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Research paper

Novel 2-aryl-4-(4′-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridines as potent DNA non-intercalative topoisomerase catalytic inhibitors

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ABSTRACT

On the basis of previous reports on the importance of thienyl, furyl or phenol group substitution on 5*H*-indeno[1,2-*b*]pyridine skeleton, a new series of rigid 2-aryl-4-(4′-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridine derivatives were systematically designed and synthesized. Topoisomerase inhibitory activity and antiproliferative activity of all the synthesized compounds were determined using human colorectal (HCT15), breast (T47D), prostate (DU145) and cervix (HeLa) cancer cells. Compounds **9**, **10**, **12**, **13**, **15**, **16**, **18** and **19** with thienyl or furyl moiety at the 2-position and hydroxyl group at the *meta* or *para* positions of 4-phenyl ring displayed strong to moderate topoisomerase IIα (topo IIα) inhibitory activity and significant antiproliferative activity. The evaluation of compound **16** to determine its mechanism of action was performed with topo IIα-DNA cleavable complex, topo IIα-mediated ATPase assay, DNA unwinding and *in vitro* and *ex vivo* topo IIα relaxation assay. Compound **16** functioned as a DNA non-intercalative topo IIα catalytic inhibitor with better potency than etoposide in T47D breast cancer cells. Molecular docking study revealed that compound **16** cannot intercalate into regularly stacked base-pairs of DNA duplex but can interact or intercalate to topo IIα-bound DNA.

1. Introduction

Despite several rationale strategies for the treatment of cancer, chemotherapy remains as one of the well-established approach for cancer therapy [1]. The human DNA topoisomerases consisting of two subtypes, I (topo I) and II (topo II), have been vitally important molecular targets for the development of anticancer drugs [2,3]. Human topo II has been considered as more important molecular target than topo I for designing anticancer agents because of its ability to cleave both strands of one DNA duplex (G-segment) to make the other DNA duplex (T-segment) pass through transient break of G-segment and thus to solve the DNA topological problems. This simultaneous cleavage function of topo II on DNA double strand endows topo II to play an essential role for DNA chromosome condensation and segregation in mitosis [4,5].

Several compounds containing indenopyridine skeleton showed important biological activities such as anticancer, anti-inflammatory, antidepressant and coronary dilating activities [6–9]. There are more reports that some similar pharmacore compounds consisting of imidazo-pyridine [10,11], 2-arylquinazolinone [12], thiophenylmethylene-thiohydantoin [13], 2-phenylnaphthalenoids and 2-phenylbenzofuranoids [14] have human topo I and/or II catalytic inhibitory activity. In our previous studies, conformationally constrained 2-hydroxyphenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines (aryl = 2- or 3-furyl or 2- or 3-thienyl) (Fig. 1a) showed dual topo I and II inhibition [15]. In addition, subtle modification in the position of hydroxyl group on the phenyl ring and aryl group attached to central pyridine altered the mechanism of action of compounds, for instance, compound **Y** (Fig. 1c) functioned as a topo II poison [16] whereas compound **Z** (Fig. 1c) was defined to be a topo II catalytic inhibitor [17]. Compounds containing thienyl or furyl groups at the 4-position of 5*H*-indeno[1,2-*b*]pyridine (Fig. 1b) exhibited strong topo II inhibitory activity [18]. Thus, we put efforts continuously to design and synthesize a new series of rigid analogs of 2-aryl-4-(4′-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridines with strategy described in Fig. 3. Hydroxylated rigid compounds **8**–**28** (Fig. 4) were newly synthesized in the

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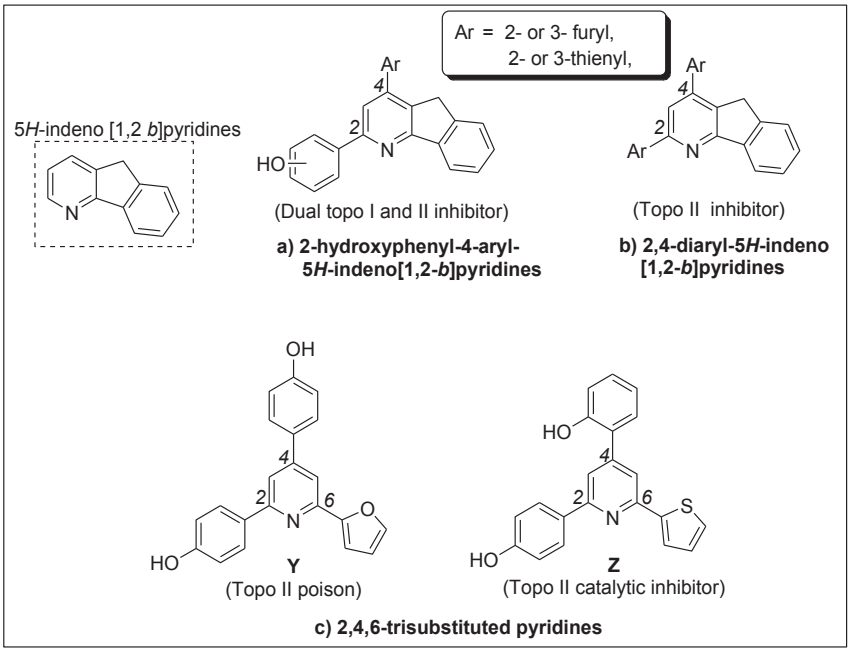


Fig. 1. Structures of previously synthesized a) 2-hydroxyphenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines, b) 2,4-diaryl-5*H*-indeno[1,2-*b*]pyridines, and c) 2,4,6-trisubstituted pyridines.

current study and their biological activities were examined. In addition, structure-activity relationship study of compounds **8**–**28** was compared with non-hydroxylated 2-aryl-4-phenyl-5*H*-indeno [1,2-*b*]pyridines (Fig. 2, compounds **1**–**7**). The most active compound **16** in *in vitro* and *ex vivo* anticancer efficacy was further carried out for molecular docking study to clarify its mechanism of action, and turned out to be a novel DNA non-intercalative topo catalytic inhibitor.

