2,6-Dithienyl-4-arylpyridines: Synthesis, Topoisomerase I and II Inhibition and Structure-activity Relationship

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Topoisomerases I and II are nuclear enzymes that play crucial roles in DNA metabolism such as replication, transcription, recombination, repair, chromatin assembly, and chromosome segregation.[1-4] Due to the crucial role of topoisomerases in the maintenance and replication of DNA during proliferation, cells become highly vulnerable when these functions are lost.[5] Consequently, topo I and II have been attractive targets for design of antitumor agents.[6]

It has been reported in our research group α-terpyridine[7,8] derivatives showed strong cytotoxicities against several human cancer cell lines and considerable topo I and II inhibitory activities.[9] We also studied for topo I and II inhibitory activities of 2,6-dithienyl-4-furylpyridines.[10] From the previous structure-activity relationship studies, we found 2,6-dithienyl-4-furylpyridines exhibited strong topo I and II inhibitory activities as well as strong cytotoxicity against several human cancer cell lines. It would be very interesting to prepare 2,6-dithienyl-4-arylpyridines and evaluate topo I and II inhibitory activities of the prepared compounds. In addition, the comparison of activities between two moieties may provide valuable information for the development of novel antitumor agents. In connection with previous studies, 2,6-dithienyl-4-arylpyridines were prepared and evaluated for their topo I and II inhibitory effects (Figure 1).

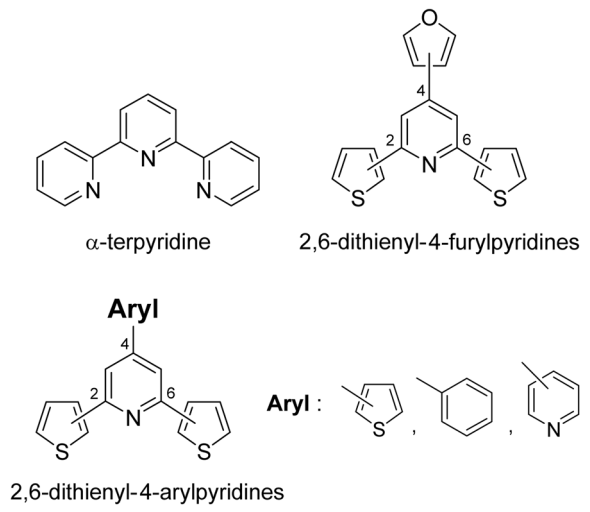


Figure 1

Experimental Section

Compounds used as starting materials and reagents were purchased from Aldrich Chemical Co., Sigma, Fluka, Junsei and used without further purification. Thin-layer chromatography (TLC) and column chromatography 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 rings, compounds 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 TMS (tetramethylsilane) was used as an internal standard. 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. 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 a 1 μL injection volume on a Waters XTerra® 3.5 μm reverse-phase C_{18} column (2.1 × 100 mm) with a gradient elution from 5% to 95% of B in A for 20 min followed by 95% B in A for 10 min at a flow rate of 200 μL/min, where mobile phase A was 100% distilled water with 50 mM ammonium formate and mobile phase B was 100% acetonitrile. MS ionization conditions were: Sheath gas flow rate: 70 arb, aux gas flow rate: 20 arb, I spray voltage: 4.5 KV, capillary temp.: 215 °C, capillary voltage: 21 V, tube lens offset: 10 V.

General method for the preparation of 3. Aryl aldehyde **2** was added to the solution of 85% KOH (1.2 eq.) in MeOH (50 mL) and H_2O (10 mL) at 0 °C. After dissolution, thienyl methyl ketone (**1**, 1.0 eq.) was added over a period of 10 min. The mixture was then stirred for 3 h at 0 °C. A solid product precipitated was filtered, washed with cold MeOH and dried to yield 47 to 99%. Utilizing the same procedure, thirteen compounds were synthesized.

