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Bioorganic & Medicinal Chemistry Letters 14 (2004) 1333–1337

# Synthesis, topoisomerase I inhibition and structure–activity relationship study of 2,4,6-trisubstituted pyridine derivatives

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Received 28 August 2003; accepted 26 November 2003

**Abstract**—For the development of new anticancer agents, phenyl, 2-pyridyl, 2-furyl, 2-thienyl, 2-furylvinyl and 2-thienylvinyl substituted derivatives on 2,4,6-position in pyridine moiety were prepared and evaluated for their topoisomerase I inhibitory activity. Among the thirteen prepared compounds, four compounds exhibited strong topoisomerase I inhibitory activity. A structure–activity relationship study indicated that the 2-thienyl-4-furylpyridine skeleton was important for topoisomerase I inhibitory activity.


## 1. Introduction

Since its discovery in 1932, the 2,2′;6′,2″-terpyridine molecule has attracted chemists due to its ability to form metal complexes.[1] In addition to such a complexation ability to act as a terdentate, its binding ability to DNA also attracted biologists and medicinal chemists as a potential anticancer agent.[2,3] We recently reported that terpyridine derivatives showed a strong cytotoxicity against several human cell lines and considerable topoisomerase I inhibitory activities.[4] Some of us also reported that terthiophene derivatives, which are bioisosteres of terpyridine, showed considerable inhibitory activity on PKC and an antitumor cytotoxicity against several human cell lines. Although the cytotoxicity of terpyridine derivatives has long been reported, a systematic study on the effects of substituted pyridines and of the substituents on the pyridine nucleus has not yet been pursued.

Such previous studies prompted us to design 2,4,6-trisubstituted pyridine derivatives as a topoisomerase I inhibitors in our endeavour to develop novel anticancer agents. We reasoned that the substituents at 2, 4, and/or 6-position may affect the conformation of the whole molecule to retain a preferably planar conformation for better conjugation with the central pyridine anchor, thus interacting with topoisomerase I effectively.[5]

In this study, we described the synthesis of 2,4,6-trisubstituted pyridine derivatives as bioisosteres of terpyridine and/or terthiophene and the inhibitory activity of the compounds prepared on topoisomerase I. The structure–activity relationship was also determined.

For the design, pyridine moiety was utilized as a basic skeleton, and hydrogen, phenyl, 2-pyridyl, 2-furyl, 2-thienyl, 2-furylvinyl and/or 2-thienylvinyl moieties were attached to the 2, 4, and/or 6 position of the pyridine structure (Fig. 1).

## 2. Chemistry

Synthetic methods for the preparation of 2,4,6-trisubstituted pyridine derivatives ($\mathbf{B_1}$–$\mathbf{B_{13}}$) are summarized in Scheme 1. Acetyl derivatives **1a–g** were treated with aldehyde derivatives **2a–d** in the presence of KOH in methanol–water (5:1), to afford intermediates **A** in a 82.4–97.1% yield. Using modified Kröhnke synthesis,[6]

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doi:10.1016/j.bmcl.2003.11.084

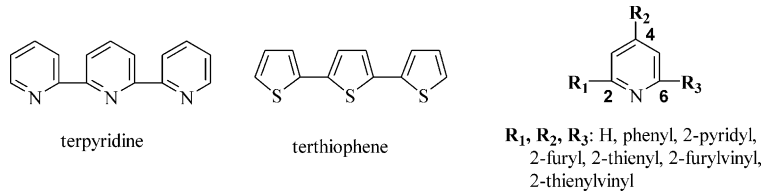
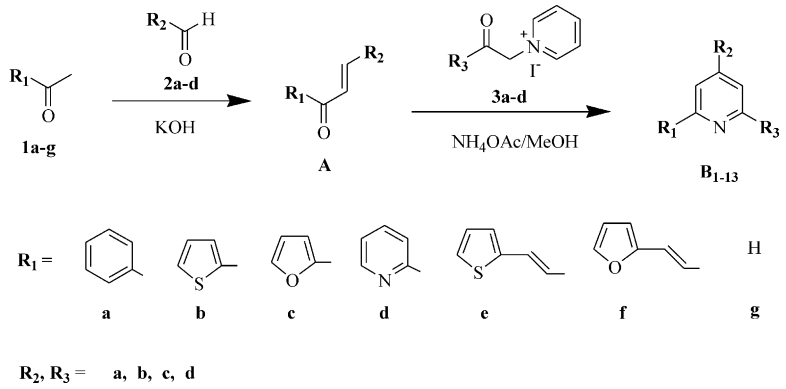



