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Synthesis of 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives and evaluation of their topoisomerase I and II inhibitory activity, cytotoxicity, and structure–activity relationship

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ABSTRACT

A series of 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives were designed, synthesized, and evaluated for their topoisomerase I and II inhibition and cytotoxic activity against several human cancer cell lines. Compounds **10**–**19** showed moderate topoisomerase I and II inhibitory activity and **20**–**29** showed significant topoisomerase II inhibitory activity. Structure–activity relationship study revealed that 4-(5-chlorofuran-2-yl)-2-(thiophen-3-yl) moiety has an important role in displaying topoisomerase II inhibition.

1. Introduction

Topoisomerases have been one of the most promising targets for the design of various anticancer agents.[1] They are nuclear enzymes that play an important role in various key cellular processes such as replication, transcription, recombination, repair, and chromatin assembly.[2] They are basically classified into two major classes: type I enzymes that make single stranded cuts in DNA, and type II enzymes that cut and pass double stranded DNA to allow a cell to change its topology.[3] Drugs from the camptothecin family[4] and etoposide[5] are clinically used to target topoisomerase I (topo I) and topoisomerase II (topo II), respectively.

α-Terpyridine is able to form metal complexes[6] and binds with RNA/DNA[7] (Fig. 1). Our research group has reported that terpyridine derivatives show strong cytotoxicity against several human cancer cell lines and considerable topo I and II inhibitory activity.[8] From previous structure–activity relationship studies, we found that the 2-thienyl-4-furyl-pyridine skeleton exhibited considerable topo I and II inhibitory activity.[8b,g,h] In addition, substitution with chloride or methyl on thienyl and/or furyl moiety increased topoisomerase inhibitory activity.[8h] It would be very interesting to observe the difference of biological activities by systematic substitution on the 2-position of 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine skeletons with various aromatic groups, and to evaluate cytotoxicity and topoisomerase I and II inhibitory activities. In addition, we anticipated that we may obtain valuable information on the correlation between positions of 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine skeletons and biological activities such as cytotoxicity and topoisomerase I or II inhibitory activity, or on the effect of branching on furyl or phenyl groups such as methyl or chloride. In continuation of previous work,[8h] we designed and systematically prepared six different series of 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives with chloride or methyl substituents at different aryl moieties for the purpose of pursuing more potent topo I and II inhibitory activity as shown in Figure 1. The compounds were evaluated for topo I and II inhibitory activity and cytotoxicity against several human cancer cell lines.

2. Results and discussion

2.1. Chemistry

2-(Thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives were synthesized on the basis of previously reported methods[9,10]

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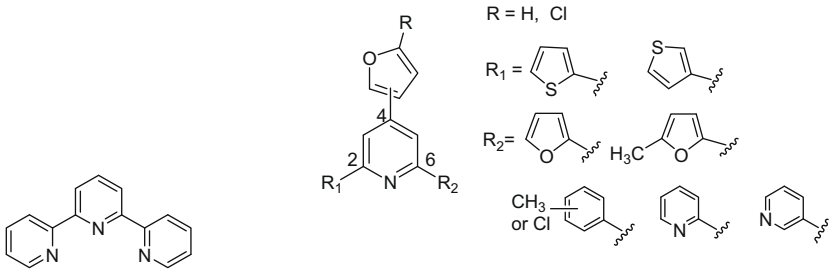
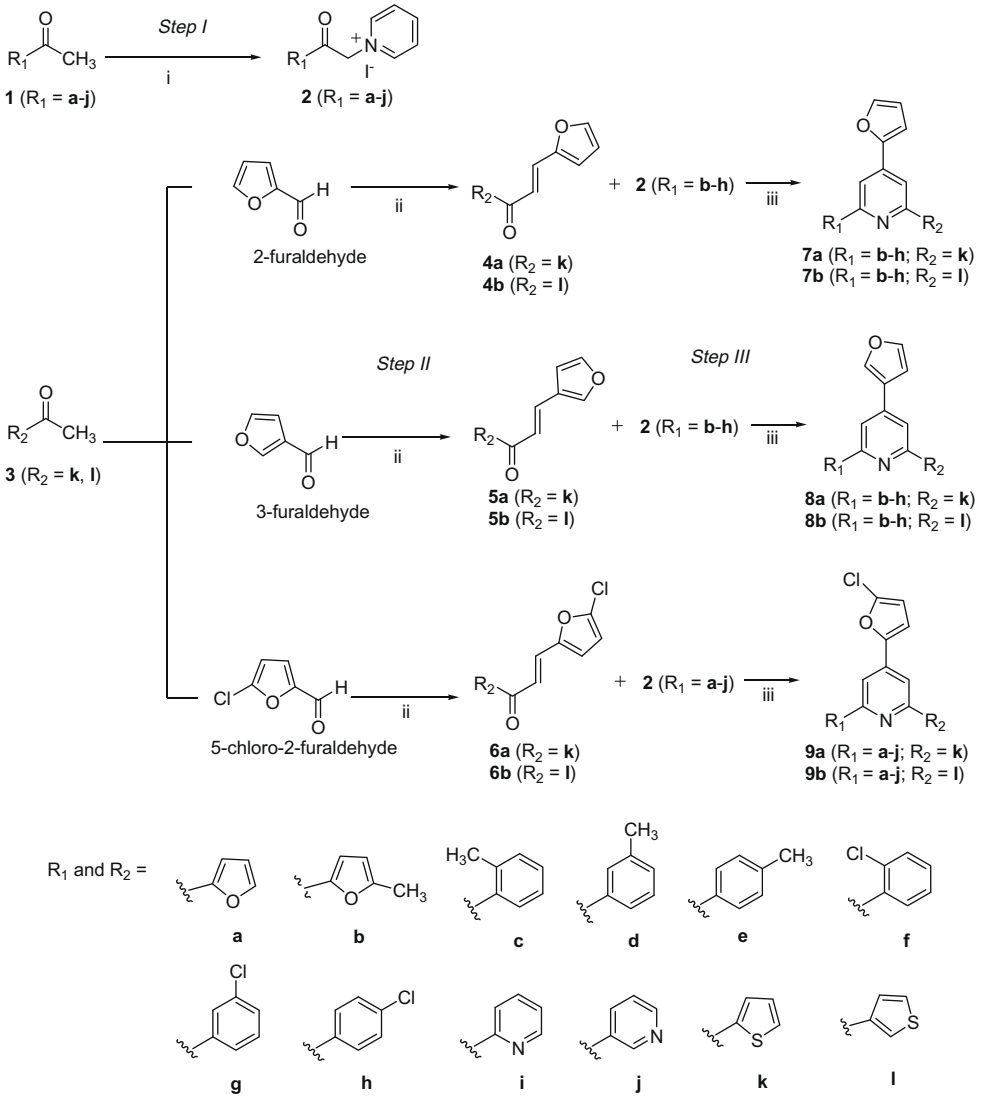


Figure 1. Structure of α-terpyridine and 2-thienyl-4-furyl-6-aryl pyridine derivatives.

as shown in Scheme 1. At first, 10 pyridinium iodide salts were prepared in 42.2–99.4% yield by refluxing 10 different aryl acetyls (**1a**–**j**) with iodine in pyridine. Propenone intermediates were synthesized by applying Claisen–Schmidt KOH catalyzed condensation reaction.[9] Two aryl ketones (**3k**, **l**) were reacted with three different aryl aldehydes to get six propenone intermediates (**4a**, **b**; **5a**, **b**; **6a**, **b**) in 74.2–91.8% yield. Finally, as per the modified Kröhnke synthesis method,[10] previously synthesized propenone intermediates and pyridinium iodide salts were reacted to give 2-thienyl-4-furyl-6-aryl pyridine derivatives in 15.1–82.4% yield. Only seven pyridinium salts **2** (R_1 = **b**–**h**) were employed for four series, **7a**, **7b**, **8a**, and **8b**, to give 28 compounds. Compounds for the remain-



Scheme 1. General synthetic scheme. Reagents and conditions: (i) pyridine, iodine (1.0 equiv), 3 h, 140 °C, 42.2–99.4%; (ii) aryl aldehydes (1.0 equiv), KOH (1.2 equiv), MeOH/H_2O (5:1), 1–3 h, 0 °C, 74.2–91.8%; (iii) NH_4OAc (10.0 equiv), dry methanol or glacial AcOH, 7–21 h, 80–100 °C, 15.1–82.4%.

ing pyridinium salts have already been reported by our research group.[8d] Similarly, 10 pyridinium salts **2** (R_1 = **a**–**j**) were used for two series, **9a**, **9b**, to give 20 compounds. As a whole, 48 compounds were synthesized. It was noticed that the yield was reduced when Cl or CH_3 substituents were placed at the ortho position of phenyl ring.[8h] The structures of compounds bearing considerable biological activities are displayed in Figure 2.

