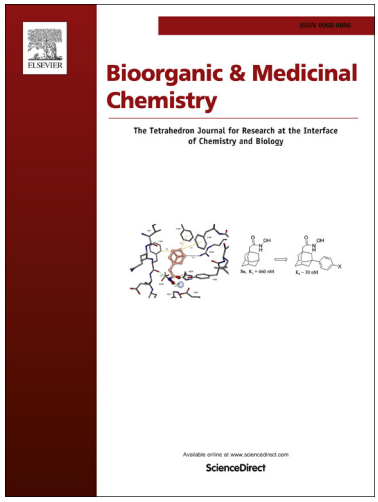


Design and synthesis of novel 2,4-diaryl-5*H*-indeno[1,2-b]pyridine derivatives, and their evaluation of topoisomerase inhibitory activity and cytotoxicity

Tara Man Kadayat, Chanmi Park, Kyu-Yeon Jun, Til Bahadur Thapa Magar, Ganesh Bist, Han Young Yoo, Youngjoo Kwon, Eung-Seok Lee

PII: S0968-0896(14)00784-6
DOI: http://dx.doi.org/10.1016/j.bmc.2014.11.010
Reference: BMC 11899

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 8 October 2014
Revised Date: 5 November 2014
Accepted Date: 6 November 2014

Please cite this article as: Kadayat, T.M., Park, C., Jun, K-Y., Magar, T.B.T., Bist, G., Yoo, H.Y., Kwon, Y., Lee, E-S., Design and synthesis of novel 2,4-diaryl-5*H*-indeno[1,2-b]pyridine derivatives, and their evaluation of topoisomerase inhibitory activity and cytotoxicity, *Bioorganic & Medicinal Chemistry* (2014), doi: http://dx.doi.org/10.1016/j.bmc.2014.11.010

'REVISED'

Design and synthesis of novel 2,4-diaryl-5*H*-indeno[1,2-b]pyridine derivatives, and their evaluation of topoisomerase inhibitory activity and cytotoxicity

Tara Man Kadayat,[a,#] Chanmi Park,[b,#] Kyu-Yeon Jun,[b] Til Bahadur Thapa Magar,[a] Ganesh Bist,[a] Han Young Yoo,[a] Youngjoo Kwon,[b,*] Eung-Seok Lee [a,*]

[a]College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Republic of Korea

[b]College of Pharmacy, Graduate School of Pharmaceutical Sciences, Ewha Global Top5 program, Ewha Womans University, Seoul 120-750, Republic of Korea

[#]Authors are equal contributors

*Corresponding Authors:

Tel: +82 2 3277-4653, fax: +82 2 3277-2851, email: ykwon@ewha.ac.kr (Y.K.)

Tel: +82 53 810-2827, fax: +82 53 810-4654, email: eslee@ynu.ac.kr (E.-S.L.)

Design and synthesis of novel 2,4-diaryl-5*H*-indeno[1,2-b]pyridine derivatives, and their evaluation of topoisomerase inhibitory activity and cytotoxicity

Tara Man Kadayat,[a,#] Chanmi Park,[b,#] Kyu-Yeon Jun,[b] Til Bahadur Thapa Magar,[a] Ganesh Bist,[a] Han Young Yoo,[a] Youngjoo Kwon,[b,*] Eung-Seok Lee [a,*]

[a]College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Republic of Korea

[b]College of Pharmacy, Graduate School of Pharmaceutical Sciences, Ewha Global Top5 program, Ewha Womans University, Seoul 120-750, Republic of Korea

Abstract

For the development of potential anticancer agents, we designed and synthesized 30 new 2,4-diaryl-5*H*-indeno[1,2-b]pyridine derivatives containing aryl moiety such as furyl, thienyl, pyridyl, and phenyl at 2- and 4-position of 5*H*-indeno[1,2-b]pyridine. They were evaluated for topoisomerase I and II inhibitory activity, and cytotoxicity against several human cancer cell lines. Among prepared 30 compounds, **7**, **8**, **9**, **10**, **12**, **14**, **16**, **19**, **20**, **22**, and **23** with 2- or 3-furyl and/or 2- or 3-thienyl either at 2- or 4-position of central pyridine showed the significant or moderate topoisomerase II inhibitory activity. Compounds **7**, **8**, **11**, **12**, **13**, and **22** with 2-furyl, 2- thienyl or 3-thienyl at 2-position of central pyridine showed the significant or moderate topoisomerase I inhibitory activity. Especially, compound **12** with strong topoisomerase II inhibitory activity at 100 μM and 20 μM, and moderate topoisomerase I inhibitory activity displayed strong cytotoxicity against several human cancer cell lines.

Keywords: 2,4-Diaryl-5*H*-indeno[1,2-b]pyridine; Topoisomerase I and II inhibition; Cytotoxicity; Anticancer agents

1. Introduction

Human DNA topoisomerases (topo) have been established as one of the most important molecular targets for the development of anticancer agents.[1] These are nuclear enzymes that transiently break one or two strands of DNA allowing to solve various DNA topological problems associated with DNA replication, transcription, recombination, and other vital cellular processes.[2] Depending on their mechanism of action, making either single or double-stranded breaks, DNA topoisomerases are generally classified into two categories, topo I and topo II.[3] In the course of several years of its discovery, topoisomerase inhibitors such as etoposide, teniposide, and camptothecin have been clinically used for treatment of cancer. However, due to their severe side effects many researchers in medicinal chemistry are attracted to develop a new class of topoisomerase inhibitors that possess less side effects and greater potency.[1,4] With the similar goal, our research group has been studying various flexible terpyridine derivatives and evaluated for topo I and/or II inhibitory activity, and cytotoxicity against several human cancer cell lines.[5,6] Previously reported study has revealed that the number of aryl groups in terpyridine derivatives is important for determining the anticancer activity.[5e]

Moreover, our group has reported that conformationally constrained rigid analogs of 2,4,6-trisubstituted pyridine [Fig. 1a] containing 5,6-dihydrobenzo[h]quinolone, 5*H*-chromeno[4,3-b]pyridine, 5,6-dihydro[1,10]phenanthroline, 5,6-dihydrothieno[2,3-h]quinolone, and benzofuro[3,2-*b*]pyridines, which showed considerable topo I and II inhibitory activities, and cytotoxicity against several human cancer cell lines.[7] It is well documented that rigidification of the flexible ring provides planar configuration to compound which facilitates molecule to intercalate into the enzyme-DNA complex.[8] In addition, as compared to flexible structures,

rigid structures have little conformational entropy, and can be more efficiently fitted into the active site of enzyme.[9] In continuation of latter work, herein we have designed and synthesized 2,4-diaryl-5*H*-indeno[1,2-b]pyridines as conformationally constrained rigid molecule in terpyridine skeleton (Fig. 1b). Furthermore, it is reported that compounds possessing indenopyridine moiety have several biological activities such as anticancer and anti-inflammatory activity.[10]

Based on reported biological activities, in this study, we designed and synthesized 30 novel rigid analogs of 2,4-diaryl-5*H*-indeno[1,2-b]pyridines employing 7 aryl groups such as 2- or 3-furyl, 2- or 3-thienyl, 2- or 3-pyridyl, and phenyl attached to the central pyridine ring at 2- and 4-position as shown in Figure 1. Our work represents the first systematic study of the 2,4-diaryl-5*H*-indeno[1,2-b]pyridines for evaluation of human topo I and II inhibitory activity, and cytotoxicity.

(Figure 1)

2. Results and discussion

2. 1. Synthetic chemistry

2,4-Diaryl-5*H*-indeno[1,2-b]pyridine derivatives (**7**-**36**) were synthesized in three steps (Scheme 1), based on the previously reported methods.[11,12] In the first step, pyridinium iodides salts **2** (R = **c**-**g**) were synthesized by the reaction of appropriate aryl methyl ketones **1** (R = **c**-**g**) with equivalent amount of iodine in pyridine for 3 h at 140 °C. Compounds **2** (R = **c**-**g**) were obtained in 38.4-98.3% yield. Secondly, 1-indanone intermediates **5** (R^1 = **a**-**f**) were prepared using the Clasien-Schmidt condensation reaction,[11] where 1-indanone (**3**) was

reacted with aryl aldehydes **4** (R^1 = **a**-**f**) in the presence of EtOH and 5% aqueous NaOH. Total 6 1-indanone intermediates **5** (R^1 = **a**-**f**) were synthesized in 90.1-95.8% yield. Finally, using modified Krönke pyridine synthesis method,[12] pyridinium iodide salts **2** (R = **c**-**g**) and 1-indanone intermediates **5** (R^1 = **a**-**f**) were reacted in the presence of NH_4OAc and glacial acetic acid for 16-24 h at 100 °C to yield 23.5-53.3% of 2,4-diaryl-5H-indeno[1,2-b]pyridines (**7-36**). Synthesized 30 compounds compounds are as shown in Figure 2. Yield (%), purity (%) by HPLC, and melting point of the compounds are shown in Table 1.

(Scheme 1)

(Figure 2)

(Table 1)

2. 2. Topoisomerase I and II inhibitory activity

We evaluated the conversion of supercoiled plasmid DNA to relaxed DNA by topo I and II in the presence of the prepared 2,4-diaryl-5*H*-indeno[1,2-b]pyridines (**7-30**) as shown in Fig. 3 and 4, and Table 2. From the preliminary study, compounds **31-36** did not show significant topo I and II inhibitory activities. Camptothecin and etoposide, well-known topo I and II inhibitors, respectively, were used as positive controls. Inhibitory activities were evaluated both at 100 μM and 20 μM.

2.2.1. Topoisomerase I inhibitory activity

Most of compounds did not show significant topo I inhibitory activity as shown in Figure 3 and Table 2. However, compounds **22** and **13** have shown significant topo I inhibitory activity

of 90.9% and 76.3%, respectively, which were stronger inhibitory activity than that of camptothecin (62.9%, 45.2%, respectively) at 100 µM. Compounds **7, 8, 11, 12,** and **27** displayed moderate topo I inhibitory activity of 38.2%, 52.5%, 41.3%, 40.7%, and 58.5% (as compared to 45.2% or 62.9% of camptothecin), respectively, at 100 µM. Topo I inhibitory activities of evaluated compounds are summarized in Table 2.

(Table 2)

(Figure 3)

2.2.2. Topoisomerase II inhibitory activity

It was observed that compounds **9, 10, 12, 14,** and **19** displayed significant topo II inhibitory activity of 83.9%, 100%, 100%, 100%, and 84.1%, respectively, at 100 µM, and 79.6%, 93.0%, 100%, 66.7%, and 68.5%, respectively, at 20 µM, which were stronger inhibitory activity than that of positive control, etoposide, at both 100 µM and 20 µM concentrations as shown in Figure 4. Especially, compound **12** displayed the most strong topo II inhibitory activity as well as moderate topo I inhibitory activity. Compounds **8, 16,** and **22** displayed 100% topo II inhibition as compared to 87.8% of etoposide at 100 µM concentration, while compounds **7** and **13** exhibited significant topo II inhibitory activity of 44.6% and 40.5%, respectively as compared to 32.4% of etoposide at 100 µM. Compound **20** displayed potent topo II inhibitory activity of 95.9% as compared to 83.7% of etoposide at 100 µM. Compounds **17** and **23** exhibited considerable topo II inhibitory activity, although weaker than that of etoposide. Table 2 summarizes the topo II inhibitory activities of evaluated compounds both at 100 and 20 µM.

(Figure 4)

Figure 5 shows structures of previously synthesized representative rigid analogs of 2,4,6-triarylsubstituted pyridines, and table 3 exhibits relative potencies of the representative compounds including 2,4-diaryl-5*H*-indeno[1,2-b]pyridines at 100 μM compared to positive controls, camptothecin (topo I) and/or etoposide (topo II) for easier comparison with previously synthesized rigid analogs of 2,4,6-triarylsubstituted pyridines and 2,4-diaryl-5*H*-indeno[1,2-b]pyridines. Generally, relative potencies of 2,4-diaryl-5*H*-indeno[1,2-b]pyridines against etoposide (topo II) is much greater than those of previously synthesized representative rigid analogs of 2,4,6-triarylsubstituted pyridines.

