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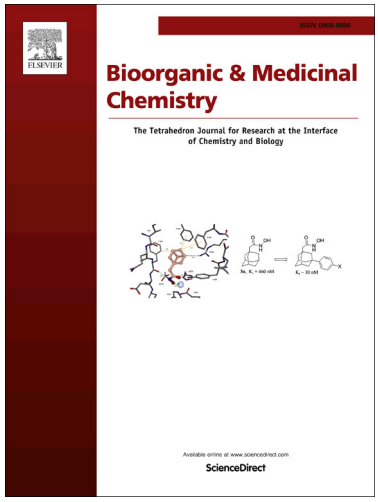


Synthesis, topoisomerase I and II inhibitory activity, cytotoxicity, and structure-activity relationship study of 2-phenyl- or hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines

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# 'REVISED'

**Synthesis, topoisomerase I and II inhibitory activity, cytotoxicity, and structure-activity relationship study of 2-phenyl- or hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines**

Tara Man Kadayat,[a,#] Chanju Song,[b,#] Somin Shin,[b] Til Bahadur Thapa Magar,[a] Ganesh Bist,[a] Aarajana Shrestha,[a] Pritam Thapa,[a] Younghwa Na,[c] 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*

[c]*College of Pharmacy, Cha University, Pochon 487-010, Republic of Korea*

*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@yu.ac.kr (E.-S.L.)

[#]Authors are equal contributors

# Synthesis, topoisomerase I and II inhibitory activity, cytotoxicity, and structure-activity relationship study of 2-phenyl- or hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines

Tara Man Kadayat,[a,#] Chanju Song,[b,#] Somin Shin,[b] Til Bahadur Thapa Magar,[a] Ganesh Bist,[a] Aarajana Shrestha,[a] Pritam Thapa,[a] Younghwa Na,[c] 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*

[c]*College of Pharmacy, Cha University, Pochon 487-010, Republic of Korea*

**Abstract**

A series of novel twenty-eight rigid 2-phenyl- or hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines were synthesized and evaluated for their topoisomerase inhibitory activity as well as their cytotoxicity against several human cancer cell lines. Generally, hydroxylated compounds (**16-18**, **22-25**, and **29-31)** containing furyl or thienyl moiety at 4-position of central pyridine exhibited strong topoisomerase I and II inhibitory activity compared to positive control, camptothecin and etoposide, respectively, in low micromolar range. Structure-activity relationship study revealed that indenopyridine compounds with hydroxyl group at 2-phenyl ring in combination with furyl or thienyl moiety at 4-position are important for topoisomerase inhibition. Compounds (**22-25**) which contain hydroxyl group at *meta* position of the 2-phenyl ring at 2-position and furanyl or thienyl substitution at 4-position of indenopyridne, showed concrete correlations between topo I and II inhibitory

activity, and cytotoxicity against evaluated human cancer cell lines.

*Keywords*: Hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines; Anticancer agents; Topoisomerase I and II inhibitor; Cytotoxicity

## Introduction

DNA topoisomerases are one of the established molecular targets for the development of anticancer agents. They have an important role in solving the DNA topological hurdles during DNA replication by their activity of transiently breaking and rejoining of one or two strands of DNA.[1-3] The human DNA topoisomerases are generally classified into topoisomerase I (topo I) and topoisomerase II (topo II).[4] In the course of several years of its discovery, topoisomerase inhibitors such as camptothecin, etoposide, and teniposide have been clinically used for treatment of cancer. However, due to their severe side effects during therapy, extensive research is going on for the development of a novel class of topoisomerase inhibitors with improved therapeutic efficacy and reduced toxicities.[5, 6]

Terpyridine, which has ability to form metal complexes, and act as DNA binding agents, has been precious in antitumor research.[7-9] In general, rigid compounds, as compared to flexible structures, have little conformational entropy, and can be more efficiently fitted into the active site of enzyme,[10] and intercalate into the enzyme-DNA complex.[11] Moreover, it has been reported that compounds containing indenopyridine moiety showed several biological activities such as anticancer and anti-inflammatory activity.[12-14] Therefore, our research group has recently synthesized 2,4-diaryl-5*H*-indeno[1,2-*b*]pyridines (Fig. 1d) as conformationally constrained rigid molecule in terpyridine skeleton, which showed moderate topoisomerase inhibitory activity and cytotoxicity.[15] As part of our continuous search for designing and

exploring newer anticancer agents, we incorporated the hydroxyl group on 2- phenyl moiety to investigate the effect on topoisomerase I and II inhibitory activities and cytotoxicity (Fig. 1f). A number of naturally occurring biologically active polyphenolic compounds such as resveratrol, curcumin, epigallocatechin gallate,[16-18] are reported to possess antioxidant, anti-inflammatory, and anticancer activity.[19-22] Therefore, they are viewed as an important pharmacophore in the field of anticancer drug discovery. It is observed that hydroxyl moiety present in the flavonoids have important role for the topoisomerase inhibitory activity.[23, 24] These promising reports on the importance of hydroxyl moiety and our previous results,[25-29] motivated us to observe the difference on biological activities by introducing hydroxyl group on 2-phenyl ring in combination with thienyl, furyl and pyridyl moiety on the 4-position of the indenopyridine skeletons.

Herein, we represent systematic design, synthesis and biological studies of twenty-eight novel rigid analogs of 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines with or without hydroxyl groups at different positions (*ortho, meta, or para*) of the 2- phenyl ring, and thienyl, furyl and pyridyl groups attached to the 4-position of central pyridine as shown in Scheme 1 and Fig. 2. The evaluation of biological activities of these compounds was carried out for topo I and II inhibitory activity, and cytotoxicity against several human cancer cell lines. Moreover, structure-activity relationship study was determined with respect to non-hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines (**8**-**14**), and 2,4-diphenyl indenopyridine (**36**), with hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines (**15**-**35**). This systematic study represents synthesis and characterization of new 2-phenyl- or hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines to develop novel anticancer agents.

(Figure 1)

## 2. Results and Discussion

### 2. 1. Synthetic chemistry

2-Phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridine (**8**-**35**) were designed and synthesized based on the previously reported methods[30, 31] as summarized in Scheme 1. At first, 1-indanone (**1**) was condensed with aryl aldehydes **2 (**$R^1$**=a-g)** to prepare intermediates **3 (**$R^1$**=a-g)** in the presence of 5% aqueous NaOH in ethanol. In the second step, four pyridinium iodide salts **4-7** were synthesized by refluxing acetophenones with iodine in pyridine. Finally, using modified Kröhnke synthesis, indanone intermediates **3 (**$R^1$**=a-g)** and pyridinium iodide salts (**4-7)** were reacted in the presence of ammonium acetate in methanol or acetic acid to give final compounds **8**-**35** in 22.3-67.5% yield.

Total twenty-eight (**8**-**35**) rigid analogs, 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridine, of 2,4,6-trisubstituted pyridines were synthesized from the reaction of seven indanone intermediates and four pyridinium iodide salts in four different series (Fig. 3). Among the twenty-eight compounds, twenty-one compounds were monohydroxylated having hydroxyl group substitution at *ortho, meta, or para* position of 2-phenyl moiety, while compound **36** has been synthesized and reported previously.[32] The substitutions of hydroxyl groups at various positions of the phenyl ring allowed us to investigate the effect of the position of hydroxyl group on biological activity. Furthermore, we were able to correlate the effect of hydroxylated and non-hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridine on biological activity. Structures of the prepared compounds with different substitution pattern together with yield, HPLC purity and melting point is illustrated in Fig. 2 and Table 1.

(Scheme 1)

(Figure 2)

(Figure 3)

(Table 1)

**2. 2. Topo I and II inhibitory activity**

Inhibition against relaxation activity of topo I and II was measured by detecting the conversion of supercoiled pBR322 DNA to its relaxed form in the presence of prepared 2-phenyl-4-aryl indenopyridines **8-35.** Camptothecin and etoposide, clinically used selective topo I and II inhibitors, respectively, were used as positive controls. Inhibitory activities were evaluated both at 100 μM and 20 μM. The reaction products were analyzed by electrophoretic mobility and developed in ethidium bromide in the presence of UV light.

Fig. 4 and Table 2 illustrates the topo I inhibitory activity of prepared compounds **8-36**. Several compounds **8, 18, 20-25, 27, 30-35** have shown significant topo I inhibitory activity at 100 μM. Interestingly, except compound **8** with significant activity of 73.0% at 100 μM (as compared to 58.0% of camptothecin), all the compounds having no hydroxyl group at 2-phenyl ring of central pyridine (**9-14**) showed very weak topo I inhibitory activity. Almost all the compounds with hydroxyl group at 2-phenyl ring exhibited significant topo I inhibitory activity at 100 μM concentration. Compounds **32** has shown the most significant topo I inhibition (90.1% as compared to 58.4% of camptothecin) at 100 μM.

As shown in Fig. 5 and Table 2, compounds **8, 16, 22-27, 29,** and **30** exhibited significant

topo II inhibition at 100 μM and 20 μM concentration which showed stronger or comparable to etoposide. Compound **22** showed the strongest topo II inhibitory activity, 78.2% and 44.5% at 100 μM and 20 μM, respectively which is stronger than that of etoposide (55.5% and 28.4%, respectively). Similarly, compound **25** displayed 77.2% and 37.0% inhibition, and compound **29** exhibited 59.9% and 44.2% inhibition at 100 μM and 20 μM, respectively. Compounds **9, 17, 18, 31, 32, 34,** and **35** showed moderate topo II inhibitory activity.

It was observed that compounds **8, 22-25**, and **30** showed both strong topo I and II inhibitory activities.

(Figure 4)

(Figure 5)

(Table 2)

**2. 3. Cytotoxicity**

All the synthesized compounds were evaluated for cytotoxicity against four different human cancer cell lines: HCT 15 (human colorectal adenocarcinoma cell line), T47D (human breast ductal carcinoma cell line), DU145 (human prostate tumor cell line), and HeLa (human cervix adenocarcinoma cell line). Adriamycin, camptothecin, and etoposide were used as positive controls. The inhibitory activities ($IC_{50}$ values) of the synthesized 2-phenyl-4-aryl indenopyridines (**8-35**) against several cancer cell lines are listed in Table 2. Most of the compounds possessing both strong topo I and II inhibitory activities showed significant cytotoxicity. Especially, compounds **8** and **22-25** showed almost equivalent cytotoxicity to positive controls in all the evaluated human cancer cell lines.

### 2.4. Structure-activity relationship study

Structure-activity relationship study was performed according to the results of topo I and II inhibitory activity of the evaluated compounds. Compounds from series 1 (**8-14**, Scheme 1), except compound **8**, all of the non-hydroxylated compounds displayed weaker topo I inhibitory activity than hydroxylated compounds at 100 μM. As shown in Table 2, hydroxylated compounds (**16-18**, **22**, **24**, **25**, **27**, and **29-31**) exhibited potent topo I and II inhibition as compared to non-hydroxylated compounds (**9-14**, and **36**). In addition, unlike previous study performed by our research group,[15] in this study, most of the hydroxylated compounds found to be potent as they showed moderate to significant topo II inhibitory activity even in low micromolar concentration with a few exceptions. Similarly, except compound **26**, all the hydroxylated compounds displayed moderate to significant topo I inhibitory activity at 100 μM concentration. From these results, it could be implied that introduction of hydroxyl moiety at phenyl ring of 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines would increase topo I and II inhibitory activities. These findings, as reported earlier,[25, 26, 28] support that introduction of hydroxyl groups on phenyl ring enhance the topo I and topo II inhibitory activity of the 2-phenyl-4-aryl indenopyridines.

Moreover, in addition to introduction of hydroxyl group of the 2-phenyl ring at 2-position of the central pyridine, substitution with furyl or thienyl group (**16-18, 22-25** and **29-31**) at 4-position of the central pyridine displayed moderate to significant topo I and II inhibitory activity. In contrast, except compound **27**, all the compounds bearing pyridyl moiety at 4-position of the central pyridine did not show any significant dual topo inhibition. This finding which was similar to previous studies,[25, 33-35] also supports the idea of importance of furyl or thienyl moiety at 4-position of central pyridine than pyridyl or phenyl moiety for significant

topo I and II inhibitory activity (Fig. 6).