2.2. Topoisomerase I and II inhibitory activity

Forty-eight 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives were prepared and evaluated for their topo I and II inhibitory activity. Figure 3 and Table 1 illustrate the human DNA topo I inhibitory activity of 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives (**10**–**29**) with camptothecin as a positive control. Compounds **15**–**19** and **27** showed moderate topo I inhibitory activity at 100 μM whereas others had very low or no activity. Figure 4 and Table 1 illustrate the human DNA topo II inhibitory activity of 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives (**10**–**29**) with etoposide as a positive control. Compounds **20**–**29** showed significant topo II inhibitory activity both at 100 μM and 20 μM ranges from the 59.6–86.5% and 34.7–75.4% inhibition ratio, respectively. Compounds **10**–**19** showed moderate topo II inhibitory activity.

Structure–activity relationship study revealed that compounds **15**–**19**, which bore moderate topo I and II inhibitory activity, possessed 4-(5-chlorofuran-2-yl)-2-(thiophen-2-yl) moiety in common. Interestingly, compounds **20**–**29**, which bore 4-(5-chlorofuran-2-yl)-2-(thiophen-3-yl) moiety, all exhibited strong topo II inhibitory activity, whereas topo I inhibitory activity was reduced or completely lost, the exception being **27**. This suggest that 4-(5-chlorofuran-2-yl)-2-(thiophen-3-yl) moiety is important for displaying significant topo

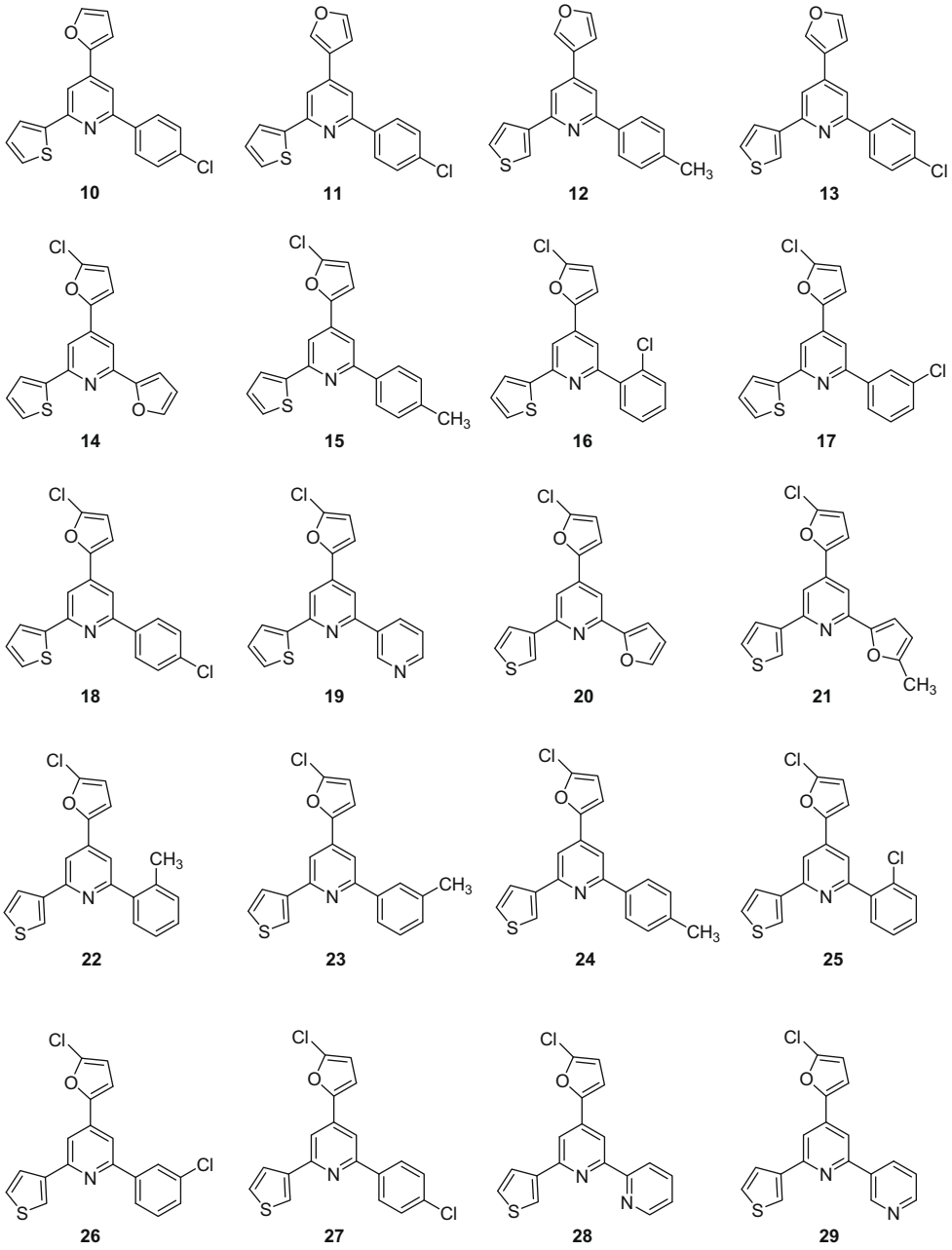
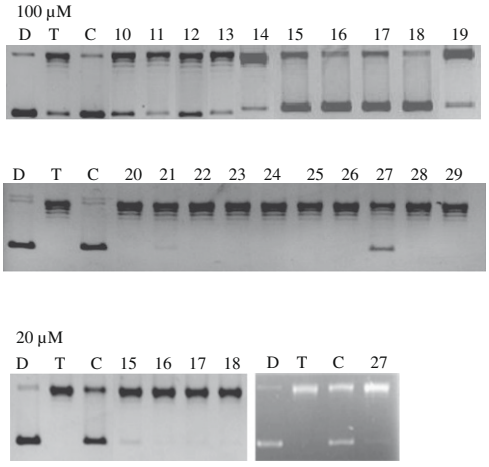


Figure 2. Structures of compounds displaying significant biological activities.



Lane D: pBR322 DNA only

Lane T: pBR322 DNA + Topo I

Lane C: pBR322 DNA + Topo I + Camptothecin

Lane 10-29: pBR322 DNA + Topo I + Compound **10-29** (100 μM)

Lane 15-18, 27: pBR322 DNA + Topo I + Compound **15-18**, **27** (20 μM)

Figure 3. Human DNA topoisomerase I inhibitory activity of compounds **10**–**29**.

II inhibitory activity (Fig. 5). Although the compounds with 4-(5-chlorofuran-2-yl)-2-(thiophen-2-yl) moiety showed moderate topo I and II inhibitory activity, further exploration on this moiety may provide a promising skeleton for the development of dual topo I and II inhibitors.

2.3. Cytotoxicity

The prepared 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives were evaluated for cytotoxicity on five different human cancer cell lines: human breast adenocarcinoma cell line (MCF-7), human cervix tumor cell line (HeLa), human prostate tumor cell line (DU145), human colorectal adenocarcinoma cell line (HCT15), and chronic myelogenous leukemia cell line (K562). Inhibitory activities were presented as micromolar concentrations of the compounds that cause 50% inhibition per unit of enzyme (IC_{50}) under the assay conditions and compared with that of adriamycin. Most of the compounds exhibited moderate cytotoxicity against human cancer cell lines (Table 1). Compounds **12**, **13**, **19**, and **24** showed the most significant cytotoxicity against HeLa, K562, HCT15, and MCF-7 cell lines, respectively, as compared to standard. Similarly, compounds **15**, **21**–**23**, **25**, **27** showed considerable cytotoxicity against MCF-7.