(Figure 5)

(Table 3)

2. 3. Cytotoxicity

Compounds **7-30** were evaluated for cytotoxicity against four different human cancer cell lines: human breast adenocarcinoma cell line (MCF-7), human cervix tumor cell line (HeLa), human colorectal adenocarcinoma cell line (HCT15), human prostate tumor cell line (DU145). The inhibitory activity (IC_{50}) is expressed as micromolar concentration as shown in Table 2. Compounds **7, 11-13,** and **27** showed considerable cytotoxicity against tested cell lines. Compound **7** possessing moderate topo I and II inhibitory activity showed moderate cytotoxicity against MCF-7 and HeLa. Compounds **11** and **27,** and **13** possessing moderate and strong topo I inhibitory activity, respectively, displayed considerable (the same or a little

weaker) cytotoxicity against tested cell lines compared to positive controls. In addition, Compounds **12** possessing strong topo II and moderate topo I inhibitory activity, displayed the strongest cytotoxicity against tested cell lines. It is interesting to note that compounds **8-10**, **14, 16, 19, 20, 22,** and **23** which have displayed strong to moderate topo I and/or II inhibitory activities did not show considerable cytotoxicity. It is common in biological study *in vitro* that cytotoxicity did not correlate directly to topoisomerase inhibitory activity.[6e]

2.4. Structure-activity relationship study (SAR)

Structure-activity relationship study was performed according to the results of topo I and II inhibitory activity of the evaluated compounds. From the prepared compounds not many compounds showed strong topo I and II inhibitory activities at both 100 µM and 20 µM concentrations, it is not facile to be determined to concrete structure-activity relationship. However, compounds **7-12** which shares the common 2-(furan-2-yl)-5*H*-indeno[1,2-*b*]pyridine skeleton possess moderate to strong topo II inhibitory activity. Similarly, compounds **13, 14,** and **16,** and compounds **19, 20,** and **22** with 2-thienyl and 3-thienyl moiety at 2-position of the central pyridine, respectively, possess significant topo II inhibitory activity. This results are similar to previous studies,[5b,d, 6a-d, 7b,f] which supports importance of 2-furyl, 2-thienyl or 3-thienyl moiety at 2-position and/or 4-position on central pyridine for significant topo II inhibitory activity. In addition, compounds with 2-furyl (**7, 13, 19**) or 3-furyl (**8, 14, 20**) at 4-position of the central pyridine also possess significant topo II inhibitory activity. Interestingly, compounds **10, 16**, and **22** which possess 3-thienyl at 4-position of the central pyridine have 100% topo II inhibitory activity at 100 µM concentration (Fig. 5). In contrast, except compound **12** and **27**, all the compounds bearing pyridyl or phenyl moiety at 2- or 4-position did not show any significant topo inhibitory activity. This indicates that

introduction of furyl or thienyl moiety at 2- or 4-position of central pyridine is crucial than pyridyl or phenyl moiety for topo II inhibitory activity.

Compounds **13** and **22** which showed strong topo I inhibitory activity and compounds **7, 8, 11, 12,** and **27** which displayed moderate topo I inhibitory activity possess 2-furyl, 2-thienyl, or 3-thienyl at 2-position of central pyridine. The results indicated that substitution of furyl or thienyl at 2-position of central pyridine is important for topo I inhibitory activity.

(Figure 6)

The concrete correlation between topo inhibitory activity and cytotoxicity was not observed since many compounds showing strong or moderate topo I and/or topo II inhibitory activities did not show significant cytotoxicity against tested cell lines. But compounds **7, 11-13,** and **27** possessing significant cytotoxicity displayed strong to moderate topo I and/or topo II inhibitory activities.

3. Conclusion

We have designed and synthesized thirty 2,4-diaryl-5*H*-indeno[1,2-b]pyridine derivatives and evaluated for their topo I and II inhibitory activity, and cytotoxicity against several human cancer cell lines. SAR study indicated that furyl or thienyl moiety at 2- or 4-position of central pyridine may play an important role for topoisomerase I and II inhibitory activity. In addition, combination of 2-(furan-2-yl)-5*H*-indeno[1,2-*b*]pyridine skeleton with furyl, or thienyl moiety rather than pyridyl or phenyl moiety at 2-position is more important for topo II inhibitory activity. No direct correlation between cytotoxicity and topoisomerase inhibitory

activity was observed but compounds possessing significant cytotoxicity displayed strong to moderate topo I and/or topo II inhibitory activities. This result may provide valuable information to researchers in medicinal chemistry, and further study including optimization may lead to develop novel topo inhibitor as anticancer drugs.

4. Experimental

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

HPLC analyses were performed using two Shimadzu LC-10AT pumps gradient-controlled HPLC system equipped with Shimadzu system controller (SCL-10A VP) and photo diode array detector (SPD-M10A VP) utilizing Shimadzu Class VP program. Sample volume of 10 µL, was run in Waters X- Terra® 5 µM reverse-phase C_{18} column (4.6 x 250 mm) with a gradient elution of 75% to 100% of B in A for 10 min followed by 100% to 75% of B in A for 20 min at a flow rate of 1.0 mL/min at 254 nm UV detection, where mobile phase A was

doubly distilled water with 50 mM ammonium formate (AF) and B was 90% ACN in water with 20 mM AF. Purity of compound is described as percent (%), and retention time is given in minutes.

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 10 μL injection volume on a Waters X Terra® 3.5 μm reverse-phase C_{18} column (2.1 x 100 mm) with a gradient elution from 10% to 95% of B in A for 10 min followed by 95% to 10% of B in A for 10 min at a flow rate of 200 μL/min, where mobile phase A was 100% distilled water with 50 mM AF and mobile phase B was 100% ACN. 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: 21V, tube lens offset: 10 V.

4. 1. General method for preparation of 2 (R = c-g)

Pyridinium iodide salts **2** (R = **c-g**) were synthesized by refluxing aryl methyl ketones **1** (R = **c-g**) with equivalent amount of iodine in pyridine at 140 °C for 3 h. Precipitate occurred during reaction was cooled to room temperature which was filtered and washed with cold pyridine followed by drying overnight to yield 38.4-98.3% of **2** (R = **c-g**). Compounds were used without further purification.

4. 2. General method for preparation of 5 (R^1 = a-f)

1-Indanone (**3**) was added in ethanol followed by the addition of equivalent amount of aryl aldehydes **4** (R^1 = **a-f**). The 5% aqueous solution of NaOH was added drop wise to the mixture at 0 °C which resulted in precipitation. The mixture was then cooled for 30 min,

filtered, washed with cold methanol, and dried to yield 90.1-95.8% solid compound. Total six 1*H*-inden-1-one derivatives **5** (R^1 **= a-f**) were synthesized following the same method.

4.2.1. Synthesis of 2-benzylidene-2,3-dihydro-1H-inden-1-one (**5a**)

The procedure described in Section 4.2 was employed with 1-indanone (1.32 g, 10 mmol) and benzaldehyde (1.01 mL) to yield 2.04g (92.9%) as a whitish yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:10) R_f = 0.27, mp: 147.9-148.6 ^oC , HPLC: Retention time: 12.76 min, purity: 95.1%; ESI LC/MS *m/z* calcd for $C_{16}H_{12}O$ $[MH]^+$ 221.10; found 221.20

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.9 (d, *J* = 7.6 Hz, 1H, indeno H-7), 7.7-7.6 (m, 3H, indeno H-5, phenyl H-2, H-6), 7.6-7.5 (m, 2H, indeno H-4, =CH-), 7.5-7.4 (m, 4H, phenyl H-3, H-4, H-5, indeno H-6), 4.06 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 194.84, 150.06, 138.35, 135.74, 135.07, 134.36, 131.14, 130.10, 129.34, 128.08, 126.59, 124.83, 32.85.

4.2.2. Synthesis of 2-(furan-3-ylmethylene)-2,3-dihydro-1H-inden-1-one (**5b**)

The procedure described in Section 4.2 was employed with 1-indanone (1.32 g, 10 mmol) and 3-furaldehyde (0.83 mL) to yield 1.98 g (94.6%) as a whitish yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.2, mp: 139.6-141.1 ^oC, HPLC: Retention time: 11.10 min, purity: 97.5%; ESI LC/MS *m/z* calcd for $C_{14}H_{10}O_2$ $[MH]^+$ 211.08; found 211.12

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.6 Hz, 1H, indeno H-7), 7.82 (s, 1H, =CH-), 7.65-7.52 (m, 4H, indeno H-4, H-5, furan H-2, H-5), 7.4 (t, *J* = 7.27 Hz, 1H, indeno H-6), 6.7 (d, *J* = 1.6 Hz, 1H, furan H-4), 3.89 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 193.87, 148.95, 145.77, 144.29 138.32, 134.47, 133.83, 127.57, 126.14, 124.21, 123.99, 122.41, 109.91, 31.89.

4.2.3. Synthesis of 2-(furan-2-ylmethylene)-2,3-dihydro-1H-inden-1-one (**5c**)

The procedure described in Section 4.2 was employed with 1-indanone (1.32 g, 10 mmol) and 2-furaldehyde (0.82 mL) to yield 1.89 g (90.1%) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.28, mp: 124.2-125.6 ^oC, HPLC: Retention time: 11.62 min, purity: 97.8%; ESI LC/MS *m/z* calcd for $C_{14}H_{10}O_2$ $[MH]^+$ 211.08; found 211.14

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.64 Hz, 1H, indeno H-7), 7.63 (s, 1H, =CH-), 7.61-7.53 (m, 2H, furan H-5, indeno H-5), 7.47-7.39 (m, 2H, indeno H-4, H-6), 6.77 (d, *J* = 3.4 Hz, 1H, furan H-3), 6.56 (br, 1H, furan H-4), 4.05 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 193.97, 152.27, 149.76, 145.35, 138.45, 134.42, 132.53, 127.44, 126.17, 124.20, 120.03, 116.61, 112.64, 32.32.

4.2.4. Synthesis of 2-(thiophen-3-ylmethylene)-2,3-dihydro-1H-inden-1-one (**5d**)

The procedure described in Section 4.2 was employed with 1-indanone (1.32 g, 10 mmol) and 3-thiophene carboxaldehyde (0.91 mL) to yield 2.1 g (95.8%) as a whitish brown solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f =0.3, mp: 147.9-148.6 ^oC, HPLC: Retention time: 12.21 min, purity: 96.9%; ESI LC/MS *m/z* calcd for $C_{14}H_{10}OS$ $[MH]^+$ 227.05; found 227.12

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.6 Hz, 1H, indeno H-7), 7.70-7.66 (m, 2H, thiophene H-2, H-5), 7.64 (dt, *J* = 7.8, 1.12 Hz, 1H, indeno H-5), 7.56 (d, *J* = 7.07 Hz, 1H, indeno H-4), 7.44-7.38 (m, 3H, =CH-, indeno H-6, thiophene H-4), 3.96 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 194.25, 149.21, 138.45, 137.76, 134.49, 133.44, 129.46, 128.30, 127.65, 127.26, 126.60, 126.16, 124.32, 32.34.

4.2.5. Synthesis of 2-(thiophen-2-ylmethylene)-2,3-dihydro-1H-inden-1-one (**5e**)

The procedure described in Section 4.2 was employed with 1-indanone (1.32 g, 10 mmol) and 2-thiophene carboxaldehyde (0.93 mL) to yield 2.16 g (95.6%) as a white solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f =0.25, mp:172.2-173.1 ^{o}C, HPLC: Retention time: 12.53 min, purity: 100%; ESI LC/MS *m/z* calcd for $C_{14}H_{10}OS$ $[MH]^+$ 227.05; found 227.13

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.90-7.88 (m, 2H, thiophene H-3, H-5), 7.62-7.55 (m, 3H, indeno H-4, H-5, H-7), 7.45-7.42 (m, 2H, =CH-, indeno H-6), 7.17 (t, *J* = 4.97 Hz, 1H, thiophene H-4), 3.93 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 193.73, 149.03, 139.94, 138.57, 134.50, 133.01, 132.80, 130.44, 128.17, 127.64, 126.51, 126.21, 124.30, 32.34.

4.2.6. Synthesis of -2-(pyridin-2-ylmethylene)-2,3-dihydro-1H-inden-1-one (**5f**)

The procedure described in Section 4.2 was employed with 1-Indanone (1.32 g, 10 mmol) and 2-pyridine carboxaldehyde (0.95 mL) to yield 2.01 g (91.9%) as a white solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.18, mp: 152.8-155.3 ^{o}C, HPLC: Retention time: 10.98 min, purity: 98.2%; ESI LC/MS *m/z* calcd for $C_{15}H_{11}NO$ $[MH]^+$ 222.09; found 222.21

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.74 (br, 1H, pyridine H-6), 7.89 (d, *J* = 7.62 Hz, 1H, indeno H-7), 7.71 (dt , *J* = 7.05, 0.9 Hz, 1H, pyridine H-3) 7.62-7.57 (m, 3H, =CH-, indeno H-4, H-5), 7.51 (d, *J* = 7.92 Hz, 1H, pyridine H-4), 7.38 (t, *J* = 7.27 Hz, 1H, indeno H-6), 7.21-7.19 (m, 1H, pyridine H-5), 4.28 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 194.89, 154.88, 151.24, 150.05, 139.11, 137.94, 136.35, 134.80, 130.94, 127.36, 127.35, 126.32, 124.37, 122.95, 33.37.

4. 3. General method for preparation of 7-36

On the basis of Kröhnke synthesis method [24], 2,4-diaryl indenopyridine derivatives (**7-36**) were synthesized by reacting previously synthesized 1-indanone derivatives **5** (R^1 = **a-f**) and pyridinium iodide salts **2** (R = **c-g**) in the presence of anhydrous ammonium acetate (10.0 equiv) and glacial acetic acid. The mixture was heated at 100 °C for 16-24 h. The reaction mixture was then extracted with ethyl acetate, washed with water and saturated NaCl. The organic layer was dried with magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure and dried in vacuum pump, which was then purified by silica gel column chromatography with the gradient elution of ethyl acetate and *n*-hexane to afford solid compounds **7-36** in 23.5-53.3% yield.

