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2,4-Diaryl-5,6-dihydro-1,10-phenanthroline and 2,4-diaryl-5,6-dihydrothieno [2,3-*h*] quinoline derivatives for topoisomerase I and II inhibitory activity, cytotoxicity, and structure–activity relationship study

Pritam Thapa [a], Radha Karki [a], Han Young Yoo [a], Pil-Hoon Park [a], Eunyoung Lee [b], Kyung-Hwa Jeon [b], Younghwa Na [c], Won-Jea Cho [d], Youngjoo Kwon [b,*], Eung-Seok Lee [a,*]

[a] College of Pharmacy, Yeungnam University, Kyongsan 712-749, Republic of Korea
[b] College of Pharmacy, Division of Life & Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, Republic of Korea
[c] College of Pharmacy, Cha University, Pochon 487-010, Republic of Korea
[d] College of Pharmacy, Chonnam National University, Kwangju 500-757, Republic of Korea

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ABSTRACT

Designed and synthesized thirty-two 2,4-diaryl-5,6-dihydro-1,10-phenanthroline and 2,4-diaryl-5,6-dihydrothieno[2,3-*h*] quinoline derivatives as rigid analogs of 2,4,6-trisubstituted pyridines 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 [2,2′;6′,2″]-terpyridine skeleton is important for the cytotoxicity against several human cancer cell lines.

1. Introduction

Our research group has been studying various flexible terpyridine derivatives for the development of anticancer agents. They have shown moderate to considerable topoisomerase (topo) I and/or II inhibitory activities, and cytotoxicity against several human cancer cell lines [1]. Recently, various rigid analogs were reported to possess moderate topoisomerase inhibitory activity and cytotoxicity against several human cancer cell lines [2].

It is well documented that planarity facilitates the molecules to intercalate into the DNA in the topo I-DNA ternary complex [3]. Intercalation of planar molecules into DNA helix allows the stabilization of the topo-DNA covalent cleavage intermediates, converting topo into a lethal DNA-damaging agent. Many clinically used drugs like topotecan (topo I inhibitor) and doxorubicin (topo II inhibitor) exert their anticancer action by the similar mechanism [4]. 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 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 [5]. Herein we have designed and synthesized various rigid analogs of flexible 2,4,6-trisubstituted pyridines. From the previous study it was found that alpha carbon linkage in terpyridine has important role in displaying cytotoxicity against several human cancer cell lines. [2,2′;6′,2″]-Terpyridine (**a**) was found to have the most significant cytotoxicity [6] (Fig. 1). This motivate us to synthesize various rigid analogs of 2,4,6-trisubstituted pyridines containing [2,2′;6′,2″]-terpyridine skeleton and other derivatives.

Moreover, 2,4-diaryl-5,6-dihydro-1,10-phenanthroline (**b**) and 2,4-diaryl-5,6-dihydrothieno[2,3-*h*] quinoline derivatives (**c**) share common quinoline core in their structure (Fig. 1). Quinoline core is of great interest in the field of medicinal chemistry as they are present in a wide range of biologically active compounds frequently condensed with other heterocycles [7]. It is the fact that well known topo I inhibitor camptothecin bears quinoline moiety. Modifications or complete replacement of the quinoline ring with an alternative ring system has been done to find the more potent compound. Though analogs with replaced quinoline ring system with other planar heterocycles showed moderate to good activities, none of these derivatives have surpassed the potency of CPT [8].

In this study, we have designed and synthesized thirty-two 2,4-diaryl-5,6-dihydro-1,10-phenanthroline (**b**) and 2,4-diaryl-5,6-dihydrothieno[2,3-*h*] quinoline derivatives (**c**) as rigid analogs of 2,4,6-trisubstituted pyridines (Fig. 1). They were evaluated for

* Corresponding authors. Fax: +82 2 3277 2851 (Y. Kwon), +82 53 810 4654 (E.-S. Lee).
E-mail addresses: ykwon@ewha.ac.kr (Y. Kwon), eslee@yu.ac.kr (E.-S. Lee).

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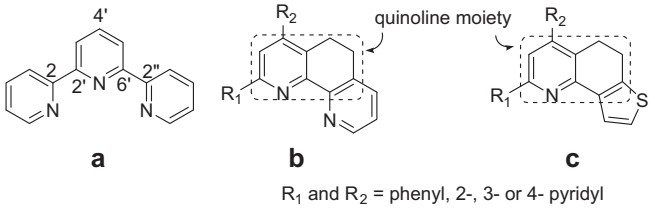


Fig. 1. Structure of 2,2′:6′,2″-terpyridine (**a**), and rigid analogs of 2,4,6-trisubstituted pyridines (**b**, **c**).

their topoisomerase I and II inhibitory activity, and cytotoxicity against several human cancer cell lines.

2. Results and discussion

2.1. Synthetic chemistry

Pyridinium iodide salts (**2a-d**) were synthesized by refluxing aryl methyl ketones (**1a-d**) with I_2 and pyridine at 140 °C for 3 h in 64.2–99.4% yields. Starting material 6,7-dihydro-5H-quinolin-8-one (**4**) was prepared according to previously reported method [9]. 6,7-Dihydroquinolin-8(5H)-one derivatives (**6a-d**) were synthesized using the Claisen–Schmidt KOH catalyzed condensation reaction [10] as illustrated in Scheme 1. Aryl aldehydes (**3a-d**) were reacted with **4** in the presence of KOH using a solution of methanol and water at 0 °C for 3–4 h to get 56.1–90.6% of **6a-d** as light yellow solid.

6,7-Dihydrobenzo[*b*]thiophen-4(5H)-one derivatives (**7a-d**) were synthesized by condensation of 6,7-dihydrobenzo[*b*]thiophen-4(5H)-one (**5**) with aryl aldehydes (**3a-d**) either in the presence of 40% aqueous KOH in ethanol (Method A) or AcOH/piperidine as a catalyst (Method B) [11] as shown in Scheme 1. Compound **7a** was synthesized according to method A, where aryl aldehyde **3a** was treated with ketone **5** in the presence of 40% aqueous KOH and ethanol at 0 °C for 3 h to yield 71.4% of **7a** as a white solid. Compounds **7b-d** was synthesized according to method B, where aryl aldehydes **3b-d** was treated with ketone **5** in the presence of catalytic amount of AcOH and piperidine at 90 °C for 5–12 h. This method affords compounds **7b-d** in 40.7–68.5% yield as white or light yellow solids.

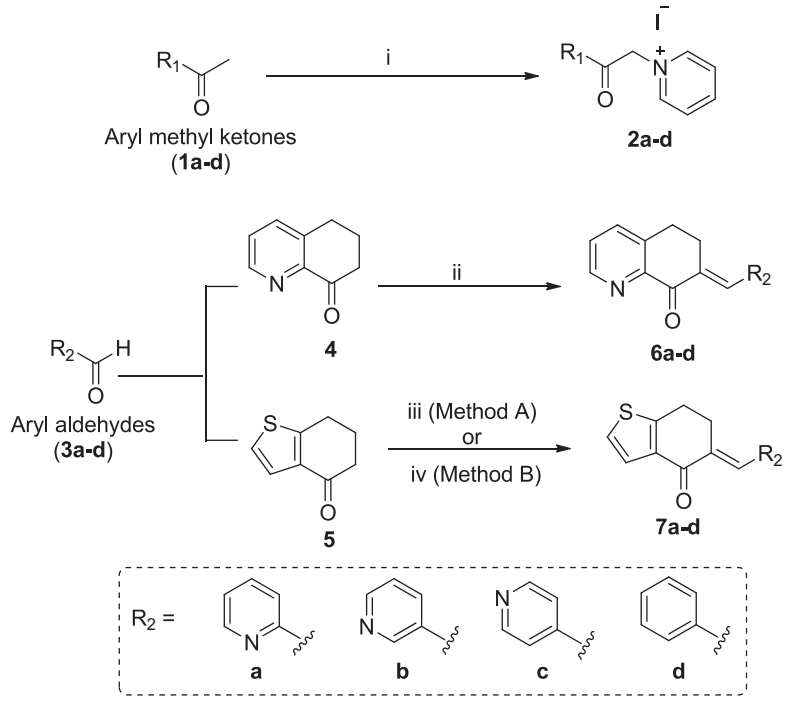
On the basis of modified KrÖhnke synthesis[12], rigid analogs of 2,4,6-trisubstituted pyridine, **8** (R_1, R_2 = **a-d**) and **9** (R_1, R_2 = **a-d**), were synthesized by treatment of 6,7-dihydroquinolin-8(5H)-one derivatives (**6a-d**) and 6,7-dihydrobenzo[*b*]thiophen-4(5H)-one derivatives (**7a-d**) respectively with pyridinium iodide salts **2a-d** in the presence of NH_4OAc in MeOH or glacial acetic acid as illustrated in Scheme 2. Thirty-two rigid analogs, 2,4-diaryl-5,6-dihydro-1,10-phenanthrolines (**10–25**) and 2,4-diaryl-5,6-dihydrothieno[2,3-*h*] quinolines (**26–41**), of 2,4,6-trisubstituted pyridines, were synthesized in 11.9–92.4% yield as shown in Fig. 2.

2.2. Topo I and II inhibitory activity

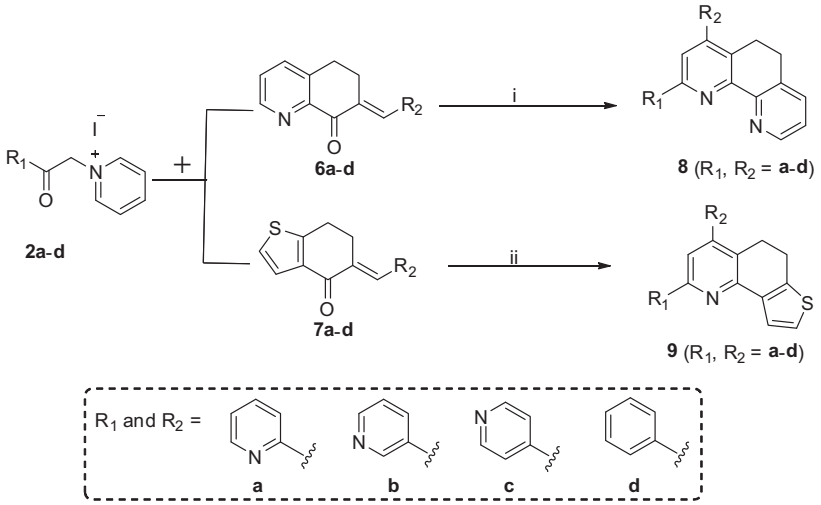
The prepared 32 compounds **10–41** were evaluated for their topo I and II inhibitory activities. Topoisomerase inhibitory activities of the tested compounds are displayed in Fig. 3 and 4 and Table 1. Percentage inhibition for compounds **10–25** are not displayed as they have not shown any topoisomerase I and II inhibitory activities.

2.2.1. Topoisomerase I inhibitory activity of the evaluated compounds

Synthesized 32 compounds **10–41** were evaluated for topo I inhibitory activity at 100 μM concentration, as compared to camptothecin, a well known topo I inhibitor. Selected compounds, possessing significant inhibitory activity at 100 μM, were evaluated for topo I inhibitory activity at 20 μM. It is found that most of the compounds were devoid of topo I inhibitory activity except **33** and **35**. The percentage of topo I inhibition for **33** and **35** was 60.0% and 25.8%, respectively at 100 μM. The topo I inhibitory activity of the evaluated compounds is summarized in Fig. 3, and Table 1.