**Figure 1.**




**Scheme 1.**

final products $\mathbf{B_{1–13}}$ were prepared by treatment of **A** with 1-(2-oxo-2-aryl-ethyl)pyridinium iodide (**3a–d**) in the presence of ammonium acetate in methanol to give $\mathbf{B_{1–13}}$ in a 46.7–88.3% yield. Pyridinium iodides **3a–d** were prepared in a quantitative yield by treatment of **1a–d** with iodine in pyridine.

Figure 2 shows the prepared 2,4,6-trisubstituted pyridine derivatives ($\mathbf{B_1}$–$\mathbf{B_{13}}$).

## 3. Results and discussion

Wheat germ topoisomerase I inhibitory activities[7] for the thirteen prepared 2,4,6-trisubstituted pyridine derivatives are shown in Figure 3. Compounds $\mathbf{B_2}$, $\mathbf{B_{10}}$ and $\mathbf{B_{13}}$ exhibited strong topoisomerase I inhibitory activities, and $\mathbf{B_6}$ exhibited moderate inhibition compared to that of camptothecin. It is generally recognized that such compounds have strong inhibitory activities as synthetic

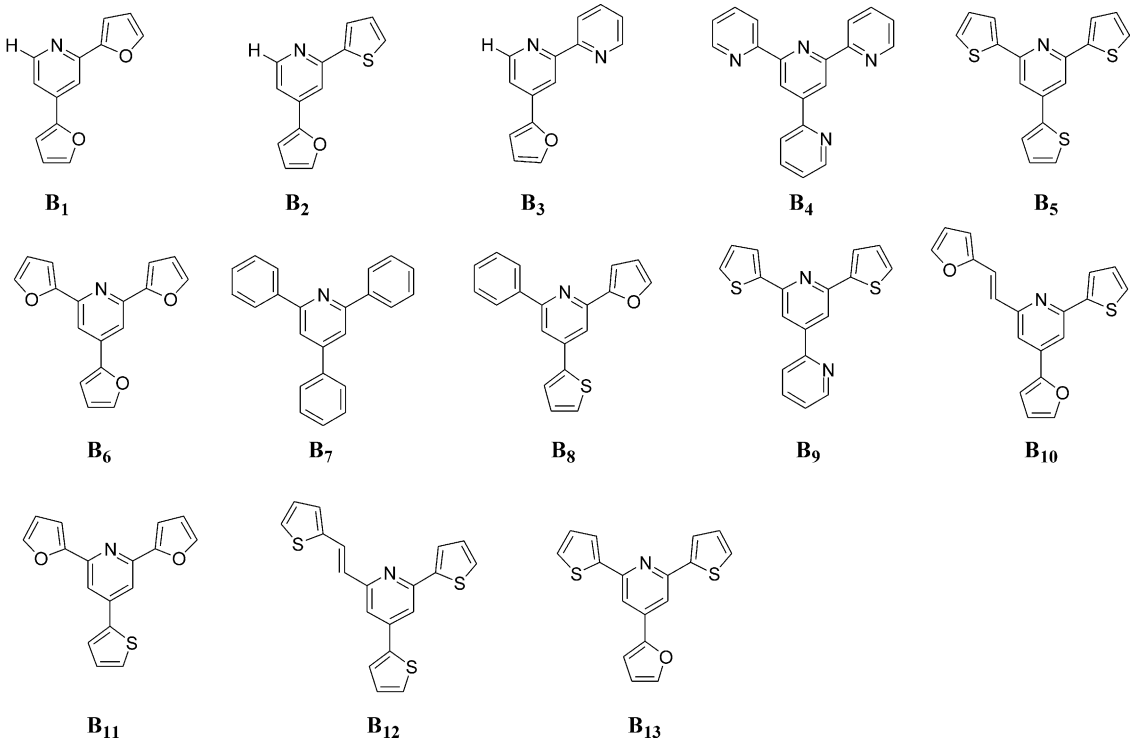
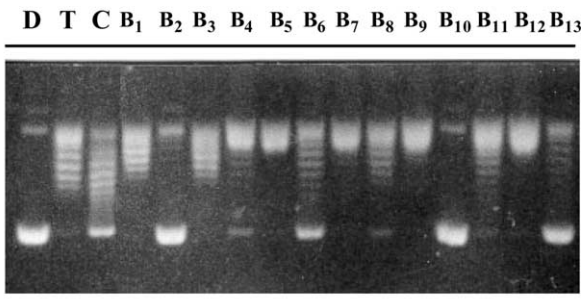



