.70, 143.55, 141.63, 140.91, 137.04, 136.93, 132.96, 128.79, 127.28, 125.01, 123.57, 121.11, 116.22, 109.38, 35.49.

*4.3.22. 4-(pyridin-2-yl)-2-(thiophen-2-yl)-5H-chromeno[4,3-b] pyridine (**35**)*

A yellow solid, Yield: 35.3%.

R_f (ethyl acetate/n-hexane 1:2 v/v): 0.40.

LC/MS/MS: retention time: 7.01 min, $[MH]^+$: 343.25.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.70 (d, J = 4.6 Hz, 1H, 4-pyridine H-6), 8.40 (dd, J = 7.6, 1.5 Hz, 1H, chromeno H-5), 7.86 (dt, J = 7.7, 1.7 Hz, 1H, 4-pyridine H-4), 7.67 (d, J = 2.3 Hz, 2H, pyridine H-3 and 2-thiophene H-3), 7.60 (d, J = 7.8 Hz, 1H, 4-pyridine H-3), 7.43–7.30 (m, 3H, chromeno H-6,7 and 2-thiophene H-5), 7.17–7.11 (m, 2H, 4-pyridine H-5 and 2-thiophene H-4), 6.98 (d, J = 7.6 Hz, 1H, chromeno H-8), 5.42 (s, 2H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.54, 155.89, 151.63, 149.49, 149.16 145.25, 144.92, 136.99, 131.30, 127.96, 127.69, 125.35, 124.67, 123.44, 123.28, 123.22, 122.80, 122.20, 117.39, 116.69, 66.23.

*4.3.23. 4-(pyridin-2-yl)-2-(thiophen-3-yl)-5H-chromeno[4,3-b] pyridine (**36**)*

A yellow solid, Yield: 52.5%.

R_f (ethyl acetate/n-hexane 1:5 v/v): 0.40.

LC/MS/MS: retention time: 6.87 min, $[MH]^+$: 343.26.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.70 (dd, J = 3.8, 1.0 Hz, 1H, 4-pyridine H-6), 8.40 (dd, J = 8.7 Hz, 1H, chromeno H-5), 8.00 (d, J = 2.8 Hz, 1H, 2-thiophene H-2), 7.90–7.78 (m, 2H, 4-pyridine H-3 and 2-thiophene H-5), 7.66 (s, 1H, pyridine H-3), 7.59 (d, J = 7.8 Hz, 1H, chromeno H-7), 7.43–7.31 (m, 3H, 2-thiophene H-4 and 4-pyridine H-4,5), 7.10 (t, J = 7.4 Hz, 2H, chromeno H-6), 6.98 (d, J = 8.0 Hz, 1H, chromeno H-8), 5.42 (s, 1H, chromeno H-2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.57, 156.06, 152.50, 149.49 145.25, 142.06, 136.97, 131.19, 126.33, 126.21, 125.24, 123.477 123.59, 123.345, 123.16, 122.68, 122.13, 118.91, 116.73, 66.25.

4.4. Biological assays

DNA topo I inhibition assay was determined following the previously reported method [14]. The prepared compounds were dissolved in DMSO at 20 mM as stock solution. The activity of DNA topo I was determined by assessing the relaxation of supercoiled DNA pBR322. The mixture of 100 ng of plasmid pBR322 DNA and 0.4 units of recombinant human DNA topo I (TopoGEN INC., USA) was incubated without and with the prepared compounds at 37 °C for 30 min in the relaxation buffer (10 mM Tris–HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin, 1 mM spermidine, 5% glycerol). The reaction in the final volume of 10 μL was terminated by adding 2.5 μL of the stop solution containing 5% sarcosyl, 0.0025% bromophenol blue, and 25% glycerol. DNA samples were then electrophoresed on a 1% agarose gel at 15 V for 7 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corporation).

DNA topo II inhibitory activity of compounds was measured as follows [15]. The mixture of 200 ng of supercoiled pBR322 plasmid DNA and 1 unit of human DNA topo IIα (TopoGEN INC., USA) was incubated without and with the prepared compounds in the assay buffer (10 mM Tris–HCl (pH 7.9) containing 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, and 15 μg/mL bovine serum albumin) for 30 min at 30 °C. The reaction in a final volume of 20 μL was terminated by the addition of 3 μL of 7 mM EDTA. Reaction products were analyzed on 1% agarose gel at 25 V for 4 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated using AlphaImager™ (Alpha Innotech Corporation).

The added amount of DMSO for each reaction mixture was same in both of enzyme only and enzyme with designated compound for topo I and II assays.

For the evaluation of cytotoxicity, five different cancer cell lines were used: human breast adenocarcinoma cell line (MCF-7), human prostate tumor cell line (DU145), human cervix tumor cell line (HeLa), chronic myelogenous leukemia cell line (K562), and human colorectal adenocarcinoma cell line (HCT15). Experiments were performed by methods previously described [15]. Cancer cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of $2-4 \times 10^4$ cells per well and incubated for overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Hyclone, USA) in 5% CO_2 incubator at 37 °C. On day 2, culture medium in each well was exchanged with 0.1 mL aliquots of medium containing graded concentrations of compounds. On day 4, each well was added with 5 μL of the cell counting kit-8 solution (Dojindo, Japan) then incubated for additional 4 h under the same condition. The absorbance of each well was determined by an Automatic Elisa Reader System (Bio-Rad 3550) at 450 nm wavelength. For determination of the IC_{50} values, the absorbance readings at 450 nm were fitted to the four-parameter logistic equation. Adriamycin, etoposide, and camptothecin were purchased from Sigma and used as positive controls.

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