4.3.1. Synthesis of 2,4-di(furan-2-yl)-5H-indeno[1,2-b]pyridine **(7)**

The compound was synthesized as described in section 4.3 with **5c** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2c** (1.26 g, 4 mmol) with glacial acetic acid (10 mL) to yield 329 mg (27.5%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.4, mp : 136.5-140.9 ^oC, HPLC: Retention time: 7.94 min, purity: 100%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NO_2$ $[MH]^+$ 300.10; found 300.25

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (dd, *J* = 6.4, 2.08 Hz, 1H, indenopyridine H-9), 7.94 (s, 1H, indenopyridine H-3), 7.65-7.58 (m, 3H, 2-furan H-5, indenopyridine H-7, H-8), 7.48-7.44 (m, 2H, 4-furan H-5, indenopyridine H-6), 7.2 (d, *J* = 3.3 Hz, 2-furan H-3), 7.00 (d, *J* = 3.46 Hz, 4-furan H-3), 6.61 (dd, *J* = 3.39, 1.7 Hz, 1H, 2-furan H-4), 6.58 (dd, *J* = 3.29, 1.7 Hz, 1H, 4-furan H-4), 4.09 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.48, 154.18, 151.44, 148.80 143.96, 143.64, 143.01, 140.58, 134.17, 129.09, 128.85, 127.13, 124.89, 121.32, 112.11, 112, 111.03, 110.70, 108.45, 35.63

4.3.2. Synthesis of 2-(furan-2-yl)-4-(furan-3-yl)-5H-indeno[1,2-b]pyridine **(8)**

The compound was synthesized as described in section 4.3 with **5b** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2c** (1.26 g, 4 mmol) with glacial acetic acid (10 mL) to yield 481 mg (40.2%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.4, mp : 176.4-178.2 ^{o}C, HPLC: Retention time: 7.80 min, purity: 98.1%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NO_2$ $[MH]^+$ 300.10; found 300.25

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (d, *J* = 6.3 Hz, 1H, indenopyridine H-9), 8.01 (s,1H, 4-furan H-2), 7.73 (s, 1H, indenopyridine H-3), 7.60-7.57 (m, 3H, 2-furan H-5, 4-furan H-5 and indenopyridine H-8), 7.50-7.40 (m, 2H, indenopyridine H-6, H-7), 7.2 (dd, *J* = 3.31 Hz, 1H, 2-furan H-3), 6.9 (s, 1H, 4-furan H-4), 6.5 (dd, *J* = 4.30, 1.74 Hz, 1H, 2-furan H-4), 3.96 (s, 2H, indeno H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.23, 154.19, 148.94, 143.81 143.53, 142.98, 141.54, 140.76, 136.81, 131.25, 128.84, 127.25, 124.90, 123.71, 121.38, 113.79, 112.05, 109.18, 108.47, 35.42.

4.3.3. Synthesis of 2-(furan-2-yl)-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine **(9)**

The compound was synthesized as described in section 4.3 with **5e** (0.226 g, 1 mmol), dry ammonium acetate (0.77 g, 10 mmol), **2c** (0.315 g, 1 mmol) with methanol (5 mL) to yield 96 mg (30.42%) of a white solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.39, mp : 141.6-142.9 ^{o}C, HPLC: Retention time: 9.42 min, purity: 96.7%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NOS$ $[MH]^+$ 316.08; found 316.25

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.17 Hz, 1H, indenopyridine H-9), 7.87 (s, 1H, indenopyridine H-3), 7.69 (d, *J* = 3.67 Hz, 1H, 2-furan H-5), 7.63-7.58 (m, 1H, 4-thiophene

H-5), 7.54-7.42 (m, 4H, indenopyridine H-6, H-7, H-8, 4-thiophene H-3), 7.26-7.22 (m, 2H, 4-thiophene H-4, 2-furan H-3), 6.60-6.58 (m, 1H, 2-furan H-4), 4.11 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.62, 154.03, 148.90, 143.75, 143.07, 140.69, 140.61, 138.40, 130.62, 128.94, 128.01, 127.29, 127.23, 127.01, 124.89, 121.43, 113.69, 112.05, 108.59, 35.78.

4.3.4. Synthesis of 2-(furan-2-yl)-4-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(10)**

The compound was synthesized as described in section 4.3 with **5d** (0.90 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2c** (1.26 g, 4 mmol) with glacial acetic acid (10 mL) to yield 471 mg (37.2%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.5, mp : 146.6-148.2 ^{o}C, purity: HPLC: Retention time: 9.02 min, purity: 96.3%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NOS$ $[MH]^+$ 316.08; found 316.23

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (dd, *J* = 7.38, 1.2 Hz, 1H, indenopyridine H-9), 7.79-7.76 (m, 2H, indenopyridine H-3, 4-thiophene H-2), 7.59-7.56 (m, 3H, 4-thiophene H-4, H-5, 2-furan H-5), 7.52-7.42 (m, 3H, indenopyridine H-6, H-7, H-8), 7.2 (d, *J* = 3.35 Hz, 1H, 2-furan H-3), 6.5 (dd, *J* = 3.4, 1.7 Hz, 1H, 2-furan H-4), 4.05 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.36, 154.16, 148.97, 143.78 143.01, 140.72, 140.18, 139.13, 131.74, 128.81, 127.20, 127.13, 126.39, 124.87, 124.53, 121.40, 114.95, 112.04, 108.45, 35.32.

4.3.5. Synthesis of 2-(furan-2-yl)-4-phenyl-5H-indeno[1,2-b]pyridine **(11)**

The compound was synthesized as described in section 4.3 with **5a** (0.87 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2c** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 603 mg (48.6%) of a whitish yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.5, mp : 129.2-130.6 ^oC, HPLC: Retention time: 9.50 min, purity: 96.2%; ESI LC/MS *m/z* calcd for $C_{22}H_{15}NO$ $[MH]^+$ 310.12; found 310.28

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (d, *J* = 6.7 Hz, 1H, indenopyridine H-9), 7.68-7.65 (m, 3H, indenopyridine H-3, 4-phenyl H-2, H-6), 7.56-7.38 (m, 7H, 4-phenyl H-3, H-4, H-5, indenopyridine H-6, H-7, H-8, 2-furan H-5), 7.2 (d, *J* = 3.3 Hz, 1H, 2-furan H-3), 6.5 (dd, *J* = 3.3, 1.7 Hz, 1H, 2-furan H-4), 3.98 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.03, 154.19, 148.99, 146.30 144.06, 143.04, 140.79, 138.59, 132.75, 128.81, 128.76, 128.57, 128.21, 127.14, 124.88, 121.46, 116.14, 112.01, 108.46, 34.50.

4.3.6. Synthesis of 2-(furan-2-yl)-4-(pyridin-2-yl)-5H-indeno[1,2-b]pyridine **(12)**

The compound was synthesized as described in section 4.3 with **5f** (0.88 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2c** (1.26 g, 4 mmol) with glacial acetic acid (10 mL) to yield 454 mg (36.4%) of a whitish yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:2) R_f = 0.46, mp : 138.1-140.7 ^oC, HPLC: Retention time: 10.51 min, purity: 97.3%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2O$ $[MH]^+$ 311.12; found 311.31

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.8 (d, *J* = 4.67 Hz, 1H, 4-pyridine H-6), 8.2 (dd, *J* = 7.17, 1.57 Hz, 1H, indenopyridine H-9), 8 (s, 1H, indenopyridine H-3), 7.89-7.86 (m, 2H, 4-pyridine H-5, H-4), 7.61-7.57 (m, 2H, 4-pyridine H-3, 2-furan H-5), 7.48-7.34 (m, 3H, indenopyridine H-6, H-7, H-8), 7.2 (d, *J* = 3.73 Hz, 1H, 2-furan H-3), 6.5 (dd, *J* = 3.3, 1.7 Hz, 1H, 2-furan H-4), 4.26 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.11, 152.07, 145.34, 143.84, 143.52, 141.49, 140.71, 136.81, 131.29, 128.82, 127.91, 127.26, 124.88, 124.42, 123.62, 121.40, 114.16, 109.12, 35.37.

4.3.7. Synthesis of 4-(furan-2-yl)-2-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine **(13)**

The compound was synthesized as described in section 4.3 with **5c** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2e** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 360 mg (23.5%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.4, mp : 136.3-138.4 ^oC, HPLC: Retention time: 11.24 min, purity: 98.4%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NOS$ $[MH]^+$ 316.08; found 316.27

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.18 (d, *J* = 6.9 Hz, 1H, indenopyridine H-9), 7.8 (s, 1H, indenopyridine H-3), 7.7 (d, *J* = 3.6 Hz, 1H, 4-furan H-5), 7.6 (m, 2H, indenopyridine H-7, 2-thiophene H-3), 7.48-7.40 (m, 3H, indenopyridine H-6, H-8, 2-thiophene H-5), 7.1 (dt, *J* = 3.9, 0.8 Hz, 1H, 4-furan H-3), 7 (d, *J* = 3.3 Hz 1H, 2-thiophene H-4), 6.61-6-58 (m, 1H, 4-furan H-4), 4 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.32, 151.96, 151.26, 145.41 143.88, 143.58, 140.56, 134.16, 129.06, 128.82, 127.90, 127.15, 127.05, 124.86, 124.49, 121.33, 112.14, 111.24, 110.74, 35.58.

4.3.8. Synthesis of 4-(furan-3-yl)-2-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine **(14)**

The compound was synthesized as described in section 4.3 with **5b** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2e** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 306 mg (24.2%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.3, mp : 153.5-155.4 ^oC, HPLC: Retention time: 9.92 min, purity: 96.3%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NOS$ $[MH]^+$ 316.08; found 316.27

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (dd, *J* = 7.35, 1.45 Hz, 1H, indenopyridine H-9), 7.99 (s, 1H, 4-furan H-2), 7.7 (dd, *J* = 3.19, 0.91 Hz, 1H, 2-thiophene H-3), 7.64 (s, 1H, indenopyridine H-3), 7.61-7.58 (m, 2H, 2-thiophene H-5, 4-furan H-5), 7.51-7.40 (m, 3H, indenopyridine H-6, H-7, H-8), 7.1 (dd, *J* = 4.9, 3.7 Hz, 1H, 2-thiophene H-4), 6.9 (d, *J* = 0.97 Hz, 1H, 4-furan H-4), 3.94 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.11, 152.07, 145.34, 143.84, 143.52, 141.49, 140.71, 136.81, 131.29, 128.82, 127.91, 127.26, 124.88, 124.42, 123.62, 121.40, 114.16, 109.12, 35.37.

4.3.9. Synthesis of 2,4-di(thiophen-2-yl)-5H-indeno[1,2-b]pyridine **(15)**

The compound was synthesized as described in section 4.3 with **5e** (0.90 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2e** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 397 mg (27.3%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.5, mp : 138.2-140.6 ^oC, HPLC: Retention time: 12.65 min, purity: 98.3%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NS_2$ $[MH]^+$ 332.06; found 332.29

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (d, *J* = 6.76 Hz, 1H, indenopyridine H-9), 7.78 (s, 1H, indenopyridine H-3), 7.72 (d, *J* = 3.49 Hz, 1H, 2-thiophene H-3), 7.65 (d, *J* = 3.5 Hz, 1H, 4-thiophene H-5), 7.6 (d, *J* = 7.11 Hz, 1H, indenopyridine H-8), 7.51 (d, *J* = 5.1 Hz, 1H, 2-thiophene H-5), 7.48-7.41 (m, 3H, 4-thiophene H-3, indenopyridine H-6, H-7), 7.26-7.13 (m, 2H, 2-thiophene H-4, 4-thiophene H-4), 4.1 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.48, 152.08, 145.19, 143.74 140.59, 138.43, 130.68, 128.90, 128.02, 127.91, 127.23, 127.16, 126.92, 124.85, 124.52, 121.45, 114.08, 35.70.

4.3.10. Synthesis of 2-(thiophen-2-yl)-4-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(16)**

The compound was synthesized as described in section 4.3 with **5d** (0.90 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2e** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 333 mg (25.1%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.31, mp : 152.5-154.9 ^oC, HPLC: Retention time: 11.32 min, purity: 99.4%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NS_2$ $[MH]^+$ 332.06; found 332.28

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (dd, *J* = 7.34, 1.14 Hz, 1H, indenopyridine H-9), 7.74-7.71 (m, 2H, 2-thiophene H-3, 4-thiophene H-2), 7.69 (s, 1H, indenopyridine H-3), 7.58-7.40 (m, 6H, indenopyridine H-6, H-7, H-8, 4-thiophene H-4, H-5, 2-thiophene H-5), 7.1 (dd, *J* = 5, 3.68 Hz, 1H, 2-thiophene H-4), 4.02 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.23, 152.13, 145.37, 143.79, 140.71, 140.27, 139.10, 131.81, 128.79, 127.93, 127.21, 127.08, 126.47, 124.84, 124.45, 124.43, 121.43, 115.30, 35.24.