Scheme 1. Synthesis of pyridinium iodide salts **2a-d**, 6,7-dihydroquinolin-8(5H)-one derivatives **6a-d** and 6,7-dihydrobenzo[*b*]thiophen-4(5H)-one derivatives **7a-d**. Reagents and conditions: (i) pyridine, iodine (1.0 equiv.), 3 h, 140 °C, 64.2–99.4%; (ii) KOH (1.2 equiv.), MeOH: H_2O (5: 1 v/v), 3–4 h, 0 °C, 56.1–90.6%; (iii) 40% aqueous KOH, EtOH, 3 h, 0 °C, 71.4%; (iv) AcOH, piperidine, 5–12 h, 90 °C, 40.7–68.5%.



Scheme 2. Synthesis of 2,4-diaryl-5,6-dihydro-1,10-phenanthroline derivatives **8** and 2,4-diaryl-5,6-dihydrothieno[2,3-*h*] quinoline derivatives **9**. Reagents and conditions: (i) NH_4OAc (10.0 equiv.), dry MeOH, 12–18 h, 80–100 °C, 26.2–92.4%; (ii) NH_4OAc (10.0 equiv.), glacial AcOH or dry MeOH, 12–48 h, 90–100 °C, 11.9–51.6%.

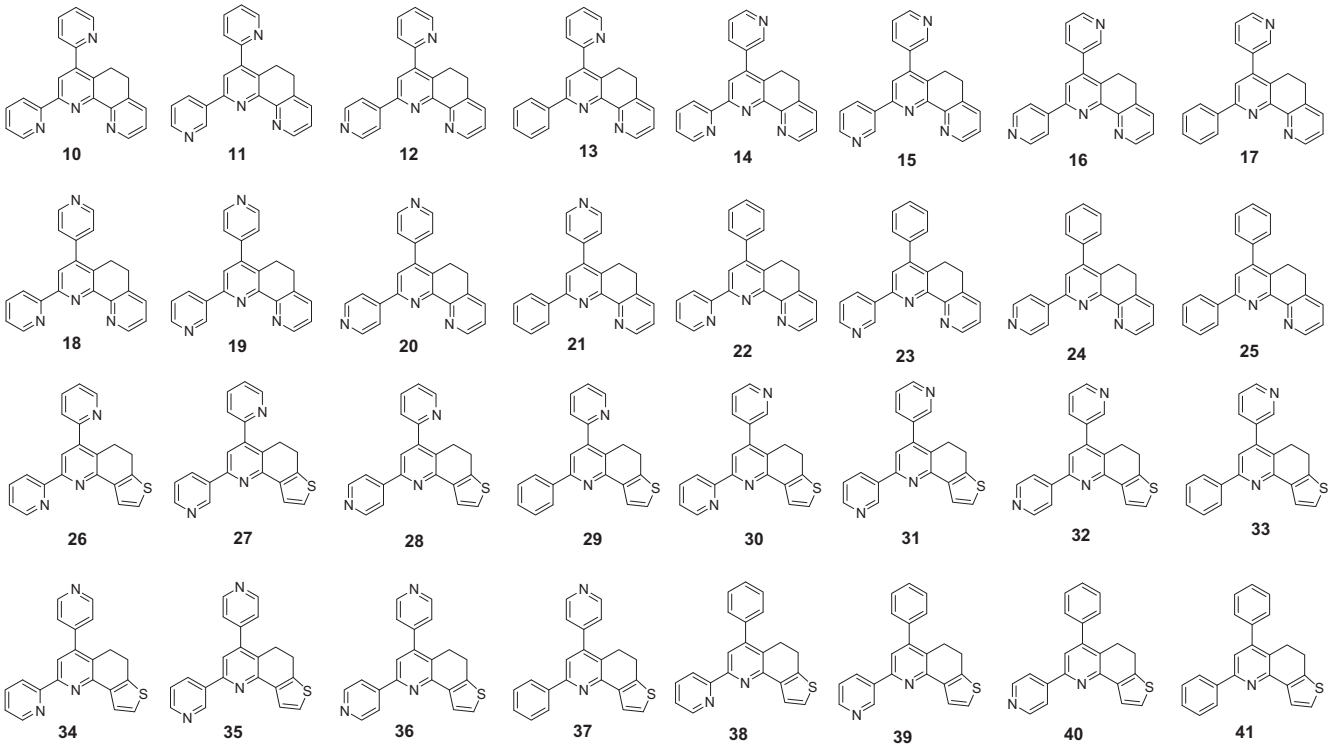


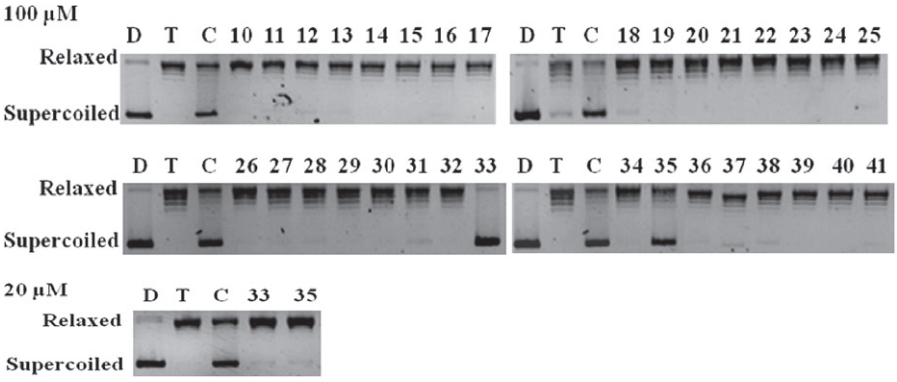
Fig. 2. Structures of synthesized 2,4-diaryl-5,6-dihydro-1,10-phenanthroline and 2,4-diaryl-5,6-dihydrothieno[2,3-*h*] quinoline derivatives.

2.2.2. Topoisomerase II inhibitory activity of the evaluated compounds

Synthesized 32 compounds **10–41** were evaluated for topo II inhibitory activity at 100 μM as compared to etoposide, a well known topo II inhibitor. Most of the compounds did not show significant topo II inhibitory activity. Several compounds such as **27–29** and **31** showed mild activity in the range of 11.5–15.7% at 100 μM. The topo II inhibitory activity of the evaluated compounds is summarized in Fig. 4 and Table 1.

2.3. Cytotoxicity

Selected nine compounds were evaluated for cytotoxicity. Selection was achieved on the basis of topo I inhibitory activity, and previously reported data by our laboratory [6]. Previously, it was found that [2,2′;6′,2″]-terpyridine has shown the most significant cytotoxicity which indicates that alpha carbon linkage in terpyridine is important for cytotoxicity. Selective compounds 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 of cell growth (IC_{50}) under the assay conditions and compared with that of adriamycin. The cytotoxicity results are summarized in Table 2.



Lane D: pBR322 only,

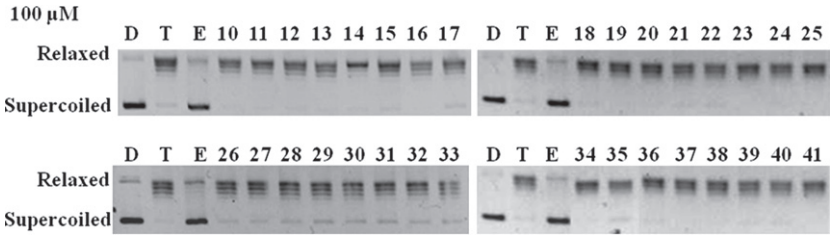
Lane T: pBR322 + Topo I,

Lane C: pBR322 + Topo I + Camptothecin,

Lane 10-41: pBR322 + Topo I + samples (**10** to **41**) at 100 μM

Lane 33 and 35: pBR322 + Topo I + samples (**33** and **35**) at 20 μM

Fig. 3. Topo I inhibitory activity of synthesized compounds at 100 μM and 20 μM.



Lane D: pBR322 only,

Lane T: pBR322 + Topo II,

Lane C: pBR322 + Topo II + Etoposide,

Lane 10-41: pBR322 + Topo II + samples (**10** to **41**) at 100 μM

Fig. 4. Topo II inhibitory activity of synthesized compounds at 100 μM.

Table 1
Topoisomerase I and II inhibitory activity of compounds 26–41.

Compounds	%Inhibition	
	Topo I 100 μM	Topo II 100 μM
Camptothecin	60.82/52.39*	
Etoposide		67.46/68.28*
26	1.88	5.53
27	N	12.45
28	N	13.95
29	N	11.51
30	N	7.44
31	N	15.78
32	0.94	5.12
33	60.01	4.95
34	3.37	5.23
35	25.84	8.27
36	1.32	5.90
37	0.93	5.97
38	2.04	4.73
39	N	4.59
40	N	2.80
41	N	1.67

* Value for compounds **34**–**41**, N: none.

As anticipated, it is found that compounds (**10**, **14** and **18**) having alpha carbons of the pyridine linked to each other showed the significant cytotoxicity against all five human cancer cell lines.

2.4. Structure–activity relationship (SAR) study

SAR was performed according to the results of topo I and II inhibitory activity, and cytotoxicity of the evaluated compounds. As most of the compounds were devoid of topo I and II inhibitory activity, the concrete correlation of the structure of compounds with topo inhibitory activity could not be determined. However, some compounds from thienoquinoline derivatives such as **33** has shown significant topo I inhibitory activity at 100 μM, and **35** has shown moderate topo I inhibitory activity at 100 μM as compared to positive control, camptothecin. Moreover, **27**–**29** and **31** has shown mild topo II inhibitory activity at 100 μM. These results, to some extent, indicate that thienoquinoline moiety may have important role for topo inhibitory activity as compared to phenanthroline moiety.

From results of cytotoxicity of the selected compounds, it is found that all compounds possessing [2,2′;6′,2″]-terpyridine skeleton has shown significant cytotoxicity against several human cancer cell lines. It signify that [2,2′;6′,2″]-terpyridine skeleton is

Table 2
Cytotoxicity of selected nine compounds against five different cancer cell lines.

Compd./cell	[a]IC_{50} (μM)				
	[b]MCF-7	[c]Hela	[d]DU145	[e]HCT15	[f]K562
Adriamycin	0.97 ± 0.07	0.98 ± 0.11	0.81 ± 0.07	1.31 ± 0.06	0.46 ± 0.12
Etoposide	3.56 ± 1.74	0.94 ± 0.17	1.04 ± 0.37	3.36 ± 1.38	0.82 ± 0.11
Camptothecin	0.63 ± 0.05	1.28 ± 0.25	1.17 ± 0.19	1.19 ± 0.38	0.71 ± 0.10
10	9.55 ± 0.96	2.11 ± 0.78	1.3 ± 0.078	0.9 ± 0.03	1.59 ± 0.40
11	>50	32.40 ± 2.75	>50	>50	>50
14	5.67 ± 0.28	0.31 ± 0.19	1.19 ± 0.20	0.86 ± 0.06	0.84 ± 0.27
15	>50	>50	>50	>50	>50
18	4.81 ± 1.30	0.34 ± 0.20	0.12 ± 0.06	0.97 ± 0.04	1.85 ± 0.10
19	>50	33.72 ± 11.10	>50	>50	>50
33	>50	>50	>50	>50	>50
34	>50	50.10 ± 11.76	>50	13.12 ± 2.48	>50
35	>50	35.69 ± 7.20	>50	4.03 ± 1.01	22.89 ± 4.00

[a] Each data represents mean ± S.D. from three different experiments performed in triplicate.
[b] MCF: human breast adenocarcinoma.
[c] HeLa: human cervix tumor.
[d] DU145: human prostate tumor.
[e] HCT15: human colorectal adenocarcinoma.
[f] K562: chronic myelogenous leukemia.

important for the cytotoxicity against several human cancer cell lines as reported earlier [6].

Interruption of resonance between five or six membered ring with central pyridine due to absence of double bond at 5,6 position is believed to be the reason behind the inactivity of most compounds as reported in earlier study [13].