Structure-activity relationship study related to topo inhibitory activities and cytotoxicity was performed. As shown in table 2, since not so many compounds showed significant cytotoxicity, it was not enough to draw out correlation between topo inhibition and cytotoxicity. However, compounds (**22** – **25**) which contain hydroxyl group at *meta* position of the 2-phenyl ring at 2-position and furanyl or thienyl substitution at 4-position of indenopyridne, showed concrete correlations between topo I and II inhibitory activity, and cytotoxicity against evaluated human cancer cell lines. The results indicated that importance of introduction of 2-phenyl ring at 2-position and furanyl or thienyl substitution at 4-position of indenopyridne, which are responsible for topo inhibitory activities and cytotoxicity. It is interesting to note that compound **8** possessing 2-phenyl ring at 2-position and 2-furanyl substitution at 4-position of indenopyridne showed strong dual topo inhibition and cytotoxicity against evaluated human cancer cell lines.

(Figure 6)

**3. Conclusion**

Total twenty-eight 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines derivatives were systematically designed and synthesized by efficient synthetic routes, and evaluated them for topo I and II inhibitory activities, and cytotoxicity against several human cancer cell lines. Evaluation of the topoisomerase inhibitory activity revealed that introduction of hydroxyl moiety on 2-phenyl ring of indenopyridines increases potency of the compounds for topo I and II inhibition. The structure-activity relationship study revealed that hydroxylated 2-

phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines with furyl or thienyl moiety at 4- position of central pyridine exhibited moderate to significant topo I and II inhibitory activity. Interestingly, compounds containing *meta*-hydroxyl moiety at 2-phenyl ring of the central pyridine in combination with furyl, or thienyl moiety at 4-position rather than pyridyl or phenyl moiety at 4-position is more important for cytotoxicity. This study may provide valuable information to medicinal chemist working in the field of cancer research.

## 4. Experimental

Compounds used as starting materials and reagents were obtained from Aldrich Chemical Co., Junsei or other chemical companies, and used 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 prepared compounds contained 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 ($\delta$) 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 injected in Waters X-Terra® 5 µM reverse-phase $C_{18}$ column (4.6 x 250 mm) with a

gradient elution of 70% to 100% of B in A for 10 min followed by 100% to 70% of B in A for 20 min at a flow rate of 0.5 mL/min at 254 nm UV detection, where mobile phase A was solution (20 mM) of ammonium formate (AF) in doubly distilled water and B was 100% acetonitrile (ACN). Purity of compounds was described as percent (%) and retention time was 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 a 8 µL injection volume on a Waters Atlantis® $T_3$ reverse-phase $C_{18}$ column (2.1 x 50 mm, 3 µm) with a gradient elution from; (A) 50% to 90% of B in A for 4 min and remained linear 90% of B in A for 1 min followed by 90% to 50% of B in A for 3 min and remained linear 50% of B in A for 7 min at flow rate of 200 µL/min; (B) 50% to 90% of B in A for 5 min followed by 90% to 50% of B in A for 5 min and remained linear 50% of B in A for 5 min at flow rate of 200 µL/min, where mobile phase A was solution (20 mM) of ammonium formate (AF) in doubly distilled water and mobile phase B was 100% ACN. MS ionization conditions were: Sheath gas flow rate: 40 arb, aux gas flow rate: 0 arb, I spray voltage: 5.3 KV, capillary temperature: 275°C, capillary voltage: 27 V, tube lens offset: 45 V.

*4. 1. General method for the preparation of **3***

Total seven 1*H*-inden-1-one derivatives **3** ($R^1$ = **a**-**g**) were synthesized by base catalyzed *Claisen-Schmidt* condensation reaction as reported previously.[15] 1-Indanone (**1**) was added in ethanol followed by the addition of equivalent amount of aryl aldehydes **2** ($R^1$ = **a**-**h**). 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 90.1-99.8% solid compound.

#### *4.1.1. Synthesis of 2-(furan-2-ylmethylene)-2,3-dihydro-1H-inden-1-one* (**3a**)

The procedure described in Section 4.1 was employed with 1-indanone (**1**, 1.32 g, 10 mmol) and 2-furaldehyde (**2a**, 0.82 mL, 10 mmol) to yield 1.89 g (9.01 mmol, 90.1%) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) $R_f$ = 0.28, mp: 124.2-125.6 $^{o}$C, HPLC: Retention time: 11.62 min, purity: 97.8%; ESI LC/MS (condition A): *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.

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

The procedure described in Section 4.1 was employed with 1-indanone (**1**, 1.32 g, 10 mmol) and 3-furaldehyde (**2b**, 0.83 mL, 10 mmol) to yield 1.98 g (9.46 mmol, 94.6%) as whitish yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:5) $R_f$ = 0.2, mp: 139.6-141.1 $^{o}$C, HPLC: Retention time: 11.10 min, purity: 97.5%; ESI LC/MS (condition A): *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.

### *4.1.3. Synthesis of 2-(thiophen-2-ylmethylene)-2,3-dihydro-1H-inden-1-one* (**3c**)

The procedure described in Section 4.1 was employed with 1-indanone (**1**, 1.32 g, 10 mmol) and 2-thiophene carboxaldehyde (**2c**, 0.93 mL, 10 mmol) to yield 2.16 g (9.56 mmol, 95.6%) as a white solid.

TLC (ethyl acetate / *n*-hexane = 1:5) $R_f$ =0.25, mp:172.2-173.1 $^oC$, HPLC: Retention time: 12.53 min, purity: 100%; ESI LC/MS (condition A): *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.

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

The procedure described in Section 4.1 was employed with 1-indanone (**1**, 1.32 g, 10 mmol) and 3-thiophene carboxaldehyde (**2d**, 0.91 mL, 10 mmol) to yield 2.1 g (9.29 mmol, 92.8%) 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 (condition A): *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.

#### *4.1.5. Synthesis of -2-(pyridin-2-ylmethylene)-2,3-dihydro-1H-inden-1-one* (3e)

The procedure described in Section 4.1 was employed with 1-Indanone (**1**, 1.32 g, 10 mmol) and 2-pyridine carboxaldehyde (**2e,** 0.95 mL, 10mmol) to yield 2.01 g (9.19 mmol, 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 (condition A): *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.

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

The procedure described in Section 4.1 was employed with 1-indanone (**1**, 1.98 g, 15 mmol) and 3-pyridine carboxaldehyde (**2f**, 1.42 mL, 15 mmol) to yield 3.31 g (11.97 mmol, 99.8 %) as a white solid.

TLC (ethyl acetate / *n*-hexane = 1:1) $R_f$ =0.23, mp: 159.3-160.7 °C, HPLC: Retention time: 11.19 min, purity: 96.4%; ESI LC/MS (condition A): *m/z* calcd for $C_{15}H_{11}NO$ $[MH]^+$ 222.09; found 222.21

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 8.93 (s, 1H, pyridine H-2), 8.63 (br, 1H, pyridine H-6), 7.98-7.91 (m, 2H, pyridine H-4, indeno H-7), 7.67-7.56 (m, 3H, =CH-, indeno H-4, H-5), 7.48-7.38 (m, 2H, pyridine H-5, indeno H-6), 4.04 (s, 2H, indeno H-3).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 193.69, 151.61, 150.04, 149.30, 137.66, 136.89, 136.77, 134.97, 131.27, 130.03, 127.89, 126.23, 124.60, 123.73, 32.33.

### *4.1.7. Synthesis of 2-(pyridin-4-ylmethylene)-2, 3-dihydro-1H-inden-1-one (***3g)**

The procedure described in Section 4.1 was employed with 1-indanone (**1**, 0.66 g, 5 mmol) and 4-pyridine carboxaldehyde (**2g**, 0.47 mL, 5 mmol) to yield 1.09 g (4.93 mmol, 99.4 %) as a white solid.

TLC (ethyl acetate / *n*-hexane = 3:1) $R_f$ =0.29, mp: 182.1-183.4 °C, HPLC: Retention time: 9.43 min, purity: 97.5%; ESI LC/MS (condition A): *m/z* calcd for $C_{15}H_{11}NO$ $[MH]^+$ 222.09; found 222.21

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 8.68 (br, 2H, pyridine, H-2, H-6), 7.80 (d, *J* = 7.6 Hz, 1H, indeno H-7), 7.76-7.65 (m, 4H, =CH-, indeno H-4, H-5, H-6), 7.51-7.48 (m, 2H, pyridine H-3, H-5), 4.17 (s, 2H, indeno H-3).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 194.05, 151.19, 151.07, 142.76, 140.41, 137.65, 136.34, 130.73, 128.77, 127.65, 125.15, 124.74, 32.63.

*4. 2. General method for the preparation of **4-7***

A mixture of equivalent amount of acetophenones and iodine in pyridine (15 equiv.) was refluxed for 3 h. After cooling the mixture to room temperature, the precipitate was filtered and washed with cold pyridine followed by drying overnight to yield 66.8-93.1% of **4-7**.

*4.3. General method for the preparation of **8-35***

Anhydrous ammonium acetate (10.0 equiv.) was mixed with glacial acetic acid or methanol followed by addition of the indanone intermediates (1.0 equiv.), **3 ($R^1$= a-h)** and pyridinium iodide salts **4-7** (1.0 equiv.). The mixture was then refluxed for 12-36 h. The reaction mixture was then extracted with ethyl acetate, washed with water and brine solution. The organic layer was dried with magnesium sulfate and filtered. The filtrate was evaporated at reduced pressure, which was then purified with silica gel column chromatography with the gradient elution of ethyl acetate/n-hexane to afford solid compounds **8-35** in 22.3-67.5% yield.

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

The same procedure described in section 4.3 was employed with **3** ($R^1$=**a**) (0.84 g, 4 mmol), anhydrous ammonium acetate (3.178 g, 40 mmol), **4** (1.3 g, 4 mmol) and glacial acetic acid (10 mL) to yield 324 mg (1.05 mmol, 26.2%) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:15) $R_f$ = 0.28, mp: 141.5-142.8 $^oC$, HPLC: Retention time: 11.49 min, purity: 96.3%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO$ $[MH]^+$ 310.12; found 310.35

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 8.25-8.16 (m, 3H, indenopyridine H-9 and 2-phenyl H-2, H-6), 7.97 (s, 1H, indenopyridine H-3), 7.64-7.44 (m, 7H, 4-furan H-5, 2-phenyl H-3, H-4, H-5

and indenopyridine H-6, H-7, H-8), 7 (d, *J* = 3.43 Hz, 1H, 4-furan H-3), 6.61 (dd, *J* = 3.4, 1.8 Hz, 1H, 4-furan H-4), 4.10 (s, 2H , indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 161.54, 156.93, 151.56, 143.95, 143.55, 140.91, 139.84, 134.24, 129.20, 128.75, 128.69 (2C), 127.37, 127.19, 127.14 (2C), 124.94, 121.93, 113.04, 112.13, 110.58, 35.52.

### *4.3.2. Synthesis of 4-(furan-3-yl)-2-phenyl-5H-indeno[1,2-b]pyridine* (9)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**b**) (0.84 g, 4 mmol), anhydrous ammonium acetate (3.17 g, 40 mmol), **4** (1.37 g, 4 mmol) and glacial acetic acid (12 mL) to yield 276 mg (0.88 mmol, 22.3%) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:15) $R_f$ = 0.29, mp: 120.1-122.4 °C, HPLC: Retention time: 9.95 min, purity: 97.5%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO$ $[MH]^+$ 310.12; found 310.33

**$^{1}$H NMR** (250 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 7.04, 1.46 Hz, 1H, indenopyridine H-9), 8.10 (dd, *J* = 7.6, 1.47 Hz, 2H, 2-phenyl H-2, H-6), 8.01 (s, 1H, indenopyridine H-3), 7.72 (s, 1H, 4-furan H-2), 7.62-7.60 (m, 2H, 4-furan H-5, indenopyridine H-7), 7.55-7.41 (m, 5H, 2-phenyl H-3, H-4, H-5, indenopyridine H-6, H-8), 6.91-6.89 (m, 1H, 4-furan H-4), 3.97 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 161.32, 157.13, 143.84, 143.6, 141.43, 141.08, 139.89, 136.83, 131.36, 128.75 (2C), 128.71 (2C), 127.28, 127.15 (3C), 124.94, 123.85, 121.30, 115.96, 109.14, 35.29.