**Figure 2.**




Lane D. pBR322 DNA only

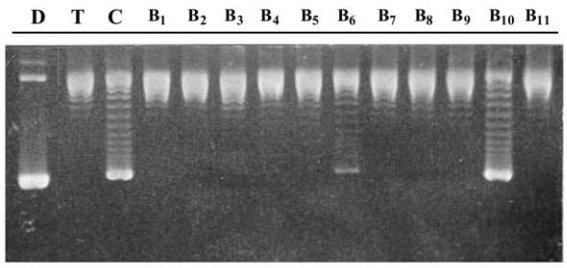
Lane T. pBR322 DNA + wheat germ Topo I

Lane C. pBR322 DNA + wheat germ Topo I + CPT (30 μM)

Lane $\mathbf{B_1}$ – $\mathbf{B_{13}}$. pBR322 DNA + wheat germ Topo I + prepared compounds ($\mathbf{B_1}$ – $\mathbf{B_{13}}$, 1 mM)

* CPT : Camptothecin

**Figure 3.** Topoisomerase I (wheat germ) inhibitory activities of prepared compounds.




Lane D. pBR322 DNA only

Lane T. pBR322 DNA + Calf thymus Topo I

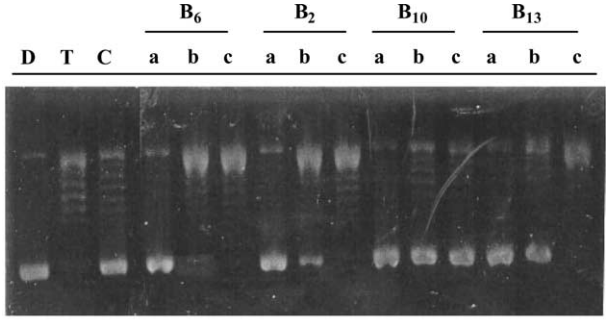
Lane C. pBR322 DNA + Calf thymus Topo I + CPT (30 μM)

Lane $\mathbf{B_1}$ – $\mathbf{B_{11}}$. pBR322 DNA + Calf thymus Topo I + prepared compounds ($\mathbf{B_1}$ – $\mathbf{B_{11}}$, 1 mM)

* CPT : Camptothecin

**Figure 5.** Topoisomerase I (calf thymus) inhibitory activities of prepared compounds.

compounds, even though they have weaker inhibitory activities than that of camptothecin. Figure 4 shows wheat germ topoisomerase I inhibitory effects in decreasing concentrations of $\mathbf{B_2}$ ($IC_{50}$: 400 μM), $\mathbf{B_6}$ ($IC_{50}$: 500 μM), $\mathbf{B_{10}}$ ($IC_{50}$: $<10$ μM) and $\mathbf{B_{13}}$ ($IC_{50}$: 50 μM).[8] This result indicated that $\mathbf{B_{10}}$ showed stronger inhibitory activity than that of camptothecin. Figure 5 shows calf thymus topoisomerase I inhibitory activities of prepared compounds. While $\mathbf{B_2}$, $\mathbf{B_{10}}$ and $\mathbf{B_{13}}$ displayed strong inhibitory effects against wheat germ topoisomerase I, $\mathbf{B_6}$ and $\mathbf{B_{10}}$ displayed considerable inhibitory effects against calf thymus topoisomerase I. Figure 6 shows calf thymus topoisomerase I inhibitory effects in decreasing concentrations of $\mathbf{B_6}$ ($IC_{50}$: $>1$ mM) and $\mathbf{B_{10}}$ ($IC_{50}$: 200 μM). In this result $\mathbf{B_{10}}$ also showed inhibitory activity in a one μM concentration since topoisomers were present in that concentration.