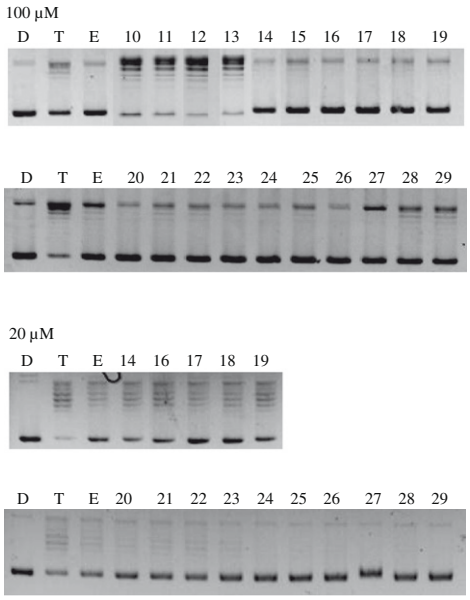
3. Conclusion

Forty-eight 2-(thienyl-2-yl or -3-yl)-4-furyl-6-aryl pyridine derivatives were designed and synthesized systematically in a total of six series by efficient synthetic routes and evaluated for topo I and II inhibitory activity and cytotoxicity against several human cancer cell lines. Structure–activity relationship study for topo inhibitory activities indicated that 4-(5-chlorofuran-2-yl)-2-(thiophen-3-yl) moiety was important to display significant topo II inhibitory activity. 4-(5-Chlorofuran-2-yl)-2-(thiophen-2-yl) moiety was also important to display moderate topo I and II inhibitory activity. Most of the compounds showed moderate cytotoxicity against several human cancer cell lines. However, we could not reach a conclusion on the concrete correlation between cytotoxic activity and topo I or II inhibitory activities, or between cytotoxic activity and structures of compounds. However, 4-(furan-3-yl)-2-(thiophen-3-yl), 4-(5-chlorofuran-2-yl)-2-(thiophen-3-yl), and 4-

Table 1
Inhibitory activity of compounds **10**–**29** against topoisomerase I and II (% inhibition ratio of relaxation) and their IC_{50} values against MCF-7, HeLa, DU145, HCT15 and K562 cell lines

Compound	% Inhibition				IC_{50}[a] (μM)				
	Topo I		Topo II		MCF-7	HeLa	DU145	HCT15	K562
	100 μM	20 μM	100 μM	20 μM					
10	2.7	NA	31.4	NA	24.60 ± 1.20	32.72 ± 10.65	>100	>100	>100
11	0	NA	30.2	NA	29.09 ± 2.34	5.53 ± 1.10	21.07 ± 9.21	50.01 ± 6.77	21.12 ± 3.54
12	13.2	NA	14.2	NA	9.32 ± 0.87	1.47 ± 0.07	13.01 ± 2.16	44.82 ± 5.20	47.81 ± 1.58
13	0	NA	20.5	NA	56.14 ± 20.30	4.14 ± 0.88	37.59 ± 9.13	>100	0.73 ± 0.04
14	16.0	NA	38.5	25.5	5.27 ± 0.90	18.80 ± 2.77	21.84 ± 4.59	>50	16.03 ± 3.38
15	41.8	9.3	21.8	NA	1.33 ± 0.40	16.3 ± 3.56	19.27 ± 3.68	9.89 ± 0.08	21.50 ± 0.94
16	41.3	8.0	35.0	15.4	1.93 ± 0.64	18.53 ± 2.14	16.82 ± 2.33	6.19 ± 2.98	17.18 ± 1.69
17	42.0	8.5	44.7	32.7	2.86 ± 1.67	12.76 ± 1.82	18.10 ± 2.72	15.50 ± 5.50	19.75 ± 3.70
18	38.9	6.3	42.5	38.1	2.45 ± 1.41	21.34 ± 2.94	19.18 ± 2.80	14.20 ± 3.51	20.88 ± 2.04
19	25.8	NA	51.5	30.9	2.87 ± 0.90	14.64 ± 3.42	12.98 ± 1.62	1.24 ± 0.15	22.30 ± 0.53
20	8.2	NA	78.6	63.8	21.28 ± 2.67	>50	18.86 ± 0.87	>50	22.35 ± 0.6
21	9.1	NA	62.6	34.7	1.42 ± 0.56	>50	18.50 ± 1.95	>50	23.29 ± 3.55
22	6.7	NA	59.6	46.3	1.23 ± 0.10	9.77 ± 2.73	24.76 ± 3.78	4.63 ± 1.78	18.38 ± 3.25
23	7.5	NA	64.1	56.7	1.28 ± 0.87	20.3 ± 2.72	17.92 ± 2.25	21.71 ± 2.37	20.16 ± 2.22
24	2.6	NA	78.9	70.2	0.63 ± 0.39	16.23 ± 4.27	21.23 ± 0.96	7.41 ± 1.45	19.86 ± 3.04
25	0.0	NA	64.1	59.5	1.71 ± 0.80	8.07 ± 1.88	19.30 ± 1.12	4.57 ± 0.79	17.24 ± 2.76
26	0.0	NA	75.9	75.4	4.26 ± 1.51	8.71 ± 2.89	10.42 ± 0.10	13.67 ± 0.97	23.04 ± 2.77
27	29.8	5.2	75.9	65.7	1.77 ± 0.56	8.41 ± 2.34	19.53 ± 3.30	5.19 ± 3.25	18.74 ± 2.73
28	0.0	NA	63.1	60.9	20.99 ± 1.08	22.46 ± 1.11	10.94 ± 1.38	>50	21.88 ± 1.52
29	0.0	NA	86.5	60.8	>50	>50	21.57 ± 1.42	>50	21.69 ± 2.49
Camptothecin (C)	75.3/81.7*	53.4/39.5*			0.05 ± 0.02	0.63 ± 0.02	0.94 ± 0.19	0.64 ± 0.03	0.08 ± 0.01
Etoposide (E)			51.0/55.1*	31.3/24.0*	1.66 ± 0.25	1.57 ± 0.43	2.45 ± 1.05	1.29 ± 0.53	2.84 ± 0.19
Adriamycin					1.25 ± 0.47	1.82 ± 0.84	1.98 ± 0.92	2.32 ± 1.35	2.45 ± 0.23

NA: not applicable.
[a] Each data represents mean ± S.D. from three different experiments performed in triplicate.
* Control value for compounds **20**–**29**.



Lane D: pBR322 DNA only

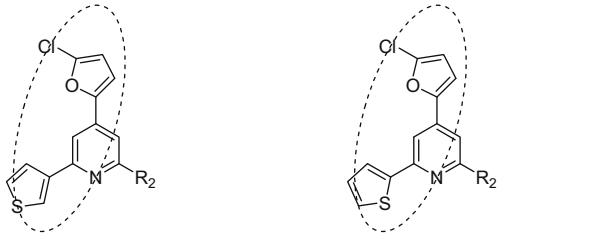
Lane T: pBR322 DNA + Topo II

Lane E: pBR322 DNA + Topo II + Etoposide

Lane 10-29: pBR322 DNA + Topo II + Compound **10-29** (100 μM)

Lane 14, 16-29: pBR322 DNA + Topo II + Compound **14**, **16-29** (20 μM)

Figure 4. Human DNA topoisomerase IIα inhibitory activity of compounds **10**–**29**.



4-(5-chlorofuran-2-yl)-2-(thiophen-3-yl) moiety

4-(5-chlorofuran-2-yl)-2-(thiophen-2-yl) moiety

Figure 5. Structure of 4-(5-chlorofuran-2-yl)-2-(thiophen-3-yl) and 4-(5-chlorofuran-2-yl)-2-(thiophen-2-yl) moiety.

(5-chlorofuran-2-yl)-2-(thiophen-2-yl) moieties, in combination with **e**, **h**, and **j** moieties, were important for cytotoxic effect. These results 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 Chemicals 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 contained aromatic rings, 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 to TMS (tetramethylsilane). 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 a gradient-controlled HPLC system with two Shimadzu LC-10AT pumps, equipped with Shimadzu system controller (SCL-10A VP) and photo diode array detector (SPD-M10A VP) that utilized the Shimadzu Class VP program. Sample volume of 10 μL was run in Waters X-Terra® 5 μM reverse-phase C_{18} column (4.6 × 250 mm) with a gradient elution of 75–100% of B in A for 10 min followed by 100–75% of B in A for 20 min at flow rate of 1.0 mL/min at 254 nm UV detection, where mobile phase A was double distilled water with 20 mM ammonium formate (AF), and B was 90% ACN in water with 20 mM AF. The purity of the compound is described as percent (%).

ESI LC/MS analyses were performed with a Finnigan LCQ Advantage® LC/MS/MS spectrometry that utilized the 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: (A) from 10% to 95% of B in A for 10 min followed by 95% to 10% of B in A for 10 min (B) from 70% to 90% of B in A for 6 min followed by 90% to 70% of B in A for 1 min and 70% of B in A for 8 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 temperature: 275 °C, capillary voltage: 27 V, tube lens offset: 45 V. Retention time is given in minutes.

4.1. General method for preparation of 2

A mixture of aryl acetyl **1**, iodine (1.0 equiv) and pyridine (50 mL) was refluxed at 140 °C for 3 h. The reaction mixture was cooled to 0 °C, the precipitate formed was filtered, washed with cold pyridine, and dried to get solid compound **2** in 42.2–99.4% yield. Following the same procedure, 10 compounds were synthesized.

4.2. General method for preparation of 4, 5, 6

To an ice cold solution of 85% KOH (1.2 equiv) in methanol (10 mL) and H_2O (2 mL), aryl acetyl **3** (1.0 equiv) was added. After dissolution, aryl aldehyde (1.0 equiv) was added slowly. The reaction mixture was then stirred for 1–3 h at 0 °C. The precipitation formed was filtered, washed with cold MeOH, and dried to get solid compound **4**, **5**, and **6** in 74.2–91.8% yield. Following the same procedure, 6 compounds were synthesized.