### *4.3.3. Synthesis of 2-phenyl-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine* (10)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**c**) (0.94 g, 4 mmol), anhydrous ammonium acetate (3.17 g, 40 mmol), **4** (3.17 g, 7 mmol) and glacial acetic acid (10 mL) to yield 311 mg (0.95 mmol, 23.9 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:10) $R_f$ = 0.31, mp: 148.6-151.5 $^oC$, HPLC: Retention time: 12.59 min, purity: 95.8%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NS$ $[MH]^+$ 326.10; found 326.33

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 8.20 (dd, *J* = 6.39, 1.86 Hz, 1H, indenopyridine H-9), 8.12-8.09 (m, 2H, 2-phenyl H-2, H-6), 7.86 (s, 1H, indenopyridine H-3), 7.67 (dd, *J* = 3.69, 0.9 Hz, 1H, 4-thiophene H-5), 7.61 (dd, *J* = 7.06, 1.12 Hz, 1H, 4-thiophene H-3), 7.51-7.42 (m, 6H, 2-phenyl H-3, H-4, H-5, indenopyridine H-6, H-7, H-8), 7.22 (dd, *J* = 5.1, 3.8 Hz, 1H, 4-thiophene H-4), 4.13 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 161.71, 157.11, 143.77, 140.95, 139.75, 138.46, 130.77, 128.84, 128.81, 128.72 (3C), 128.04, 127.27, 127.16 (3C), 126.84, 124.93, 121.36, 115.92, 35.65.

### *4.3.4. Synthesis of 2-phenyl-4-(thiophen-3-yl)-5H-indeno[1,2-b]pyridine* (11)

The same procedure described in section 4.3 was employed with **3** ($R^1$=d) (1.58 g, 7 mmol), anhydrous ammonium acetate (5.56 g, 70 mmol), **4** (2.27 g, 7 mmol) and glacial acetic acid (12 mL) to yield 686 mg (2.11 mmol, 30.1%) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:15) $R_f$ = 0.29, mp: 159.5-160.3 $^oC$, HPLC: Retention time: 11.38 min, purity: 96.9%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NS$ $[MH]^+$ 326.10; found 326.34

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 8.2 (dd, *J* = 7.27, 1.38 Hz, 1H, indenopyridine H-9), 8.12-8.09 (m, 2H, 2-phenyl H-2, H-6), 7.78 (s, 1H, indenopyridine H-3), 7.5 (dd, *J* = 3.7, 1.41 Hz, 1H, 4-thiophene H-2), 7.51-7.40 (m, 8H, 2-phenyl H-3, H-4, H-5, indenopyridine H-6, H-7, H-8, 4-thiophene H-4, H-5), 4.13 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 161.44, 157.15, 143.81, 141.08, 140.30, 139.87, 139.38, 131.87, 128.71 (3C), 127.24, 127.14 (3C), 127.11, 126.44, 124.91, 124.34, 121.32, 117.09, 35.17.

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

The same procedure described in section 4.3 was employed with **3** ($R^1$=**e**) (0.88 g, 4 mmol), anhydrous ammonium acetate (3.17 g, 40 mmol), **4** (1.3 g, 4 mmol) and glacial acetic acid (10 mL) to yield 458 mg (1.42 mmol, 35.7 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:10) $R_f$ = 0.16, mp : 160.9-163.1 °C, HPLC: Retention time: 13.72 min, purity: 97.8%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2$ $[MH]^+$ 321.14; found 321.35

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 8.8 (dt, *J* = 4.7, 1.11 Hz, 1H, 4-pyridine H-6), 8.28-8.19 (m, 3H, indenopyridine H-9, 2-phenyl H-2, H-6), 8.04 (s, 1H, indenopyridine H-3), 7.87-7.84 (m, 2H, 2-phenyl H-3, H-5), 7.6-7.3 (m, 7H, 4-pyridine H-3, H-4, H-5, 2-phenyl H-4, indenopyridine H-6, H-7, H-8), 4.24 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 161.80, 157.16, 156.43, 149.84, 144.63, 143.87, 140.88, 139.84, 136.75, 133.23, 128.73, 128.67 (3C), 127.17, 127.05 (2C), 124.90, 123.20, 122.73, 121.29, 117.04, 35.47.

#### *4.3.6. Synthesis of 2-phenyl-4-(pyridin-3-yl)-5H-indeno[1,2-b]pyridine* **(13)**

The same procedure described in section 4.3 was employed with **3** ($R^1$=**f**) (0.88 g, 4 mmol), anhydrous ammonium acetate (3.17 g, 40 mmol), **4** (1.3 g, 4 mmol) and glacial acetic acid (8 mL) to yield 398 mg (1.24 mmol, 31.1 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:10) $R_f$ = 0.21, mp : 157.8-159.2 $^oC$, HPLC: Retention time: 13.46 min, purity: 96.3%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2$ $[MH]^+$ 321.14; found 321.35

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 9.02 (s, 1H, 4-pyridine H-2), 8.78 (d, *J* = 4.9 Hz, 1H, 4-pyridine H-6), 8.18-8.14 (m, 3H, indenopyridine H-9, 2-phenyl H-2, H-6), 7.98 (s, 1H, indenopyridine H-3), 7.86-7.84 (m, 2H, 2-phenyl H-3, H-5), 7.66-7.28 (m, 6H, 4-pyridine H-4, H-5, 2-phenyl H-4, indenopyridine H-6, H-7, H-8), 4.22 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 161.24, 159.44, 157.16, 150.34, 149.84, 145.63, 143.87, 141.68, 136.70, 132.22, 129.32, 128.73 (2C), 128.37, 127.57 (2C), 127.12, 124.88, 123.32, 122.65, 121.11, 117.24, 35.36.

#### *4.3.7. Synthesis of 2-phenyl-4-(pyridin-4-yl)-5H-indeno[1,2-b]pyridine* **(14)**

The same procedure described in section 4.3 was employed with **3** ($R^1$=**g**) (0.88 g, 4 mmol), anhydrous ammonium acetate (3.17 g, 40 mmol), **4** (1.3 g, 4 mmol) and glacial acetic acid (10 mL) to yield 356 mg (1.11 mmol, 27.8 %) as a light yellow solid.

TLC (ethyl acetate / *n*-hexane = 1: 10) $R_f$ = 0.28, mp : 162.5-164.2 $^oC$, HPLC: Retention time: 14.92 min, purity: 95.9%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2$ $[MH]^+$ 321.14; found 321.35

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 8.74 (br, 2H, 4-pyridine H-2, H-6), 8.16-8.06 (m, 3H, indenopyridine H-9, 2-phenyl H-2, H-6), 7.93-7.84 (m, 3H, indenopyridine H-3, 4-pyridine H-3, H-5), 7.68-7.42 (m, 6H, indenopyridine H-6, H-7, H-8, 2-pheny H-3, H-4, H-5), 4.19 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 161.34, 159.46, 157.13, 151.02, 146.23, 143.87, 141.32, 136.42, 132.22, 129.44 (2C), 128.71, 128.46, 127.21, 127.12 (2C), 124.88, 123.32, 122.35, 121.61, 117.10, 116.23, 35.63.

### *4.3.8. Synthesis of 2-(4-(Furan-2-yl)-5H-indeno [1, 2-b] pyridin-2-yl) phenol* (15)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**a**) (1.05 g, 5 mmol), anhydrous ammonium acetate (3.85 g, 50 mmol), **5** (1.71 g, 5 mmol) and methanol (20 mL) to yield 436 mg (1.32 mmol, 26.7 %) as a light solid.

TLC (ethyl acetate / *n*-hexane = 1:10) $R_f$ = 0.23, mp: 211.2-212.9 °C, HPLC: Retention time: 12.75 min, purity: 98.7%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.43

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 14.51 (s, 1H, 2-phenyl 2-OH), 8.06 (s, 1H, indenopyridine H-3), 8.01-7.98 (m, 1H, indenopyridine H-9), 7.91 (dd, *J* = 7.95, 0.95 Hz, 1H, 2-phenyl H-6), 7.65 (br, 1H, 4-furan H-5), 7.60-7.57 (m, 1H, indenopyridine H-6), 7.46-7.43 (m, 2H, indenopyridine H-8, H-7), 7.33 (td, *J* = 8.27, 1.3 Hz, 1H, 2-phenyl H-4), 7.06 (d, *J* = 8 Hz, 1H, 2-phenyl H-3), 6.98 (d, *J* = 3.47 Hz, 1H, 4-furan H-3), 6.92 (t, *J* = 7.77 Hz, 1H, 2-phenyl H-5), 6.62-6.60 (m, 1H, 4-furan H-4), 4.02 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 159.94, 158.09, 156.89, 150.98, 144.01, 143.98, 139.18,

135.26, 131.12, 129.31, 128.87, 127.41, 126.43, 125.06, 120.89, 119.44, 118.80, 118.55, 112.30, 111.45, 111.22, 35.69.

### *4.3.9. Synthesis of 2-(4-(Furan-3-yl)-5H-indeno[1, 2-b]pyridin-2-yl)phenol* (16)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**b**) (1.05 g, 5 mmol), anhydrous ammonium acetate (3.85 g, 50 mmol), **5** (1.71 g, 5 mmol) and methanol (20 mL) at 100°C for 24 h to yield 574 mg (1.76 mmol, 35.4 %) as a dark brown solid.

TLC (ethyl acetate / *n*-hexane = 1:10) $R_f$= 0.21, mp: 196.0-197.4 °C, HPLC: Retention time: 11.51 min, purity: 96.4%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.42

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 14.48 ( s, 1H, 2-phenyl 2-OH), 8.02-7.98 (m, 2H, indenopyridine H-9, H-3), 7.87 (dd, *J* = 8.02, 1.3 Hz, 1H, 2-phenyl H-6), 7.82 (s, 1H, 4-furan H-2), 7.61-7.56 (m, 2H, indenopyridine H-6, 4-furan H-5), 7.49-7.41 (m, 2H, indenopyridine H-8, H-7), 7.32 (td, *J* = 8.25, 1.57 Hz, 1H, 2-phenyl H-4), 7.08 (dd, *J* = 8.17, 0.8 Hz, 1H, 2-phenyl H-3), 6.96-6.89 (m, 2H, 2-phenyl H-5, 4-furan H-4), 3.91 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 159.94, 157.85, 156.97, 144.05, 143.63, 141.79, 139.33, 138.23, 131.16 (2C), 129.32, 127.53, 126.30, 125.09, 123.60, 120.96, 119.37, 118.80, 118.59, 114.13, 109.08, 35.44.

### *4.3.10. Synthesis of 2-(4-(Thiophen-2-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (17)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**c**) (1.13 g, 5 mmol), dry ammonium acetate (3.85 g, 50 mmol), **5** (1.71 g, 5 mmol) and methanol (20 mL) at

100°C for 24 h to yield 568 mg (1.66 mmol, 35.2 %) as a grey solid.

TLC (ethyl acetate / *n*-hexane = 1:10) $R_f$ =0.26, mp: 243.2-244.8 °C, HPLC: Retention time: 13.39 min, purity: 97.2%; ESI LC/MS (condition B): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.10; found 342.43

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 14.50 (s, 1H, 2-phenyl 2-OH), 8.01-7.98 (m, 1H, indenopyridine H-9), 7.95 (s, 1H, indenopyridine H-3), 7.85 (dd, *J* = 8.01, 1.47 Hz, 1H, 2-phenyl H-6), 7.64 (dd, *J* = 3.7, 1.07 Hz, 1H, 4-thiophene H-3), 7.59-7.56 (m, 1H, indenopyridine H-6), 7.53 (dd, *J* = 5.07, 1.05 Hz, 1H, 4-thiophene H-5) 7.46-7.43 (m, 2H, indenopyridine H-8, H-7), 7.32 (td, *J* = 8.6, 1.17 Hz, 1H, 2-phenyl H-4), 7.22 (t, *J* = 5.1 Hz, 1H, 4-thiophene H-4), 7.04 (dd, *J* = 8.25, 1.17 Hz, 1H, 2-phenyl H-3), 6.93 (td, *J* = 7.22, 1.27 Hz, 1H, 2-phenyl H-5), 4.04 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 159.88, 158.19, 156.84, 143.82, 140.27, 139.63, 139.14, 131.19, 130.50, 129.38, 128.17, 127.79, 127.47, 127.41, 126.32, 125.05, 120.97, 119.24, 118.83, 118.55, 114.02, 35.81.