1,3-Dithiophen-3-yl-propenone {3 (R^1 = b, R^2 = b)}. A white solid (85%), TLC (EtOAc:*n*-hexane = 1:2) R_f = 0.5, mp 77.1-78.2 °C, ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.14 (dd, J = 2.8, 1.2 Hz, 1H, 1-thiophen H-2), 7.80 (d, J = 15.5 Hz, 1H,

-CO-C=CH-), 7.65 (dd, *J* = 5.0, 1.2 Hz, 1H, 1-thiophen H-5), 7.59 (dd, *J* = 2.7, 1.1 Hz, 1H, 3-thiophen H-2), 7.41 (dd, *J* = 5.0, 1.1 Hz, 1H, 3-thiophen H-5), 7.35 (dd, *J* = 5.0, 2.8 Hz, 1H, 1-thiophen H-4), 7.34 (dd, *J* = 5.0, 2.7 Hz, 1H, 3-thiophen H-4), 7.22 (d, *J* = 15.5 Hz, 1H, -CO-CH=C-).

General method for the preparation of 6-23. A mixture of **3** (R^1 = **a-c**, R^2 = **a-g**), dry ammonium acetate and **4** (R^3 = **a-b**) in dry MeOH was heated at 80 °C for 12 to 24 h under nitrogen atmosphere. The solvent was removed by evaporation under reduced pressure, and the residue was diluted with ethyl acetate (100 mL), washed with water (75 mL × 2) and saturated NaCl solution (50 mL). The organic layer was dried with magnesium sulfate and filtered. The filtrate was evaporated at reduced pressure, which was purified by silica gel column chromatography with a gradient elution of ethyl acetate/n-hexane to afford a white solid in 54 to 93% yields. Utilizing the same procedure, eighteen compounds were synthesized.

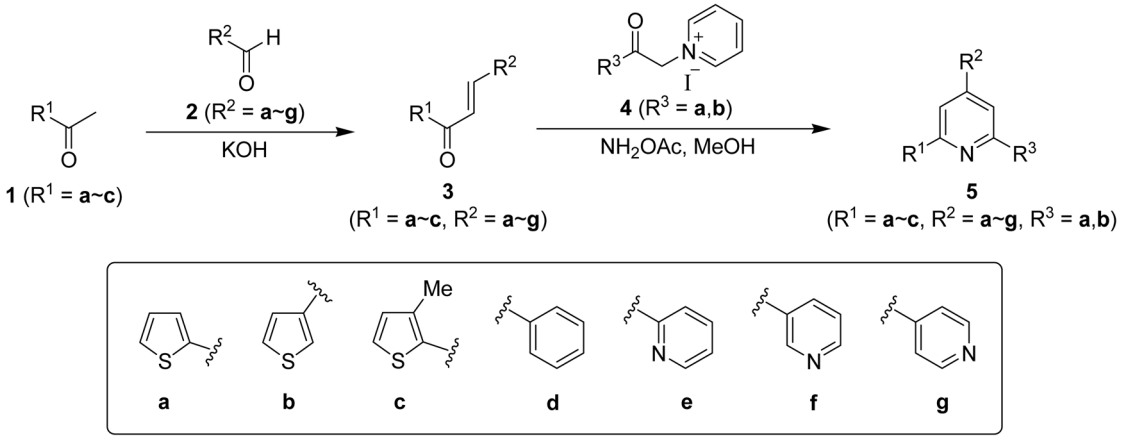
4-(Thiophen-2-yl)-2,6-di(thiophen-3-yl)pyridine (10). A white solid (54%), TLC (EtOAc/*n*-hexane = 1:5), R_f = 0.4, mp 155.9-156.5 °C, ESI LC/MS/MS: Retention time: 6.84 min, $[MH]^+$: 326.2, ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 2.98, 1.18 Hz, 2H, 2-thiophene H-2,6"-thiophene H-2), 7.79 (dd, *J* = 5.03, 1.16 Hz, 2H, 2-thiophene H-5,6"-thiophene H-5), 7.68 (s, 2H, pyridine H-3, H-5), 7.58 (dd, *J* = 3.57, 0.98 Hz, 1H, 4'-thiophene H-5), 7.44 (dd, *J* = 5.01, 0.99 Hz, 1H, 4'-thiophene H-3), 7.42 (dd, *J* = 5.01, 3.03 Hz, 2H, 2-thiophene H-3,6"-thiophene H-3), 7.17 (dd, *J* = 5.03, 3.67 Hz, 1H, 4'-thiophene H-4), ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.77, 142.87, 142.18, 141.69, 128.36, 126.90, 126.39, 126.17, 125.21, 123.92, 114.76.