4.2.1. 3-(Furan-2-yl)-1-(thiophen-2-yl)-propenone (4a)

The same procedure described in Section 4.2 was employed with **3** (R_2 = **k**) and 2-furaldehyde to yield yellow solid (81.1%).

R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.30; mp 68.2–69.3 °C.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.83 (dd, J = 3.8, 1.0 Hz, 1H, 1-thiophene H-3), 7.64 (dd, J = 4.8, 1.0 Hz, 1H, 1-thiophene H-5), 7.58 (d, J = 15.3 Hz, 1H, –CO–C═C*H*–), 7.51 (d, J = 1.4 Hz, 1H, 3-furan H-5), 7.30 (d, J = 15.3 Hz, 1H, –CO–*CH*═C–), 7.15 (dd, J = 4.9, 3.9 Hz, 1H, 1-thiophene H-4), 6.71 (d, J = 3.4 Hz, 1H, 3-furan H-3), 6.50 (dd, J = 3.4, 1.8 Hz, 1H, 3-furan H-4).

4.2.2. 3-(Furan-2-yl)-1-(thiophen-3-yl)-propenone (4b)

The same procedure described in Section 4.2 was employed with **3** (R_2 = **l**) and 2-furaldehyde to yield a yellow solid (82.5%).

R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.30; mp 51.7–52.1 °C.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.15 (dd, J = 2.8, 1.2 Hz, 1H, 1-thiophene H-2), 7.63 (dd, J = 5.2, 1.2 Hz, 1H, 1-thiophene H-4), 7.56 (d,

J = 15.3 Hz, 1H, –CO–C=C*H*–), 7.50 (br, 1H, 3-furan H-5), 7.32 (dd, *J* = 5.2, 2.8 Hz, 1H, 1-thiophene H-5), 7.29 (d, *J* = 15.3 Hz, 1H, –CO–C*H*=C–), 6.69 (d, *J* = 3.4 Hz, 1H, 3-furan H-3), 6.49 (dd, *J* = 3.4, 1.8 Hz, 1H, 3-furan H-4).

4.2.3. 3-(Furan-3-yl)-1-(thiophen-2-yl)-propenone (5a)

The same procedure described in Section 4.2 was employed with **3** (R_2 = **k**) and 3-furaldehyde to yield a creamy white solid (74.2%).

R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.30; mp 83.3–84.5 °C.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.81 (d, *J* = 3.8 Hz, 1H, 1-thiophene H-3), 7.73 (d, *J* = 15.4 Hz, 1H, –CO–C=C*H*–), 7.72 (br, 1H, 3-furan H-2), 7.65 (d, *J* = 4.8 Hz, 1H, 1-thiophene H-5), 7.45 (br, 1H, 3-furan H-5), 7.17 (dd, *J* = 5.7, 2.9 Hz, 1H, 1-thiophene H-4), 7.11 (d, *J* = 15.7 Hz, 1H, –CO–C*H*=C–), 6.68 (br, 1H, 3-furan H-4).

4.2.4. 3-(Furan-3-yl)-1-(thiophen-3-yl)-propenone (5b)

The same procedure described in Section 4.2 was employed with **3** (R_2 = **l**) and 3-furaldehyde to yield a creamy white solid (79.5%).

R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.35; mp 96.3–97.4 °C.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.11 (dd, *J* = 2.8, 1.2 Hz, 1H, 1-thiophene H-2), 7.70 (d, *J* = 15.4 Hz, 1H, –CO–C=C*H*–), 7.71 (br, 1H, 3-furan H-2), 7.63 (d, *J* = 5.0 Hz, 1H, 1-thiophene H-4), 7.45 (br, 1H, 3-furan H-5), 7.33 (dd, *J* = 5.0, 2.8 Hz, 1H, 1-thiophene H-5), 7.09 (d, *J* = 15.5 Hz, 1H, –CO–C*H*=C), 6.67 (br, 1H, 3-furan H-4).

4.2.5. 3-(5-Chlorofuran-2-yl)-1-(thiophen-2-yl)-propenone (6a)

The same procedure described in Section 4.2 was employed with **3** (R_2 = **k**) and 5-chloro-2-furaldehyde to yield a yellow solid (91.8%).

R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.33; mp 109.4–110.5 °C.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 3.8, 1.1 Hz, 1H, 1-thiophene H-3), 7.69 (dd, *J* = 4.9, 1.1 Hz, 1H, 1-thiophene H-5), 7.49 (d, *J* = 15.1 Hz, 1H, –CO–C=C*H*–), 7.29 (d, *J* = 15.0 Hz, 1H, –CO–C*H*=C–), 7.18 (dd, *J* = 4.9, 3.8 Hz, 1H, 1-thiophene H-4), 6.69 (d, *J* = 3.5 Hz, 1H, 3-furan H-3), 6.32 (d, *J* = 3.5 Hz, 1H, 3-furan H-4).

4.2.6. 3-(5-Chlorofuran-2-yl)-1-(thiophen-3-yl)-propenone (6b)

The same procedure described in Section 4.2 was employed with **3** (R_2 = **l**) and 5-chloro-2-furaldehyde to yield a yellow solid (90.8%).

R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.33; mp 109.8–110.8 °C.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.19 (dd, *J* = 2.9, 1.2 Hz, 1H, 1-thiophene H-2), 7.67 (dd, *J* = 5.1, 1.2 Hz, 1H, 1-thiophene H-4), 7.47 (d, *J* = 15.3 Hz, 1H, –CO–C=C*H*–), 7.36 (dd, *J* = 5.1, 2.9 Hz, 1H, 1-thiophene H-5), 7.29 (d, *J* = 15.3 Hz, 1H, –CO–C*H*=C–), 6.68 (d, *J* = 3.5 Hz, 1H, 3-furan H-3), 6.31 (d, *J* = 3.5 Hz, 1H, 3-furan H-4).

4.3. General method for preparation of 7, 8, 9

A mixture of **2** (R_1 = **a**–**j**), **4**, **5** or **6** (R_2 = **k**, **l**), and dry ammonium acetate in glacial acetic acid or dry methanol was heated to 80–100 °C for 7–21 h under nitrogen gas. The solvent was evaporated and the residue was extracted with ethyl acetate (80 mL), washed with water (50 mL × 3) and saturated NaCl solution (30 mL), and dried over $MgSO_4$. After filtration, the filtrate was concentrated and purified by silica gel column chromatography with a gradient elution of ethyl acetate/*n*-hexane to afford a white or yellow solid compound **7**, **8**, or **9** in 15.1–82.4% yield. Following the same procedure, 48 compounds were synthesized.

4.3.1. 2-(4-Chlorophenyl)-4-(furan-2-yl)-6-(thiophen-2-yl) pyridine (10)

The same procedure described in Section 4.3 was employed with **4a** (0.61 g, 3.00 mmol), dry ammonium acetate (2.31 g, 30.00 mmol), **2** (R_1 = **h**) (1.07 g, 3.00 mmol), and glacial acetic acid (5.00 mL) to yield a white solid (204 mg, 20.1%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.26; mp 125.8–127.2 °C, purity: 100%.

LC/MS/MS (condition A): retention time: 13.76 min; [MH^+]: 338.2 (100%), [MH+2]: 340.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.10 (dd, *J* = 8.5, 2.0 Hz, 2H, 2-phenyl H-2, H-6), 7.81 (s, 1H, pyridine H-3), 7.80 (s, 1H, pyridine H-5), 7.71 (dd, *J* = 3.7, 1.0 Hz, 1H, 6-thiophene H-3), 7.59 (dd, *J* = 1.7, 0.6 Hz, 1H, 4-furan H-5), 7.47 (dd, *J* = 8.5, 2.0 Hz, 2H, 2-phenyl H-3, H-5), 7.43 (dd, *J* = 5.0, 1.0 Hz, 1H, 6-thiophene H-5), 7.15 (dd, *J* = 5.0, 3.7 Hz, 1H, 6-thiophene H-4), 6.97 (d, *J* = 3.4 Hz, 1H, 4-furan H-3), 6.57 (dd, *J* = 3.4, 1.7 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.46, 153.26, 151.88, 145.51, 144.22, 139.53, 137.70, 135.71, 129.28, 128.66, 128.44, 128.28, 125.23, 112.80, 112.62, 111.80, 109.26.

4.3.2. 2-(4-Chlorophenyl)-4-(furan-3-yl)-6-(thiophen-2-yl) pyridine (11)

The same procedure described in Section 4.3 was employed with **5a** (0.51 g, 2.50 mmol), dry ammonium acetate (1.92 g, 25.00 mmol), **2** (R_1 = **h**) (0.90 g, 2.50 mmol), and glacial acetic acid (5.00 mL) to yield a white solid (428 mg, 50.7%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.29; mp 119.2–120.0 °C, purity: 96.9%.