### *4.3.11. Synthesis of 2-(4-(Thiophen-3-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (18)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**d**) (1.13 g, 5 mmol), anhydrous ammonium acetate (3.85 g, 50 mmol), **5** (2.05 g, 6 mmol) and methanol (20 mL) at 100°C for 24 h to yield 875 mg (2.56 mmol, 51.3 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:10) $R_f$= 0.24, mp: 231.3-232.7 °C, HPLC: Retention time: 12.75 min, purity: 97.4%; ESI LC/MS (condition C): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$

342.10; found 342.43

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 14.52 (s, 1H, 2-phenyl 2-OH), 8.0-7.97 (m, 1H, indenopyridine H-9), 7.86 (s, 1H, indenopyridine H-3), 7.82 (s, 1H, 4-thiophene H-2), 7.72-7.71 (m, 1H, 2-phenyl H-6), 7.54 (d, *J* = 5.45 Hz, 1H, 4-thiophene H-4), 7.52-7.50 (m, 1H, indenopyridine H-6), 7.49 (d, *J* = 2.6 Hz, 1H, 4-thiophene H-5), 7.44-7.41 (m, 2H, indenopyridine H-8, H-7), 7.3 (t, *J* = 8.15 Hz, 1H, 2-phenyl H-4), 7.06 (d, *J* = 8.15 Hz, 1H, 2-phenyl H-3), 6.9 (t , *J* = 7.25 Hz, 1H, 2-phenyl H-5), 3.96 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 159.93, 157.94, 156.99, 143.89, 141.55, 139.31, 138.90, 131.68, 131.12, 129.26, 127.45, 127.01, 126.68, 126.32, 125.04, 124.94, 120.95, 119.39, 118.79, 118.56, 115.29, 35.33.

### *4.3.12. Synthesis of 2-(4-(Pyridin-2-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (19)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**e**) (0.66 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **5** (1.535 g, 4.5 mmol) and methanol (12 mL) at 95-100 °C for 24 h to yield 502 mg (1.72 mmol, 58.2 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane =1:10) $R_f$= 0.13, mp: 223.3-224.6 °C, HPLC: Retention time: 11.36 min, purity: 95.6%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.45

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 13.99 (s, 1H, 2-phenyl 2-OH), 8.85 (br, 1H, 4-pyridine H-6), 8.52 (s, 1H, indenopyridine H-3), 8.31 (d, *J* = 7.75 Hz, 1H, 2-phenyl H-6), 8.24 (d, *J* = 7.82 Hz, 1H, indenopyridine H-9), 8.08-7.97 (m, 2H, 4-pyridine H-3, H-4), 7.76-7.74 (m, 1H, indenopyridine H-6), 7.58-7.53 (m, 3H, indenopyridine H-8, H-7, 4-pyridine H-5), 7.35 (t, *J*

= 8.25 Hz, 1H, 2-phenyl H-4), 7.01-6.94 (m, 2H, 2-phenyl H-3, H-5), 4.39 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 159.70, 158.69, 157.18, 155.55, 150.64, 145.92, 145.75, 139.25, 138.34, 134.13, 132.11, 130.46, 128.53, 128.32, 126.54, 125.07, 124.49, 120.98, 120.46, 119.97, 118.76, 117.03, 36.64.

### *4.3.13. Synthesis of 2-(4-(Pyridin-3-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (20)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**f**) (0.66 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **5** (1.54 g, 4.5 mmol) and methanol (15 mL) at 95-100 °C for 24 h to yield 432 mg (1.29 mmol, 42.3 %) as a light brown solid.

TLC (ethyl acetate / *n*-hexane = 1:2) $R_f$ = 0.18, mp: 222.3-224.7 °C, HPLC: Retention time: 8.31 min, purity: 97.9%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.46

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 14.43 (s, 1H, 2-phenyl 2-OH), 8.94 (br, 1H, 4-pyridine H-2), 8.76 (br, 1H, 4-pyridine H-6), 8.10-8.07 (m, 1H, indenopyridine H-9), 7.97 (d, *J* = 7.75 Hz, 1H, 4-pyridine H-4), 7.90 (br, 1H, 2-phenyl H-6), 7.81 (s, 1H, indenopyridine H-3), 7.60-7.46 (m, 4H, indenopyridine H-6, H-8, H-7, 4-pyridine H-5), 7.33 (td, *J* = 8.25, 1.42 Hz, 1H, 2-phenyl H-4), 7.09 (d, *J* = 8.25 Hz, 1H, 2-phenyl H-3), 6.93 (t, *J* = 7.87 Hz, 1H, 2-phenyl H-5), 4.0 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 160.39, 158.64, 157.93, 150.61, 149.51, 144.80, 144.47, 139.65, 136.02, 133.21, 131.96 (2C), 130.11, 128.17, 126.64, 125.73, 121.71, 119.65, 119.49, 119.16, 116.85 (2C), 34.91.

*4.3.14. Synthesis of 2-(4-(Pyridin-4-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* **(21)**

The same procedure described in section 4.3 was employed with **3** ($R^1$=**g**) (0.66 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **5** (1.54 g, 4.5 mmol) and methanol (15 mL) at 95-100°C for 24 h to yield 672 mg (1.98 mmol, 66.6 %) as a light yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:1) $R_f$ =0.25, mp: 251.2-252.3 °C, HPLC: Retention time: 8.43 min, purity: 97.7%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.45

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 13.84 (s, 1H, 2-phenyl 2-OH), 8.79 (br, 2H, 4-pyridine H-2, H-6), 8.22 (br, 2H, indenopyridine H-3, H-9), 7.99-7.91 (m, 3H, 2-phenyl H-6, 4-pyridine H-3, H-5), 7.70 (br, 1H, indenopyridine H-6), 7.56-7.53 (m, 2H, indenopyridine H-8, H-7), 7.34 (t, *J* = 7.7 Hz, 1H, 2-phenyl H-4), 7.0-6.92 (m, 2H, 2-phenyl H-3, H-5), 4.22 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 158.94, 157.23, 156.68, 150.43 (3C), 145.08, 144.82 (2C), 138.61, 133.35, 131.51, 129.87, 128.07, 127.74, 125.86, 123.39, 120.42, 119.70, 119.27, 118.02, 117.26, 34.30.

*4.3.15. Synthesis of 3-(4-(Furan-2-yl)-5H-indeno[1, 2-b]pyridin-2-yl)phenol* **(22)**

The same procedure described in section 4.3 was employed with **3** ($R^1$=**a**) (1.05 g, 5 mmol), anhydrous ammonium acetate (3.85 g, 50 mmol), **6** (1.71 g, 5 mmol) and methanol (20 mL) at 100°C for 48 h to yield 834 mg (2.58 mmol, 51.3 %) as a light yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:3) $R_f$ = 0.26, mp: 228.2-229.4 °C, HPLC: Retention time:

9.80 min, purity: 99.8%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.43

**$^1$H NMR** (250 MHz, $CDCl_3$) δ 8.23 (dd, *J* = 6.02, 2.57 Hz, 1H, indenopyridine H-9), 7.96 (s, 1H, indenopyridine H-3), 7.81 (d, *J* = 1.42 Hz, 1H, 2-phenyl H-6), 7.67-7.62 (m, 3H, 2-phenyl H-2, indenopyridine H-6, 4-furan H-5), 7.47-7.43 (m, 2H, indenopyridine H-8, H-7), 7.38 (t, *J* = 7.85 Hz, 1H, 2-phenyl H-5), 7.02 (d, *J* = 3.45 Hz, 1H, 4-furan H-3), 6.93-6.89 (m, 1H, 2-phenyl H-4), 6.65- 6.63 (m, 1H, 4-furan H-4), 4.12 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, $CDCl_3$) δ 161.58, 156.82, 156.45, 151.54, 144.01, 143.70, 141.36, 140.67, 134.49, 129.92, 129.56, 128.90, 127.32, 124.94, 121.39, 119.38, 116.10, 114.52, 113.53, 112.17, 110.76, 35.42.

### *4.3.16. Synthesis of 3-(4-(Furan-3-yl)-5H-indeno[1, 2-b]pyridin-2-yl)phenol* (23)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**b**) (1.05 g, 5 mmol), anhydrous ammonium acetate (3.85 g, 50 mmol), **6** (1.71 g, 5 mmol) and methanol (20 mL) at 100°C for 24 h to yield 724 mg (2.22 mmol, 44.6 %) as a light yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:10) $R_f$= 0.26, mp: 231.4-232.1 °C, HPLC: Retention time: 8.17 min, purity: 98.5%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.42

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.58 ( s, 1H, 2-phenyl 3-OH), 8.53 (s, 1H, 4-furan H-2), 8.07-8.06 (m, 1H, indenopyridine H-9), 7.99 (s, 1H, indenopyridine H-3), 7.93-7.91 (m, 1H, 4-furan H-5), 7.73-7.68 (m, 3H, 2-phenyl H-2, H-6, indenopyridine H-6), 7.51-7.47 (m, 2H, indenopyridine H-8, H-7), 7.38 (d, *J* = 1.17 Hz, 1H, 4-furan H-4), 7.32 (t, *J* = 7.9 Hz, 1H, 2-

phenyl H-5), 6.86 (dd, *J* = 7.87, 1.6 Hz, 1H, 2-phenyl H-4), 4.15 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 160.44, 157.93, 156.0, 144.62, 144.33, 143.11, 140.61 (2C), 137.08, 131.69, 129.83, 129.07, 127.41, 125.59, 123.42, 120.62, 117.93, 116.13, 115.70, 113.93, 109.76, 35.76.

***4.3.17. Synthesis of 3-(4-(Thiophen-2-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (24)**

The same procedure described in section 4.3 was employed with **3** ($R^1$=**c**) (0.68 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **6** (1.54 g, 4.5 mmol) and methanol (12 mL) at 100 °C for 36 h to yield 606 mg (1.77 mmol, 59.1 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:3) $R_f$ = 0.28, mp: 227.9-229.1 °C, HPLC: Retention time: 10.75 min, purity: 98.5%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.10; found 342.44

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.56 (s, 1H, 2-phenyl 3-OH), 8.09-8.05 (m, 1H, indenopyridine H-9), 8.03 (d, *J* = 3.72 Hz, 1H, 4-thiophene H-3), 8.0 (s, 1H, indenopyridine H-3), 7.87 (d, *J* = 5.0 Hz, 1H, 4-thiophene H-5), 7.74-7.65 (m, 3H, 2-phenyl H-2, H-6 indenopyridine H-6), 7.52-7.49 (m, 2H, indenopyridine H-8, H-7), 7.36-7.30 (m, 2H, 4-thiophene H-4, 2-phenyl H-5), 6.88 (dd, *J* = 7.87, 1.12 Hz, 1H, 2-phenyl H-4), 4.23 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 161.45, 158.56, 156.75, 144.90, 140.97, 140.50, 139.04, 131.43, 130.50, 129.81, 129.45, 129.29 (2C), 129.02, 128.06, 126.06, 120.04, 118.43, 116.86, 115.78, 114.46, 36.03.