Lane D. pBR322 DNA only

Lane T. pBR322 DNA + wheat germ Topo I

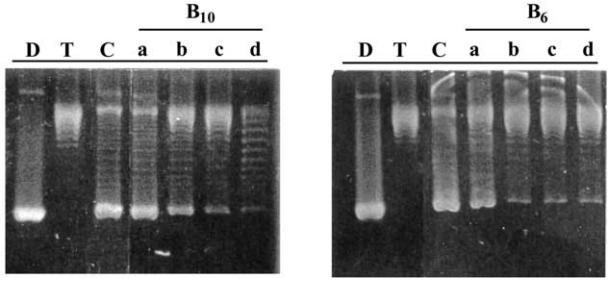
Lane C. pBR322 DNA + wheat germ Topo I + CPT (30 μM)

Lane **a, b, c**. **a** : 1 mM, **b** : 100 μ M, **c** : 10 μ M

* CPT : Camptothecin

**Figure 4.** Topoisomerase I (wheat germ) inhibitory effects in decreasing concentrations of $B_2$, $B_6$, $B_{10}$ and $B_{13}$.

A structure–activity relationship study of prepared compounds for topoisomerase I inhibition indicated that the 2-thienyl-4-furylpyridine skeleton (Fig. 7) is important for topoisomerase I inhibitory activity, since $\mathbf{B_2}$, $\mathbf{B_{10}}$ and $\mathbf{B_{13}}$ possess that moiety. It is interesting to notice that a slight modification of the 2-thienyl-4-furylpyridine skeleton dramatically decreased the inhibitory activity, as seen in $\mathbf{B_2}$ versus $\mathbf{B_1}$ and $\mathbf{B_3}$, $\mathbf{B_{13}}$ versus $\mathbf{B_{11}}$,




Lane D. pBR322 DNA only

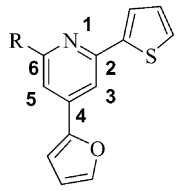
Lane T. pBR322 DNA + calf thymus Topo I

Lane C. pBR322 DNA + calf thymus Topo I + CPT (30 μM)

Lane **a, b, c, d**. **a** : 1 mM, **b** : 100 μ M, **c** : 10 μ M, **d** : 1 μ M

* CPT : Camptothecin

**Figure 6.** Topoisomerase I (calf thymus) inhibitory effects in decreasing concentrations of $B_6$ and $B_{10}$.




2-thienyl-4-furylpyridine skeleton

**Figure 7.**

and $\mathbf{B_{10}}$ versus $\mathbf{B_{12}}$. On the other hand, $\mathbf{B_6}$ maintained moderate topoisomerase I inhibitory activity on both wheat germ and calf thymus topoisomerase I. In addition, the 6-substituent effect on topoisomerase I inhibition was not evident.

For the evaluation of antitumor cytotoxicity,[9] four different human tumor cell lines were utilized: A-549 (human lung carcinoma), HCT-15 (human colon adenocarcinoma), SK-OV-3 (human ovary adenocarcinoma), SK-MEL-2 (human malignant melanoma). Cytotoxicity of prepared compounds against human cancer cell lines indicated that moderate cytotoxicities ($EC_{50}$ value of 10.4–118.1 μM) were observed for all prepared compounds except $\mathbf{B_4}$ ($EC_{50}$ value of 0.01–0.03 μM).