3. Conclusion

We have designed and synthesized thirty-two 2,4-diaryl-5,6-dihydro-1,10-phenanthroline and 2,4-diaryl-5,6-dihydrothieno[2,3-*h*] quinoline derivatives as rigid analogs of 2,4,6-trisubstituted pyridines, and evaluated their topo I and II inhibitory activity, and cytotoxicity against several human cancer cell lines. Compound **33** showed considerable topo I inhibitory activity of 60.0% at 100 μM as compared to camptothecin (60.8% at 100 μM). Interestingly, it is found that phenanthroline derivatives having [2,2′;6′,2″]-terpyridine skeleton has shown significant cytotoxicity against all tested human cancer cell lines. This indicates that α-carbon linkage in terpyridine is important for possessing cytotoxicity against several human cancer cell lines. Therefore, in conclusion, we believe that [2,2′;6′,2″]-terpyridine skeleton has an important role for displaying the cytotoxic effects.

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 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 to solvent peaks. 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 run in Waters X-Terra® 5 μM reverse-phase C_{18} column (4.6 × 250 mm) with a gradient elution of (A) 20–100% of B in A for 20 min followed by 100–20% of B in A for 10 min; (B) 50–100% of B in A for 10 min followed by 100–50% 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 20 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 a 5 μL injection volume on a Waters Atlantis T3, 3 μm reverse-phase C_{18} column (2.1 × 50 mm) with a gradient elution from 20% to 100% of B in A for 6.5 min followed by 100–20% of B in A for 6 min and 20% of B in A for 2.5 min at a flow rate of 200 μL/min, where mobile phase A was 100% distilled water with 20 mM ammonium formate (AF) and mobile phase B was 100% acetonitrile (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 was given in minutes.

4.1. General method for preparation of ***2a-d***

Aryl methyl ketone (**1a-d**) with equivalent amount of iodine in pyridine was refluxed at 140 °C for 3 h. Reaction mixture was cooled to room temperature resulting precipitation. It was filtered and washed with cold pyridine followed by drying overnight to yield 64.2–99.4% of **2a-d**.

4.2. General method for the preparation of ***6a-d***

Aryl aldehydes (**3a-d**) (1.0 equiv.) were reacted with **4** (1.0 equiv.) in the presence of KOH (1.2 equiv.) with methanol and water at 0 °C for 3–4 h. After completion of reaction as monitored by TLC, methanol was evaporated under reduced pressure. The mixture was diluted with water, extracted with chloroform, dried over $MgSO_4$, filtered and concentrated to get yellow solid. It was further purified by recrystallization in EtOAc and *n*-hexane to afford 56.1–90.6% of **6a-d** as light yellow solid.

4.2.1. 7-(Pyridin-2-ylmethylene)-6,7-dihydroquinolin-8(5H)-one (***6a***)

The same procedure described at Section 4.2 was employed with **3a** (0.09 mL, 1.0 mmol) and **4** (0.14 g, 1.0 mmol) to yield 0.16 g (0.67 mmol, 67.7%) of **6a** as a yellow solid.

Mp 138.1–138.8 °C; R_f (dichloromethane/methanol 20:1 v/v): 0.28.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.78 (dd, J = 4.5, 1.0 Hz, 1H, dihydroquinolinone H-2), 8.71 (dd, J = 5.0, 1.2 Hz, 1H, pyridine H-6), 7.82 (s, 1H, =CH—), 7.74 (td, J = 7.7, 1.8 Hz, 1H, pyridine H-4), 7.69 (dd, J = 7.8, 0.8 Hz, 1H, dihydroquinolinone H-4), 7.47 (d, J = 7.8 Hz, 1H, pyridine H-3), 7.41 (dd, J = 7.8, 4.5 Hz, 1H, dihydroquinolinone H-3), 7.24 (ddd, J = 7.6, 4.8, 1.0 Hz, 1H, pyridine H-5), 3.73 (td, J = 6.7, 1.6 Hz, 2H, 6-CH_2), 3.05 (t, J = 6.7 Hz, 2H, 5-CH_2).

4.2.2. 7-(Pyridin-3-ylmethylene)-6,7-dihydroquinolin-8(5H)-one (**6b**)

The same procedure described at Section 4.2 was employed with **3b** (0.09 mL, 1.0 mmol) and **4** (0.14 g, 1.0 mmol) to yield 0.19 g (0.82 mmol, 82.5%) of **6b** as a light yellow solid.

Mp 135.8–136.5 °C; R_f (dichloromethane/methanol 20:1 v/v): 0.28.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.79 (d, J = 3.5 Hz, 1H, dihydroquinoline H-2), 8.71 (s, 1H, pyridine H-2), 8.60 (d, J = 3.5 Hz, 1H, pyridine H-6), 7.92 (s, 1H, =CH—), 7.75 (d, J = 7.8 Hz, 1H, pyridine H-4), 7.68 (dd, J = 7.8, 0.7 Hz, 1H, dihydroquinoline H-4), 7.43 (dd, J = 7.8, 4.5 Hz, 1H, dihydroquinoline H-3), 7.38 (dd, J = 7.9, 4.8 Hz, 1H, pyridine H-5), 3.18 (t, J = 6.5 Hz, 2H, 6-CH_2), 3.03 (t, J = 6.2 Hz, 2H, 5-CH_2).

4.2.3. 7-(Pyridin-4-ylmethylene)-6,7-dihydroquinolin-8(5H)-one (**6c**)

The same procedure described at Section 4.2 was employed with **3c** (0.13 mL, 1.36 mmol) and **4** (0.20 g, 1.36 mmol) to yield 0.29 g (1.23 mmol, 90.6%) of **6c** as a light yellow solid.

Mp 172.9–173.5 °C; R_f (dichloromethane/methanol 20:1 v/v): 0.33.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.78 (dd, J = 4.5, 1.5 Hz, 1H, dihydroquinolinone H-2), 8.69 (dd, J = 4.5, 1.6 Hz, 2H, pyridine H-2, H-6), 7.84 (s, 1H, =CH—), 7.69 (dd, J = 7.8, 1.3 Hz, 1H, dihydroquinolinone H-4), 7.45 (dd, J = 7.8, 4.5 Hz, 1H, dihydroquinoline H-3), 7.29 (dd, J = 5.8, 1.5 Hz, 2H, pyridine H-3, H-5), 3.73 (td, J = 7.0, 1.7 Hz, 2H, 6-CH_2), 3.05 (t, J = 6.8 Hz, 2H, 5-CH_2).

4.2.4. 7-Benzylidene-6,7-dihydroquinolin-8(5H)-one (**6d**)

The same procedure described at Section 4.2 was employed with **3d** (0.20 mL, 2.00 mmol) and **4** (0.29 g, 2.00 mmol) to yield 0.26 g (1.12 mmol, 56.1%) of **6d** as a light yellow solid.

Mp 125.3–125.9 °C; R_f (ethyl acetate/hexane 3:1 v/v): 0.18.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.77 (dd, J = 4.3, 1.2 Hz, 1H, dihydroquinoline H-2), 7.98 (s, 1H, =CH—), 7.65 (d, J = 7.7 Hz, 1H, dihydroquinolinone H-4), 7.44–7.36 (m, 5H, Phenyl H-2, H-3, H-4, H-5, H-6), 7.41 (dd, J = 7.8, 4.4 Hz, 1H, dihydroquinolinone H-3), 3.18 (td, J = 6.7, 1.5 Hz, 2H, 6-CH_2), 3.05 (t, J = 6.7 Hz, 2H, 5-CH_2).

4.3. General method for the preparation of **7a-d**

Compounds **7a**-**d** were synthesized by condensation of compound **5** with aryl aldehydes (**3a**-**d**) either in the presence of 40% aqueous KOH in ethanol (method A) or AcOH/piperidine as a catalyst (method B) [11] as shown in Scheme 1.

Compound **7a** was synthesized by following method A where aryl aldehyde **3a** was treated with **5** in the presence of 40% aqueous KOH in ethanol at 0 °C for 3 h, and precipitation was observed. It was filtered and washed with H_2O and cold methanol to yield 71.4% of **7a** as an off-white solid.

In method B, aryl aldehydes (**3b**-**d**) and **5** were reacted in the presence of catalytic amount of AcOH and piperidine at 90 °C for 5–12 h. The reaction mixture was diluted with H_2O, and extracted with $CHCl_3$, dried over $MgSO_4$, filtered, and concentrated in reduced pressure to yield brown viscous solid. Flash chromatography with ethyl acetate and *n*-hexane afford 40.7–68.5% of **7b**-**d** as white or light yellow solid.

4.3.1. 5-(Pyridin-2-ylmethylene)-6,7-dihydrobenzo[b]thiophen-4(5H)-one (**7a**)

The same procedure described at Section 4.3 was employed with **3a** (0.28 mL, 3.00 mmol) and **5** (0.30 g, 2.00 mmol) to yield 0.34 g (1.43 mmol, 71.4%) of **7a** as an off-white solid.

Mp 146.5–147.4 °C; R_f (ethyl acetate/*n*-hexane 3:1 v/v): 0.18.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.70 (d, J = 3.4 Hz, 1H, pyridine H-6), 7.73 (td, J = 7.6, 1.7 Hz, 1H, pyridine H-4), 7.66 (s, 1H, =CH—), 7.52 (d, J = 5.3 Hz, 1H, dihydrobenzothiophenone H-2), 7.43 (d, J = 7.7 Hz, 1H, pyridine H-3), 7.22 (dd, J = 7.5, 4.8 Hz, 1H, pyridine H-5), 7.12 (d, J = 5.2 Hz, 1H, dihydrobenzothiophenone H-3), 3.75 (td, J = 6.3, 1.5 Hz, 2H, 6-CH_2), 3.13 (t, J = 6.3 Hz, 2H, 7-CH_2).

4.3.2. 5-(Pyridin-3-ylmethylene)-6,7-dihydrobenzo[b]thiophen-4(5H)-one (**7b**)

The same procedure described at Section 4.3 was employed with **3b** (0.37 mL, 4.00 mmol) and **5** (0.30 g, 2.00 mmol) to yield 0.19 g (0.81 mmol, 40.7%) of **7b** as a white solid.

Mp 113.4–114.8 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.17.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.67 (d, J = 0.8 Hz, 1H, pyridine H-2), 8.57 (dd, J = 4.7, 1.2 Hz, 1H, pyridine H-6), 7.72 (s, 1H, =CH—), 7.70 (dt, J = 8.2, 1.6 Hz, 1H, pyridine H-4), 7.52 (d, J = 5.3 Hz, 1H, dihydrobenzothiophenone H-2), 7.35 (dd, J = 7.9, 4.9 Hz, 1H, pyridine H-5), 7.14 (d, J = 5.3 Hz, 1H, dihydrobenzothiophenone H-3), 3.21–3.07 (m, 4H, 6-CH_2, 7-CH_2).

4.3.3. 5-(Pyridin-4-ylmethylene)-6,7-dihydrobenzo[b]thiophen-4(5H)-one (**7c**)

The same procedure described at Section 4.3 was employed with **3c** (0.34 mL, 3.60 mmol) and **5** (0.30 g, 2.00 mmol) to yield 0.20 g (0.84 mmol, 42.2%) of **7c** as a white solid.

Mp 142.5–143.0 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.17.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.67 (dd, J = 4.5, 1.6 Hz, 2H, pyridine H-2, H-6), 7.66 (s, 1H, =CH—), 7.51 (d, J = 5.3 Hz, 1H, dihydrobenzothiophenone H-2), 7.27 (dd, J = 4.5, 1.0 Hz, 2H, pyridine H-3, H-5), 7.14 (d, J = 5.3 Hz, 1H, dihydrobenzothiophenone H-3), 3.18–3.09 (m, 4H, 6-CH_2, 7-CH_2).