### *4.3.18. Synthesis of 3-(4-(Thiophen-3-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (25)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**d**) (1.13 g, 5 mmol), anhydrous ammonium acetate (3.85 g, 50 mmol), **6** (3.41 g, 10 mmol) and glacial acetic acid (10 mL) at 100°C for 24 h to yield 1.120 g (3.28 mmol, 65.6 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:3) $R_f$ = 0.27, mp: 244.0-245.0 °C, HPLC: Retention time: 9.74 min, purity: 95.9%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.10; found 342.42

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.51 ( s, 1H, 2-phenyl 3-OH), 8.29 (s, 1H, 4-thiophene H-2), 8.09-8.05 (m, 1H, indenopyridine H-9), 7.98 (s, 1H, indenopyridine H-3), 7.87-7.85 (m, 1H, 4-thiophene H-4), 7.79-7.76 (m, 1H, 4-thiophene H-5), 7.72-7.67 (m, 3H, 2-phenyl H-2, H-6, indenopyridine H-6), 7.5-7.48 (m, 2H, indenopyridine H-8, H-7), 7.32 (t, *J* = 7.87 Hz, 1H, 2-phenyl H-5), 6.86 (dd, *J* = 7.9, 1.6 Hz, 1H, 2-phenyl H-4), 4.22 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 161.27, 158.52, 156.72, 145.06, 141.22, 141.19, 140.79, 139.35, 132.67, 130.40, 129.60, 128.38, 127.95, 127.88, 126.76, 126.15, 121.21, 118.50, 117.28, 116.72, 114.53, 35.64.

### *4.3.19. Synthesis of 3-(4-(Pyridin-2-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (26)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**e**) (0.66 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **6** (1.56 g, 4.5 mmol) and methanol (12 mL) at 95-100 °C for 24 h to yield 558 mg (1.66 mmol, 55.3 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:2) $R_f$ = 0.24, mp: 202.1-203.9 °C, HPLC: Retention time:

7.88 min, purity: 98.2%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.46

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.59 ( s, 1H, 2-phenyl 3-OH), 8.83 (br, 1H, 4-pyridine H-6), 8.24 (br, 1H, indenopyridine H-9), 8.23 (s, 1H, indenopyridine H-3), 8.11-8.08 (m, 1H, 4-pyridine H-3), 8.02 (td, *J* = 7.90, 1.72 Hz, 1H, 4-pyridine H-4), 7.72-7.68 (m, 3H, 2-phenyl H-2, H-6, indenopyridine H-6), 7.55-7.48 (m, 3H, indenopyridine H-8, H-7, 4-pyridine H-5), 7.33 (t, *J* = 7.5 Hz, 1H, 2-phenyl H-5), 6.86 (dd, *J* = 7.9, 1.27 Hz, 1H, 2-phenyl H-4), 4.34 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 161.72, 158.65, 156.79, 155.98, 150.59, 145.63, 144.45, 141.16, 140.98, 138.24, 134.01, 130.63, 129.81, 128.01, 126.31, 124.73, 124.16, 121.31, 118.49, 117.36, 116.89, 114.45, 36.27.

### *4.3.20. Synthesis of 3-(4-(Pyridin-3-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (27)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**f**) (0.66 g, 3 mmol), dry ammonium acetate (2.31 g, 30 mmol), **6** (1.54 g, 4.5 mmol) and glacial acetic acid (12 mL) at 95-100°C for 24 h to yield 336 mg (0.99 mmol, 36.3 %) as a light brown solid.

TLC (ethyl acetate / *n*-hexane = 3:1) $R_{f=}$ 0.31, mp: 252.6-253.7 °C, HPLC: Retention time: 5.74 min, purity: 95.1%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.47

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.57 (s, 1H, 2-phenyl 3-OH), 9.07 (s, 1H, 4-pyridine H-2), 8.71 (br, 1H, 4-pyridine H-6), 8.32 (d, *J* = 7.85 Hz, 1H, 4-pyridine H-4), 8.09 (br, 1H, indenopyridine H-9), 7.90 (s, 1H, indenopyridine H-3) 7.73-7.57 (m, 4H, 2-phenyl H-2, H-6,

indenopyridine H-6, 4-pyridine H-5), 7.51-7.48 (m, 2H, indenopyridine H-8, H-7), 7.31 (t, *J* = 7.85 Hz, 1H, 2-phenyl H-5), 6.85 (dd, *J* = 6.25, 1.77 Hz, 1H, 2-phenyl H-4), 4.17 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 160.54, 157.96, 156.29, 149.90, 149.21, 144.63, 143.03, 140.42, 140.38, 136.17, 133.92, 133.51, 129.92, 129.17, 127.45, 125.71, 124.06, 120.75, 117.97, 117.85, 116.27, 113.91, 34.06.

#### *4.3.21. Synthesis of 3-(4-(Pyridin-4-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (28)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**g**) (0.66 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **6** (1.54 g, 4.5 mmol) and methanol (20 mL) at 95-100°C for 24 h to yield 420 mg (1.26 mmol, 41.7%) as a light brown solid.

TLC (ethyl acetate / *n*-hexane = 1: 1) $R_f$ = 0.19, mp: 296.1-297.0 °C, HPLC: Retention time: 5.77 min, purity: 98.9%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.46

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.60 ( s, 1H, 2-phenyl 3-OH), 8.77 (d, *J* = 4.7 Hz, 2H, 4-pyridine H-2, H-6), 8.11-8.08 (m, 1H, indenopyridine H-9), 7.91-7.90 (m, 3H, indenopyridine H-3, 2-phenyl H-2, H-6), 7.70-7.67 (m, 3H, 4-pyridine H-3, H-5, indenopyridine H-6), 7.55-7.46 (m, 2H, indenopyridine H-8, H-7), 7.32 (t, *J* = 7.72 Hz, 1H, 2-phenyl H-5), 6.87 (d, *J* = 7.0 Hz, 1H, 2-phenyl H-4), 4.18 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 161.38, 158.61, 157.06, 151.04 (2C), 146.21, 145.23, 144.08, 140.93 (2C), 133.90, 130.56, 129.92, 128.11, 126.31, 123.93 (2C), 121.39, 118.58, 118.05, 116.98, 114.55, 34.69.

***4.3.22. Synthesis of 4-(4-(Furan-2-yl)-5H-indeno[1, 2-b]pyridin-2-yl)phenol* (29)**

The same procedure described in section 4.3 was employed with **3** ($R^1$=**a**) (1.05 g, 5 mmol), anhydrous ammonium acetate (3.85 g, 50 mmol), **7** (2.05 g, 6 mmol) and methanol (20 mL) at 100°C for 24 h to yield 656 mg (2.02 mmol, 40.4 %) as a grey solid.

TLC (ethyl acetate / *n*-hexane = 1:3), $R_f$ = 0.19, mp: 316.1-318.2 °C, HPLC: Retention time: 9.87 min, purity: 98.7%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.42

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 8.12 (d, *J* = 8.62 Hz, 2H, 2-phenyl H-2, H-6), 8.07-8.01 (m, 2H, indenopyridine H-9, 4-furan H-5), 7.99 (s, 1H, indenopyridine H-3), 7.71-7.68 (m, 1H, indenopyridine H-6), 7.49-7.44 (m, 2H, indenopyridine H-8, H-7), 7.4 (d, *J* = 3.37 Hz, 1H, 4-furan H-3), 6.92 (d, *J* = 8.62, Hz, 2H, 2-phenyl H-3, H-5), 6.79-6.77 (m, 1H, 4-furan H-4), 4.18 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 160.66, 158.90, 156.01, 150.95, 144.82, 144.41, 140.49, 134.14, 129.70, 128.92, 128.21 (2C), 128.14, 127.24, 125.51, 120.52, 115.68 (2C), 112.67, 111.72, 111.19, 35.18.

***4.3.23. Synthesis of 4-(4-(Furan-3-yl)-5H-indeno[1, 2-b]pyridin-2-yl)phenol* (30)**

The same procedure described in section 4.3 was employed with **3** ($R^1$=**b**) (1.05 g, 5 mmol), anhydrous ammonium acetate (3.85 g, 50 mmol), **7** (2.05 g, 6 mmol) and methanol (20 mL) at 100°C for 24 h to yield 849 mg (2.61 mmol, 52.2 %) as a brown solid.

TLC (ethyl acetate / *n*-hexane = 1: 3) $R_f$ = 0.26, mp: 319.0-320.3 °C, HPLC: Retention time:

8.17 min, purity: 95.2%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.41

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.72 ( s, 1H, 2-phenyl 4-OH), 8.51 (s, 1H, 4-furan H-2), 8.17 (d, *J* = 8.67, 2H, 2-phenyl H-2, H-6), 8.09-8.06 (m, 1H, indenopyridine H-9), 7.96 (s, 1H, indenopyridine H-3), 7.92-7.90 (m, 1H, 4-furan H-5), 7.70-7.67 (m, 1H, indenopyridine H-6), 7.5-7.48 (m, 2H, indenopyridine H-8, H-7), 7.37-7.36 (m, 1H, 4-furan H-4), 6.92 (d, *J* = 8.67 Hz, 2H, 2-pheny H-3, H-5), 4.13 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 160.21, 158.58, 156.0, 144.45, 144.21, 142.86, 140.72, 136.90, 130.48, 130.02, 128.81 (2C), 128.33, 127.24, 125.43, 123.48, 120.53, 115.54 (2C), 114.43, 109.68, 34.96.

### *4.3.24. Synthesis of 4-(4-(Thiophen-2-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (31)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**c**) (1.13 g, 5 mmol), dry ammonium acetate (3.85 g, 50 mmol), **7** (2.05 g, 6 mmol) and methanol (20 mL) at 100°C for 24 h to yield 390 mg (1.12 mmol, 37.6 %) as a grey solid.

TLC (ethyl acetate / *n*-hexane = 1:3) $R_f$= 0.27, mp: 321.3-322.7 °C, HPLC: Retention time: 9.89 min, purity: 97.7%; ESI LC/MS (condition B): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.10; found 342.43

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.78 ( s, 1H, 2-phenyl 4-OH), 8.13 (d, *J* = 8.65 Hz, 2H, 2-phenyl H-2, H-6), 8.07 (d, *J* = 3.8 Hz, 1H, 4-thiophene H-3), 8.05-8.01 (m, 1H, indenopyridine H-9), 7.98 (s, 1H, indenopyridine H-3), 7.86 (d, *J* = 5.02 Hz, 1H, 4-thiophene H-5), 7.72-7.68 (m, 1H, indenopyridine H-6), 7.50-7.47 (m, 2H, indenopyridine H-8, H-7),

7.32 (t, *J* = 3.77 Hz, 1H, 4-thiophene H-4), 6.92 (d, *J* = 8.65 Hz, 2H, 2-pheny H-3, H-5), 4.19 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 160.84, 158.76, 156.16, 144.28, 140.57, 140.12, 138.38, 129.84, 129.73, 129.10, 128.75, 128.65, 128.44 (2C), 128.28, 127.43, 125.65, 120.71, 115.70 (2C), 114.0, 35.39.

### *4.3.25. Synthesis of 4-(4-(Thiophen-3-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (32)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**d**) (0.68 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **7** (1.54 g, 4.5 mmol) and methanol (12 mL) at 100°C for 24 h to yield 455 mg (1.33 mmol, 44.4 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:3) $R_f$ = 0.28, mp: 308.0-309.6 °C, HPLC: Retention time: 9.87 min, purity: 95.2%; ESI LC/MS (condition B): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.10; found 342.40

**$^{1}$H NMR** (250 MHz, DMSO-$d_6$) δ 9.69 ( s, 1H, 2-phenyl 4-OH), 8.27 (br, 1H, 4-thiophene H-2), 8.15 (d, *J* = 8.67 Hz, 2H, 2-phenyl H-2, H-6), 8.09-8.05 (m, 1H, indenopyridine H-9), 7.95 (s, 1H, indenopyridine H-3), 7.86 (dd, *J* = 5.07, 1.25 Hz, 1H, 4-thiophene H-4), 7.79-7.75 (m, 1H, 4-thiophene H-5), 7.68-7.65 (m, 1H, indenopyridine H-6), 7.49-7.45 (m, 2H, indenopyridine H-8, H-7), 6.91 (d, *J* = 8.7 Hz, 2H, 2-pheny H-3, H-5), 4.19 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 161.10, 159.24, 156.78, 145.04, 141.36, 140.71, 139.54, 131.54, 130.73, 129.46, 129.02 (2C), 128.42, 127.89, 127.81, 126.59, 126.11, 121.22, 116.22 (2C), 116.13, 35.60.