In conclusion, we have designed an efficient synthetic route to prepare thirteen 2,4,6-trisubstituted pyridine derivatives and evaluated them for their inhibitory activity of topoisomerase I. This was the first report of the topoisomerase I inhibitory activity of 2,4,6-trisubstituted pyridine compounds. The structure–activity relationship analysis revealed that the 2-thienyl-4-furylpyridine skeleton exhibited strong topoisomerase I inhibitory activity. Further structure–activity relationship studies are on progress. This study may provide valuable information to the researchers who are working on the development of antitumor agents, especially that of topoisomerase I inhibitors.

## Acknowledgements

This work was supported by a grant (KRF-2002-005-E00019) from the Korea Research Foundation, R.O.K.

## References and notes

1. (a) Mukkala, V. M.; Helenius, M.; Hemmila, I.; Kankare, J.; Takalo, H. *Helv. Chim. Acta* **1993**, *76*, 1361. (b) Mukkala, V. M.; Kwiatkowski, M.; Kankare, J.; Takalo, H. *Helv. Chim. Acta* **1993**, *76*, 893. (c) Lowe, G.; Droz, A. S.; Park, J. J.; Weaver, G. W. *Bioorg. Chemistry* **1999**, *27*, 477.
2. (a) Jennette, K.; Lippard, S. J.; Vassiliades, G.; Bauer, W. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3839. (b) Liu, H. Q.; Cheung, T. C.; Peng, S. M.; Che, C. M. *J. Chem. Soc., Chem. Commun.* **1995**, 1787. (c) McCoubrey, A.; Latham, H. C.; Cook, P. R.; Rodger, A.; Lower, G. *FEBS Letters* **1996**, *380*, 73. (d) Vliet, P. M. V.; Toekimin, M. S.; Haasnoot, J. G.; Reedijk, J.; Nováková, O.; Vrána, O.; Brabec, V. *Inorg. Chim. Acta* **1995**, *231*, 57. (e) Carter, P. J.; Cheng, C. C.; Thorp, H. H. *J. Am. Chem. Soc.* **1998**, *120*, 632.
3. Lowe, G.; Droz, A. S.; Vilaivan, T.; Weaver, G. W.; Tweedale, L.; Pratt, J. M.; Rock, P.; Yardley, V.; Croft, S. L. *J. Med. Chem.* **1999**, *42*, 999.
4. (a) Kim, D. S. H. L.; Ashendel, C. L.; Zhou, Q.; Chang, C. t.; Lee, E. S.; Chang, C.-j. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2695. (b) Xu, W. C.; Zhou, Q.; Ashendel, C. L.; Chang, C. t.; Chang, C.-j. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2279. (c) Zhao, L. X.; Kim, T. S.; Ahn, S. H.; Kim, T. H.; Kim, E. k.; Cho, W. J.; Choi, H.; Lee, C. S.; Kim, J. A.; Jeong, T. C.; Chang, C.-j.; Lee, E. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2659.
5. (a) Cozzarelli, N. R. *Cell* **1980**, *22*, 327. (b) Liu, L. F. *Crit. Rev. Biochem.* **1983**, *15*, 1. (c) Kafiani, C. A.; Bronstein, I. B.; Timofeen, A. V.; Gromova, I. I.; Terskikh, V. V. *Adv. Enzyme Regul* **1986**, *25*, 439. (d) Zijlstra, J. G.; de Jong, S.; de Vries, E. G. E.; Mulder, N. H. *Med. Oncol. Tumor Pharmacother.* **1990**, *7*, 11.
6. (a) Zecher, W.; Kröhnke, F. *Chem. Ber.* **1961**, *94*, 690. (b) Kröhnke, F. *Angew. Chem., Int. Ed.* **1963**, *2*, 380. (c) Kröhnke, F. *Synthesis* **1976**, 1.