4.3.11. Synthesis of 4-phenyl-2-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine **(17)**

Same procedure described as above for **17** was employed with **5a** (0.33 g, 1.5 mmol), dry ammonium acetate (1.156 g, 15 mmol), **2e** (0.99 g, 3 mmol) with methanol (15 mL) to yield 164 mg (33.3%) of a white solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.47, mp : 132.3-134.4 ^oC, HPLC: Retention time: 10.32 min, purity: 98.1%; ESI LC/MS *m/z* calcd for $C_{22}H_{15}NS$ $[MH]^+$ 326.10; found 326.46

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.21 (d, *J* = 6.87 Hz, 1H, indenopyridine H-9), 7.69 (dd, *J* = 3.62, 0.95 Hz, 1H, 2-thiophene H-3), 7.65-7.61 (m, 2H, 4-phenyl H-2, H-6), 7.57 (s, 1H, indenopyridine H-3), 7.56-7.36 (m, 7H, indenopyridine H-6, H-7, H-8, 2-thiophene H-5, 4-phenyl H-3, H-4, H-5), 7.12 (t, *J* = 3.67 Hz, 1H, 2-thiophene H-4), 4.94 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 160.95, 152.22, 146.36, 145.49, 144.08, 140.87, 138.68, 132.77, 128.85, 128.73, 128.58, 128.19, 127.89, 127.16, 127.05, 124.86, 124.47, 121.51, 116.42, 34.47.

4.3.12. Synthesis of 4-(pyridin-2-yl)-2-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine **(18)**

The compound was synthesized as described in section 4.3 with **5f** (0.88 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2e** (32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 385 mg (29.4%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 2:1) R_f = 0.4, mp : 169.4-172.6 ^{o}C, HPLC: Retention time: 13.57 min, purity: 96.8%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2S$ $[MH]^+$ 327.10; found 327.29

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.8 (d, *J* = 4.54 Hz, 1H, 2-pyridine H-6), 8.2 (d, *J* = 6.6 Hz, 1H, indenopyridine H-9), 7.96 (s, 1H, indenopyridine H-3), 7.88-7.83 (m, 2H, 4-pyridine H-4, 2-thiophene H-3), 7.7 (dd, *J* = 3.2, 0.80 Hz, 1H, 2-thiophene H-3), 7.58 (d, *J* = 6.88 Hz, 1H, 2-pyridine H-5), 7.51-7.34 (m, 4H, indenopyridine H-6, H-7, H-8, 2-pyridine H-3), 7.1 (dd, *J* = 5.9, 3.7 Hz, 1H, 2-thiophene H-4), 4.21 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.62, 156.19, 152.28, 149.87 145.44, 144.61, 143.88, 140.54, 136.78, 133.10, 128.82, 127.92, 127.08, 124.85, 124.58, 123.28, 122.75, 121.42, 115.35, 35.55.

4.3.13. Synthesis of 4-(furan-2-yl)-2-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(19)**

Same procedure described as above for **19** was employed with **5c** (0.105 g, 0.5 mmol), dry ammonium acetate (0.385 g, 5 mmol), **2d** (0.165 g, 0.5 mmol) with methanol (5 mL) to yield 84 mg (53.3%) of a white solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.36, mp : 149.4-151.7 ^oC, HPLC: Retention time: 9.43 min, purity: 97.4%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NOS$ $[MH]^+$ 316.08; found 316.44

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.19 (d, *J* = 7.11 Hz, 1H, indenopyridine H-9), 8.07-8.06 (m, 1H, 2-thiophene H-2), 7.84 (s, 1H, indenopyridine H-3), 7.82 (d, *J* = 4.32 Hz, 1H, 4-furan H-5), 7.64-7.59 (m, 2H, 2-thiophene H-4, H-5), 7.50-7.40 (m, 3H, indenopyridine H-6, H-7, H-8), 6.97 (d, *J* = 3.45 Hz, 1H, 4-furan H-3), 6.62-6.60 (m, 1H, 4-furan H-4), 4.04 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.39, 152.92, 151.42, 143.91, 143.50, 142.61, 140.83, 134.17, 128.87, 128.73, 127.14, 126.52, 126.07, 124.91, 123.37, 121.15, 112.64, 112.12, 110.59, 35.53

4.3.14. Synthesis of 4-(furan-3-yl)-2-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(20)**

The compound was synthesized as described in section 4.3 with **5b** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2d** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 452 mg (35.8%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.3, mp : 145.4-146.8 ^oC, HPLC: Retention time: 9.71 min, purity: 98.9%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NOS$ $[MH]^+$ 316.08; found 316.28

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.18 (d, *J* = 6.9 Hz, 1H, indenopyridine H-9), 8 (d, *J* = 2.97 Hz, 1H, 2-thiophene H-2), 7.98 (s, 1H, indenopyridine H-3), 7.79 (d, *J* = 5.03 Hz, 1H, 2-thiophene H-5), 7.60-7.58 (m, 3H, 4-furan H-2, H-5, 2-thiophene H-4), 7.51-7.40 (m, 3H, indenopyridine H-6, H-7, H-8), 6.9 (dd, *J* = 1.3, 0.8 Hz, 1H, 4-furan H-4), 3.9 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.21, 153.12, 143.83, 143.57 142.62, 141.42, 141.02, 136.83, 131.10, 128.74, 127.26, 126.51, 126.14, 124.93, 123.77, 123.36, 121.25, 115.60, 35.31.

4.3.15. Synthesis of 4-(thiophen-2-yl)-2-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(21)**

Same procedure described as above for **21** was employed with **5e** (0.45 g, 2 mmol), dry ammonium acetate (1.54 g, 20 mmol), **2d** (0.99 g, 3 mmol) with methanol (15 mL) to yield 193 mg (29.1%) of a light yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.44, mp : 155.2-157.5 ^oC, HPLC: Retention time: 11.11 min, purity: 96.7%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NS_2$ $[MH]^+$ 332.06; found 332.44

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.18 (d, *J* = 6.65 Hz, 1H, indenopyridine H-9), 8.04 (s, 1H, 2-thiophene H-2), 7.80 (d, *J* = 4.87 Hz, 1H, 4-thiophene H-5), 7.64 (s, 1H, indenopyridine H-3), 7.62-7.51 (m, 2H, 2-thiophene H-5, 4-thiophene H-3), 7.49-7.40 (m, 4H, indenopyridine H-6, H-7, H-8, 4-thiophene H-4), 7.24-7.19 (m, 1H, 2-thiophene H-4), 4.06 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.62, 156.19, 152.28, 149.87 145.44, 144.61, 143.88, 140.54, 136.78, 133.10, 128.82, 127.92, 127.08, 124.85, 124.58, 123.28, 122.75, 121.42, 115.35, 35.55.

4.3.16. Synthesis of 2,4-di(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(22)**

The compound was synthesized as described in section 4.3 with **5d** (0.90, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2d** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 389 mg (30.8%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.3, mp : 145.4-146.8 ^oC, HPLC: Retention time: 10.57 min, purity: 95.6%; ESI LC/MS *m/z* calcd for $C_{20}H_{13}NS_2$ $[MH]^+$ 332.06; found 332.28

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (d, *J* = 7.51 Hz, 1H, indenopyridine H-9), 8 (dd, *J* = 3.03, 1.12 Hz, 1H, 2-thiophene H-2), 7.8 (d, *J* = 5.02 Hz, 1H, 2-thiophene H-5), 7.7 (dd, *J* = 4.78, 1.4 Hz, 1H, 4-thiophene H-2), 7.67 (s, 1H, indenopyridine H-3), 7.61-7.40 (m, 6H, 2-thiophene H-4, 4-thiophene H-4, H-5, indenopyridine H-6, H-7, H-8), 4.05 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.33, 153.16, 143.82, 143.57 142.61, 141, 141.31, 139.29, 131.62, 128.72, 127.22, 126.50, 126.46, 123.17, 124.91, 124.34, 123.36, 121.27, 116.76, 35.20.

4.3.17. Synthesis of 4-phenyl-2-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(23)**

The compound was synthesized as described in section 4.3 with **5a** (0.88 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2d** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 386 mg (29.4%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.3, mp : 144.8-146.4 ^oC, HPLC: Retention time: 11.22 min, purity: 97.1%; ESI LC/MS *m/z* calcd for $C_{22}H_{15}NS$ $[MH]^+$ 326.10; found 326.32

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (d, *J* = 7.03 Hz, 1H, indenopyridine H-9), 8 (dd, J = 2.4, 1.14 Hz, 1H, 2-thiophene H-2), 7.8 (dd, *J* = 4.5, 1 Hz, 1H, 2-thiophene H-5), 7.6 (dd, *J* = 7.4, 1.5 Hz, 2H, 4-phenyl H-2, H-6), 7.56-7.47 (m, 6H, indenopyridine H-3, 4-phenyl H-3, H-4, H-5, indenopyridine H-7, H-8), 7.45-7.41 (m, 2H, 2-thiophene H-4 and indenopyridine H-6), 3.9 (s, 2H, indenopyridine H-5)

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161, 153.14, 146.33, 144.07 142.62, 141.06, 138.77, 132.55, 128.83, 128.65, 128.53, 128.18, 127.15, 126.51, 126.14, 124.92, 123.34, 121.30, 117.85, 34.39.

4.3.18. Synthesis of 4-(pyridin-2-yl)-2-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(24)**

The compound was synthesized as described in section 4.3 with **5f** (0.88 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2d** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 341 mg (26.1%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 2:1) R_f = 0.4, mp : 179.5-181.6 ^oC, HPLC: Retention time: 13.21 min, purity: 95.4%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2S$ $[MH]^+$ 327.10; found 327.30

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.8 (dt, *J* = 4.5, 1.3 Hz, 1H, 4-pyridine H-6), 8.2 (dd, *J* = 7.34, 1.37 Hz, 1H, indenopyridine H-9), 8.1 (dd, *J* = 3, 1.2 Hz, 1H, 2-thiophene H-2), 7.94 (s, 1H, indenopyridine H-3), 7.94-7.84 (m, 3H, 4-pyridine H-4, 2-thiophene H-4, H-5), 7.6 (d, *J* = 7 Hz, 1H, 4-pyridine H-5), 7.51-7.35 (m 4H, 4-pyridine H-3, indenopyridine H-6, H-7, H-8), 4.22 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.67, 156.31, 153.23, 149.86, 144.59, 143.88, 142.61, 140.78, 136.77, 132.87, 128.73, 127.04, 126.58 126.08, 124.90, 123.41, 123.23, 122.76, 121.23, 116.76, 35.46.

4.3.19. Synthesis of 4-(furan-2-yl)-2-(pyridin-2-yl)-5H-indeno[1,2-b]pyridine **(25)**

The compound was synthesized as described in section 4.3 with **5c** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2f** (1.30 g, 4 mmol) with glacial acetic acid (12 mL) to yield 367 mg (29.6%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.44, mp : 167.2-168.1 ^oC, HPLC: Retention time: 12.99 min, purity: 97.7%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2O$ $[MH]^+$ 311.12; found 311.35

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.7 (dd, *J* = 4.74, 0.8 Hz, 1H, 2-pyridine H-6), 8.69-8.66 (m, 2H, 2-pyridine H-3, indenopyridine H-3), 8.2 (dd, *J* = 7.7, 1.7 Hz, 1H, indenopyridine H-9), 7.8 (dt, *J* = 7.7, 1.7 Hz, 1H, 2-pyridine H-4), 7.66-7.62 (m, 2H, 4-furan H-5, indenopyridine H-7), 7.5-7.4 (m, 2H, indenopyridine H-6, H-8), 7.3 (ddd, *J* = 7.16, 5.07, 1.09 Hz, 1H, 2-

pyridine H-5), 7 (d, *J* = 3.4 Hz, 1H, 4-furan H-3), 6.6 (dd, *J* = 3.4, 1.7 Hz, 1H, 4-furan H-4), 4.16 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.29, 156.56, 155.44, 151.82, 149.07, 144.12, 143.65, 140.78, 136.89, 134.48, 130.89, 128.82, 127.17, 125.02, 123.55, 121.29, 121.08, 113.57, 112.07, 110.66, 35.73.

4.3.20. Synthesis of 4-(furan-3-yl)-2-(pyridin-2-yl)-5H-indeno[1,2-b]pyridine **(26)**

The compound was synthesized as described in section 4.3 with **5b** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2f** (1.30 g, 4 mmol) with glacial acetic acid (12 mL) to yield 502 mg (38.4%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.44, mp : 157.3-160.3 ^{o}C, HPLC: Retention time: 11.59 min, purity: 97.8%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2O$ $[MH]^+$ 311.12; found 311.33

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.7 (dd, *J* = 4.3, 1.83 Hz, 1H, 2-pyridine H-6), 8.6 (d, *J* = 7.9 Hz, 1H, 2-pyridine H-3), 8.48 (s, 1H, 4-furan H-2), 8.2 (dd, *J* = 7.34, 1.23 Hz, 1H, indenopyridine H-9), 8.05 (s, 1H, indenopyridine H-3), 7.8 (dt, *J* = 7.7, 1.7 Hz, 1H, 2-pyridine H-4), 7.63-7.59 (m, 2H, 4-furan H-5, indenopyridine H-8), 7.52-7.41 (m, 2H, indenopyridine H-6, H-7), 7.3 (ddd, *J* = 7.3, 4.8, 1 Hz, 1H, 2-pyridine H-5), 7 (d, *J* = 0.98 Hz, 1H, 4-furan H-4), 3.99 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 160.94, 156.56, 155.51, 148.98, 143.70, 143.55, 141.63, 140.91, 137.04, 136.93, 132.96, 128.79, 127.28, 125.01, 123.85, 123.57, 121.35, 121.11, 116.22, 109.38, 35.86.