### *4.3.26. Synthesis of 4-(4-(Pyridin-2-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (33)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**e**) (0.66 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **7** (1.54 g, 4.5 mmol) and methanol (12 mL) at 95-100 °C for 24 h to yield 681 mg (2.02 mmol, 67.5 %) as a yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:2) $R_f$= 0.31, mp: 285.9-287.1 °C, HPLC: Retention time: 8.01 min, purity: 96.0%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.45

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.76 (s, 1H, 2-phenyl 4-OH), 8.83 (br, 1H, 4-pyridine H-6), 8.25-8.07 (m, 5H, 2-phenyl H-2, H-6, indenopyridine H-3, H-9, 4-pyridine H-3), 8.01 (td, *J* = 7.87, 1.62 Hz, 1H, 4-pyridine H-4), 7.70-7.67 (m, 1H, indenopyridine H-6), 7.53-7.46 (m, 3H, indenopyridine H-8, H-7, 4-pyridine H-5), 6.92 (d, *J* = 8.57 Hz, 2H, 2-pheny H-3, H-5), 4.30 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 161.53, 159.37, 156.89, 156.23, 150.54, 145.62, 144.41, 141.15, 138.19, 132.84, 130.68, 129.64, 129.07 (2C), 127.93, 126.25, 124.63, 124.08, 121.30, 116.38 (2C), 116.24, 36.22.

### *4.3.27. Synthesis of 4-(4-(Pyridin-3-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (34)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**f**) (0.66 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **7** (1.54 g, 4.5 mmol) and methanol (20 mL) at 95-100 °C for 24 h to yield 438 mg (1.30 mmol, 43.4 %) as a light yellow solid.

TLC (ethyl acetate / *n*-hexane = 1:1) $R_f$ = 0.25, mp: 302.9-304.1 °C, HPLC: Retention time:

5.69 min, purity: 96.0%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.46

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.76 ( s, 1H, 2-phenyl 4-OH), 9.06 (s, 1H, 4-pyridine H-2), 8.70 (br, 1H, 4-pyridine H-6), 8.30 (d, *J* = 7.92, 1H, 4-pyridine H-4), 8.16 (d, *J* = 8.60 Hz, 2H, 2-phenyl H-2, H-6) 8.10-8.07 (m, 1H, indenopyridine H-9), 7.86 (s, 1H, indenopyridine H-3), 7.67-7.57 (m, 2H, indenopyridine H-6, 4-pyridine H-4), 7.49-7.47 (m, 2H, indenopyridine H-8, H-7), 6.90 (d, *J* = 8.57 Hz, 2H, 2-pheny H-3, H-5), 4.13 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 161.24, 159.45, 157.06, 150.51, 149.87, 145.28, 143.65, 141.28, 136.83, 132.98, 130.58, 129.71, 129.54, 129.21 (2C), 128.06, 126.25, 124.73, 121.44, 117.36, 116.36 (2C), 34.55.

### *4.3.28. Synthesis of 4-(4-(Pyridin-4-yl)-5H-indeno[1,2-b]pyridin-2-yl)phenol* (35)

The same procedure described in section 4.3 was employed with **3** ($R^1$=**g**) (0.44 g, 2 mmol), anhydrous ammonium acetate (1.54 g, 20 mmol), **7** (1.02 g, 3.0 mmol) and methanol (20 mL) at 95-100°C for 24 h to yield 200 mg (0.60 mmol, 29.8 %) as a light brown solid.

TLC (ethyl acetate / *n*-hexane = 1:1) $R_f$ = 0.23, mp: 359.4-360.2 °C, HPLC: Retention time: 7.53 min, purity: 96.1%; ESI LC/MS (condition B): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.13; found 337.47

**$^1$H NMR** (250 MHz, DMSO-$d_6$) δ 9.75 ( s, 1H, 2-phenyl 4-OH), 8.76 (br, 2H, 4-pyridine H-2, H-6), 8.16-8.07 (m, 3H, 2-phenyl H-2, H-6, indenopyridine H-9), 7.88-7.85 (m, 3H, 4-pyridine H-3, H-5, indenopyridine H-3), 7.69-7.47 (m, 3H, indenopyridine H-6, H-8, H-7), 6.91 (d, *J* = 8.65 Hz, 2H, 2-pheny H-3, H-5), 4.13 (s, 2H, indenopyridine H-5).

**$^{13}$C NMR** (62.5 MHz, DMSO-$d_6$) δ 161.23, 159.46, 157.17, 151.01 (2C), 146.43, 145.20, 144.03, 141.11, 132.68, 130.47, 129.77, 129.49, 129.16 (2C), 128.05, 126.27, 123.94, 121.41, 116.90 , 116.36 (2C), 34.63.

### 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,[36] with minor modifications. The test compounds were dissolved in DMSO at 20 mM as stock solutions. 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 1 unit 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$^{TM}$ (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.[37] 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**

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 like adriamycin, etoposide, and camptothecin were purchased from Sigma and used as positive controls.

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**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 compounds **8-36.**

**Figure captions**

**Figure 1.** Structures of a) Terpyridine, b) 2,4,6-trisubstituted pyridine, c) 2,4-diphenyl 5H-indeno[1,2-b]pyridines, d) 2,4-diaryl 5H-indeno[1,2-b]pyridines, e) 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines, and f) hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines

**Figure 2.** Structure of the prepared compounds.

**Figure 3.** Strategy for the design of compounds 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines

**Figure 4.** Human DNA topo II inhibitory activity of the prepared compounds **8-35.**

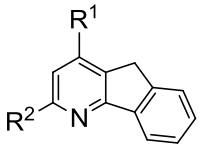
**Figure 5.** Human DNA topo II inhibitory activity of the prepared compounds **8-35.**

**Figure 6.** Hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines containing furyl or thienyl moiety at 4- position of central pyridine displaying moderate to significant topo I and II inhibitory activity.

**Scheme caption**

**Scheme 1.** General synthetic scheme of 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines. Reagents and conditions: i) aq. NaOH, EtOH, 1-3 h, room temperature, 90.1- 99.8% yield ii) **4-7** (1.0 equiv.), $NH_4OAc$ (10.0 equiv.), glacial acetic acid or methanol, 12-36 h, 95-100 $^{o}$C, 22.3-67.5% yield.

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




| Entry | $R^1$ | $R^2$ | Yield (%) | Purity (%) | mp (°C) |
|---|---|---|---|---|---|
| 8 | 2-Furyl | Phenyl | 26.2 | 96.3 | 141.5-142.8 |
| 9 | 3-Furyl | Phenyl | 22.3 | 97.5 | 120.1-122.4 |
| 10 | 2-Thienyl | Phenyl | 23.9 | 95.8 | 148.6-151.5 |
| 11 | 3-Thienyl | Phenyl | 30.1 | 96.9 | 159.5-160.3 |
| 12 | 2-Pyridyl | Phenyl | 35.7 | 97.8 | 160.9-163.1 |
| 13 | 3-Pyridyl | Phenyl | 31.1 | 96.3 | 157.8-159.2 |
| 14 | 4-Pyridyl | Phenyl | 27.8 | 95.9 | 162.5-164.2 |
| 15 | 2-Furyl | 2’-OH phenyl | 26.7 | 98.7 | 211.2-212.9 |
| 16 | 3-Furyl | 2’-OH phenyl | 35.5 | 96.4 | 196.0-197.4 |
| 17 | 2-Thienyl | 2’-OH phenyl | 35.2 | 97.2 | 243.2-244.8 |
| 18 | 3-Thienyl | 2’-OH phenyl | 51.3 | 97.4 | 231.3-232.7 |
| 19 | 2-Pyridyl | 2’-OH phenyl | 58.2 | 95.6 | 223.3-224.6 |
| 20 | 3-Pyridyl | 2’-OH phenyl | 42.3 | 97.9 | 222.3-224.7 |
| 21 | 4-Pyridyl | 2’-OH phenyl | 66.6 | 97.7 | 251.2-252.3 |
| 22 | 2-Furyl | 3’-OH phenyl | 51.3 | 99.8 | 228.2-229.4 |
| 23 | 3-Furyl | 3’-OH phenyl | 44.6 | 98.5 | 231.4-232.1 |
| 24 | 2-Thienyl | 3’-OH phenyl | 59.1 | 98.5 | 227.9-229.1 |

| | | | | | |
|---|---|---|---|---|---|
| 25 | 3-Thienyl | 3'-OH phenyl | 65.6 | 95.9 | 244.0-245.0 |
| 26 | 2-Pyridyl | 3'-OH phenyl | 55.3 | 98.2 | 202.1-203.9 |
| 27 | 3-Pyridyl | 3'-OH phenyl | 36.3 | 95.1 | 252.6-253.7 |
| 28 | 4-Pyridyl | 3'-OH phenyl | 41.7 | 98.9 | 296.1-297.0 |
| 29 | 2-Furyl | 4'-OH phenyl | 40.4 | 98.7 | 316.1-318.2 |
| 30 | 3-Furyl | 4'-OH phenyl | 52.2 | 95.2 | 319.0-320.3 |
| 31 | 2-Thienyl | 4'-OH phenyl | 37.6 | 97.7 | 321.3-322.7 |
| 32 | 3-Thienyl | 4'-OH phenyl | 44.4 | 95.2 | 308.8-309.6 |
| 33 | 2-Pyridyl | 4'-OH phenyl | 67.5 | 96.0 | 285.9-287.1 |
| 34 | 3-Pyridyl | 4'-OH phenyl | 43.4 | 96.0 | 302.9-304.1 |
| 35 | 4-Pyridyl | 4'-OH phenyl | 29.8 | 96.1 | 359.4-360.2 |