4.3.4. 5-Benzylidene-6,7-dihydrobenzo[b]thiophen-4(5H)-one (**7d**)

The same procedure described at Section 4.3 was employed with **3d** (0.36 mL, 3.60 mmol) and **5** (0.30 g, 2.00 mmol) to yield 0.33 g (1.37 mmol, 68.5%) of **7d** as a light yellow solid.

Mp 105.7–106.0 °C; R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.36.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.77 (s, 1H, =CH—), 7.49 (d, J = 5.3 Hz, 1H, dihydrobenzothiophenone H-2), 7.40–7.30 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.09 (d, J = 5.3 Hz, 1H, dihydrobenzothiophenone H-3), 3.20 (td, J = 5.9, 1.5 Hz, 2H, 6-CH_2) 3.05 (t, J = 6.1 Hz, 2H, 7-CH_2).

4.4. General method for the preparation of **8** (R_1, R_2 = **a-d**) (**10–25**)

A mixture of **6a**-**d** (1.0 equiv.), **2a**-**d** (1.0 equiv.) and NH_4OAc (10.0 equiv.) in dry methanol was refluxed at 80–100 °C for 12–18 h under nitrogen gas. Thereafter, MeOH was evaporated under reduced pressure. The residue was diluted with H_2O (30 mL) and extracted with $CHCl_3$ (15 mL × 3). The organic layer was collected, dried over $MgSO_4$ (1 spatula), and filtered. Filtrate was evaporated at reduced pressure, dried and purified by alumina chromatography with a gradient elution of ethyl acetate and *n*-hexane to yield 26.2–92.4% of compounds **10**–**25**.

4.4.1. 2,4-Di(pyridin-2-yl)-5,6-dihydro-1,10-phenanthroline (**10**)

Procedure described at Section 4.4 was employed with **6a** (0.10 g, 0.42 mmol), dry ammonium acetate (0.32 g, 4.20 mmol), **2a** (0.13 g, 0.42 mmol), and dry methanol (2.5 mL) at 100 °C for 12 h to yield 60 mg (0.19 mmol, 42.6%) of **10** as a light yellow solid.

Mp 158.1–159.2 °C; R_f (Al_2O_3) (ethyl acetate/*n*-hexane 2:1 v/v): 0.11; purity (condition A): 97.2%.

LC/MS/MS: retention time: 5.99 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (dd, J = 4.8, 1.5 Hz, 1H, phenanthroline H-9), 8.76 (d, J = 8.0 Hz, 1H, 2-pyridine H-3), 8.75 (ddd, J = 4.1, 1.7, 0.9 Hz, 1H, 4-pyridine H-6), 8.66 (ddd, J = 4.7, 1.6, 0.8 Hz, 1H, 2-pyridine H-6), 8.54 (s, 1H, phenanthroline H-3), 7.85 (td, J = 7.8, 1.7 Hz, 2H, 2-pyridine H-4, 4-pyridine H-4), 7.60 (d, J = 7.9 Hz, 2H, 4-pyridine H-3, phenanthroline H-7), 7.37 (ddd, J = 7.5, 4.9, 1.0 Hz, 1H, 2-pyridine H-5), 7.32 (ddd, J = 8.0, 4.7, 1.2 Hz, 1H, 4-pyridine H-5), 7.28 (dd, J = 7.5, 4.8 Hz, 1H, phenanthroline H-8), 3.14 (t, J = 7.9 Hz, 2H, 5-CH_2), 2.95 (t, J = 7.8 Hz, 2H, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.25, 155.86, 154.82, 152.09, 152.04, 149.42, 149.03, 148.82, 147.93, 136.84, 136.59, 135.54, 134.09, 132.15, 124.22, 123.70, 123.56, 122.82, 121.96, 121.65, 27.45, 24.61.

4.4.2. 4-(Pyridin-2-yl)-2-(pyridin-3-yl)-5, 6-dihydro-1, 10-phenanthroline (**11**)

Procedure described at Section 4.4 was employed with **6a** (0.09 g, 0.40 mmol), dry ammonium acetate (0.31 g, 4.00 mmol), **2b** (0.13 g, 0.40 mmol), and dry methanol (2.5 mL) at 100 °C for 12 h to yield 92 mg (0.27 mmol, 68.3%) of **11** as a white solid.

Mp 201.2–201.9 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.22; purity (condition A): 100%.

LC/MS/MS: retention time: 5.57 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.28 (d, J = 1.8 Hz, 1H, 2-pyridine H-2), 8.81–8.77 (m, 2H, phenanthroline H-9, 4-pyridine H-6), 8.65 (dd, J = 4.7, 1.5 Hz, 1H, 2-pyridine H-6), 8.57 (dt, J = 8.0, 2.0 Hz, 1H, 2-pyridine H-4), 7.88 (s, 1H, phenanthroline H-3), 7.87 (td, J = 7.8, 1.7 Hz, 1H, 4-pyridine H-4), 7.60 (d, J = 7.5 Hz, 1H, phenanthroline H-7), 7.52 (d, J = 7.8 Hz, 1H, 4-pyridine H-3), 7.44–7.38 (m, 2H, 2-pyridine H-5, 4-pyridine H-5), 7.29 (dd, J = 7.6, 4.8 Hz, 1H, phenanthroline H-8), 3.11 (t, J = 7.8 Hz, 2H, 5-CH_2), 2.95 (t, J = 7.6 Hz, 2H, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.62, 153.66, 152.91, 151.81, 149.82, 149.75, 149.13, 148.29, 147.97, 136.64, 135.56, 135.01, 134.93, 134.03, 131.00, 124.11, 123.73, 123.49, 123.09, 121.26, 27.38, 24.59.

4.4.3. 4-(Pyridin-2-yl)-2-(pyridin-4-yl)-5,6-dihydro-1,10-phenanthroline (**12**)

Procedure described at Section 4.4 was employed with **6a** (0.08 g, 0.34 mmol), dry ammonium acetate (0.26 g, 3.40 mmol), **2c** (0.11 g, 0.34 mmol) and dry methanol (2.0 mL) at 100 °C for 12 h to yield 72 mg (0.21 mmol, 63.4%) of **12** as a white solid.

Mp 225.5–226.8 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.22; purity (condition A): 100%.

LC/MS/MS: retention time: 5.55 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (br, 2H, phenanthroline H-9, 4-pyridine H-6), 8.72 (d, J = 5.9 Hz, 2H, 2-pyridine H-2, H-6), 8.10 (d, J = 5.9 Hz, 2H, 2-pyridine H-3, H-5), 7.93 (s, 1H, phenanthroline H-3), 7.88 (td, J = 7.6, 1.5 Hz, 1H, 4-pyridine H-4), 7.60 (d, J = 7.2 Hz, 1H, phenanthroline H-7), 7.52 (d, J = 7.7 Hz, 1H, 4-pyridine H-3), 7.42 (dd, J = 7.4, 4.9 Hz, 1H, 4-pyridine H-5), 7.30 (dd, J = 7.5, 4.8 Hz, 1H, phenanthroline H-8), 3.13 (d, J = 7.7 Hz, 2H, 5-CH_2), 2.94 (t, J = 7.6 Hz, 2H, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.47, 153.41, 152.96, 151.71, 150.26, 149.87, 149.20, 148.04, 146.25, 136.64, 135.59, 134.05, 132.01, 124.13, 123.83, 123.16, 121.46, 121.42, 27.33, 24.71.

4.4.4. 2-Phenyl-4-(pyridin-2-yl)-5,6-dihydro-1,10-phenanthroline (**13**)

Procedure described at Section 4.4 was employed with **6a** (0.09 g, 0.40 mmol), dry ammonium acetate (0.31 g, 4.00 mmol), **2d** (0.13 g, 0.40 mmol), and dry methanol (2.5 mL) at 100 °C for 12 h to yield 124 mg (0.37 mmol, 92.4%) of **13** as a white solid.

Mp 224.8–225.9 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.34; purity (condition A): 100%.

LC/MS/MS: retention time: 7.03 min; [MH^+]: 336.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80–8.78 (m, 2H, phenanthroline H-9, 4-pyridine H-6), 8.20 (d, J = 8.3 Hz, 2H, 2-phenyl H-2, H-6), 7.86 (s, 1H, phenanthroline H-3), 7.85 (td, J = 7.8, 1.8 Hz, 1H, 4-pyridine H-4), 7.58 (dd, J = 7.5, 0.9 Hz, 1H, phenanthroline H-7), 7.51 (d, J = 7.8 Hz, 1H, 4-pyridine H-3), 7.47–7.36 (m, 4H, 2-phenyl H-3, H-4, H-5, 4-pyridine H-5), 7.26 (dd, J = 7.5, 4.8 Hz, 1H, phenanthroline H-8), 3.09–2.95 (m, 2H, 5-CH_2), 2.93–2.90 (m, 2H, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.12, 156.23, 152.39, 152.17, 149.69, 149.09, 147.66, 139.23, 136.56, 135.43, 133.96, 130.18, 128.74, 128.49, 127.27, 124.12, 123.46, 122.90, 121.16, 27.52, 24.53.

4.4.5. 2-(Pyridin-2-yl)-4-(pyridin-3-yl)-5,6-dihydro-1,10-phenanthroline (**14**)

Procedure described at Section 4.4 was employed with **6b** (0.08 g, 0.34 mmol), dry ammonium acetate (0.26 g, 3.40 mmol), **2a** (0.11 g, 0.34 mmol), and dry methanol (2.0 mL) at 100 °C for 12 h to yield 52 mg (0.15 mmol, 45.4%) of **14** as a white solid.

Mp 213.4–214.2 °C; R_f (Al_2O_3) (ethyl acetate/*n*-hexane 2:1 v/v): 0.13; purity (condition A): 96.3%.

LC/MS/MS: retention time: 5.65 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (d, J = 5.4 Hz, 1H, phenanthroline H-9), 8.79 (d, J = 1.1 Hz, 1H, 4-pyridine H-2), 8.74 (d, J = 7.8 Hz, 1H, 2-pyridine H-3), 8.71 (dd, J = 4.9, 1.5 Hz, 1H, 4-pyridine H-6), 8.66 (d, J = 4.1 Hz, 1H, 2-pyridine H-6), 8.41 (s, 1H, phenanthroline H-3), 7.87 (td, J = 7.8, 1.7 Hz, 1H, 2-pyridine H-4), 7.81 (dt, J = 8.1, 1.8 Hz, 1H, 4-pyridine H-4), 7.60 (d, J = 7.4 Hz, 1H, phenanthroline H-7), 7.45 (dd, J = 7.4, 4.9 Hz, 1H, 4-pyridine H-5), 7.33 (d, J = 5.0 Hz, 1H, 2-pyridine H-5), 7.28 (dd, J = 7.5, 4.8 Hz, 1H, phenanthroline H-8), 3.05–2.95 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 155.65, 154.94, 152.02, 151.91, 149.48, 149.43, 149.17, 148.90, 146.10, 136.95, 136.28, 135.63, 134.48, 133.86, 131.74, 123.89, 123.72, 123.29, 122.05, 121.85, 27.44, 24.88.

4.4.6. 2,4-Di(Pyridin-3-yl)-5,6-dihydro-1,10-phenanthroline (**15**)

Procedure described at Section 4.4 was employed with **6b** (0.07 g, 0.30 mmol), dry ammonium acetate (0.23 g, 3.00 mmol), **2b** (0.10 g, 0.30 mmol), and dry methanol (2.0 mL) at 100 °C for 12 h to yield 58 mg (0.17 mmol, 57.5%) of **15** as a white solid.

Mp 217.5–218.2 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.25; purity (condition A): 98.9%.