LC/MS/MS (condition A): retention time: 13.24 min; [MH^+]: 338.2 (100%), [MH+2]: 340.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 8.7, 1.9 Hz, 2H, 2-phenyl H-2, H-6), 7.95 (dd, *J* = 1.3, 1.0 Hz, 1H, 4-furan H-2), 7.67 (dd, *J* = 3.7, 1.0 Hz, 1H, 6-thiophene H-3), 7.63 (d, *J* = 1.3 Hz, 1H, pyridine H-3), 7.61 (d, *J* = 1.3 Hz, 1H, pyridine H-5), 7.55 (t, *J* = 1.8 Hz, 1H, 4-furan H-5), 7.45 (dd, *J* = 8.7, 2.0 Hz, 2H, 2-phenyl H-3, H-5), 7.42 (dd, *J* = 5.0, 1.0 Hz, 1H, 6-thiophene H-5), 7.13 (dd, *J* = 5.0, 3.7 Hz, 1H, 6-thiophene H-4), 6.81 (dd, *J* = 1.8, 0.9 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.15, 152.91, 145.08, 144.38, 141.70, 140.40, 137.35, 135.29, 128.86, 128.24, 127.97, 127.83, 124.72, 124.59, 115.05, 114.03, 108.46.

4.3.3. 4-(Furan-3-yl)-2-(thiophen-3-yl)-6-p-tolyl pyridine (12)

The same procedure described in Section 4.3 was employed with **5b** (1.02 g, 5.00 mmol), dry ammonium acetate (3.85 g, 50.00 mmol), **2** (R_1 = **e**) (1.69 g, 5.00 mmol), and methanol (30.00 mL) to yield a white solid (912 mg, 57.5%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.35; mp 93.2–94.1 °C, purity: 97.9%.

LC/MS/MS (condition A): retention time: 13.50 min; [MH^+]: 318.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.9 Hz, 2H, 6-phenyl H-2, H-6), 8.02 (br, 1H, 2-thiophene H-2), 7.94 (br, 1H, 4-furan H-2), 7.79 (dd, *J* = 5.0, 1.0 Hz, 1H, 2-thiophene H-4), 7.65 (d, *J* = 1.1 Hz, 1H, pyridine H-5), 7.58 (d, *J* = 1.1 Hz, 1H, pyridine H-3), 7.54 (t, *J* = 1.5 Hz, 1H, 4-furan H-5), 7.40 (dd, *J* = 5.0, 3.0 Hz, 1H, 2-thiophene H-5), 7.30 (d, *J* = 7.9 Hz, 2H, 6-phenyl H-3, H-5), 6.82 (d, *J* = 0.9 Hz, 1H, 4-furan H-4), 2.41 (s, 3H, 6-phenyl 4-CH_3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.50, 153.58, 144.23, 142.44, 141.33, 140.20, 139.06, 136.52, 129.36, 126.85, 126.41, 126.10, 124.84, 123.73, 115.09, 115.03, 108.49, 21.31.

4.3.4. 2-(4-Chlorophenyl)-4-(furan-3-yl)-6-(thiophen-3-yl) pyridine (13)

The same procedure described in Section 4.3 was employed with **5b** (1.02 g, 5.00 mmol), dry ammonium acetate (3.85 g, 50.00 mmol), **2** (R_1 = **h**) (1.79 g, 5.00 mmol), and methanol (30.00 mL) to yield a white solid (683 mg, 40.4%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.36; mp 130.8–132.0 °C, purity: 100%.

LC/MS/MS (condition A): retention time: 13.86 min; [MH^+]: 338.2 (100%), [MH+2]: 340.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.08 (d, J = 8.6 Hz, 2H, 2-phenyl H-2, H-6), 8.02 (dd, J = 2.9, 1.0 Hz, 1H, 6-thiophene H-2), 7.94 (s, 1H, 4-furan H-2), 7.76 (dd, J = 5.0, 0.8 Hz, 1H, 6-thiophene H-4), 7.63 (s, 1H, pyridine H-3), 7.61 (s, 1H, pyridine H-5), 7.55 (t, J = 1.5 Hz, 1H, 4-furan H-5), 7.46 (d, J = 8.6 Hz, 2H, 2-phenyl H-3, H-5), 7.40 (dd, J = 5.0, 3.0 Hz, 1H, 6-thiophene H-5), 6.81 (br, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.27, 153.77, 144.36, 142.17, 141.63, 140.31, 137.73, 135.13, 128.82, 128.26, 126.34, 126.27, 124.66, 123.93, 115.58, 115.07, 108.44.

4.3.5. 4-(5-Chlorofuran-2-yl)-2-(furan-2-yl)-6-(thiophen-2-yl) pyridine (14)

The same procedure described in Section 4.3 was employed with **6a** (0.59 g, 2.50 mmol), dry ammonium acetate (1.92 g, 25.00 mmol), **2** (R_1 = **a**) (0.78 g, 2.50 mmol), and glacial acetic acid (5.00 mL) to yield a white solid (336 mg, 41.0%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.30; mp 142.5–143.5 °C, purity: 100%.

LC/MS/MS (condition B): retention time: 6.49 min; [MH^+]: 328.2 (100%), [MH+2]: 330.1 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.69 (d, J = 1.2 Hz, 1H, pyridine H-3), 7.67 (dd, J = 3.5, 1.1 Hz, 1H, 6-thiophene H-3), 7.65 (d, J = 1.4 Hz, 1H, pyridine H-5), 7.54 (dd, J = 1.7, 0.8 Hz, 1H, 2-furan H-5), 7.41 (dd, J = 5.0, 1.1 Hz, 1H, 6-thiophene H-5), 7.19 (dd, J = 3.4, 0.7 Hz, 1H, 2-furan H-3), 7.12 (dd, J = 5.0, 3.7 Hz, 1H, 6-thiophene H-4), 6.93 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.54 (dd, J = 3.4, 1.7 Hz, 1H, 2-furan H-4), 6.33 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.43, 152.92, 150.88, 149.53, 144.67, 143.37, 138.13, 137.79, 127.96, 127.88, 125.00, 112.14, 110.79, 110.41, 110.11, 109.42, 108.87.

4.3.6. 4-(5-Chlorofuran-2-yl)-2-(thiophen-2-yl)-6-p-tolyl pyridine (15)

The same procedure described in Section 4.3 was employed with **6a** (0.47 g, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **2** (R_1 = **e**) (0.67 g, 2.00 mmol), and glacial acetic acid (5.00 mL) to yield a white solid (106 mg, 15.1%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.34; mp 121.7–122.3 °C, purity: 99.1%.

LC/MS/MS (condition B): retention time: 8.80 min; [MH^+]: 352.2 (100%), [MH+2]: 354.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.05 (d, J = 8.2 Hz, 2H, 6-phenyl H-2, H-6), 7.74 (d, J = 1.2 Hz, 1H, pyridine H-5), 7.71 (dd, J = 3.4, 1.4 Hz, 1H, 2-thiophene H-3), 7.70 (d, J = 2.0 Hz, 1H, pyridine H-3), 7.42 (dd, J = 5.0, 1.0 Hz, 1H, 2-thiophene H-5), 7.30 (d, J = 8.2 Hz, 2H, 6-phenyl H-3, H-5), 7.14 (dd, J = 5.0, 3.7 Hz, 1H, 2-thiophene H-4), 6.93 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.35 (d, J = 3.5 Hz, 1H, 4-furan H-4), 2.42 (s, 3H, 6-phenyl 4-CH_3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.38, 152.74, 151.19, 145.27, 139.35, 137.97, 137.87, 135.89, 129.41, 127.93, 127.76, 126.81, 124.71, 111.71, 110.47, 110.33, 108.82, 21.34.

4.3.7. 4-(5-Chlorofuran-2-yl)-2-(2-chlorophenyl)-6-(thiophen-2-yl) pyridine (16)

The same procedure described in Section 4.3 was employed with **6a** (0.71 g, 3.00 mmol), dry ammonium acetate (2.31 g, 30.00 mmol), **2** (R_1 = **f**) (1.07 g, 3.00 mmol), and glacial acetic acid (5.00 mL) to yield a light yellow solid (172 mg, 15.4%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.31; mp 122.0–123.0 °C, purity: 100%.

LC/MS/MS (condition B): retention time: 7.44 min; [M^+]: 372.1 (100%), [M+2]: 374.1 (38%), [M+4]: 376.1 (15%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.80 (d, J = 1.4 Hz, 1H, pyridine H-3), 7.74–7.71 (m, 1H, 2-phenyl H-6), 7.73 (dd, J = 3.7, 1.1 Hz, 1H, 6-thiophene H-3), 7.69 (d, J = 1.4 Hz, 1H, pyridine H-5), 7.51–7.48 (m, 1H, 2-phenyl H-3), 7.41 (dd, J = 5.1, 1.1 Hz, 1H, 6-thiophene H-5), 7.39–7.34 (m, 2H, 2-phenyl H-4, H-5), 7.14 (dd, J = 5.1, 3.7 Hz, 1H, 6-thiophene H-4), 6.92 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.35 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.02, 153.01, 150.88, 144.67, 138.70, 138.21, 137.14, 132.28, 131.77, 130.22, 129.74, 127.99, 127.87, 127.01, 125.03, 116.70, 110.95, 110.82, 108.89.