Pharmacology. The topoisomerase I inhibitory activity was carried out as followings:[11] The activity of DNA topoisomerase I was determined by measuring the relaxation of supercoiled DNA pBR322. For measurement of topoisomerase I activity, the reaction mixture was comprised of 35 mM Tris-HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mM spermidine, 0.01% bovine serum albumin, 200 ng pBR322, 0.3 U calf thymus DNA topoisomerase I (Amersham), and topoisomerase I inhibitors (prepared compounds) in a final volume of 10 μL. The reaction mixture was incubated at 37 °C for 30 min. The reactions were terminated by adding 2.5 μL of solution comprising 10% SDS, 0.2% bromophenol blue, 0.2% xylene cyanol and 30% glycerol. The mixture was applied to 1% agarose gel and electrophoresed for 10 h with a running buffer of Tris-borate-EDTA. Gels were stained for 30 min in a aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated by an image analyzer and LabWork 4.5 software (UVP).

The topoisomerase II inhibitory activity was carried out as followings: DNA topoisomerase II inhibition was measured by assessing relaxation of supercoiled pBR322 plasmid DNA. The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol, 30 μg/mL bovine serum albumin, 0.2 μg pBR322 plasmid DNA, 0.3 U human DNA topoisomerase IIα (TopoGEN), and topoisomerase II inhibitors (prepared compounds) in a final volume of 20 μL. The reactions were incubated for 30 min at 37 °C and terminated by the addition of 3 μL of solution containing 0.77% sodium dodecyl sulfate, 77 mM EDTA. Samples were mixed with 2 μL of solution containing of 30% sucrose, 0.5% bromophenol blue and 0.5% xylene cyanol, and subjected to electrophoresis on a 1% agarose gel at 1.5 V/cm for 10 h with a running buffer of Tris-borate-EDTA. Gels were stained for 30 min in a aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated by an image analyzer and LabWork 4.5 software (UVP).

Results and Discussion

Chemistry. Synthetic methods for the preparation of 2,6-dithienyl-4-aryl pyridines (**6-23**) are summarized in Scheme 1. Acetylthiophenes **1a-c** were treated with arylcarboxaldehydes **2a-g** in the presence of KOH in methanol-water (5:1), to afford intermediates **3** in a 47-99% yields. Using modified Kröhnke synthesis,[12] 2,6-dithienyl-4-arylpyridines were prepared by treatment of **3** with 1-(2-oxo-2-thienylethyl)pyridinium iodide (**4a-b**) in the presence of ammoni-