LC/MS/MS: retention time: 5.29 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.27 (d, J = 1.2 Hz, 1H, 2-pyridine H-2), 8.80 (d, J = 3.5 Hz, 1H, phenanthroline H-9), 8.75 (dd, J = 5.8, 1.3 Hz, 1H, 2-pyridine H-6), 8.73 (br, 1H, 4-pyridine H-2), 8.67 (dd, J = 4.5, 1.1 Hz, 1H, 4-pyridine H-6), 8.55 (dt, J = 7.8, 1.6 Hz, 1H, 2-pyridine H-4), 7.79 (dt, J = 7.7, 1.8 Hz, 1H, 4-pyridine H-4), 7.69 (s, 1H, phenanthroline H-3), 7.61 (d, J = 6.9 Hz, 1H, phenanthroline H-7), 7.49 (dd, J = 7.6, 4.8 Hz, 1H, 2-pyridine H-5), 7.44 (dd, J = 7.8, 4.8 Hz, 1H, 4-pyridine H-5), 7.30 (dd, J = 7.5, 4.8 Hz, 1H, phenanthroline H-8), 3.03–2.93 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.82, 152.90, 151.67, 149.95, 149.69, 149.36, 149.26, 148.25, 146.14, 136.14, 135.67, 135.05, 134.73, 134.22, 133.87, 130.87, 123.91, 123.59, 123.43, 121.47, 27.36, 24.74.

4.4.7. 4-(Pyridin-3-yl)-2-(pyridin-4-yl)-5,6-dihydro-1,10-phenanthroline (**16**)

Procedure described at Section 4.4 was employed with **6b** (0.12 g, 0.50 mmol), dry ammonium acetate (0.38 g, 5.00 mmol),

2c (0.16 g, 0.50 mmol), and dry methanol (2.5 mL) at 100 °C for 12 h to yield 58 mg (0.17 mmol, 34.5%) of **16** as a white solid.

Mp 284.2–284.8 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.22; purity (condition A): 99.4%.

LC/MS/MS: retention time: 5.26 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (dd, J = 4.6, 1.0 Hz, 1H, phenanthroline H-9), 8.74–8.72 (m, 4H, 2-pyridine H-2, H-6, 4-pyridine H-2, H-6), 8.06 (dd, J = 4.5, 1.2 Hz, 2H, 2-pyridine H-3, H-5), 7.76 (dt, J = 7.8, 1.7 Hz, 1H, 4-pyridine H-4), 7.72 (s, 1H, phenanthroline H-3), 7.61 (d, J = 7.5 Hz, 1H, phenanthroline H-7), 7.48 (dd, J = 7.7, 4.8 Hz, 1H, 4-pyridine H-5), 7.31 (dd, J = 7.4, 4.7 Hz, 1H, phenanthroline H-8), 3.02–2.92 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.57, 152.93, 151.51, 150.34, 149.74, 149.31, 149.28, 146.23, 146.06, 136.16, 135.75, 134.13, 133.91, 131.92, 124.05, 123.46, 121.67, 121.41, 27.28, 24.83.

*4.4.8. 2-Phenyl-4-(pyridin-3-yl)-5,6-dihydro-1,10-phenanthroline (**17**)*

Procedure described at Section 4.4 was employed with **6b** (0.12 g, 0.50 mmol), dry ammonium acetate (0.38 g, 5.00 mmol), **2d** (0.16 g, 0.50 mmol), and dry methanol (2.5 mL) at 100 °C for 12 h to yield 127 mg (0.38 mmol, 76.0%) of **17** as a white solid.

Mp 221.2–221.9 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.30; purity (condition A): 100%.

LC/MS/MS: retention time: 6.72 min; [MH^+]: 336.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (dd, J = 4.7, 1.3 Hz, 1H, phenanthroline H-9), 8.74–8.71 (m, 2H, 4-pyridine H-2, H-6), 8.17 (dd, J = 8.2, 1.4 Hz, 2H, 2-phenyl H-2, H-6), 7.77 (dt, J = 7.8, 1.7 Hz, 1H, 4-pyridine H-4), 7.67 (s, 1H, phenanthroline H-3), 7.59 (dd, J = 7.5, 0.9 Hz, 1H, phenanthroline H-7), 7.52–7.39 (m, 4H, 4-pyridine H-5, 2-phenyl H-3, H-4, H-5), 7.27 (dd, J = 7.6, 4.8 Hz, 1H, phenanthroline H-8), 3.00–2.90 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.36, 152.35, 151.98, 149.45, 149.40, 149.16, 145.76, 138.98, 136.19, 135.56, 134.59, 133.79, 130.01, 128.97, 128.63, 127.24, 123.66, 123.35, 121.41, 27.47, 24.68.

*4.4.9. 2-(Pyridin-2-yl)-4-(pyridin-4-yl)-5,6-dihydro-1,10-phenanthroline (**18**)*

Procedure described at Section 4.4 was employed with **6c** (0.07 g, 0.30 mmol), dry ammonium acetate (0.23 g, 3.00 mmol), **2a** (0.10 g, 0.30 mmol), and dry methanol (2.5 mL) at 100 °C for 12 h to yield 41 mg (0.12 mmol, 40.6%) of **18** as a white solid.

Mp 210.2–210.8 °C; R_f (Al_2O_3) (ethyl acetate/*n*-hexane 2:1 v/v): 0.17; purity (condition A): 97.7%.

LC/MS/MS: retention time: 5.62 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (dd, J = 4.7, 1.5 Hz, 1H, phenanthroline H-9), 8.77 (d, J = 8.5 Hz, 1H, 2-pyridine H-3), 8.75 (dd, J = 4.4, 1.5 Hz, 2H, 4-pyridine H-2, H-6), 8.65 (d, J = 4.7 Hz, 1H, 2-pyridine H-6), 8.37 (s, 1H, phenanthroline H-3), 7.88 (td, J = 7.7, 1.7 Hz, 1H, 2-pyridine H-4), 7.61 (dd, J = 7.5, 1.3 Hz, 1H, phenanthroline H-7), 7.37 (dd, J = 4.4, 1.6 Hz, 2H, 4-pyridine H-3, H-5), 7.35 (ddd, J = 7.8, 4.9, 1.0 Hz, 1H, 2-pyridine H-5), 7.32 (dd, J = 7.6, 4.8 Hz, 1H, phenanthroline H-8), 3.03–2.92 (s, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 155.52, 154.99, 151.99, 151.72, 149.97, 149.12, 148.92, 146.92, 146.58, 137.00, 135.69, 133.87, 131.16, 123.95, 123.81, 123.64, 122.00, 121.19, 27.35, 24.79.

*4.4.10. 2-(Pyridin-3-yl)-4-(pyridin-4-yl)-5,6-dihydro-1,10-phenanthroline (**19**)*

Procedure described at Section 4.4 was employed with **6c** (0.07 g, 0.30 mmol), dry ammonium acetate (0.23 g, 3.00 mmol), **2b** (0.10 g, 0.30 mmol), and dry methanol (2.5 mL) at 80 °C for 12 h to yield 47 mg (0.14 mmol, 46.6%) of **19** as a yellow solid.

Mp 278.7–279.3 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.29; purity (condition A): 95.3%.

LC/MS/MS: retention time: 5.20 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.26 (d, J = 1.7 Hz, 1H, 2-pyridine H-2), 8.80 (d, J = 4.3 Hz, 1H, phenanthroline H-9), 8.78 (dd, J = 4.4, 1.5 Hz, 2H, 4-pyridine H-2, H-6), 8.67 (dd, J = 4.8, 1.6 Hz, 1H, 2-pyridine H-6), 8.55 (dt, J = 8.0, 2.0 Hz, 1H, 2-pyridine H-4), 7.66 (s, 1H, phenanthroline H-3), 7.61 (dd, J = 7.6, 1.4 Hz, 1H, phenanthroline H-7), 7.44 (dd, J = 7.9, 4.8 Hz, 1H, 2-pyridine H-5), 7.37 (dd, J = 4.4, 1.5 Hz, 2H, 4-pyridine H-3, H-5), 7.33 (dd, J = 7.6, 4.8 Hz, 1H, phenanthroline H-8), 2.96 (s, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.87, 152.90, 151.51, 150.17, 149.97, 149.24, 148.19, 147.00, 146.29, 135.73, 135.06, 134.65, 133.89, 130.30, 124.00, 123.62, 123.50, 120.77, 27.29, 24.66.

*4.4.11. 2,4-Di(pyridin-4-yl)-5,6-dihydro-1,10-phenanthroline (**20**)*

Procedure described at Section 4.4 was employed with **6c** (0.08 g, 0.35 mmol), dry ammonium acetate (0.27 g, 3.50 mmol), **2c** (0.11 g, 0.35 mmol), and dry methanol (2.5 mL) at 95 °C for 14 h to yield 66 mg (0.19 mmol, 56.1%) of **20** as a white solid.

Mp 284.1–284.6 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.20; purity (condition A): 98.9%.

LC/MS/MS: retention time: 5.21 min; [MH^+]: 337.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80–8.78 (m, 3H, phenanthroline H-9, 4-pyridine H-2, H-6), 8.73 (dd, J = 4.7, 1.4 Hz, 2H, 2-pyridine H-2, H-6), 8.07 (dd, J = 4.6, 1.4 Hz, 2H, 2-pyridine H-3, H-5), 7.70 (s, 1H, phenanthroline H-3), 7.62 (d, J = 6.5 Hz, 1H, phenanthroline H-7), 7.36 (dd, J = 4.5, 1.5 Hz, 2H, 4-pyridine H-3, H-5), 7.32 (dd, J = 7.6, 4.7 Hz, 1H, phenanthroline H-8), 2.97 (s, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.72, 153.02, 151.47, 150.38, 150.23, 149.34, 147.11, 146.23, 145.98, 135.69, 133.90, 131.29, 124.06, 123.44, 121.38, 120.93, 27.27, 24.80.

*4.4.12. 2-Phenyl-4-(pyridin-4-yl)-5,6-dihydro-1,10-phenanthroline (**21**)*

Procedure described at Section 4.4 was employed with **6c** (0.08 g, 0.35 mmol), dry ammonium acetate (0.27 g, 3.50 mmol), **2d** (0.11 g, 0.35 mmol), and dry methanol (2.5 mL) at 95 °C for 14 h to yield 70 mg (0.21 mmol, 60.2%) of **21** as a white solid.

Mp 173.5–174.5 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.35; purity (condition A): 99.5%.

LC/MS/MS: retention time: 6.67 min; [MH^+]: 336.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (dd, J = 4.9, 1.5 Hz, 1H, phenanthroline H-9), 8.77 (dd, J = 4.4, 1.5 Hz, 2H, 4-pyridine H-2, H-6), 8.16 (dd, J = 7.8, 1.1 Hz, 2H, 2-phenyl H-2, H-6), 7.64 (s, 1H, phenanthroline H-3), 7.60 (dd, J = 7.5, 1.3 Hz, 1H, phenanthroline H-7), 7.52–7.42 (m, 3H, 2-phenyl H-3, H-4, H-5), 7.36 (dd, J = 4.4, 1.3 Hz, 2H, 4-pyridine H-3, H-5), 7.29 (dd, J = 7.6, 4.8 Hz, 1H, phenanthroline H-8), 2.94 (s, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.50, 152.43, 151.94, 150.09, 149.20, 146.77, 146.68, 138.96, 135.52, 133.79, 129.39, 129.02, 128.64, 127.25, 123.68, 123.56, 120.66, 27.46, 24.65.

*4.4.13. 4-Phenyl-2-(pyridin-2-yl)-5,6-dihydro-1,10-phenanthroline (**22**)*

Procedure described at Section 4.4 was employed with **6d** (0.09 g, 0.40 mmol), dry ammonium acetate (0.30 g, 4.00 mmol), **2a** (0.13 g, 0.40 mmol), and dry methanol (2.5 mL) at 100 °C for 16 h to yield 88 mg (0.26 mmol, 65.6%) of **22** as a white solid.