4.3.21. Synthesis of 2-(pyridin-2-yl)-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine **(27)**

The compound was synthesized as described in section 4.3 with **5e** (0.90 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2f** (1.30 g, 4 mmol) with glacial acetic acid (12 mL) to yield 377 mg (28.9%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.44, mp : 169.2-172.4 ^{o}C, HPLC: Retention time: 14.76 min, purity: 95.1%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2S$ $[MH]^+$ 327.10; found 327.35

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.7 (ddd, *J* = 4.7, 1.7, 0.89 Hz, 1H, 2-pyridine H-6), 8.68 (d, J = 7.83 Hz, 1H, 2-pyridine H-3), 8.64 (s, 1H, indenopyridine H-3), 8.2 (dd, *J* = 7.42, 1.47 Hz, 1H, indenopyridine H-9), 7.8 (dt, *J* = 7.73, 1.7 Hz, 1H, 2-pyridine H-4), 7.7 (dd, *J* = 3.7, 1.7 Hz, 1H, 4-thiophene H-5), 7.6 (dd, *J* = 6.44, 1.17 Hz, 1H, indenopyridine H-7), 7.54-7.45 (m, 3H, 4-thiophene H-3, indenopyridine H-6, H-8), 7.3 (ddd, *J* = 7.4, 4.8, 1.2 Hz, 1H, 2-pyridine H-5), 7.2 (dt, *J* = 6.02, 2.29 Hz, 1H, 4-thiophene H-4), 4.16 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.35, 156.45, 155.57, 149.07, 143.79, 140.86, 140.82, 138.67, 136.89, 132.31, 128.88, 127.93, 127.28, 127.15, 125, 123.59, 121.32, 121.18, 116.15, 35.85.

4.3.22. Synthesis of 2-(pyridin-2-yl)-4-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(28)**

The compound was synthesized as described in section 4.3 with **5d** (0.90 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2f** (1.30 g, 4 mmol) with glacial acetic acid (12 mL) to yield 583 mg (42.6%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.44, mp : 178.4-181.1 ^{o}C, HPLC: Retention time: 13.22 min, purity: 100%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2S$ $[MH]^+$ 327.10; found 327.35

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.72-8.70 (m, 2H, 2-pyridine H-3, H-6), 8.55 (s, 1H, 4-thiophene H-2), 8.2 (d, *J* = 6.9 Hz, 1H, indenopyridine H-9), 8.08 (s, 1H, indenopyridine H-3), 7.9 (dd, *J* = 7.8, 1.7 Hz, 1H, 2-pyridine H-4), 7.8 (dd, *J* = 1.27, 3 Hz, 1H, 4-thiophene H-

5), 7.67 (dd, *J* = 5.06, 1.2 Hz, 1H, 4-thiophene H-4), 7.6 (dd, *J* = 6.71, 0.87 Hz, 1H, indenopyridine H-8), 7.51-7.44 (m, 3H, indenopyridine H-6, H-7, thiophene H-4), 7.3 (ddd, *J* = 6.87, 4.36, 1.06 Hz, 1H, 2-pyridine H-5), 4.33 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.57, 156.59, 156.55, 155.67, 149.59, 149.04, 144.95, 143.88, 140.70, 136.90, 136.69, 135.29, 128.78, 126.98, 125, 123.53, 123.14, 122.88, 121.30, 121.10, 117.28, 35.36.

4.3.23. Synthesis of 4-phenyl-2-(pyridin-2-yl)-5H-indeno[1,2-b]pyridine **(29)**

The compound was synthesized as described in section 4.3 with **5a** (0.88 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2f** (1.30 g, 4 mmol) with glacial acetic acid (12 mL) to yield 581 mg (45.3%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:3) R_f = 0.28, mp : 153.7-155.4 ^{o}C, HPLC: Retention time: 14.15 min, purity: 95.4%; ESI LC/MS *m/z* calcd for $C_{23}H_{16}N_2$ $[MH]^+$ 321.14; found 321.40

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.71-8.68 (m, 2H, 2-pyridine H-3, H-6), 8.43 (s, 1H, indenopyridine H-3), 8.2 (dd, *J* = 7.33, 0.9 Hz, 1H, indenopyridine H-9), 7.87 (dd, *J* = 7.60, 1.56 Hz, 1H, 2-pyridine H-4), 7.73-7.69 (m, 2H, indenopyridine H-6, H-7), 7.56-7.39 (m, 6H, 4-phenyl H-2, H-3, H-4, H-5, H-6, indenopyridine H-8), 7.3 (ddd, *J* = 7.46, 4.66, 1.31 Hz, 1H, 2-pyridine H-5), 4.04 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 160.37, 156.65, 155.66, 149.06, 146.56, 144.09, 141.01, 138.70, 136.88, 134.52, 128.71, 128.48, 128.234, 127.19, 125, 123.51, 121.33, 121.21, 118.58, 34.57.

4.3.24. Synthesis of 2,4-di(pyridin-2-yl)-5H-indeno[1,2-b]pyridine **(30)**

The compound was synthesized as described in section 4.3 with **5f** (0.88 g, 4 mmol), dry ammonium acetate (3.178 g, 40 mmol), **2f** (1.3 g, 4 mmol) with glacial acetic acid (10 mL) to yield 447 mg (34.8 %) of a white solid.

TLC (ethyl acetate / *n*-hexane = 1:5) R_f = 0.44, mp : 166.5-167.7 ^{o}C, HPLC: Retention time: 11.78 min, purity: 100%; ESI LC/MS *m/z* calcd for $C_{22}H_{15}N_3$ $[MH]^+$ 322.13; found 322.37

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.8 (dd, *J* = 4.4, 0.57 Hz, 1H, 2-pyridine H-3), 8.73-8.69 (m, 3H, indenopyridine H-3, 2-pyridine H-6 and 4-pyridine H-6), 8.2 (dd, *J* = 7.3, 1.5 Hz, 1H, indenopyridine H-9), 7.98 (d, *J* = 7.93 Hz, 1H, 4-pyridine H-4), 7.9 (dd, *J* = 7.19, 1.74 Hz, 1H, 2-pyridine H-4), 7.8 (td, *J* = 7.6, 1.75 Hz, 1H, 2-pyridine H-5), 7.6 (dd, *J* = 7.3, 1.56 Hz, 1H, 4-pyridine H-5), 7.5 (dd, *J* = 6.84, 0.82 Hz, 1H, 4-pyridine H-3), 7.4 (td, *J* = 7.29, 1.27 Hz, 1H, indenopyridine H-7), 7.37-7.30 (m, 2H, indenopyridine H-6, H-8), 4.33 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.57, 156.59, 155.67, 149.59, 149.04, 144.95, 143.88, 140.70, 136.90, 136.69, 135.29, 128.78, 126.98, 125, 123.53, 123.14, 122.88, 121.30, 121.10, 117.28, 35.86.

4.3.25. ***4-(furan-2-yl)-2-(pyridin-3-yl)-5H-indeno[1,2-b]pyridine*** **(31)**

The compound was synthesized as described in section 4.3 with **5c** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2g** (1.32 g, 4 mmol) with glacial acetic acid (10 mL) to yield 237 mg (38.2%) of a yellow solid.

TLC (ethyl acetate / *n*-hexane = 2:1) R_f = 0.38, mp : 164.8-165.2 ^{o}C, HPLC: Retention time: 9.61 min, purity: 96.6%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2O$ $[MH]^+$ 311.12; found 311.34

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.3 (s, 1H, 2-pyridine H-2), 8.6 (dd, *J* = 4.7, 1.45 Hz, 1H, 2-pyridine H-6), 8.5 (dt, *J* = 7.99, 1.9 Hz, 1H, 2-pyridine H-4), 8.21-8.19 (br, 1H,

indenopyridine H-9), 7.97 (s, 1H, indenopyridine H-3), 7.67-7.62 (m, 2H, 4-furan H-5 and indenopyridine H-7), 7.52-7.42 (m, 3H, 2-pyridine H-5, indenopyridine H-6, H-8), 7 (d, *J* = 3.37 Hz, 1H, 4-furan H-3), 6.6 (dd, *J* = 3.4, 1.7 Hz, 1H, 4-furan H-4), 4.2 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.83, 154.30, 149.69, 148.41, 143.79, 140.70, 140.52, 138.96, 135.30, 134.53, 132.57, 129.02, 127.36, 127, 126.66, 124.98, 124.63, 124.56, 121.31, 116.94, 35.22.

4.3.26. 4-(furan-3-yl)-2-(pyridin-3-yl)-5H-indeno[1,2-b]pyridine **(32)**

The compound was synthesized as described in section 4.3 with **5b** (0.84 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2g** (1.30 g, 4 mmol) with glacial acetic acid (10 mL) to yield 498 mg (40.1%) of a yellowish white solid.

TLC (ethyl acetate / *n*-hexane = 1:2) R_f = 0.1, mp : 178.7-179.8 ^{o}C, HPLC: Retention time: 9.06 min, purity: 97.9%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2O$ $[MH]^+$ 311.12; found 311.31

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.3 (s, 1H, 2-pyridine H-2), 8.6 (dd, *J* = 4.8, 1.6 Hz, 1H, 2-pyridine H-6), 8.4 (dt, *J* = 7.95, 2 Hz, 1H, 2-pyridine H-4), 8.2 (dd, *J* = 7.3, 1.7 Hz, 1H, indenopyridine H-9), 8 (s, 1H, indenopyridine H-3), 7.72 (s, 1H, 4-furan H-2), 7.63-7.61 (m, 2H, 4-furan H-5 and indenopyridine H-7), 7.53-7.42 (m, 3H, indenopyridine H-6, H-8 and 2-pyridine H-5), 6.9 (dd, *J* = 1.7, 0.8 Hz, 1H, 4-furan H-4), 3.9 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.69, 154.26, 149.71, 148.41 143.99, 143.51, 140.70, 137.09, 135.30, 134.54, 132.02, 129.04, 127.40, 125.01, 123.55, 121.28, 115.80, 109.03, 35.33.

4.3.27. 2-(pyridin-3-yl)-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine **(33)**

The compound was synthesized as described in section 4.3 with **5e** (0.90 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2g** (1.30 g, 4 mmol) with glacial acetic acid (10 mL) to yield 394 mg (30.2%) of a whitish yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:2) R_f = 0.15, mp : 178.2-181.4 ^oC, HPLC: Retention time: 10.58 min, purity: 95.9%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2S$ $[MH]^+$ 327.10; found 327.33

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.3 (s, 1H, 2-pyridine H-2), 8.6 (dd, *J* = 4.7, 1.56 Hz, 1H, 2-pyridine H-6), 8.48 (td, *J* = 7.9, 1.9 Hz, 1H, 2-pyrindine H-4), 8.2 (dd, *J* = 6.90, 1.99 Hz, 1H, indenopyridine H-9), 7.85 (s, 1H, indenopyridine H-3), 7.67 (dd, *J* = 3.6, 1 Hz, 1H, 4-thiophene H-5), 7.62 (dd, *J* = 6.9, 1.7 Hz, 1H, indenopyridine H-7), 7.5 (dd, *J* = 5.09, 0.9 Hz, 1H, 4-thiophene H-3), 7.53-7.43 (m, 3H, indenopyridine H-6, H-8, 2-pyridine H-5), 7.2 (dd, *J* = 4.28, 2.94 Hz, 1H, 4-thiophene H-4), 4.12 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 162.51, 154.68, 150.18, 148.87 144.17, 141.01, 140.90, 139.10, 135.60, 134.96, 131.84, 129.56, 128.56, 127.90, 127.49, 125.42, 123.98, 121.76, 116.15, 36.11.

4.3.28. 2-(pyridin-3-yl)-4-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine **(34)**

The compound was synthesized as described in section 4.3 with **5d** (0.90 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2g** (1.30 g, 4 mmol) with glacial acetic acid (10 mL) to yield 308 mg (23.6%) of a yellowish brown solid.

TLC (ethyl acetate / *n*-hexane = 1:1) R_f = 0.26, mp : 198.5-199.6 ^oC, HPLC: Retention time: 10.35 min, purity: 95.5%; ESI LC/MS *m/z* calcd for $C_{21}H_{14}N_2S$ $[MH]^+$ 327.10; found 327.34

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.3 (s, 1H, 2-pyridine H-2), 8.6 (dd, *J* = 4.7, 1.5 Hz, 1H, 2-pyridine H-6), 8.5 (td, *J* = 7.94, 2.14, 1.6 Hz, 1H, 2-pyridine H-4), 8.2 (d, *J* = 6.95 Hz, 1H, indenopyridine H-9), 7.77-7.76 (m, 2H, indenopyridine H-3, 4-thiophene H-2), 7.62-7.42 (m,

6H, indenopyridine H-6, H-7, H-8, 4-thiophene H-5, H-4, 2-pyridine H-5), 4.07 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.83, 154.30, 149.69, 148.41, 143.79, 140.70, 140.52, 138.96, 135.30, 134.53, 132.57, 129.02, 127.36, 127, 126.66, 124.98, 124.63, 124.56, 121.31, 116.94, 35.22.