**Table 2**. Topo I and II inhibitory activities, and cytotoxicity of the compounds **8-36**

| Compounds | Topo I (% Inhibition) | | Topo II (% Inhibition) | | $IC_{50}$ ** (μM) | | | |
|---|---|---|---|---|---|---|---|---|
| | 100 μM | 20 μM | 100 μM | 20 μM | [a]HCT15 | [b] T47D | [c] DU145 | [d]HeLa |
| [e]**Adriamycin** | | | | | 1.21 ± 0.02/ 1.23 ± 0.00* | 0.41 ± 0.02/ 1.34 ± 0.03* | 1.02 ± 0.14/ 0.52 ± 0.28* | 1.01 ± 0.07/ 0.88 ± 0.08* |
| [f]**Etoposide** | | | 40.5/55.5* | 22.3/28.4* | 1.10 ± 0.02/ 2.82 ± 0.24* | 0.7 ± 0.06/ 1.84 ± 0.44* | 0.5 ± 0.03/ 0.02 ± 0.00* | 1.41 ± 0.15/ 0.18 ± 0.02* |
| [g]**Camptothecin** | 58/58.4* | 20.9/27.6* | | | 0.52 ± 0.06/ 18.87 ±0.34* | 0.31 ± 0.13/ 13.7 ± 0.81* | 0.40 ± 0.16/ 2.09 ± 0.11* | 0.41 ± 0.13/ 7.32 ± 0.15* |
| **8** | 73.0 | 8.7 | 44.5 | 25.9 | 11.2± 1.18 | 10.62± 0.31 | 5.00 ± 0.81 | 1.42 ± 0.02 |
| **9** | 0.0 | ND | 19.6 | 15.1 | 1.52±0.08 | 35.26± 1.06 | 46.52 ± 0.66 | 23.76 ± 1.09 |
| **10** | 3.7 | ND | 16.8 | ND | > 50 | 26.67± 0.53 | 9.34± 3.36 | 30.84 ± 0.41 |
| **11** | 3.4 | ND | 16.7 | ND | 31.44± 0.93 | > 50 | 1.62 ± 0.16 | 29.82 ± 1.82 |
| **12** | 3.6 | ND | 16.4 | ND | > 50 | 44.52 ± 1.74 | 13.18 ± 1.99 | 18.67 ± 0.22 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **13** | 4.1 | ND | ND | ND | > 50 | > 50 | > 50 | > 50 |
| **14** | 3.3 | ND | ND | ND | > 50 | > 50 | > 50 | > 50 |
| **15** | 58.3 | 2.4 | 11.7 | ND | > 50 | > 50 | > 50 | > 50 |
| **16** | 50.6 | 0.5 | 51.7 | 39.2 | > 50 | > 50 | > 50 | > 50 |
| **17** | 51.1 | 0.0 | 31.5 | 21.9 | > 50 | > 50 | > 50 | > 50 |
| **18** | 87.8 | 0.0 | 46.8 | 23.2 | > 50 | > 50 | 36.53 ± 0.05 | > 50 |
| **19** | 52.8 | 4.9 | 9.1 | ND | > 50 | > 50 | > 50 | > 50 |
| **20** | 70.9 | 3.8 | 3.5 | ND | > 50 | > 50 | > 50 | > 50 |
| **21** | 68.7 | 2.3 | 5.1 | ND | > 50 | > 50 | 39.58 ± 0.10 | > 50 |
| **22** | 59.7 | 5.5 | 78.2 | 44.5 | 1.59 ± 0.04 | 6.74 ± 0.02 | 4.38 ± 0.04 | 3.66±0.07 |
| **23** | 69.2 | 0.0 | 56.2 | 19.3 | > 50 | > 50 | 28.94 ± 0.16 | 0.94 ± 0.04 |
| **24** | 55.6 | 0.0 | 72.2 | 32.0 | 2.32 ± 0.09 | 4.07 ± 0.04 | 4.38 ± 0.11 | 1.47±0.07 |
| **25** | 76.9 | 0.0 | 77.2 | 37.0 | 6.73 ± 0.04 | 0.94 ± 0.00 | 2.30 ± 0.05 | 1.74±0.08 |
| **26** | 5.9 | ND | 48.5 | 19.7 | > 50 | > 50 | > 50 | 3.09±0.07 |
| **27** | 68.5 | 1.7 | 50.6 | 30.6 | > 50 | > 50 | > 50 | 4.67±0.27 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **28** | 49.8 | 1.5 | 8.4 | ND | > 50 | > 50 | > 50 | > 50 |
| **29** | 54.8 | 2.7 | 59.9 | 44.2 | > 50 | > 50 | > 50 | > 50 |
| **30** | 63.5 | 0.0 | 40.4 | 36.9 | > 50 | > 50 | > 50 | > 50 |
| **31** | 69.1 | 0.0 | 40.1 | 21.4 | > 50 | > 50 | > 50 | > 50 |
| **32** | 90.1 | 0.0 | 36.9 | 0.0 | > 50 | > 50 | > 50 | > 50 |
| **33** | 61.2 | 4.7 | 18.2 | ND | > 50 | > 50 | > 50 | > 50 |
| **34** | 59.7 | 2.4 | 28.3 | ND | > 50 | > 50 | > 50 | > 50 |
| **35** | 72.4 | 0.7 | 27.1 | ND | > 50 | > 50 | > 50 | > 50 |
| **36*** ** | 0.0 | ND | 17.8 | ND | > 50 | 26.8 ± 0.26 | 7.40 ± 0.95 | 17.3 ± 0.56 |

**ND: Not determined**

**[a]HCT15: human colorectal adenocarcinoma; [b]T47D: Human breast ductal carcinoma; [c]DU145: human prostate tumor; [d]HeLa: human cervix adenocarcinoma cell line; [e]Adriamycin: positive control for cytotoxicity; [f]Etoposide: positive control for topo II and cytotoxicity; [g]Camptothecin: positive control for topo I and cytotoxicity.**

***Control value for compounds 15-35.**

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

*****Previously reported result.[32]**

**Figure 1.** Structures of a) Terpyridine, b) 2,4,6-trisubstituted pyridine, c) 2,4-diphenyl 5*H*-indeno[1,2-b]pyridines, d) 2,4-diaryl 5*H*-indeno[1,2-b]pyridines, e) 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines, and f) hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines

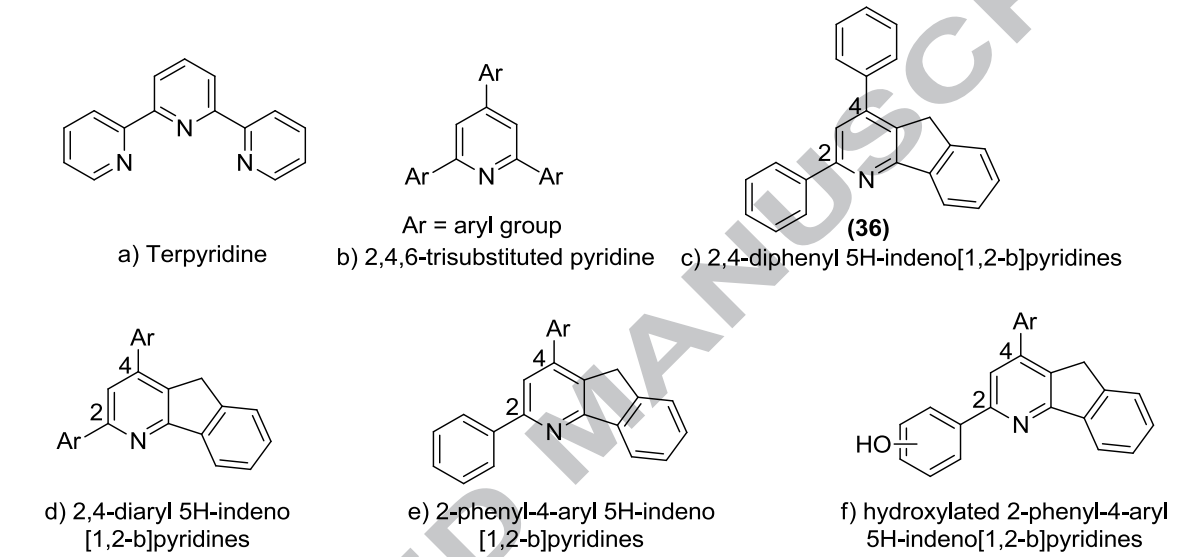


**Figure 2.** Structure of the prepared compounds

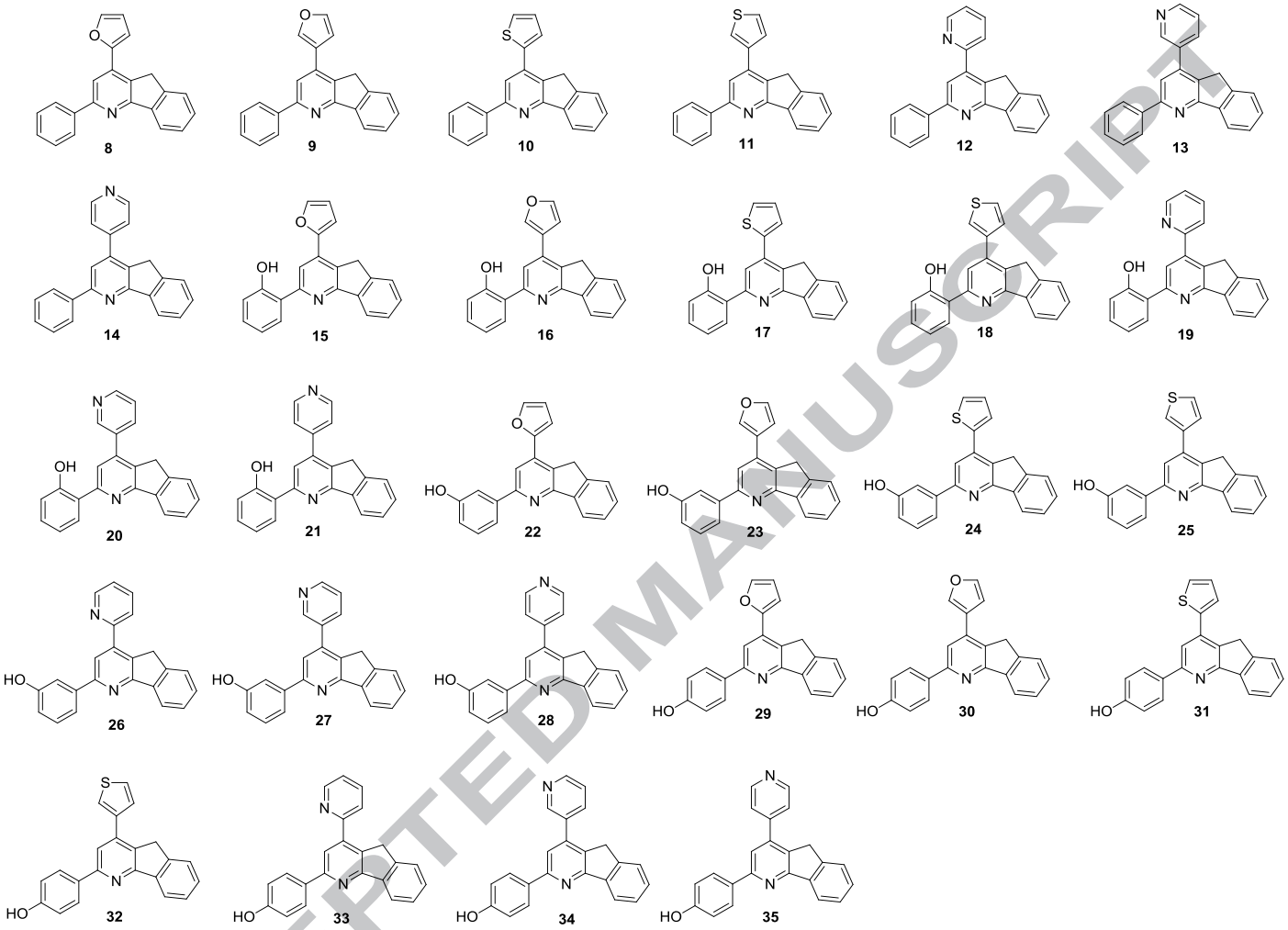


**Figure 3.** Strategy for the design of compounds 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines

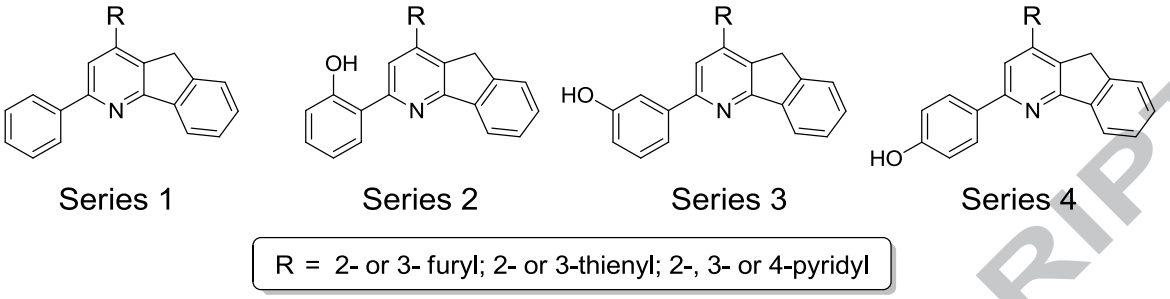
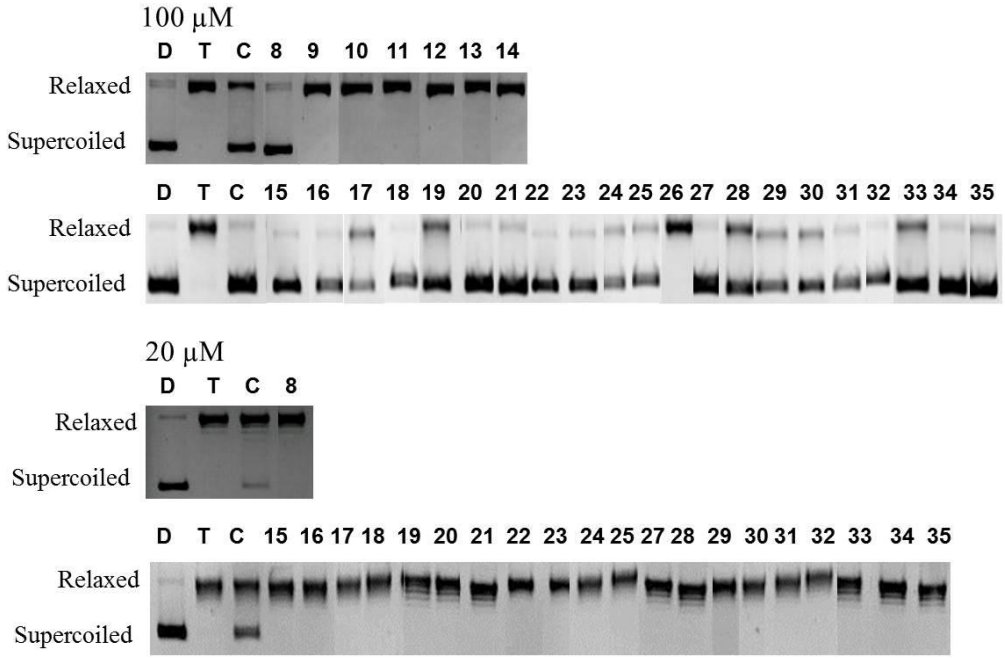