Scheme 1

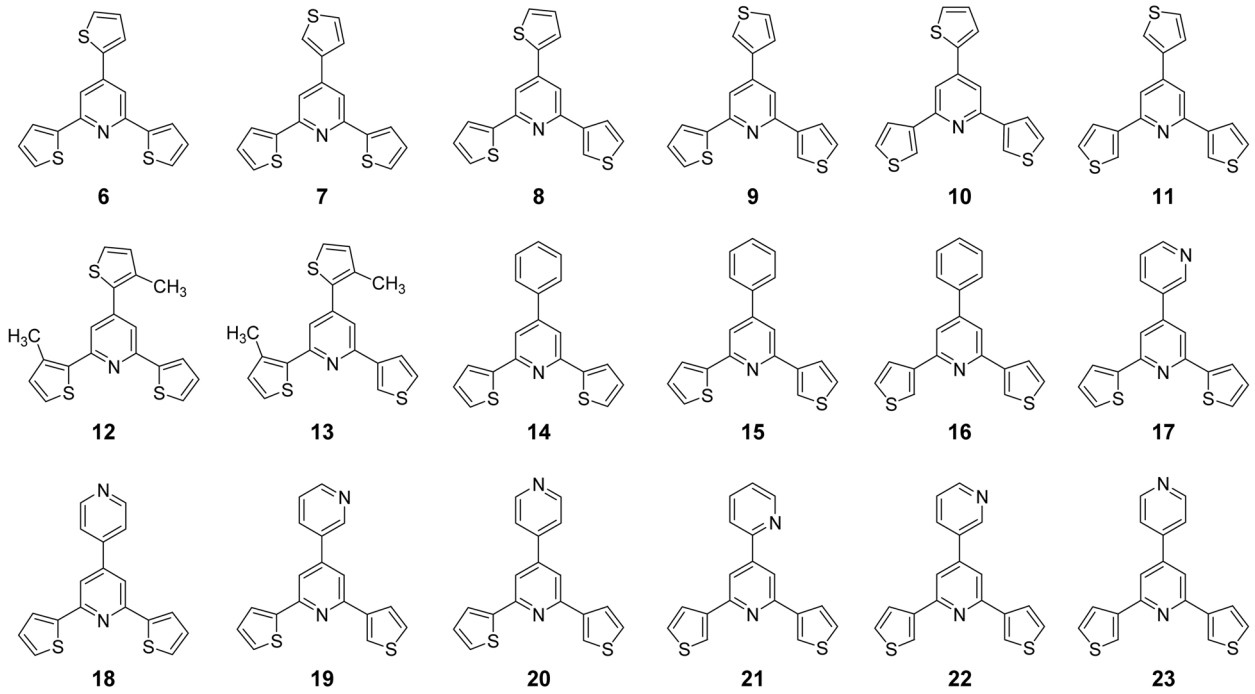
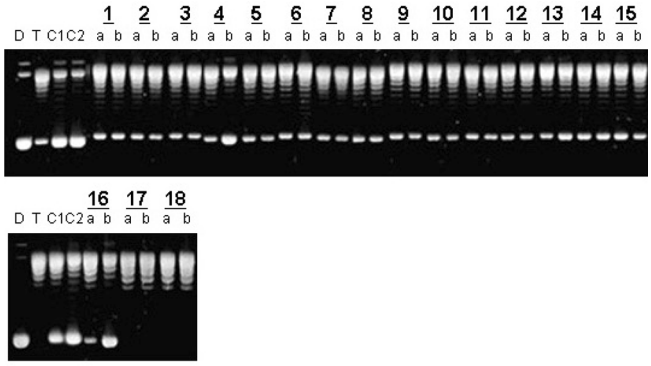


Figure 2. The prepared compounds.

um acetate in methanol to give **6-23** in a 54-93% yields. Pyridinium iodides **4a-b** were prepared in a quantitative yield by treatment of **1a-b** with iodine in pyridine. Figure 2 shows the prepared 2,6-dithienyl-4-arylpyridines (**6-23**).