4.3.29. 4-phenyl-2-(pyridin-3-yl)-5H-indeno[1,2-b]pyridine **(35)**

The compound was synthesized as described in section 4.3 with **5a** (0.88 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2g** (1.30 g, 4 mmol) with glacial acetic acid (10 mL) to yield 480 mg (37.5%) of a whitish yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:2) R_f = 0.11, mp : 152.1-155.1 ^{o}C, HPLC: Retention time: 10.49 min, purity: 97.2%; ESI LC/MS *m/z* calcd for $C_{23}H_{16}N_2$ $[MH]^+$ 321.14; found 321.38

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.3 (s, 1H, 2-pyridine H-2), 8.6 (dd, *J* = 4.7, 1. 6 Hz, 1H, 2-pyridine H-6), 8.5 (dt, *J* = 8, 1.2 Hz, 1H, 2-pyridine H-4), 8.2 (d, *J* = 6.8 Hz, 1H, indenopyridine H-9), 7.69-7.65 (m, 3H, indenopyridine H-3, 4-phenyl H-2, H-6), 7.59-7.42 (m, 7H, 4-phenyl H-3, H-4, H-5, indenopyridine H-6, H-7, H-8 and 2-pyridine H-5), 4.03 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.53, 154.32, 149.70, 148.48 146.62, 144.07, 140.79, 138.46, 135.30, 134.52, 133.60, 128.98, 128.94, 128.74, 128.19, 127.31, 125.01, 123.55, 121.37, 118.08, 34.43.

4.3.30. 4-(pyridin-2-yl)-2-(pyridin-3-yl)-5H-indeno[1,2-b]pyridine **(36)**

The compound was synthesized as described in section 4.3 with **5f** (0.88 g, 4 mmol), dry ammonium acetate (3.17 g, 40 mmol), **2g** (1.30 g, 4 mmol) with glacial acetic acid (10 mL) to yield 458 mg (35.7%) of a white solid.

TLC (ethyl acetate / *n*-hexane = 2:1) R_f = 0.21, mp : 200.1-202.5 ^oC, HPLC: Retention time: 8.16 min, purity: 98.4%; ESI LC/MS *m/z* calcd for $C_{22}H_{15}N_3$ $[MH]^+$ 322.13; found 322.36

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.4 (1H, 2-pyridine H-2), 8.8 (dt, *J* = 4.3, 1.6 Hz, 1H, 4-pyridine H-6), 8.6 (dd, *J* = 4.4, 0.7 Hz, 1H, 2-pyridine H-6) 8.5 (dt, *J* = 7.9, 1.5 Hz, 1H, 2-pyridine H-4), 8.2 (dd, *J* = 7.39, 1.3 Hz, 1H, indenopyridine H-9), 8.01 (s, 1H, indenopyridine H-3), 7.90-7.87 (m, 2H, 4-pyridine H-4, H-5), 7.6 (d, *J* = 6.4 Hz, 1H, 4-pyridine H-3), 7.53-7.36 (m, 4H, 2-pyridine H-5, indenopyridine H-6, H-7, H-8), 4.27 (s, 2H, indenopyridine H-5).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 162.25, 156.02, 154.40, 149.97 149.70, 148.52, 144.61, 144.13, 140.56, 136.85, 135.33, 134.55, 133.96, 129.07, 127.21, 124.99, 123.53, 123.40, 122.78, 121.33, 116.96, 35.55.

4.4. Pharmacology

4.4.1. Assay for DNA topoisomerase I inhibition *in vitro*

DNA topo I inhibition assay was determined following the previously reported method.[13] 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 topoisomerase I (TopoGEN INC., USA) was incubated without and with the prepared compounds at 37 °C for 30 min in the relaxation buffer (10 mM Tris-HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin, 1 mM spermidine, 5% glycerol). The reaction in the final volume of 10 μL was terminated by adding 2.5 μL of the stop solution containing 5% sarcosyl, 0.0025% bromophenol blue and 25% glycerol. DNA samples were then electrophoresed on a 1% agarose gel at 15 V for 7 h with a running buffer of TAE. Gels were stained for 30 min in an

aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corporation).

4.4.2. Assay for DNA Topoisomerase II Inhibition *in vitro*

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

4.4.3. Cytotoxicity Assay

For the evaluation of cytotoxicity, four different human cancer cell lines were used: human breast adenocarcinoma cell line (MCF-7), human cervix tumor cell line (HeLa), human colorectal adenocarcinoma cell line (HCT15), and human prostate tumor cell line (DU145). Experiments were performed according to previously described methods.[13,14] Cancer cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of 2~4 x 10^4 cells per well and incubated for overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Hyclone, USA) in 5% CO_2 incubator at 37 °C. On day 2, after

FBS starvation for 4 h, culture medium in each well was exchanged with 0.1 mL aliquots of medium containing graded concentrations of compounds. On day 4, each well was added with 5 μL of the cell counting kit-8 solution (Dojindo, Japan) then incubated for additional 4 h under the same condition. The absorbance of each well was determined by an Automatic Elisa Reader System (Bio-Rad 3550) at 450 nm wavelength. For determination of the IC_{50} values, the absorbance readings at 450 nm were fitted to the four-parameter logistic equation. The compounds such as adriamycin, etoposide, and camptothecin were purchased from Sigma and used as positive controls.

Acknowledgements.

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012R1A2A2A01046188).

References

1. Pommier, Y. *Chem. Rev.* **2009,** *109*, 2894.

2. (a) Wang, J. C. *Annu. Rev. Biochem.* **1996,** *65*, 635. (b) Nitiss, J. L. *Biochim. Biophys. Acta* **1998,** *1400*, 63.

3. Forterre, P.; Gribaldo, S.; Gadelle, D.; Serre, M. C. *Biochimie* **2007**, *89*, 427.

4. (a) Cummings, J.; Smyth, J.F. *Ann. Oncol.* **1993**, *4*, 533. (b) Chen, A. Y.; Liu, L.F. *Ann. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191. (c) Nitiss, J. L. *Nat. Rev. Cancer* **2009**, *5*, 338.

(d) Nitiss, J. L. *Nat. Rev. Cancer* **2009**, *5*, 327.

5. (a) Zhao, L. X.; Kim, T. S.; Ahn, S. H.; Kim, T. H.; Kim, E. K.; Cho, W. J.; Choi, H. S.; Lee, C. S.; Kim, J. A.; Jeong, T. C.; Chang, C. J.; Lee, E. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2659. (b) Zhao, L. X.; Moon, Y. S.; Basnet, A.; Kim, E. K.; Jahng, Y.; Park, J. G.; Jeong, T. C.; Cho, W. J.; Choi, S. U.; Lee, C.O.; Lee, S. Y.; Lee, C. S.; Lee, E. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1333. (c) Zhao, L. X.; Sherchan, J.; Park, J. K.; Jahng, Y.; Jeong, B. S.; Jeong, T. C.; Lee, C. S.; Lee, E.S. *Arch. Pharm. Res.* **2006,** *29*, 1091. (d) Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, B. S.; Jeong, T. C.; Lee, C. S.; Lee, E. S. *Bioorg. Med. Chem.* **2007**, *15*, 4351. (e) Son, J. K.; Zhao, L. X.; Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, T. C.; Jeong, B. S.; Lee, C. S.; Lee, E. S. *Eur. J. Med. Chem.* **2008**, *43*, 675. (f) Thapa, P.; Karki, R.; Basnet, A.; Thapa, U.; Choi, H. Y.; Na, Y.; Jahng, Y.; Lee, C. S.; Kwon, Y.; Jeong, B. S.; Lee, E. S. *Bull. Korean Chem. Soc.* **2008**, *29*, 1605.

6. (a) Basnet, A.; Thapa, P.; Karki, R.; Choi, H. Y.; Choi, J. H.; Yun, M.; Jeong, B. S.; Jahng, Y.; Na, Y.; Cho, W. J.; Kwon, Y.; Lee, C. S.; Lee, E. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 42. (b) Thapa, P.; Karki, R.; Thapa, U.; Jahng, Y.; Jung, M. J.; Nam, J. M.; Na, Y.; Kwon, Y.; Lee, E. S. *Bioorg. Med. Chem.* **2010**, *18*, 377. (c) Thapa, P.; Karki, R.; Choi, H. Y.; Choi, J. H.; Yun, M.; Jeong, B. S.; Jung, M. J.; Nam, J. M.; Na, Y; Cho, W. J.; Kwon, Y.; Lee, E. S. *Bioorg. Med. Chem.* **2010**, *18*, 2245. (d) Karki, R.; Thapa, P.; Kang, M. J.; Jeong, T. C.; Nam, J. M.; Kim, H. L.; Na, Y.; Cho, W. J.; Kwon, Y.; Lee, E. S. *Bioorg. Med. Chem.* **2010**, *18*, 3066. (e) Karki, R.; Thapa, P.; Yoo, H. Y.; Kadayat, T. M.; Park, P. H.; Na, Y.; Lee, E.; Jeon, K. H.; Cho, W. J.; Choi,

H.; Kwon, Y.; Lee, E.S. *Eur. J. Med. Chem.* **2012**, *49*, 219. (f) Thapa, P.; Karki, R.; Minho, Y.; Kadayat, T. M.; Lee, E.; Kwon, H. B.; Na, Y.; Cho, W. J.; Kim, N. D.; Jeong, B. S.; Kwon, Y.; Lee, E. S. *Eur. J. Med. Chem.* **2012**, *52*, 123. (g) Karki, R.; Park, C.; Jun, K.Y.; Jee, J.G.; Lee, J.H.; Thapa, P.; Kadayat, T. M.; Kwon, Y.; Lee, E.S. *Eur. J. Med. Chem.* **2014**, *84*, 555.

7. (a) Jeong, B. S.; Choi, H. Y.; Thapa, P.; Karki, R.; Jung, M. J.; Nam, J. M.; Na, Y.; Ha, E. M.; Kwon, Y.; Lee, E. S. *Bull. Korean Chem. Soc.* **2011**, *32*, 303. (b) Thapa, U.; Thapa, P.; Karki, R.; Yun, M.; Choi, J.H.; Jahng, Y.; Lee, E.; Jeon, K. H.; Na, Y.; Ha, E. M.; Cho, W. J.; Kwon, Y.; Lee, E. S. *Eur. J. Med. Chem.* **2011**, *46*, 3201. (c) Thapa, P.; Karki, R.; Yun, M.; Yoo, H. Y.; Park, P. H.; Lee, E.; Jeon, K. H.; Na, Y.; Cho, W. J.; Kwon, Y.; Lee, E. S. *Bioorg. Chem.* **2012**, *40*, 67. (d) Thapa, P.; Lee, E. S. *Bull. Korean Chem. Soc.* **2012**, *33*, 1769. (e) Thapa, P.; Lee, E. S. *Bull. Korean Chem. Soc.* **2012**, *33*, 3103. (f) Thapa, P.; Jhang, Y.; Park, P. H.; Kwon, Y.; Lee, E. S. *Bull. Korean Chem. Soc.* **2013**, *34*, 3073.

8. Xiao, X. S.; Cushman, M.; *J. Am. Chem. Soc.* **2005,** *127*, 9960.

9. Van, H.T.M.; Cho, W. J.; *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2551.

10. (a) Utsugi, T.; Aoyagi, K.; Asao, T.; Okazaki, S.; Aoyagi, Y.; Sano, M.; Wierzba, K.; Yamada, Y. *Jpn. J. Cancer Res.* **1997**, *88*, 992. (b) Manpadi, M.; Uglinskii, P. Y.; Rastogi, S. K.; Cotter, K. M.; Wong, Y. S.; Anderson, L.A.; Ortega, A. J.; Van Slambrouck, S.; Steelant, W.F.; Rogelj, S.; Tongwa, P.; Antipin, M.Y.; Magedov, I. V.;

Kornienko, A.; *Org. Biomol. Chem.* **2007,** *5***,** 3865. (c) Ghorab, M. M.; Al-Said, M. S.; *Arch. Pharm. Res.* **2012,** *35*, 987.

11. Jahng, Y.; Zhao, L. X.; Moon, Y. S.; Basnet, A.; Kim, E. K.; Chang, H. W.; Ju, H. K.; Jeong, T. C.; Lee, E. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2559.

12. (a) Kröhnke, F. *Synthesis* **1976,** 1. (b) Kröhnke, F. *Angew. Chem. Internat. Edit.* **1963**, *2*, 380.

13. Fukuda, M.; Nishio, K.; Kanzawa, F.; Ogasawara, H.; Ishida, T.; Arioka, H.; Bonjanowski, K.; Oka, M.; Saijo, N. *Cancer Res.* **1996**, *56*, 789.

14. Kang, D. H.; Kim, J. S.; Jung, M. J.; Lee, E. S.; Jhang, Y.; Kwon, Y.; Na, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1520.