4.3.8. 4-(5-Chlorofuran-2-yl)-2-(3-chlorophenyl)-6-(thiophen-2-yl) pyridine (17)

The same procedure described in Section 4.3 was employed with **6a** (0.47 g, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **2** (R_1 = **g**) (0.72 g, 2.00 mmol), and glacial acetic acid (5.00 mL) to yield a white solid (298 mg, 40.0%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.29; mp 147.0–147.8 °C, purity: 99.3%.

LC/MS/MS (condition B): retention time: 9.40 min; [M^+]: 372.1 (100%), [M+2]: 374.1 (38%), [M+4]: 376.1 (15%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.12 (br, 1H, 2-phenyl H-2), 8.03–7.99 (m, 1H, 2-phenyl H-6), 7.74 (d, J = 5.9 Hz, 1H, 6-thiophene H-3), 7.72 (d, J = 1.3 Hz, 2H, pyridine H-3, H-5), 7.45–7.41 (m, 2H, 2-phenyl H-4, H-5), 7.43 (dd, J = 4.3, 1.1 Hz, 1H, 6-thiophene H-5), 7.15 (dd, J = 4.9, 3.7 Hz, 1H, 6-thiophene H-4), 6.95 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.36 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 155.87, 152.99, 150.81, 144.77, 140.46, 138.27, 138.10, 134.76, 129.92, 129.25, 128.05, 128.02, 127.08, 125.03, 112.04, 111.08, 110.82, 108.92.

4.3.9. 4-(5-Chlorofuran-2-yl)-2-(4-chlorophenyl)-6-(thiophen-2-yl) pyridine (18)

The same procedure described in Section 4.3 was employed with **6a** (0.47 g, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **2** (R_1 = **h**) (0.72 g, 2.00 mmol), and glacial acetic acid (5.00 mL) to yield a white solid (190 mg, 25.5%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.32; mp 134.1–134.9 °C, purity: 99.6%.

LC/MS/MS (condition B): retention time: 9.44 min; [M^+]: 372.1 (100%), [M+2]: 374.1 (38%), [M+4]: 376.1 (15%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.09 (d, J = 8.7 Hz, 2H, 2-phenyl H-2, H-6), 7.73 (d, J = 1.2 Hz, 1H, pyridine H-3), 7.72 (d, J = 1.2 Hz, 1H, pyridine H-5), 7.70 (dd, J = 4.2, 1.1 Hz, 1H, 6-thiophene H-3), 7.46 (d, J = 8.7 Hz, 2H, 2-phenyl H-3, H-5), 7.42 (dd, J = 5.1, 1.1 Hz, 1H, 6-thiophene H-5), 7.15 (dd, J = 5.1, 3.7 Hz, 1H, 6-thiophene H-4), 6.94 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.36 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.12, 152.92, 150.88, 144.88, 138.21, 138.06, 137.07, 135.39, 128.86, 128.21, 128.01, 127.99, 124.93, 111.74, 110.77, 110.74, 108.90.

4.3.10. 4-(5-Chlorofuran-2-yl)-6-(thiophen-2-yl)-2,3′-bipyridine (19)

The same procedure described in Section 4.3 was employed with **6a** (0.32 g, 1.35 mmol), dry ammonium acetate (1.04 g, 13.50 mmol), **2** (R_1 = **j**) (0.44 g, 1.35 mmol), and glacial acetic acid (4.00 mL) to yield a yellow solid (181 mg, 39.6%).

R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.24; mp 178.6–179.6 °C, purity: 100%.

LC/MS/MS (condition B): retention time: 5.23 min; [MH^+]: 339.2 (100%), [MH+2]: 341.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.33 (d, J = 1.7 Hz, 1H, 2-pyridine H-2′), 8.68 (d, J = 4.7 Hz, 1H, 2-pyridine H-6′), 8.46 (td, J = 8.0, 1.7 Hz, 1H, pyridine H-4′), 7.78 (d, J = 0.8 Hz, 1H, pyridine H-3), 7.77 (d, J = 0.8 Hz, 1H, pyridine H-5), 7.73 (dd, J = 3.7, 0.9 Hz, 1H, 6-thio-

phene H-3), 7.45 (dd, J = 5.1, 1.0 Hz, 1H, 6-thiophene H-5), 7.43 (t, J = 7.8 Hz, 1H, pyridine H-5′), 7.16 (dd, J = 4.9, 3.7 Hz, 1H, 6-thiophene H-4), 6.97 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.37 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 154.88, 153.32, 150.71, 150.18, 148.33, 144.65, 138.23, 134.43, 134.24, 128.20, 128.06, 125.14, 123.55, 112.01, 111.23, 110.98, 108.97.

4.3.11. 4-(5-Chlorofuran-2-yl)-2-(furan-2-yl)-6-(thiophen-3-yl) pyridine (20)

The same procedure described in Section 4.3 was employed with **6b** (0.31 g, 1.30 mmol), dry ammonium acetate (1.00 g, 13.00 mmol), **2** (R_1 = **a**) (0.40 g, 1.30 mmol), and glacial acetic acid (3.00 mL) to yield a white solid (351 mg, 82.4%).

R_f (ethyl acetate/n-hexane 1:10 v/v): 0.34; mp 155.5–156.3 °C, purity: 99.7%.

LC/MS/MS (condition B) retention time: 6.23 min; [MH^+]: 328.1 (100%), [MH+2]: 330.1 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.03 (dd, J = 3.0, 1.2 Hz, 1H, 6-thiophene H-2), 7.77 (dd, J = 5.2, 1.2 Hz, 1H, 6-thiophene H-4), 7.75 (d, J = 1.3 Hz, 1H, pyridine H-3), 7.65 (d, J = 1.3 Hz, 1H, pyridine H-5), 7.56 (dd, J = 1.6, 0.7 Hz, 1H, 2-furan H-5), 7.42 (dd, J = 5.0, 3.0 Hz, 1H, 6-thiophene H-5), 7.20 (d , J = 3.4 Hz, 1H, 2-furan H-3), 6.94 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.57 (dd, J = 3.4, 1.75 Hz, 1H, 2-furan H-4), 6.35 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.84, 153.73, 151.06, 149.68, 143.29, 141.92, 138.04, 137.82, 126.36, 126.21, 124.13, 112.10, 111.98, 110.63, 110.15, 109.12, 108.84.

4.3.12. 4-(5-Chlorofuran-2-yl)-2-(5-methylfuran-2-yl)-6-(thiophen-3-yl) pyridine (21)

The same procedure described in Section 4.3 was employed with **6b** (0.31 g, 1.30 mmol), dry ammonium acetate (1.00 g, 13.00 mmol), **2** (R_1 = **b**) (0.42 g, 1.30 mmol), and glacial acetic acid (3.00 mL) to yield a white solid (228 mg, 51.3%).

R_f (ethyl acetate/n-hexane 1:10 v/v): 0.37; mp 129.7–130.5 °C, purity: 100%.

LC/MS/MS (condition B): retention time: 7.30 min; [MH^+]: 342.1 (100%), [MH+2]: 344.1 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.02 (dd, J = 3.0, 1.2 Hz, 1H, 6-thiophene H-2), 7.75 (dd, J = 5.1, 1.2 Hz, 1H, 6-thiophene H-4), 7.68 (d, J = 1.4 Hz, 1H, pyridine H-3), 7.61 (d, J = 1.4 Hz, 1H, pyridine H-5), 7.41 (dd, J = 5.1, 3.0 Hz, 1H, 6-thiophene H-5), 7.09 (d, J = 3.2 Hz, 1H, 2-furan H-3), 6.94 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.34 (d, J = 3.5 Hz, 1H, 4-furan H-4), 6.15 (dd, J = 3.2, 0.9 Hz, 1H, 2-furan H-4), 2.43 (s, 3H, 2-furan 5-CH_3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.75, 153.59, 152.11, 151.22, 149.87, 142.05, 137.90, 137.73, 126.38, 126.13, 124.03, 111.49, 110.52, 110.28, 109.64, 108.79, 108.39, 13.96.

4.3.13. 4-(5-Chlorofuran-2-yl)-2-(thiophen-3-yl)-6-o-tolyl pyridine (22)

The same procedure described in Section 4.3 was employed with **6b** (0.47 g, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **2** (R_1 = **c**) (0.67 g, 2.00 mmol), and glacial acetic acid (3.00 mL) to yield a white solid (171 mg, 24.3%).

R_f (ethyl acetate/n-hexane 1:10 v/v): 0.35; mp 110.7–111.4 °C, purity: 98.9%.