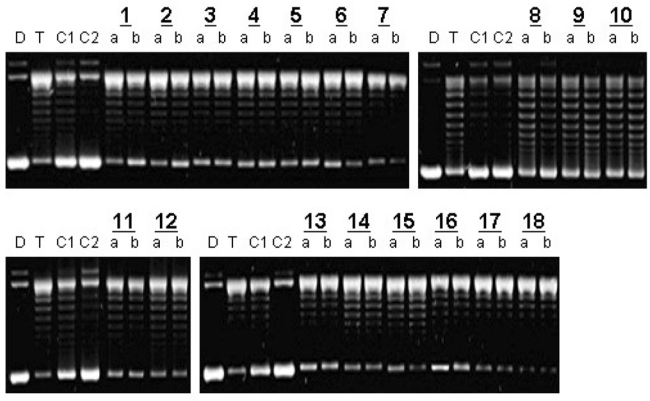
Pharmacology. Wheat germ topoisomerase I and human DNA topoisomerase IIa inhibitory activities[11] for the eighteen prepared 2,6-dithienyl-4-arylpyridines are shown in Figures 3 and 4, and summarized in Table 1. Compounds **6-10** exhibited moderate topo I and/or II inhibitory activities. Especially compound **6** indicates both moderate topo I and II inhibitory activities (33% and 31% inhibition at 100 μM, respectively) and compound **10** has moderate topo I inhibitory activity (35% inhibition at 100 μM). It is generally recognized that those compounds indicate considerable topo I or II inhibitory activities as synthetic compounds, although they have weaker inhibitory activities than those of camptothecin or etoposide. Besides the thienyl derivatives on 4-



Lane D: pBR322 DNA only
Lane T: pBR322 DNA + Topo I
Lane C1: pBR322 DNA + Topo I + Camptothecin 20 μM
Lane C2: pBR322 DNA + Topo I + Camptothecin 100 μM
Lane 1−18 Lane a: pBR322 DNA + Topo I + Compounds 20 μM
Lane 1−18 Lane b: pBR322 DNA + Topo I + Compounds 100 μM

lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
compound	**7**	**8**	**9**	**10**	**11**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**6**	**12**	**13**

Figure 3. Topoisomerase I inhibitory activity of the prepared compounds.



Lane D: pBR322 DNA only
Lane T: pBR322 DNA + Topo II
Lane C1: pBR322 DNA + Topo II + Etoposide 20 μM
Lane C2: pBR322 DNA + Topo II + Etoposide 100 μM
Lane 1−18 Lane a: pBR322 DNA + Topo II + Compounds 20 μM
Lane 1−18 Lane b: pBR322 DNA + Topo II + Compounds 100 μM

lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
compound	**7**	**8**	**9**	**10**	**11**	**16**	**18**	**6**	**12**	**13**	**15**	**19**	**14**	**17**	**20**	**21**	**22**	**23**

Figure 4. Topoisomerase II inhibitory activity of the prepared compounds.

Table 1. Topoisomerase I and II inhibitory activities of the prepared compounds

Compounds	Topo I (% Inhibition)		Topo II (% Inhibition)	
	20 μM	100 μM	20 μM	100 μM
6	12	33	5	31
7	7	12	0	17
8	6	13	0	11
9	10	6	5	2
10	15	35	2	5
11	0	0	0	3
12	0	0	0	0
13	0	0	0	3
14	0	0	10	7
15	0	0	12	15
16	0	0	5	0
17	0	0	0	0
18	0	0	0	0
19	0	0	2	0
20	0	0	0	0
21	0	7	13	7
22	1	1	0	0
23	8	0	0	0
Camptothecin	41	54		
Etoposide			65	73

position, other compounds **11**-**23** did not show considerable inhibitory activity, which indicate that phenyl or pyridyl derivatives as well as methyl thienyl on 4-position in pyridine ring did not increase the inhibitory activity.

In conclusion, we have designed an efficient synthetic route to prepare eighteen 2,6-dithienyl-4-arylpyridines and evaluated them for their topo I and II inhibitory activity. From structure-activity relationship study, 2,4,6-trithienylpyridines show better inhibitory activities than 2,6-dithienyl-4-phenyl or 2,6-dithienyl-4-pyridylpyridines, but lower inhibitory activities than 2,6-dithienyl-4-furylpyridines.[10] This study may provide valuable information to the researchers who are working on the development of antitumor agents, especially that of topoisomerase I or II inhibitors.

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