Table captions

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

Table 2. Topo I and II inhibitory activities, and cytotoxicity of the prepared compounds **7**-**30**

Table 3. Relative potency of the representative compounds

Figure captions

Figure 1. Structures of (a) Previously synthesized rigid analogs of 2,4,6-triarylsubstituted pyridines, and (b) 2,4-diaryl-5*H*-indeno[1,2-*b*]pyridines

Figure 2. Structures of the prepared compounds

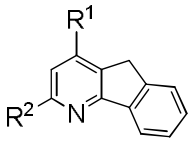
Figure 3.Topoisomerase I inhibitory activity of the prepared compounds **7-30**

Figure 4. Topoisomerase II inhibitory activity of the prepared compounds **7-30**

Figure 5. Structures of previously synthesized representative rigid analogs of 2,4,6-triarylsubstituted pyridines

Figure 6. Structure-activity relationship study of 2,4-diaryl-5*H*-indeno[1,2-*b*]pyridines

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



Entry	R^1	R^2	Yield (%)	Purity (%)	mp (°C)
7	2-furyl	2'-furyl	27.5	100.0	136.5-140.9
8	3-furyl	2'-furyl	40.2	98.1	176.4-178.2
9	2-thienyl	2'-furyl	30.4	96.7	141.6-142.9
10	3-thienyl	2'-furyl	37.2	96.3	146.6-148.2
11	Phenyl	2'-furyl	48.6	96.2	129.2-130.6
12	2-pyridyl	2'-furyl	36.4	97.3	138.1-140.7
13	2-furyl	2'-thienyl	23.5	98.4	136.3-138.4
14	3-furyl	2'-thienyl	24.2	96.3	153.5-155.4
15	2-thienyl	2'-thienyl	27.3	98.3	138.2-140.6
16	3-thienyl	2'-thienyl	25.1	99.4	152.5-154.9
17	Phenyl	2'-thienyl	33.3	98.1	132.3-134.4
18	2-pyridyl	2'-thienyl	29.4	96.8	169.4-172.6
19	2-furyl	3'-thienyl	53.3	97.4	149.4-151.7
20	3-furyl	3'-thienyl	35.8	98.9	145.4-146.8
21	2-thienyl	3'-thienyl	29.1	96.7	155.2-157.5
22	3-thienyl	3'-thienyl	30.8	95.6	145.4-146.8
23	Phenyl	3'-thienyl	29.4	97.1	144.8-146.4
24	2-pyridyl	3'-thienyl	26.1	95.4	179.5-181.6
25	2-furyl	2'-pyridyl	29.6	97.7	167.2-168.1
26	3-furyl	2'-pyridyl	38.4	97.8	157.3-160.3
27	2-thienyl	2'-pyridyl	28.9	95.1	169.2-172.4
28	3-thienyl	2'-pyridyl	42.6	100.0	178.4-181.1
29	Phenyl	2'-pyridyl	45.3	95.4	153.7-155.4
30	2-pyridyl	2'-pyridyl	34.8	100.0	166.5-167.7
31	2-furyl	3'-pyridyl	38.2	96.6	164.8-165.2

32	3-furyl	3’-pyridyl	40.1	97.9	178.7-179.8
33	2-thienyl	3’-pyridyl	30.2	95.9	178.2-181.4
34	3-thienyl	3’-pyridyl	23.6	95.5	198.5-199.6
35	Phenyl	3’-pyridyl	37.5	97.2	152.1-155.1
36	2-pyridyl	3’-pyridyl	35.7	98.4	200.1-202.5

Table 2. Topo I and II inhibitory activities, and cytotoxicity of the prepared compounds **7**-**30**

Compounds	%Inhibition				IC_{50} (μM)			
	Topo II		Topo I		[d]MCF-7	[e] HeLa	[f] DU145	[g] HCT15
	100 μM	20 μM	100 μM	20 μM				
[h]Camptothecin (C)			45.2[a]	25.9[a]	0.31±0.01[a]	0.40±0.13[a]	0.42±0.16[a]	0.50±0.06[a]
			51.5[b]		3.91±0.66[b]	1.01±0.01[b]	2.37±0.55[b]	0.26±0.04[b]
			62.9[c]	19.6[c]	0.08±0.07[c]	1.30±0.14[c]	0.11±0.05[c]	0.31±0.09[c]
[i]Etoposide (E)	32.4[a]	20.2[a]			0.73±0.06[a]	1.40±0.15[a]	0.50±0.03[a]	1.10±0.02[a]
	83.7[b]	42.1[b]			3.25±0.04[b]	3.02±0.50[b]	2.94±0.04[b]	1.33±0.10[b]
	87.8[c]	46.7[c]			0.94±0.06[c]	1.26±0.03[c]	13.1±1.71[c]	2.6±1.681[c]
[j]Adriamycin					0.40±0.02[a]	1.00±0.07[a]	1.0±0.14[a]	1.21±0.02[a]
					3.69±0.13[b]	1.45±0.01[b]	0.86±0.04[b]	1.28±0.07[b]
					1.88±1.41[c]	1.20±0.20[c]	0.95±1.05[c]	1.08±0.10[c]
7	44.6	15.0	38.2	6.4	5.40±0.08	8.14±1.85	20.62±4.63	>50
8	100.0	0.0	52.5	1.5	>50	NA	>50	20.21±0.41

9	83.9	79.6	2.0	NA	>50	>50	>50	23.61±0.69
10	100.0	93.0	0.0	NA	>50	>50	>50	>50
11	18.7	NA	41.3	5.7	>50	5.22±1.04	7.74±0.85	16.60±1.22
12	100.0	100.0	40.7	0.5	3.08±0.07	4.13±0.21	>50	8.50±0.04
13	40.5	14.6	76.3	3.9	4.11±1.40	3.20±0.49	6.71±0.25	37.72±4.17
14	100.0	66.7	1.7	NA	>50	>50	37.29±2.72	34.62±6.21
15	9.8	NA	4.7	NA	>50	NA	>50	>50
16	100.0	0.0	10.1	NA	>50	NA	>50	>50
17	56.0	0.0	5.8	NA	>50	NA	>50	>50
18	18.1	NA	4.5	NA	43.28±0.88	NA	>50	9.63±0.21
19	84.1	68.5	2.8	NA	>50	>50	>50	31.60±4.66
20	95.9	10.9	1.2	NA	>50	>50	>50	20.61±4.11
21	17.8	NA	4.1	NA	>50	NA	>50	>50
22	100.0	0.0	90.9	2.9	>50	NA	>50	16.36±0.31
23	72.5	0.0	0.5	NA	>50	NA	>50	>50

24	3.2	NA	3.5	NA	>50	NA	>50	>50
25	17.7	NA	0.6	NA	NA	NA	NA	NA
26	17.5	NA	8.6	NA	NA	NA	NA	NA
27	9.0	NA	58.5	12.0	11.01±0.25	20.50±0.32	6.7±1.58	9.1±1.00
28	15.0	NA	1.1	NA	NA	NA	NA	NA
29	12.3	NA	0.0	NA	NA	NA	NA	NA
30	10.2	NA	6.2	NA	NA	NA	NA	NA

Each data represents mean ± S.D. from three different experiments performed in triplicate.

NA: not applicable, [a]value for compounds **7, 11, 13, 25-27, 29, 30**; [b]value for compounds **9, 10, 14, 19, 20;** [c]value for compounds **8, 12, 15-18, 21-24, 28.**

[d]MCF-7: human breast adenocarcinoma cell line.

[e]HeLa: human cervix tumor cell line.

[f]DU145: human prostate tumor cell line.

[g]HCT15 human colorectal adenocarcinoma cell line.

[h]Camptothecin: positive control for topo I and cytotoxicity.

[i]Etoposide: positive control for topo II and cytotoxicity.

[j]Adriamycin: positive control for cytotoxicity.

Table 3. Relative potency of the representative compounds

Relative potency* compared to positive controls (Topo II: Etoposide; Topo I: Camptothecin)					
Compounds	Topo II 100 μM	Topo I 100 μM	Compounds	Topo II 100 μM	Topo I 100 μM
A[7a]	N**	0.97	**P**[7f]	1.24	N
B[7a]	N	0.53	**7**	1.38	0.85
C[7b]	N	0.83	**8**	1.14	0.83
D[7b]	0.28	0.80	**9**	1.00	N
E[7e]	0.89	1.20	**10**	1.19	N
F[7e]	0.90	1.42	**11**	0.58	0.91
G[7e]	N	1.58	**12**	1.14	0.65
H[7e]	1.13	1.67	**13**	2.35	0.90
I[7d]	N	1.25	**14**	1.19	N
J[7d]	N	1.36	**16**	1.14	N
K[7c]	N	1.15	**19**	1.00	N
L[7d]	N	0.49	**20**	1.15	N
M[7f]	N	1.15	**22**	1.14	1.45
N[7f]	1.11	N	**23**	0.83	N
O[7f]	1.11	0.60			

* Relative potency: % inhibition of compounds / % inhibition of positive control
**N: Value less than 0.2

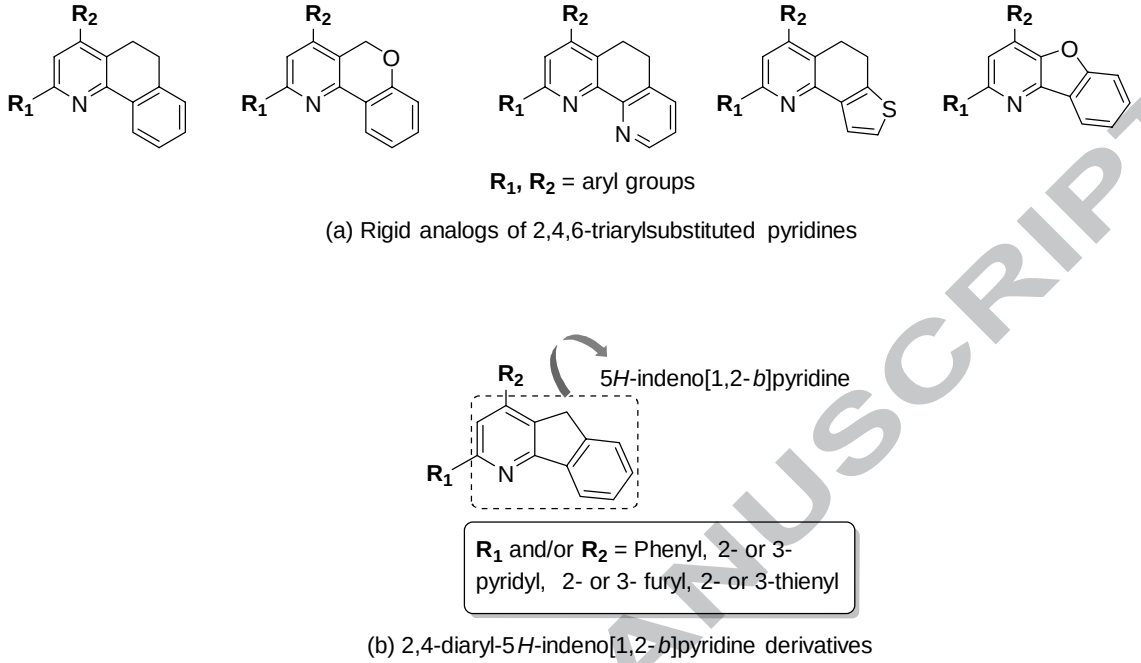


Figure 1. Structures of (a) Previously synthesized rigid analogs of 2,4,6-triarylsubstituted pyridines, and (b) 2,4-diaryl-5*H*-indeno[1,2-*b*]pyridines