LC/MS/MS (condition B): retention time: 7.59 min; [MH^+]: 352.2 (100%), [MH+2]: 354.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.01 (dd, J = 3.0, 1.2 Hz, 1H, 2-thiophene H-2), 7.76 (dd, J = 4.9, 1.2 Hz, 1H, 2-thiophene H-4), 7.75 (d, J = 1.3 Hz, 1H, pyridine H-3), 7.51–7.47 (m, 1H, 6-phenyl H-6), 7.46 (d, J = 1.3 Hz, 1H, pyridine H-5), 7.40 (dd, J = 5.0, 3.0 Hz, 1H, 2-thiophene H-5), 7.34–7.28 (m, 3H, 6-phenyl H-3, H-4, H-5), 6.90 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.34 (d, J = 3.5 Hz, 1H, 4-furan H-4), 2.48 (s, 3H, 6-phenyl 2-CH_3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 160.54, 153.35, 151.17, 142.15, 140.20, 138.00, 137.50, 136.21, 130.89, 129.58, 128.43, 126.40, 126.17, 125.88, 124.04, 115.75, 111.66, 110.44, 108.81, 20.60.

4.3.14. 4-(5-Chlorofuran-2-yl)-2-(thiophen-3-yl)-6-m-tolyl pyridine (23)

The same procedure described in Section 4.3 was employed with **6b** (0.23 g, 1.00 mmol), dry ammonium acetate (0.77 g, 10.00 mmol), **2** (R_1 = **d**) (0.34 g, 1.00 mmol), and glacial acetic acid (2.50 mL) to yield a white solid (162 mg, 46.0%).

R_f (ethyl acetate/n-hexane 1:10 v/v): 0.35; mp 121.7–122.5 °C, purity: 99.8%.

LC/MS/MS (condition B): retention time: 8.56 min; [MH^+]: 352.2 (100%), [MH+2]: 354.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.07 (dd, J = 3.0, 1.2 Hz, 1H, 2-thiophene H-2), 7.96 (br, 1H, 6-phenyl H-2), 7.94 (d, J = 8.2 Hz, 1H, 6-phenyl H-6), 7.81 (dd, J = 5.0, 1.2 Hz, 1H, 2-thiophene H-4), 7.77 (d, J = 1.3 Hz, 1H, pyridine H-5), 7.71 (d, J = 1.3 Hz, 1H, pyridine H-3), 7.42 (dd, J = 5.0, 3.0 Hz, 1H, 2-thiophene H-5), 7.39 (t, J = 7.6 Hz, 1H, 6-phenyl H-5), 7.25 (d, J = 7.6 Hz, 1H, 6-phenyl H-4), 6.94 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.35 (d, J = 3.5 Hz, 1H, 4-furan H-4), 2.47 (s, 3H, 6-phenyl 3-CH_3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.79, 153.75, 151.34, 142.29, 139.12, 138.29, 137.95, 137.92, 129.96, 128.57, 127.68, 126.45, 126.15, 124.15, 124.02, 112.20, 112.15, 110.37, 108.81, 21.60.

4.3.15. 4-(5-Chlorofuran-2-yl)-2-(thiophen-3-yl)-6-p-tolyl pyridine (24)

The same procedure described in Section 4.3 was employed with **6b** (0.31 g, 1.30 mmol), dry ammonium acetate (1.00 g, 13.00 mmol), **2** (R_1 = **e**) (0.44 g, 1.30 mmol), and glacial acetic acid (3.00 mL) to yield a white solid (198 mg, 43.3%).

R_f (ethyl acetate/n-hexane 1:10 v/v): 0.39; mp 147.2–148.0 °C, purity: 100%.

LC/MS/MS (condition B): retention time: 8.67 min; [MH^+]: 352.2 (100%), [MH+2]: 354.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.06 (br, 1H, 2-thiophene H-2), 8.04 (d, J = 8.3 Hz, 2H, 6-phenyl H-2, H-6), 7.82 (dd, J = 5.0, 1.1 Hz, 1H, 2-thiophene H-4), 7.76 (d, J = 1.1 Hz, 1H, pyridine H-5), 7.69 (d, J = 1.1 Hz, 1H, pyridine H-3), 7.42 (dd, J = 5.0, 3.0 Hz, 1H, 2-thiophene H-5), 7.30 (d, J = 8.3 Hz, 2H, 6-phenyl H-3, H-5), 6.92 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.34 (d, J = 3.5 Hz, 1H, 4-furan H-4), 2.42 (s, 3H, 6-phenyl 4-CH_3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.57, 153.67, 151.39, 142.33, 139.22, 137.90, 136.35, 129.38, 126.86, 126.44, 126.11, 123.95, 111.92, 111.78, 110.30, 108.78, 21.31.

4.3.16. 4-(5-Chlorofuran-2-yl)-2-(2-chlorophenyl)-6-(thiophen-3-yl) pyridine (25)

The same procedure described in Section 4.3 was employed with **6b** (0.41 g, 1.75 mmol), dry ammonium acetate (1.34 g, 17.50 mmol), **2** (R_1 = **f**) (0.63 g, 1.75 mmol), and glacial acetic acid (3.00 mL) to yield a white solid (249 mg, 38.3%).

R_f (ethyl acetate/n-hexane 1:10 v/v): 0.35; mp 101.0–101.6 °C, purity: 100%.

LC/MS/MS (condition B): retention time: 7.47 min; [M^+]: 372.1 (100%), [M+2]: 374.1 (71%), [M+4]: 376.1 (15%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.02 (dd, J = 3.0, 1.3 Hz, 1H, 6-thiophene H-2), 7.78 (d, J = 1.4 Hz, 1H, pyridine H-3), 7.75 (dd, J = 5.1, 1.3 Hz, 1H, 6-thiophene H-4), 7.72–7.69 (m, 1H, 2-phenyl H-6), 7.68 (d, J = 1.3 Hz, 1H, pyridine H-5), 7.52–7.48 (m, 1H, 2-phenyl H-3), 7.40 (dd, J = 5.1, 3.0 Hz, 1H, 6-thiophene H-5), 7.39–7.34 (m, 2H, 2-phenyl H-4, H-5), 6.91 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.34 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.25, 153.93, 151.06, 141.98, 139.13, 138.14, 137.21, 132.33, 131.67, 130.19, 129.67, 126.94, 126.41, 126.23, 124.20, 116.56, 112.44, 110.67, 108.86.

4.3.17. 4-(5-Chlorofuran-2-yl)-2-(3-chlorophenyl)-6-(thiophen-3-yl) pyridine (26)

The same procedure described in Section 4.3 was employed with **6b** (0.31 g, 1.30 mmol), dry ammonium acetate (1.00 g, 13.00 mmol), **2** (R_1 = **g**) (0.46 g, 1.30 mmol), and glacial acetic acid (3.00 mL) to yield a white solid (302 mg, 62.4%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.35; mp 153.8–154.6 °C, purity: 99.6%.

LC/MS/MS (condition B): retention time: 9.26 min; [M^+]: 372.1 (100%), [M+2]: 374.1 (71%), [M+4]: 376.1 (15%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.15–8.14 (m, 1H, 2-phenyl H-2), 8.07 (dd, J = 3.0, 1.2 Hz, 1H, 6-thiophene H-2), 8.02–7.98 (m, 1H, 2-phenyl H-6), 7.81 (dd, J = 5.1, 1.2 Hz, 1H, 6-thiophene H-4), 7.74 (d, J = 1.3 Hz, 1H, pyridine H-3), 7.73 (d, J = 1.2 Hz, 1H, pyridine H-5), 7.43 (dd, J = 5.1, 3.0 Hz, 1H, 6-thiophene H-5), 7.45–7.41 (m, 2H, 2-phenyl H-4, H-5), 6.95 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.36 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.02, 153.86, 150.97, 141.92, 140.89, 138.16, 138.11, 134.74, 129.88, 129.13, 127.13, 126.34, 126.31, 125.03, 124.23, 112.62, 112.08, 110.66, 108.89.

4.3.18. 4-(5-Chlorofuran-2-yl)-2-(4-chlorophenyl)-6-(thiophen-3-yl) pyridine (27)

The same procedure described in Section 4.3 was employed with **6b** (0.31 g, 1.30 mmol), dry ammonium acetate (1.00 g, 13.00 mmol), **2** (R_1 = **h**) (0.46 g, 1.30 mmol), and glacial acetic acid (3.00 mL) to yield a white solid (316 mg, 65.4%).

R_f (ethyl acetate/*n*-hexane 1:10 v/v): 0.35; mp 128.4–130.0 °C, purity: 99.4%.