7. Fukuda, M.; Nishio, K.; Kanzawa, F.; Ogasawara, H.; Ishida, T.; Arioka, H.; Bojanowski, K.; Oka, M.; Saijo, N. *Cancer Res.* **1996**, *56*, 789. The topoisomerase I inhibitory activity was carried out as follows: The activity of DNA topoisomerase was determined by measuring the relaxation of supercoiled DNA pBR322. For measurement of topoisomerase activity, the reaction mixture was comprised of 35 mM Tris–HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine, 0.01% bovine serum albumin (BSA), 1 μg pBR322, and 2 U DNA topoisomerase. Topoisomerase I inhibitors (prepared compounds) were added to the above component for measuring the inhibition of DNA relaxation. The reaction mixture was incubated at 37 °C for 30 min. The reactions were terminated by adding dye solution comprising 1% SDS, 0.02% bromophenol blue and 50% glycerol. The mixture was applied to 1% agarose gel and electrophoresed for 1 h with a running buffer of Tris-acetate EDTA. The gel was stained with ethidium bromide.
8. The spectral data of $\mathbf{B_2}$, $\mathbf{B_6}$, $\mathbf{B_{10}}$ and $\mathbf{B_{13}}$:
$\mathbf{B_2}$: TLC (EtOAc:*n*-hexane = 1:5, v:v), $R_f = 0.2$, mp 83.1–84.6 °C.
$^1$H NMR (250 MHz, $CDCl_3$) δ 8.53 (dd, $J = 5.3$, 0.8 Hz, 1H, pyridine H-6), 7.87 (dd, $J = 1.5$, 0.8 Hz, 1H, pyridine H-3), 7.65 (dd, $J = 3.7$, 1.1 Hz, 1H, thiophene H-5), 7.55 (dd, $J = 1.8$, 0.6 Hz, 1H, furan H-5), 7.40 (dd, $J = 5.1$, 1.1 Hz, thiophene H-3), 7.34 (dd, $J = 5.3$, 1.5 Hz, pyridine H-5), 7.12 (dd, $J = 5.1$, 3.7 Hz, 2H, thiophene H-4), 6.90 (dd, $J = 3.4$, 0.6 Hz, 1H, furan H-3), 6.53 (dd, $J = 3.4$, 1.8 Hz, 1H, furan H-4).
$^{13}$C NMR (62.5 MHz, $CDCl_3$) δ 153.03, 151.21, 149.87, 144.72, 143.76, 138.07, 127.97, 127.60, 124.63, 116.06, 112.68, 112.08, 108.79.
ESI LC/MS $[MH]^+$ 228.
HPLC condition: column: C18 reverse phased, 1.5×150 mm, 5 μm, GL science, flow rate: 180 μL/min, injection volume: 5 μL of 100 μM solution, mobile phase: 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B), 10% B in A to 90% B in A for 10 min and retaining for 10 min at 90% B in A, retention time: 15 min.
MS ionization condition: 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.
The other compounds were followed the same above conditions for ESI LC/MS, and showed almost the same retention time for each compound.
$\mathbf{B_6}$: TLC (EtOAc:*n*-hexane = 1:5, v:v), $R_f = 0.3$, mp 129.8–130.8 °C.
$^1$H NMR (250 MHz, $CDCl_3$) δ 7.80 (s, 2H, pyridine H-3, H-5), 7.57–7.55 (m, 3H, 2-furan H-5, 4-furan H-5, 6-furan H-5), 7.17 (d, $J = 3.4$ Hz, 2H, 2-furan H-3, 6-furan H-3), 6.96 (d, $J = 3.3$ Hz, 1H, 4-furan H-3), 6.56–6.53 (m, 3H, 2-furan H-4, 4-furan H-4, 6-furan H-4).
$^{13}$C NMR (62.5 MHz, $CDCl_3$) δ 153.63, 151.51, 149.66, 143.72, 143.29, 138.73, 112.10, 112.02, 110.85, 109.10, 108.87.
ESI LC/MS $[MH]^+$ 278.
$\mathbf{B_{10}}$: TLC (EtOAc:*n*-hexane = 1:5, v:v), $R_f = 0.2$, mp 102.5–104.3 °C.