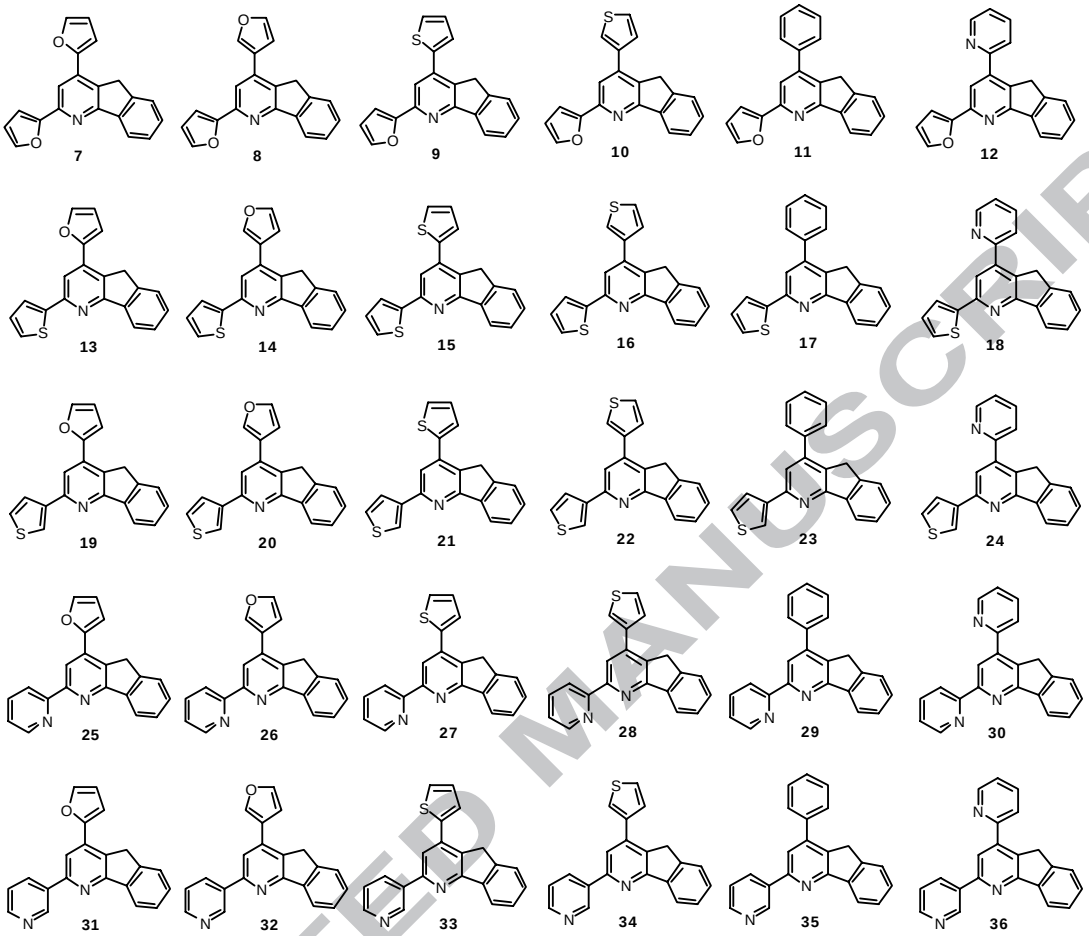
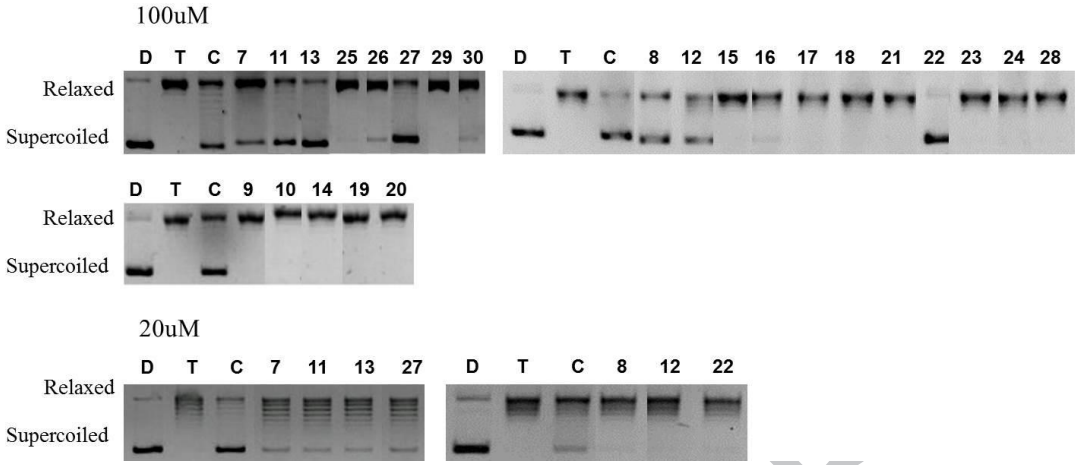


Figure 2. Structures of the prepared compounds



Lane D: pBR322 only,

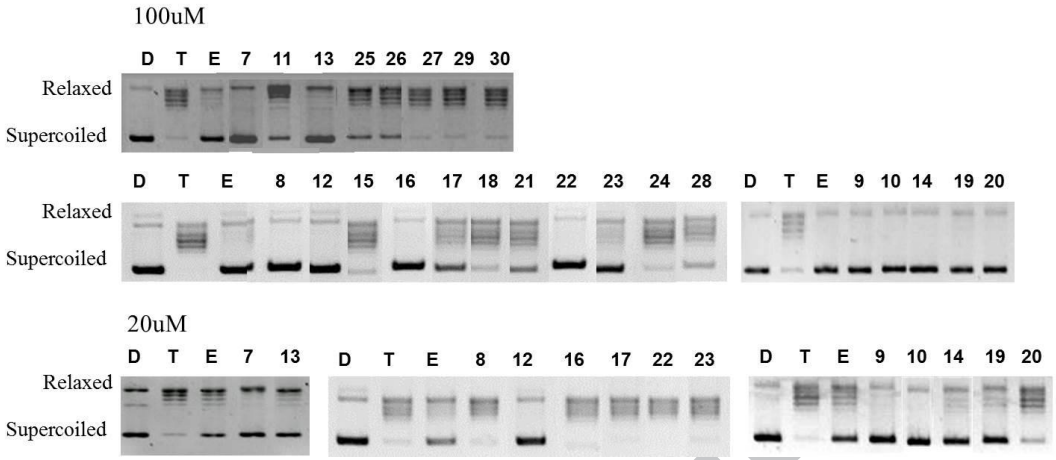
Lane T: pBR322 + Topo I,

Lane C: pBR322 + Topo I + Camptothecin

Lane 7-30 (100uM): pBR322 + Topo I + compounds **7-30**

Lane 7-27 (20uM): pBR322 + Topo I + compounds **7-27**

Figure 3. Topoisomerase I inhibitory activity of the prepared compounds **7-30**



Lane D: pBR322 only,

Lane T: pBR322 + Topo II,

Lane E: pBR322 + Topo II + Etoposide,

Lane 7-30 (100uM): pBR322 + Topo II + compounds **7-30**

Lane 7-23 (20uM): pBR322 + Topo II + compounds **7-23**

Figure 4. Topoisomerase II inhibitory activity of the prepared compounds **7-30**

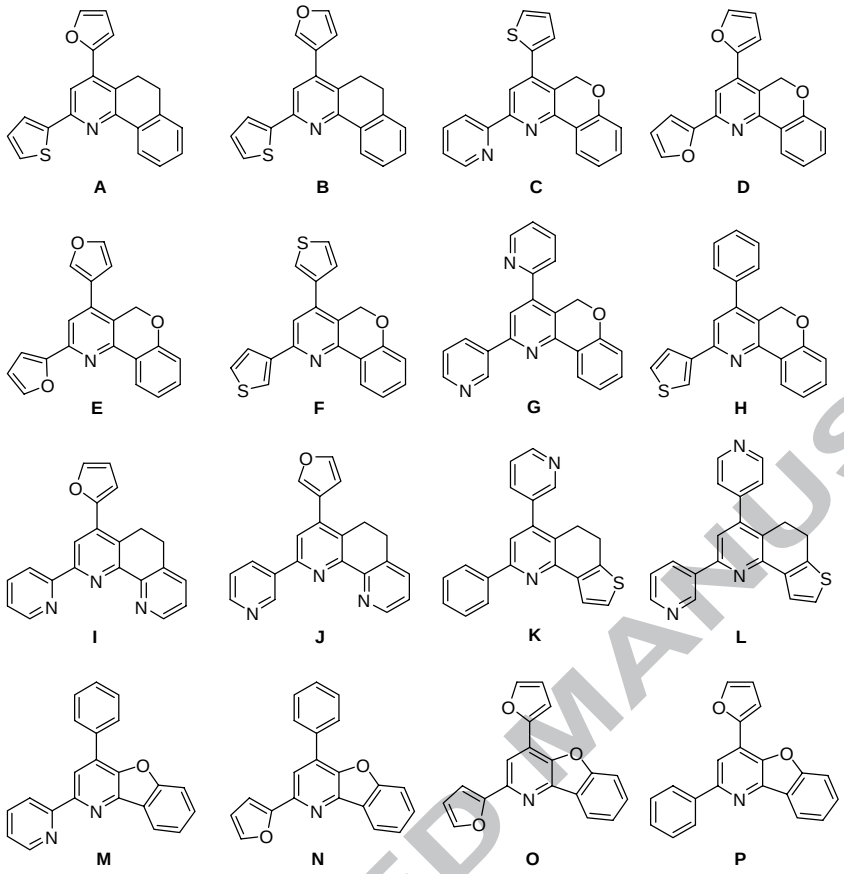


Figure 5. Structures of previously synthesized representative rigid analogs of 2,4,6-triarylsubstituted pyridines

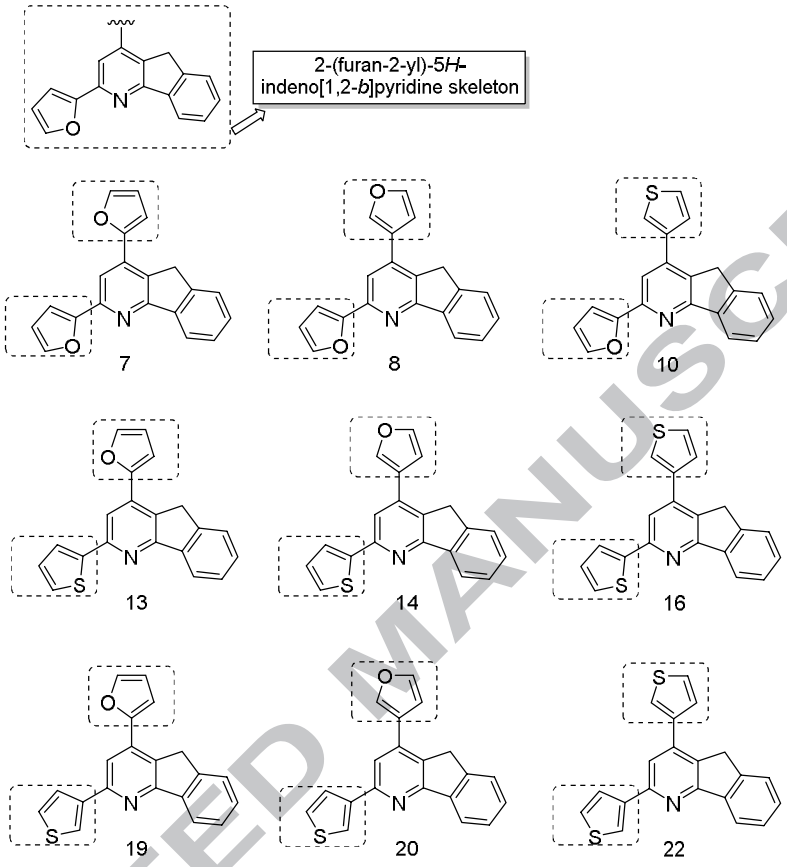
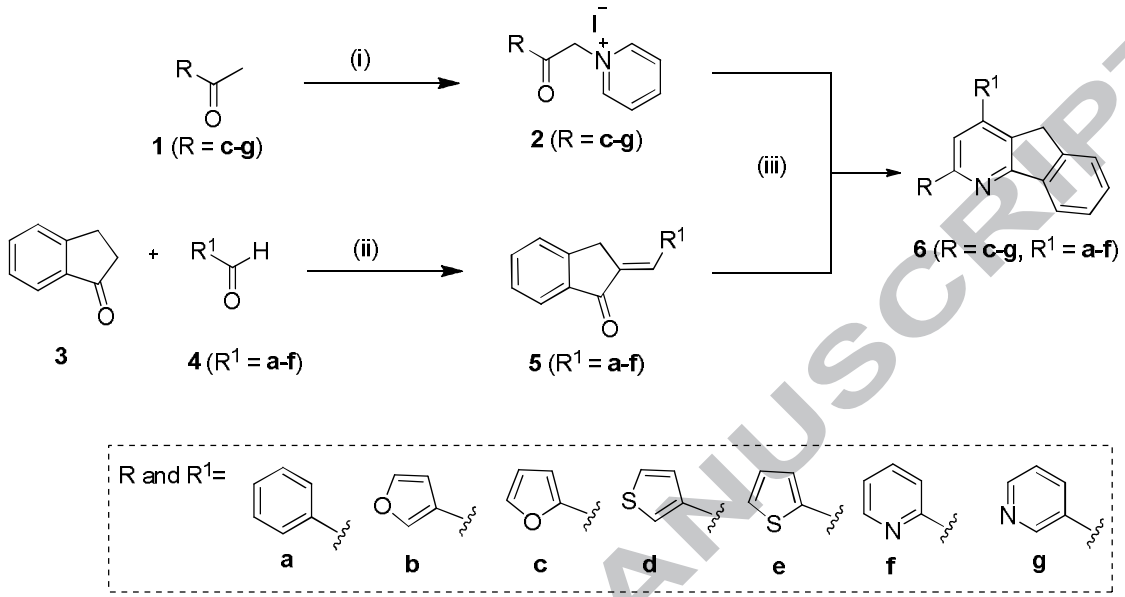


Figure 6. Structure-activity relationship study of 2,4-diaryl-5*H*-indeno[1,2-*b*]pyridines



Scheme 1. Synthesis of pyridinium iodide salts **2** (R = **c**-**g**), 1*H*-inden-1-one derivatives **5** (R^1 = **a**-**f**) and 2,4-diaryl 5*H*-indeno[1,2-*b*]pyridine derivatives **6** (R = **c**-**g,** R^1 = **a**-**f**); Reagents and conditions: i) iodine (1.0 equiv), pyridine, 3 h, 140 °C, 38.4-98.3% yield; ii) aq. NaOH (1.25 equiv), EtOH, 1-2 h, 0 °C, 90.1-95.8% yield; iii) NH_4OAc (10.0 equiv), glacial acetic acid, 16-24 h, 100 °C, 23.5-53.3% yield.

Graphical abstract