Mp 179.2–179.8 °C; R_f (Al_2O_3) (ethyl acetate/*n*-hexane 2:1 v/v): 0.28; purity (condition A): 95.5%.

LC/MS/MS: retention time: 7.58 min; [MH^+]: 336.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (dd, J = 4.8, 1.3 Hz, 1H, phenanthroline H-9), 8.75 (d, J = 8.0 Hz, 1H, 2-pyridine H-3), 8.65 (ddd, J = 4.8, 1.6, 0.8 Hz, 1H, 2-pyridine H-6), 8.40 (s, 1H, phenanthroline H-3), 7.85 (td, J = 7.5, 1.7 Hz, 1H, 2-pyridine H-4), 7.56 (dd, J = 7.5, 0.9 Hz, 1H, phenanthroline H-7), 7.51–7.40 (m, 5H, 4-phenyl H-2, H-3, H-4, H-5, H-6), 7.31 (ddd, J = 7.4, 4.8, 1.1 Hz, 1H,

2-pyridine H-5), 7.26 (dd, J = 7.3, 5.1 Hz, 1H, phenanthroline H-8), 3.05–2.88 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.05, 154.58, 152.23, 151.71, 149.77, 149.01, 148.85, 138.69, 136.85, 135.53, 133.98, 131.76, 128.85, 128.36, 128.10, 123.64, 123.50, 121.99, 27.57, 24.96.

4.4.14. 4-Phenyl-2-(pyridin-3-yl)-5,6-dihydro-1,10-phenanthroline (**23**)

Procedure described at Section 4.4 was employed with **6d** (0.09 g, 0.40 mmol), dry ammonium acetate (0.30 g, 4.00 mmol), **2b** (0.13 g, 0.40 mmol), and dry methanol (2.5 mL) at 100 °C for 16 h to yield 81 mg (0.24 mmol, 60.3%) of **23** as a white solid.

Mp 202.1–202.8 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.20; purity (condition A): 99.3%.

LC/MS/MS: retention time: 7.08 min; [MH^+]: 336.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.27 (d, J = 1.7 Hz, 1H, 2-pyridine H-2), 8.77 (dd, J = 4.6, 1.3 Hz, 1H, phenanthroline H-9), 8.65 (dd, J = 4.7, 1.5 Hz, 1H, 2-pyridine H-6), 8.54 (dt, J = 7.9, 1.9 Hz, 1H, 2-pyridine H-4), 7.69 (s, 1H, phenanthroline H-3), 7.57 (d, J = 7.5 Hz, 1H, phenanthroline H-7), 7.54–7.40 (m, 5H, 4-phenyl H-2, H-3, H-4, H-5, H-6), 7.41 (dd, J = 7.7, 4.3 Hz, 1H, 2-pyridine H-5), 7.27 (dd, J = 7.5, 4.8 Hz, 1H, phenanthroline H-8), 3.05–2.87 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.33, 152.51, 151.91, 149.69, 149.62, 149.01, 148.20, 138.34, 135.48, 134.97, 134.92, 133.89, 130.80, 128.62, 128.52, 128.30, 123.59, 123.43, 121.54, 27.41, 24.73.

4.4.15. 4-Phenyl-2-(pyridin-4-yl)-5,6-dihydro-1,10-phenanthroline (**24**)

Procedure described at Section 4.4 was employed with **6d** (0.07 g, 0.30 mmol), dry ammonium acetate (0.23 g, 3.00 mmol), **2c** (0.10 g, 0.30 mmol), and dry methanol (2.5 mL) at 100 °C for 16 h to yield 26 mg (0.08 mmol, 26.2%) of **24** as a white solid.

Mp 215.8–216.3 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.19; purity (condition A): 99.8%.

LC/MS/MS: retention time: 7.10 min; [MH^+]: 336.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (dd, J = 4.7, 1.5 Hz, 1H, phenanthroline H-9), 8.72 (dd, J = 4.6, 1.6 Hz, 2H, 2-pyridine H-2, H-6), 8.05 (dd, J = 4.6, 1.6 Hz, 2H, 2-pyridine H-3, H-5), 7.74 (s, 1H, phenanthroline H-3), 7.60 (dd, J = 7.6, 1.4 Hz, 1H, phenanthroline H-7), 7.53–7.48 (m, 3H, 4-phenyl H-3, H-4, H-5), 7.42 (dd, J = 7.8, 1.6 Hz, 2H, 4-phenyl H-2, H-6), 7.29 (dd, J = 7.6, 4.8 Hz, 1H, phenanthroline H-8), 3.04–2.89 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.18, 152.64, 151.88, 150.26, 149.87, 149.17, 146.41, 138.34, 135.61, 134.01, 131.91, 128.69, 128.64, 128.46, 123.79, 121.83, 121.43, 27.44, 24.92.

4.4.16. 2,4-Diphenyl-5,6-dihydro-1,10-phenanthroline (**25**)

Procedure described at Section 4.4 was employed with **6d** (0.08 g, 0.35 mmol), dry ammonium acetate (0.27 g, 3.50 mmol), **2d** (0.11 g, 0.35 mmol), and dry methanol (2.5 mL) at 100 °C for 18 h to yield 85 mg (0.25 mmol, 73.0%) of **25** as a white solid.

Mp 210.5–211.0 °C; R_f (dichloromethane/methanol 15:1 v/v): 0.19; purity (condition A): 98.2%.

LC/MS/MS: retention time: 8.63 min; [MH^+]: 335.3 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.78 (dd, J = 4.7, 1.5 Hz, 1H, phenanthroline H-9), 8.17 (dd, J = 7.9, 1.6 Hz, 2H, 2-phenyl H-2, H-6), 7.68 (s, 1H, phenanthroline H-3), 7.56 (dd, J = 7.7, 1.7 Hz, 1H, phenanthroline H-7), 7.51–7.39 (m, 6H, 2-phenyl H-3, H-4, H-5, 4-phenyl H-3, H-4, H-5), 7.42 (dd, J = 7.7, 1.7 Hz, 2H, 4-phenyl H-2, H-6), 7.24 (dd, J = 7.5, 4.8 Hz, 1H, phenanthroline H-8), 3.01–2.89 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.02, 152.36, 152.07, 149.42, 149.06, 139.37, 138.88, 135.43, 133.90, 130.04, 128.76, 128.71, 128.54, 128.50, 128.16, 127.26, 123.42, 121.59, 27.63, 24.79.

4.5. General method for the preparation of 9 (R_1, R_2 = a-d) (**26–41**)

A mixture of **7a-d** (1.0 equiv.), **2a-d** (1.0 equiv.) and NH_4OAc (10.0 equiv.) in glacial acetic acid or dry MeOH was refluxed at 90–100 °C for 12–48 h under nitrogen gas. Thereafter, mixture was extracted with EtOAc, washed with H_2O, and saturated NaCl. The organic layer was collected, dried over $MgSO_4$ and filtered. The filtrate was concentrated at reduced pressure, dried in vacuum pump and purified by silica gel chromatography with a gradient elution of ethyl acetate and *n*-hexane to yield 11.9–51.6% of **26–41**.

4.5.1. 2,4-Di(pyridin-2-yl)-5,6-dihydrothieno[2,3-h]quinoline (**26**)

Procedure described at Section 4.5 was employed with **7a** (0.10 g, 0.41 mmol), dry ammonium acetate (0.32 g, 4.10 mmol), **2a** (0.13 g, 0.41 mmol), and glacial acetic acid (1.5 mL) at 100 °C for 20 h to yield 43 mg (0.12 mmol, 31.0%) of **26** as an off-white solid.

Mp 128.8–129.3 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.17; purity (condition B): 96.0%.

LC/MS/MS: retention time: 8.77 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.75 (d, J = 4.1 Hz, 1H, 2-pyridine H-6), 8.65 (d, J = 3.9 Hz, 1H, 4-pyridine H-6), 8.61 (d, J = 8.0 Hz, 1H, 2-pyridine H-3), 8.33 (s, 1H, thienoquinoline H-3), 7.87 (d, J = 5.0 Hz, 1H, thienoquinoline H-8), 7.84 (td, J = 7.7, 1.8 Hz, 1H, 2-pyridine H-4), 7.82 (td, J = 7.7, 1.8 Hz, 1H, 4-pyridine H-4), 7.56 (d, J = 7.8 Hz, 1H, 4-pyridine H-3), 7.35 (ddd, J = 7.5, 4.9, 1.0 Hz, 1H, 2-pyridine H-5), 7.31 (ddd, J = 7.6, 4.9, 1.1 Hz, 1H, 4-pyridine H-5), 7.24 (d, J = 5.1 Hz, 1H, thienoquinoline H-9), 3.24 (t, J = 8.0 Hz, 2H, 5-CH_2), 3.03 (t, J = 8.0 Hz, 2H, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.80, 156.23, 153.23, 151.29, 149.33, 148.95, 147.53, 141.09, 137.48, 136.79, 136.53, 127.37, 124.94, 124.17, 123.46, 122.71, 122.66, 121.00, 119.34, 25.94, 23.08.

4.5.2. 4-(Pyridin-2-yl)-2-(pyridin-3-yl)-5,6-dihydrothieno[2,3-h] quinoline (**27**)

Procedure described at Section 4.5 was employed with **7a** (0.06 g, 0.25 mmol), dry ammonium acetate (0.19 g, 2.50 mmol), **2b** (0.08 g, 0.25 mmol), and glacial acetic acid (0.5 mL) at 100 °C for 20 h to yield 43 mg (0.13 mmol, 51.6%) of **27** as a light yellow solid.

Mp 155.2–156.0 °C; R_f (ethyl acetate/*n*-hexane 2:1 v/v): 0.13; purity (condition B): 96.0%.

LC/MS/MS: retention time: 7.80 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.33 (d, J = 1.6 Hz, 1H, 2-pyridine H-2), 8.79 (d, J = 4.1 Hz, 1H, 4-pyridine H-6), 8.64 (dd, J = 4.7, 1.5 Hz, 1H, 2-pyridine H-6), 8.46 (dt, J = 7.9, 1.9 Hz, 1H, 2-pyridine H-4), 7.88 (td, J = 8.3, 1.7 Hz, 1H, 4-pyridine H-4), 7.84 (d, J = 5.1 Hz, 1H, thienoquinoline H-8), 7.68 (s, 1H, thienoquinoline H-3), 7.49 (d, J = 7.8 Hz, 1H, 4-pyridine H-3), 7.41 (dd, J = 7.9, 5.2 Hz, 1H, 2-pyridine H-5), 7.39 (dd, J = 7.6, 4.9 Hz, 1H, 4-pyridine H-5), 7.21 (d, J = 5.1 Hz, 1H, thienoquinoline H-9), 3.20 (t, J = 7.9 Hz, 2H, 5-CH_2), 3.00 (t, J = 8.0 Hz, 2H, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.22, 151.98, 151.82, 149.74, 149.59, 148.19, 147.54, 141.33, 137.30, 136.58, 134.73, 134.13, 126.25, 124.95, 124.05, 123.45, 122.92, 118.69, 25.89, 23.05.

4.5.3. 4-(Pyridin-2-yl)-2-(pyridin-4-yl)-5,6-dihydrothieno[2,3-h] quinoline (**28**)

Procedure described at Section 4.5 was employed with **7a** (0.14 g, 0.60 mmol), dry ammonium acetate (0.46 g, 6.00 mmol), **2c** (0.19 g, 0.60 mmol), and glacial acetic acid (1.0 mL) at 100 °C for 20 h to yield 87 mg (0.25 mmol, 42.5%) of **28** as a light yellow solid.

Mp 161.2–162.1 °C; R_f (ethyl acetate/*n*-hexane 6:1 v/v): 0.10; purity (condition B): 97.6%.