LC/MS/MS (condition B): retention time: 9.23 min; [M^+]: 372.1 (100%), [M+2]: 374.1 (71%), [M+4]: 376.1 (15%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.09 (d, J = 8.7 Hz, 2H, 2-phenyl H-2, H-6), 8.05 (dd, J = 3.0, 1.2 Hz, 1H, 6-thiophene H-2), 7.80 (dd, J = 5.1, 1.2 Hz, 1H, 6-thiophene H-4), 7.75 (d, J = 1.3 Hz, 1H, pyridine H-3), 7.72 (d, J = 1.3 Hz, 1H, pyridine H-5), 7.46 (d, J = 8.7 Hz, 2H, 2-phenyl H-3, H-5), 7.42 (dd, J = 5.1, 3.0 Hz, 1H, 6-thiophene H-5), 6.94 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.36 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.29, 153.81, 151.05, 142.01, 138.12, 138.08, 137.51, 135.27, 128.83, 128.25, 126.33, 126.29, 124.13, 112.36, 111.81, 110.58, 108.87.

4.3.19. 4-(5-Chlorofuran-2-yl)-6-(thiophen-3-yl)-2,2′-bipyridine (28)

The same procedure described in Section 4.3 was employed with **6b** (0.23 g, 1.00 mmol), dry ammonium acetate (0.77 g, 10.00 mmol), **2** (R_1 = **i**) (0.32 g, 1.00 mmol), and glacial acetic acid (2.00 mL) to yield a white solid (173 mg, 51.3%).

R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.28; mp 167.6–168.3 °C, purity: 100%.

LC/MS/MS (condition B): retention time: 6.18 min; [MH^+]: 339.2 (100%), [MH+2]: 341.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.72 (td, J = 4.8, 1.2 Hz, 1H, 2-pyridine H-6′), 8.60 (d, J = 8.0 Hz, 1H, 2-pyridine H-3′), 8.45 (d, J = 1.5 Hz, 1H, pyridine H-3), 8.08 (dd, J = 3.0, 1.2 Hz, 1H, 6-thiophene H-2), 7.86 (dt, J = 8.0, 1.8 Hz, 1H, 2-pyridine H-4′), 7.84 (d, J = 1.4 Hz, 1H, pyridine H-5), 7.83 (dd, J = 5.0, 1.2 Hz, 1H, 6-thiophene H-4), 7.44 (dd, J = 5.0, 3.0 Hz, 1H, 6-thiophene H-5), 7.35 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H, 2-pyridine H-5′), 7.03 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.35 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.18, 155.86, 153.45, 151.23, 148.99, 142.10, 138.21, 137.98, 136.91, 126.39, 126.26, 123.97, 121.38, 113.65, 112.71, 110.88, 108.87.

4.3.20. 4-(5-Chlorofuran-2-yl)-6-(thiophen-3-yl)-2,3′-bipyridine (29)

The same procedure described in Section 4.3 was employed with **6b** (0.28 g, 1.20 mmol), dry ammonium acetate (0.92 g, 12.00 mmol), **2** (R_1 = **j**) (0.39 g, 1.20 mmol), and glacial acetic acid (2.50 mL) to yield a yellow solid (238 mg, 58.6%).

R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.23; mp 188.6–189.0 °C, purity: 100%.

LC/MS/MS (condition B): retention time: 4.29 min; [MH^+]: 339.2 (100%), [MH+2]: 341.2 (38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.34 (d, J = 1.6 Hz, 1H, 2-pyridine H-2′), 8.68 (dd, J = 4.7, 1.3 Hz, 1H, 2-pyridine H-6′), 8.45 (td, J = 8.0, 1.9 Hz, 1H, 2-pyridine H-4′), 8.07 (dd, J = 3.0, 1.2 Hz, 1H, 6-thiophene H-2), 7.81 (dd, J = 5.1, 1.2 Hz, 1H, 6-thiophene H-4), 7.79 (d, J = 1.3 Hz, 1H, pyridine H-3), 7.76 (d, J = 1.2 Hz, 1H, pyridine H-5), 7.45 (ddd, J = 8.0, 4.7, 1.8 Hz, 1H, 2-pyridine H-5′), 7.42 (dd, J = 5.0, 2.9 Hz, 1H, 6-thiophene H-5), 6.96 (d, J = 3.5 Hz, 1H, 4-furan H-3), 6.37 (d, J = 3.5 Hz, 1H, 4-furan H-4).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 154.98, 154.14, 150.85, 150.04, 148.35, 141.82, 138.33, 138.22, 134.63, 134.42, 126.39, 126.29, 124.30, 123.51, 112.75, 112.06, 110.82, 108.93.

4.4. Pharmacology

DNA topo I inhibition assay was determined by following the method previously reported.[11] The test compounds were dissolved in DMSO at 20 mM as the stock solution. The activity of DNA topoisomerase 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 topoisomerase 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.[11] The mixture of 200 ng of supercoiled pBR322 plasmid DNA and two units of human DNA topo IIα (Amersham, USA) was incubated with and without 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 a 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).

For the evaluation of cytotoxicity, five different cancer cell lines were used: human breast adenocarcinoma cell line (MCF-7), human cervix tumor cell line (HeLa), human prostate tumor cell line (DU145), human colorectal adenocarcinoma cell line (HCT15), and chronic myelogenous leukemia cell line (K562). Experiments were performed by methods previously described.[11] 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, the 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) with a 450 nm wavelength. For determination of the IC_{50} values, the absorbance readings at 450 nm were fitted to a four-parameter logistic equation. The compounds adriamycin, etoposide, and camptothecin were purchased from Sigma and used as positive controls.

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References and notes

1. Holden, J. A. *Curr. Med. Chem.-Anti-Cancer Agents* **2001**, *1*, 1.
2. Nitiss, J. L. *Biochim. Biophys. Acta* **1998**, *1400*, 63.
3. Campoux, J. J. *Annu. Rev. Biochem.* **2001**, *70*, 369.
4. Pommier, Y. *Nat. Rev. Cancer* **2006**, *6*, 789.
5. Hande, K. R. *Update Cancer Ther.* **2006**, *1*, 3.
6. Hofmeier, H.; Schubert, U. S. *Chem. Soc. Rev.* **2004**, *33*, 373.
7. (a) McCoubrey, A.; Latham, H. C.; Cook, P. R.; Rodger, A.; Lowe, G. *FEBS Lett.* **1996**, *380*, 73; (b) Carter, P. J.; Cheng, C. C.; Thorp, H. H. *J. Am. Chem. Soc.* **1998**, *120*, 632.
8. (a) Zhao, L. X.; Kim, T. S.; Ahn, S. H.; Kim, T. H.; Kim, E. K.; Cho, W. J.; Choi, H. S.; Lee, C. S.; Kim, J. A.; Jeong, T. C.; Chang, C.-J.; Lee, E.-S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2659; (b) Zhao, L. X.; Moon, Y. S.; Basnet, A.; Kim, E. K.; Jahng, Y.; Park, J. G.; Jeong, T. C.; Cho, W. J.; Choi, S. U.; Lee, C. O.; Lee, S. Y.; Lee, C. S.; Lee, E.-S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1333; (c) Zhao, L. X.; Sherchan, J.; Park, J. K.; Jahng, Y.; Jeong, B. S.; Jeong, T. C.; Lee, C. S.; Lee, E.-S. *Arch. Pharm. Res.* **2006**, *29*, 1091; (d) Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, B. S.; Jeong, T. C.; Lee, C. S.; Lee, E.-S. *Bioorg. Med. Chem.* **2007**, *15*, 4351; (e) Son, J. K.; Zhao, L. X.; Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, T. C.; Jeong, B. S.; Lee, C. S.; Lee, E.-S. *Eur. J. Med. Chem.* **2008**, *43*, 675; (f) Thapa, P.; Karki, R.; Basnet, A.; Thapa, U.; Choi, H. Y.; Na, Y.; Jahng, Y.; Lee, C. S.; Kwon, Y.; Jeong, B. S.; Lee, E.-S. *Bull. Korean Chem. Soc.* **2008**, *29*, 1605; (g) Basnet, A.; Thapa, P.; Karki, R.; Choi, H.; Choi, J. H.; Yun, M.; Jeong, B.-S.; Jahng, Y.; Na, Y.; Won-Jea Cho, W.-J.; Kwon, Y.; Lee, C.-S.; Lee, E.-S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 42; (h) Thapa, P.; Karki, R.; Thapa, U.; Jahng, Y.; Jung, M. J.; Nam, J. M.; Na, Y.; Kwon, Y.; Lee, E.-S. *Bioorg. Med. Chem.* **2010**, *18*, 377.
9. Jahng, Y.; Zhao, L. X.; Moon, Y. S.; Basnet, A.; Kim, E. K.; Chang, H. W.; Ju, H. K.; Jeong, T. C.; Lee, E.-S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2559.
10. Kröhnke, F. *Synthesis* **1976**, 1.
11. Kang, D. H.; Kim, J. S.; Jung, M. J.; Lee, E.-S.; Jahng, Y.; Kwon, Y.; Na, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1520.