**Figure 4.** Human DNA topo I inhibitory activity of the prepared compounds **8-35**




Lane D: pBR322 only,

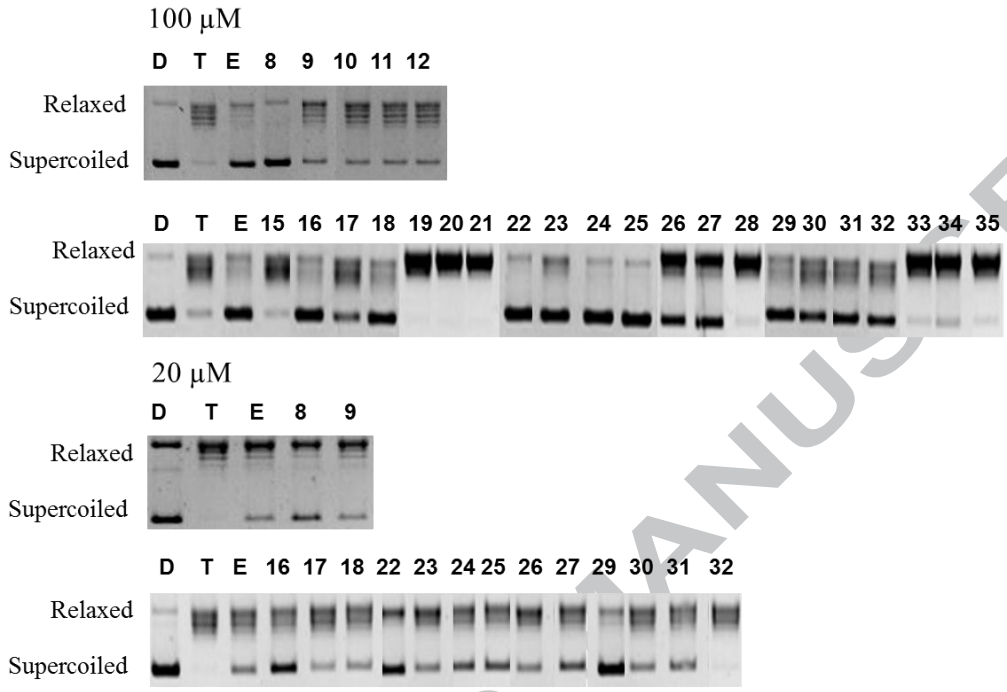
Lane T: pBR322 + Topo I,

Lane C: pBR322 + Topo I + Camptothecin

Lane 8-35 (100 μM): pBR322 + Topo I + compounds **8-35**

Lane 8-35 (20 μM): pBR322 + Topo I + compounds **8-35**

**Figure 5.** Human DNA topo II inhibitory activity of the prepared compounds **8-35**




Lane D: pBR322 only,

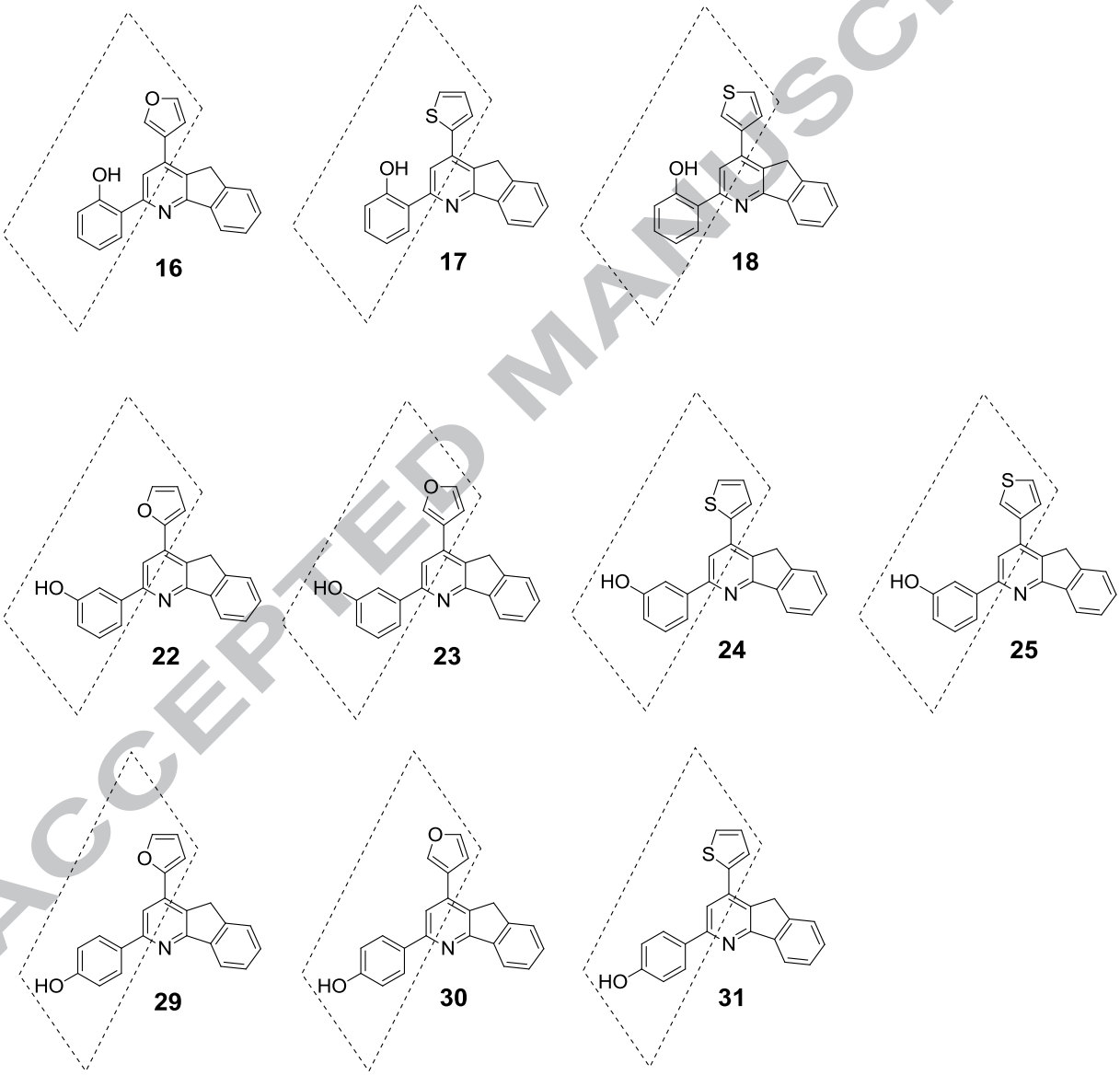
Lane T: pBR322 + Topo II,

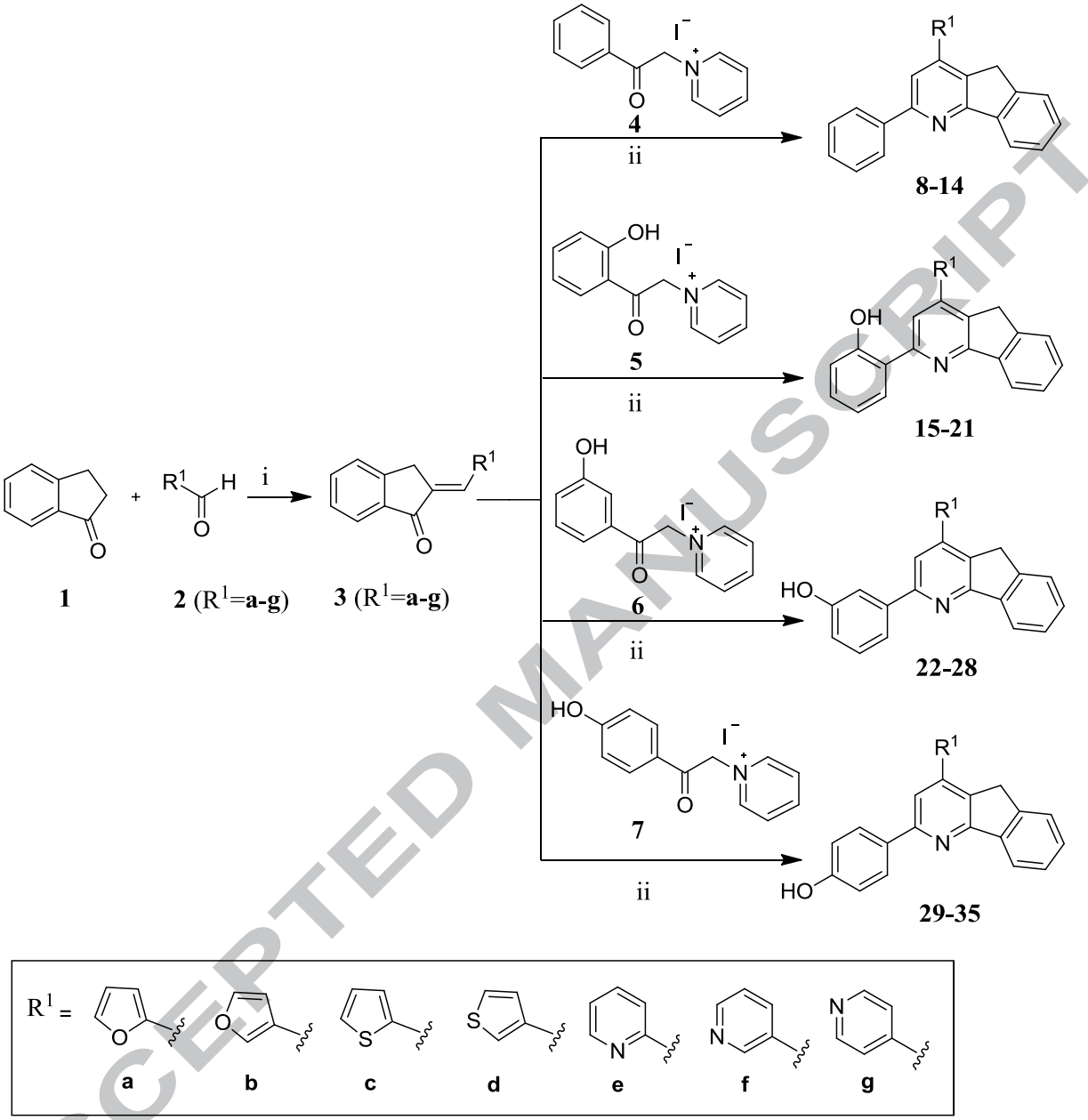
Lane E: pBR322 + Topo II + Etoposide,

Lane 8-35 (100 µM): pBR322 + Topo II + compounds **8-35**

Lane 8-32 (20 µM): pBR322 + Topo II + compounds **8-32**

**Figure 6.** Hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines containing furyl or thienyl moiety at 4-position of central pyridine displaying moderate to significant topo I and II inhibitory activity.






**Scheme 1.** General synthetic scheme of 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines. Reagents and conditions: i) aq. NaOH, EtOH, 1-3 h, room temperature, 90.1- 99.8% yield ii) **4-7** (1.0 equiv.), $NH_4OAc$ (10.0 equiv.), glacial acetic acid or methanol, 12-36 h, 95-100 $^oC$, 22.3-67.5% yield.

**Graphical abstract**

Novel Twenty-eight 2-phenyl- or hydroxylated 2-phenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines were designed, synthesized, and evaluated for topo I and II inhibitory activity, and cytotoxicity.