$^1$H NMR (250 MHz, $CDCl_3$) δ 7.72 (d, $J$ = 1.2 Hz, 1H, pyridine H-3), 7.69 (dd, $J$ = 3.7, 1.0 Hz, 1H, thiophene H-5), 7.63 (d, $J$ = 15.7 Hz, 1H, -CH = C**H**-furan), 7.57 (d, $J$ = 1.7 Hz, 1H, 4-furan H-5), 7.45 (d, $J$ = 1.7 Hz, 1H, 2-furan H-5), 7.41 (dd, $J$ = 5.0, 1.0 Hz, 1H, thiophene H-3), 7.37 (d, $J$ = 1.2 Hz, 1H, pyridine H-5), 7.13 (dd, $J$ = 5.0, 3.7 Hz, 1H, thiophene H-4), 7.09 (d, $J$ = 15.7 Hz, 1H, -C**H** = CH-furan), 6.92 (d, $J$ = 3.3 Hz, 1H, 2-furan H-3), 6.55 (dd, $J$ = 3.7, 1.7 Hz, 1H, 2-furan H-4), 6.52 (d, $J$ = 3.3 Hz, 1H, 4-furan H-3), 6.46 (dd, $J$ = 3.7, 1.7 Hz, 1H, 4-furan H-4).

$^{13}$C NMR (62.5 MHz, $CDCl_3$) δ 155.71, 153.51, 153.13, 151.92, 145.76, 144.04, 143.03, 139.07, 128.36, 128.06, 126.23, 125.23, 121.18, 115.52, 112.53, 112.37, 111.25, 111.09, 109.08.

ESI LC/MS $[MH]^+$ 320.

**$B_{13}$**: TLC (EtOAc:*n*-hexane = 1:5, v:v), $R_f$ = 0.3, mp 120.4–122.2 °C.

$^1$H NMR (250 MHz, $CDCl_3$) δ 7.72 (s, 2H, pyridine H-3, H-5), 7.70 (dd, $J$ = 3.7, 1.1 Hz, 2H, 2-thiophene H-5, 4-thiophene H-5), 7.56 (dd, $J$ = 1.8, 0.5 Hz, 1H, furan H-5), 7.42 (dd, $J$ = 5.0, 1.1 Hz, 2H, 2-thiophene H-3, 4-thiophene H-3), 7.13 (dd, $J$ = 5.0, 3.7 Hz, 2H, 2-thiophene H-4, 4-thiophene H-4), 6.95 (dd, $J$ = 3.4, 0.5 Hz, 1H, furan H-3), 6.56 (dd, $J$ = 3.4, 1.8 Hz, 1H, furan H-4).

$^{13}$C NMR (62.5 MHz, $CDCl_3$) δ 152.61, 151.38, 144.76, 143.73, 138.88, 127.92, 127.82, 124.85, 112.13, 110.92, 108.79.

ESI LC/MS $[MH]^+$ 310.

9. Skehan, P.; Streng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warrenm, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107. All experimental procedures were followed up the NCI's protocol based on the Sulforhodamine B (SRB) method. Briefly, tumor cells were cultured to maintain logarithmic growth by changing the medium 24 h before cytotoxicity assay. On the day of the assay, the cells were harvested by trysinization, counted, diluted in media and added to 96-well plates. The concentrations of tumor cells used were $5\times10^3$ (A549, HCT15), $1\times10^4$ (SK-MEL-2), and $2\times10^4$ cells/well (SK-OV-3). The cells were then preincubated for 24 h in 5% $CO_2$ incubator at 37 °C. The compounds dissolved in DMSO were added to the wells in six 3-fold dilutions starting from the highest concentrations, and incubated for 48 h in 5% $CO_2$ incubator at 37 °C. The final DMSO concentration was $<0.5\%$. At the termination of the incubation, the culture medium in each well was removed, and the cells were fixed with cold 10% trichloroacetic acid (TCA) for 1 h at 4 °C. The microplates were washed, dried, and stained with 0.4% SRB in 1% acetic acid for 30 min at room temperature. The cells were washed again and the bound stain was solubilized with 10 mM Tris base solution (pH 10.5), and the absorbances were measured spectrophotometrically at 520 nm on a microtiter plate reader (Molecular Devices, Sunnyvale, CA). The data was transformed into MS Excel format and survival fractions were calculated by regression analysis (plotting the cell viability versus the concentration of the test compound). The $EC_{50}$ values represent the concentrations of the compounds that inhibit 50% of cell growth. All data represent the average values for a minimum of three wells.