LC/MS/MS: retention time: 7.84 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (d, J = 4.1 Hz, 1H, 4-pyridine H-6), 8.72 (dd, J = 4.6, 1.5 Hz, 2H, 2-pyridine H-2, H-6), 8.04 (dd, J = 4.6, 1.5 Hz, 2H, 2-pyridine H-3, H-5), 7.87 (td, J = 7.5, 1.7 Hz, 1H, 4-pyridine H-4), 7.84 (d, J = 5.0 Hz, 1H, thienoquinoline H-8), 7.74 (s, 1H, thienoquinoline H-3), 7.49 (d, J = 7.8 Hz, 1H, 4-pyridine H-3), 7.39 (ddd, J = 7.6, 4.9, 0.8 Hz, 1H, 4-pyridine H-5), 7.21 (d, J = 5.1 Hz, 1H, thienoquinoline H-9), 3.21 (t, J = 8.0 Hz, 2H, 5-CH_2), 3.00 (t, J = 8.0 Hz, 2H, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.10, 152.05, 151.57, 150.29, 149.80, 147.57, 146.30, 141.45, 137.23, 136.58, 127.39, 124.95, 124.04, 122.98, 120.86, 119.08, 26.02, 23.01.

4.5.4. 2-Phenyl-4-(pyridin-2-yl)-5,6-dihydrothieno[2,3-h]quinoline (**29**)

Procedure described at Section 4.5 was employed with **7a** (0.07 g, 0.30 mmol), dry ammonium acetate (0.23 g, 3.00 mmol), **2d** (0.10 g, 0.30 mmol), and glacial acetic acid (0.5 mL) at 100 °C for 12 h to yield 52 mg (0.15 mmol, 50.7%) of **29** as an off-white solid.

Mp 168.0–168.8 °C; R_f (ethyl acetate/*n*-hexane 1:3 v/v): 0.27; purity (condition B): 96.7%.

LC/MS/MS: retention time: 9.50 min; [MH^+]: 341.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.78 (ddd, J = 4.8, 1.6, 0.9 Hz, 1H, 4-pyridine H-6), 8.14 (dd, J = 7.9, 1.5 Hz, 2H, 2-phenyl H-2, H-6), 7.87 (d, J = 5.2 Hz, 1H, thienoquinoline H-8), 7.83 (td, J = 7.7, 1.8 Hz, 1H, 4-pyridine H-4), 7.66 (s, 1H, thienoquinoline H-3), 7.48 (d, J = 7.8 Hz, 1H, 4-pyridine H-3), 7.47–7.42 (m, 3H, 2-phenyl H-3, H-4, H-5), 7.36 (ddd, J = 7.5, 4.9, 1.0 Hz, 1H, 4-pyridine H-5), 7.19 (d, J = 5.1 Hz, 1H, thienoquinoline H-9), 3.18 (t, J = 8.1 Hz, 2H, 5-CH_2), 2.99 (t, J = 8.0 Hz, 2H, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.71, 154.46, 151.58, 149.63, 147.32, 140.94, 139.29, 137.66, 136.51, 128.67, 128.57, 126.76, 125.39, 125.14, 124.06, 122.74, 122.63, 118.67, 25.85, 23.15.

4.5.5. 2-(Pyridin-2-yl)-4-(pyridin-3-yl)-5,6-dihydrothieno[2,3-h]quinoline (**30**)

Procedure described at Section 4.5 was employed with **7b** (0.10 g, 0.40 mmol), dry ammonium acetate (0.30 g, 4.00 mmol), **2a** (0.13 g, 0.40 mmol), and dry MeOH (2.5 mL) at 90 °C for 21 h to yield 45 mg (0.13 mmol, 32.3%) of **30** as a white solid.

Mp 147.6–148.8 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.23; purity (condition B): 99.2%.

LC/MS/MS: retention time: 8.40 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.69 (dd, J = 5.0, 1.8 Hz, 1H, 2-pyridine H-6), 8.70–8.63 (m, 2H, 4-pyridine H-2, H-6), 8.61 (d, J = 8.0 Hz, 1H, 2-pyridine H-3), 8.19 (s, 1H, thienoquinoline H-3), 7.86 (d, J = 5.3 Hz, 1H, thienoquinoline H-8), 7.85 (td, J = 7.9, 1.8 Hz, 1H, 2-pyridine H-4), 7.77 (dt, J = 7.8, 1.9 Hz, 1H, 4-pyridine H-4), 7.43 (dd, J = 7.8, 4.8 Hz, 1H, 4-pyridine H-5), 7.32 (ddd, J = 7.4, 4.8, 1.04 Hz, 1H, 2-pyridine H-5), 7.22 (d, J = 5.1 Hz, 1H, thienoquinoline H-9), 3.10–2.98 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 155.99, 153.34, 151.14, 149.43, 149.20, 148.99, 145.64, 141.01, 137.39, 136.88, 136.21, 135.02, 127.02, 124.86, 123.64, 123.24, 123.00, 121.07, 119.55, 26.24, 23.08.

4.5.6. 2,4-Di(pyridin-3-yl)-5,6-dihydrothieno[2,3-h]quinoline (**31**)

Procedure described at Section 4.5 was employed with **7b** (0.15 g, 0.62 mmol), dry ammonium acetate (0.48 g, 6.20 mmol), **2b** (0.30 g, 0.93 mmol), and dry methanol (5 mL) at 100 °C for 24 h to yield 25 mg (0.07 mmol, 12.0%) of **31** as a white solid.

Mp 208.8–209.4 °C; R_f (ethyl acetate/*n*-hexane 10:1 v/v): 0.11; purity (condition B): 96.3%.

LC/MS/MS: retention time: 7.50 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.32 (d, J = 1.7 Hz, 1H, 2-pyridine H-2), 8.72 (dd, J = 4.9, 1.5 Hz, 1H, 4-pyridine H-6), 8.70 (d, J = 1.7 Hz, 1H, 4-pyridine H-2), 8.66 (dd, J = 4.8, 1.5 Hz, 1H, 2-pyridine H-6), 8.43 (dt, J = 8.0, 1.8 Hz, 1H, 2-pyridine H-4), 7.84 (d, J = 5.1 Hz, 1H, thienoquinoline H-8), 7.75 (dt, J = 7.8, 1.8 Hz, 1H, 4-pyridine H-4), 7.49 (s, 1H, thienoquinoline H-3), 7.46 (dd, J = 7.7, 5.8 Hz, 1H, 2-pyridine H-5), 7.42 (dd, J = 8.0, 7.8 Hz, 1H, 4-pyridine H-5), 7.22 (d, J = 5.1 Hz, 1H, thienoquinoline H-9), 3.09–2.98 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 152.01, 151.93, 149.82, 149.48, 149.34, 148.20, 145.66. 141.36, 137.22, 136.07, 134.84, 134.52, 134.12, 126.19, 124.92, 123.52, 123.38, 123.17, 118.92, 26.08, 23.06.

4.5.7. 4-(Pyridin-3-yl)-2-(pyridin-4-yl)-5,6-dihydrothieno[2,3-h]quinoline (**32**)

Procedure described at Section 4.5 was employed with **7b** (0.07 g, 0.29 mmol), dry ammonium acetate (0.22 g, 2.90 mmol), **2c** (0.09 g, 0.29 mmol), and glacial acetic acid (0.5 mL) at 90 °C for 14 h to yield 32 mg (0.09 mmol, 31.7%) of **32** as a light yellow solid.

Mp 205.2–205.9 °C; R_f (ethyl acetate/*n*-hexane 3:1 v/v): 0.15; purity (condition B): 95.0%.

LC/MS/MS: retention time: 7.49 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.73–8.70 (m, 4H, 2-pyridine H-2, H-6, 4-pyridine H-2, H-6), 8.01 (d, J = 4.4 Hz, 2H, 2-pyridine H-3, H-5), 7.85(d, J = 5.0 Hz, 1H, thienoquinoline H-8), 7.74 (d, J = 7.6, 1H, 4-pyridine H-4), 7.55 (s, 1H, thienoquinoline H-3), 7.47 (dd, J = 6.9, 4.7 Hz, 1H, 4-pyridine H-5), 7.23 (d, J = 4.9 Hz, 1H, thienoquinoline H-9), 3.08–3.02 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 151.96, 151.66, 150.30, 149.50, 149.27, 146.08, 145.64, 141.47, 137.08, 136.06, 134.69, 127.34, 124.87, 123.38, 123.23, 120.83, 119.28, 26.16, 22.97.

4.5.8. 2-Phenyl-4-(pyridin-3-yl)-5,6-dihydrothieno[2,3-h]quinoline (**33**)

Procedure described at Section 4.5 was employed with **7b** (0.10 g, 0.41 mmol), dry ammonium acetate (0.31 g, 4.10 mmol), **2d** (0.13 g, 0.41 mmol), and dry methanol (5 mL) at 100 °C for 24 h to yield 36 mg (0.10 mmol, 26.1%) of **33** as a white solid.

Mp 199.0–199.7 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.19; purity (Condition B): 100.0%.

LC/MS/MS: retention time: 9.23 min; [MH^+]: 341.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.71–8.69 (m, 2H, 4-pyridine H-2, H-6), 8.12 (dd, J = 6.7, 1.6 Hz, 2H, 2-phenyl H-2, H-6), 7.87 (d, J = 5.2 Hz, 1H, thienoquinoline H-8), 7.74 (dt, J = 7.8, 1.8 Hz, 1H, 4-pyridine H-4), 7.52–7.41 (m, 3H, 2-phenyl H-3, H-4, H-5), 7.48 (s, 1H, thienoquinoline H-3), 7.44 (ddd, J = 7.6, 5.3, 0.9 Hz, 1H, 4-pyridine H-5), 7.20 (d, J = 5.2 Hz, 1H, thienoquinoline H-9), 3.06–2.96 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 154.59, 151.49, 149.42, 149.28, 145.36, 140.93, 139.04, 137.55, 136.10, 135.17, 128.88, 128.66, 126.73, 125.28, 125.08, 123.28, 122.88, 118.92, 26.04, 23.12.

4.5.9. 2-(Pyridin-2-yl)-4-(pyridin-4-yl)-5,6-dihydrothieno[2,3-h]quinoline (**34**)

Procedure described at Section 4.5 was employed with **7c** (0.07 g, 0.30 mmol), dry ammonium acetate (0.23 g, 3.00 mmol), **2a** (0.10 g, 0.30 mmol), and glacial acetic acid (0.5 mL) at 100 °C for 12 h to yield 37 mg (0.11 mmol, 36.1%) of **34** as a light yellow solid.

Mp 166.8–167.5 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.18; purity (condition B): 95.8%.

LC/MS/MS: retention time: 8.43 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.74 (dd, J = 4.5, 1.5 Hz, 2H, 4-pyridine H-2, H-6), 8.65 (d, J = 3.9 Hz, 1H, 2-pyridine H-6), 8.61 (d,

J = 7.9 Hz, 1H, 2-pyridine H-3), 8.17 (s, 1H, thienoquinoline H-3), 7.87 (td, *J* = 7.7, 1.7 Hz, 1H, 2-pyridine H-4), 7.86 (d, *J* = 5.1 Hz, 1H, thienoquinoline H-8), 7.37 (dd, *J* = 4.5, 1.5 Hz, 2H, 4-pyridine H-3, H-5), 7.33 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H, 2-pyridine H-5), 7.22 (d, *J* = 5.2 Hz, 1H, thienoquinoline H-9), 3.08–2.98 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 155.92, 153.46, 151.18, 149.94, 149.02, 147.20, 146.52, 141.07, 137.33, 136.91, 126.42, 124.86, 123.70, 123.65, 123.05, 121.07, 118.83, 26.17, 23.05.

4.5.10. 2-(Pyridin-3-yl)-4-(pyridin-4-yl)-5,6-dihydrothieno[2,3-h]quinoline (**35**)

Procedure described at Section 4.5 was employed with **7c** (0.05 g, 0.22 mmol), dry ammonium acetate (0.17 g, 2.20 mmol), **2b** (0.07 g, 0.22 mmol), and glacial acetic acid (0.5 mL) at 100 °C for 14 h to yield 26 mg (0.07 mmol, 34.6%) of **35** as a white solid.

Mp 186.9–187.4 °C; R_f (dichloromethane/methanol 20:1 v/v): 0.13; purity (condition B): 95.1%.

LC/MS/MS: retention time: 7.47 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.31 (d, *J* = 1.8 Hz, 1H, 2-pyridine H-2), 8.78 (dd, *J* = 5.7, 1.3 Hz, 2H, 4-pyridine H-2, H-6), 8.65 (dd, *J* = 4.5, 1.1 Hz, 1H, 2-pyridine H-6), 8.42 (dt, *J* = 7.9, 1.8 Hz, 1H, 2-pyridine H-4), 7.84 (d, *J* = 5.1 Hz, 1H, thienoquinoline H-8), 7.47 (s, 1H, thienoquinoline H-3), 7.43 (dd, *J* = 7.9, 4.8 Hz, 1H, 2-pyridine H-5), 7.35 (dd, *J* = 5.8, 1.4 Hz, 2H, 4-pyridine H-3, H-5), 7.22 (d, *J* = 5.1 Hz, 1H, thienoquinoline H-9), 3.08–2.97 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 152.08, 151.94, 150.13, 149.87, 148.18, 146.92, 146.52, 141.41, 137.13, 134.42, 134.09, 125.55, 124.89, 123.48, 123.20, 118.14, 26.00, 23.02.

4.5.11. 2,4-Di(pyridin-4-yl)-5,6-dihydrothieno[2,3-h]quinoline (**36**)

Procedure described at Section 4.5 was employed with **7c** (0.07 g, 0.30 mmol), dry ammonium acetate (0.23 g, 3.00 mmol), **2c** (0.10 g, 0.30 mmol), and glacial acetic acid (0.5 mL) at 100 °C for 16 h to yield 28 mg (0.08 mmol, 27.6%) of **36** as an off-white solid.

Mp 224.8–225.5 °C; R_f (dichloromethane/methanol 20:1 v/v): 0.26; purity (condition B): 95.2%.

LC/MS/MS: retention time: 7.46 min; [MH^+]: 342.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.78 (dd, *J* = 4.5, 1.5 Hz, 2H, 2-pyridine H-2, H-6), 8.73 (dd, *J* = 5.0, 0.9 Hz, 2H, 4-pyridine H-2, H-6), 8.01 (dd, *J* = 4.5, 1.4 Hz, 2H, 2-pyridine H-3, H-5), 7.85 (d, *J* = 5.1 Hz, 1H, thienoquinoline H-8), 7.52 (s, 1H, thienoquinoline H-3), 7.34 (dd, *J* = 4.6, 1.4 Hz, 2H, 4-pyridine H-3, H-5), 7.23 (d, *J* = 5.1 Hz, 1H, thienoquinoline H-9), 3.09–3.01 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 152.00, 151.78, 150.36, 150.14, 146.81, 146.54, 145.98, 141.57, 137.01, 126.73, 124.86, 123.47, 123.29, 120.82, 118.53, 26.10, 22.95.

4.5.12. 2-Phenyl-4-(pyridin-4-yl)-5,6-dihydrothieno[2,3-h]quinoline (**37**)

Procedure described at Section 4.5 was employed with **7c** (0.08 g, 0.35 mmol), dry ammonium acetate (0.27 g, 3.50 mmol), **2d** (0.11 g, 0.35 mmol), and glacial acetic acid (0.5 mL) at 90 °C for 14 h to yield 25 mg (0.07 mmol, 20.7%) of **37** as a white solid.

Mp 218.0–218.5 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.24; purity (condition B): 95.3%.

LC/MS/MS: retention time: 9.28 min; [MH^+]: 341.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.73 (dd, *J* = 4.4, 1.5 Hz, 2H, 4-pyridine H-2, H-6), 8.10 (dd, *J* = 7.9, 1.2 Hz, 2H, 2-phenyl H-2, H-6), 7.86 (d, *J* = 5.2 Hz, 1H, thienoquinoline H-8), 7.51–7.40 (m, 3H, 2-phenyl H-3, H-4, H-5), 7.50 (s, 1H, thienoquinoline H-3), 7.30 (dd, *J* = 4.4, 1.6 Hz, 2H, 4-pyridine H-3, H-5), 7.19 (d, *J* = 5.2 Hz, 1H, thienoquinoline H-9), 3.06–2.93 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 154.74, 151.57, 150.03, 147.42, 146.31, 140.99, 139.01, 137.54, 128.94, 128.67, 126.75, 125.12, 124.64, 123.57, 122.90, 118.12, 26.00, 23.12.

4.5.13. 4-Phenyl-2-(pyridin-2-yl)-5,6-dihydrothieno[2,3-h]quinoline (**38**)

Procedure described at Section 4.5 was employed with **7d** (0.12 g, 0.50 mmol), dry ammonium acetate (0.38 g, 5.00 mmol), **2a** (0.16 g, 0.50 mmol), and dry methanol (5 mL) at 100 °C for 48 h to yield 45 mg (0.13 mmol, 26.6%) of **38** as a white solid.

Mp 118.5–118.9 °C; R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.37; purity (condition B): 98.8%.

LC/MS/MS: retention time: 10.50 min; [MH^+]: 341.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.64 (ddd, *J* = 4.7, 1.6, 0.8 Hz, 1H, 2-pyridine H-6), 8.60 (d, *J* = 8.0 Hz, 1H, 2-pyridine H-3), 8.20 (s, 1H, thienoquinoline H-3), 7.86 (d, *J* = 5.1 Hz, 1H, thienoquinoline H-8), 7.83 (td, *J* = 7.8, 1.8 Hz, 1H, 2-pyridine H-4), 7.46–7.40 (m, 5H, 4-phenyl H-2, H-3, H-4, H-5, H-6), 7.29 (ddd, *J* = 7.4, 4.8, 1.1 Hz, 1H, 2-pyridine H-5), 7.20 (d, *J* = 5.1 Hz, 1H, thienoquinoline H-9), 3.11–2.96 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.40, 153.00, 150.90, 149.37, 148.96, 140.88, 139.30, 137.66, 136.78, 128.80, 128.30, 127.88, 126.95, 124.92, 123.41, 122.78, 121.03, 119.78, 26.34, 23.19.

4.5.14. 4-Phenyl-2-(pyridin-3-yl)-5,6-dihydrothieno[2,3-h]quinoline (**39**)

Procedure described at Section 4.5 was employed with **7d** (0.09 g, 0.37 mmol), dry ammonium acetate (0.28 g, 3.70 mmol), **2b** (0.12 g, 0.37 mmol), and glacial acetic acid (1 mL) at 100 °C for 20 h to yield 30 mg (0.09 mmol, 24.2%) of **39** as a light yellow solid.

Mp 163.6–164.2 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.18; purity (condition B): 95.6%.

LC/MS/MS: retention time: 9.49 min; [MH^+]: 341.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.31 (d, *J* = 1.6 Hz, 1H, 2-pyridine H-2), 8.64 (dd, *J* = 4.7, 1.5 Hz, 1H, 2-pyridine H-6), 8.43 (dt, *J* = 8.0, 1.9 Hz, 1H, 2-pyridine H-4), 7.84 (d, *J* = 5.2 Hz, 1H, thienoquinoline H-8), 7.51 (s, 1H, thienoquinoline H-3), 7.47–7.38 (m, 3H, 4-phenyl H-3, H-4, H-5), 7.42 (dd, *J* = 7.9, 1.6 Hz, 2H, 4-phenyl H-2, H-6), 7.42 (ddd, *J* = 8.1, 4.8, 0.9 Hz, 1H, 2-pyridine H-5), 7.20 (d, *J* = 5.2 Hz, 1H, thienoquinoline H-9), 3.11–2.94 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 151.64, 151.58, 149.57, 149.31, 148.19, 141.20, 139.08, 137.43, 134.82, 134.09, 128.64, 128.53, 128.14, 126.07, 124.94, 123.46, 122.94, 119.16, 26.14, 23.14.

4.5.15. 4-Phenyl-2-(pyridin-4-yl)-5,6-dihydrothieno[2,3-h]quinoline (**40**)

Procedure described at Section 4.5 was employed with **7d** (0.09 g, 0.37 mmol), dry ammonium acetate (0.28 g, 3.70 mmol), **2c** (0.12 g, 0.37 mmol), and dry methanol (2.5 mL) at 100 °C for 36 h to yield 23 mg (0.06 mmol, 18.0%) of **40** as a white solid.

Mp 165.0–165.3 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.17; purity: (condition B): 96.0%.

LC/MS/MS: retention time: 9.57 min; [MH^+]: 341.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.72 (dd, *J* = 4.6, 1.4 Hz, 2H, 2-pyridine H-2, H-6), 8.02 (dd, *J* = 4.5, 1.5 Hz, 2H, 2-pyridine H-3, H-5), 7.85 (d, *J* = 5.2 Hz, 1H, thienoquinoline H-8), 7.57 (s, 1H, thienoquinoline H-3), 7.52–7.46 (m, 3H, 4-phenyl H-3, H-4, H-5), 7.40 (dd, *J* = 7.8, 1.5 Hz, 2H, 4-phenyl H-2, H-6), 7.22 (d, *J* = 5.2 Hz, 1H, thienoquinoline H-9), 3.13–2.95 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 151.72, 151.30, 150.30, 149.35, 146.43, 141.34, 138.97, 137.36, 128.63, 128.57, 128.22, 127.23, 124.93, 123.02, 120.85, 119.55, 26.26, 23.09.

*4.5.16. 2,4-Diphenyl-5,6-dihydrothieno[2,3-h]quinoline (**41**)*

Procedure described at Section 4.5 was employed with **7d** (0.10 g, 0.41 mmol), dry ammonium acetate (0.32 g, 4.16 mmol), **2d** (0.13 g, 0.41 mmol), and dry methanol (5 mL) at 100 °C for 48 h to yield 16 mg (0.05 mmol, 11.9%) of **41** as a white solid.

Mp 174.6–175.0 °C; R_f (ethyl acetate/*n*-hexane 1:5 v/v): 0.52; purity (condition B): 98.2%.

LC/MS/MS: retention time: 11.48 min; [MH^+]: 340.2 (100%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.12 (dd, J = 8.4, 1.5 Hz, 2H, 2-phenyl H-2, H-6), 7.87 (d, J = 5.2 Hz, 1H, thienoquinoline H-8), 7.50 (s, 1H, thienoquinoline H-3), 7.49–7.46 (m, 3H, 2-phenyl H-3, H-4, H-5), 7.44–7.37 (m, 5H, 4-phenyl H-2, H-3, H-4, H-5, H-6), 7.18 (d, J = 5.2 Hz, 1H, thienoquinoline H-9), 3.10–2.92 (m, 4H, 5-CH_2, 6-CH_2).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 154.23, 151.25, 149.06, 140.80, 139.52, 139.42, 137.81, 128.71, 128.64, 128.59, 128.44, 127.94, 126.74, 125.20, 125.14, 122.66, 119.18, 26.14, 23.24.

4.6. Biological assays

DNA topo I inhibition assay was determined following the method reported by Fukuda M. et al. with minor modifications [14]. The test 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 2 units of human DNA topo IIα (Amersham, 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 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 AlphaImagerTM (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 as described previously [15]. Cancer cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of 2∼4 × 104 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) with a 450 nm wavelength. For determination of the IC_{50} values, the absorbance readings at 450 nm were fitted to the four-parameter logistic equation. The compounds of adriamycin, etoposide, and camptothecin were purchased from Sigma and used as positive controls.

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