2. Results and discussion

2.1. Design and synthesis

Twenty-eight compounds (**1**–**28**) in seven different series were prepared (Fig. 3). Compounds **1**–**7** (Fig. 2) are non-hydroxylated 2-aryl-4-phenyl-5*H*-indeno[1,2-*b*]pyridines whereas each of compounds **8**–**28** has a hydroxyl group at *ortho, meta* or *para* position of the 4-phenyl ring (Fig. 4). Synthesis of compounds **1**–**3, 5, 6,** and **8**–**10** was reported earlier [12,19]. The synthetic route for compounds **4, 7**–**28** is outlined in Scheme 1. In the first step (i), 1-indanone (**I**) was condensed with aryl aldehydes **II** (R = **a-d)** in the presence of 5% NaOH in ethanol (EtOH) to obtain indanone intermediates **III** (R = **a-d)** using *Clasien-Schmidt* condensation reaction [13,20]. In the second step (ii), acetophenones **IV** (R^1 = **e-k)** were refluxed with iodine in pyridine to yield seven pyridinium iodide salts **V** (R^1 = **e-k)**. In the last step (iii), indanone intermediates **III** (R = **a-d)** were reacted with pyridinium iodide salts **V** (R^1 = **e-k)** in the presence of ammonium acetate in methanol or acetic acid using modified Kröhnke synthesis [21,22] to obtain final compounds **4, 7**–**28** in the yields of 20–83%. The yields (%), melting points (°C), purities (%), and retention time of the prepared compounds are listed in Table S1 (Supplementary Data).

On the basis of the previously reported biological results, the present investigation was undertaken in order to determine the effect of thienyl or furyl moieties at the 2-position and phenol moiety at the 4-position of 5*H*-indeno[1,2-*b*]pyridine skeleton

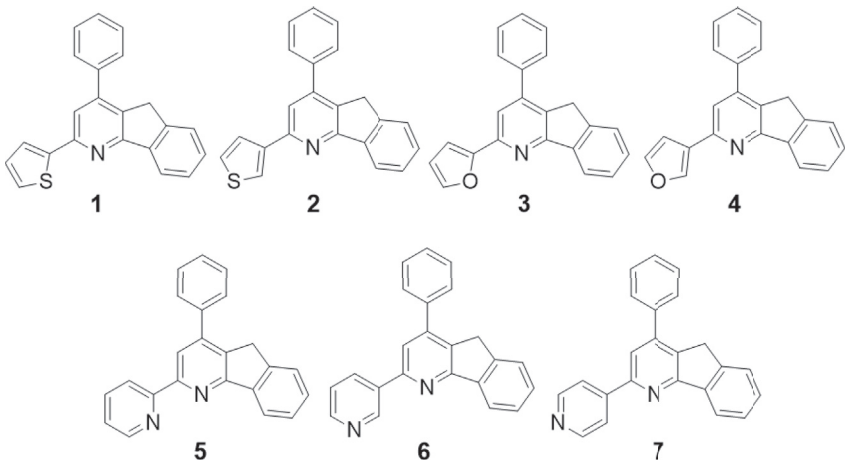


Fig. 2. Structures of non-hydroxylated 2-aryl-4-phenyl-5*H*-indeno[1,2-*b*]pyridines.

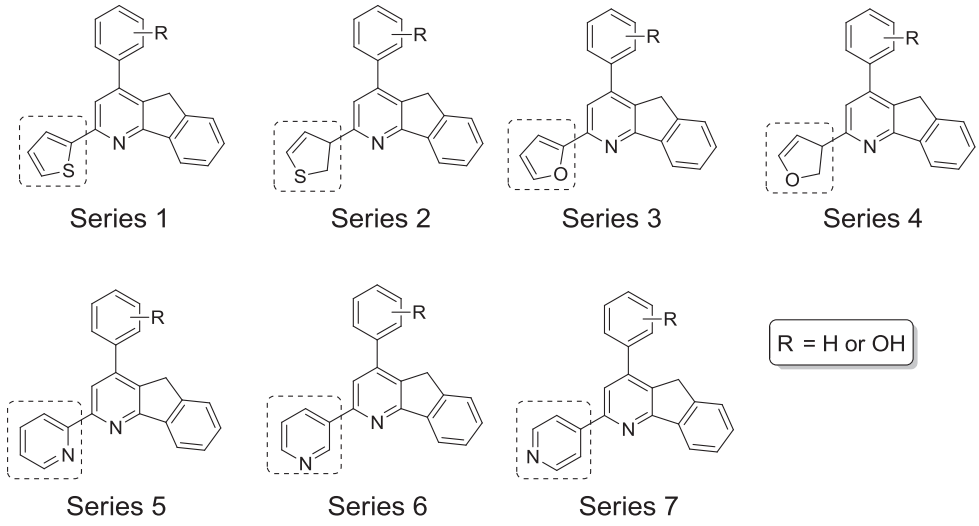


Fig. 3. Strategy for the design of compounds 2-aryl-4-phenyl or hydroxyphenyl-5*H*-indeno[1,2-*b*]pyridines.

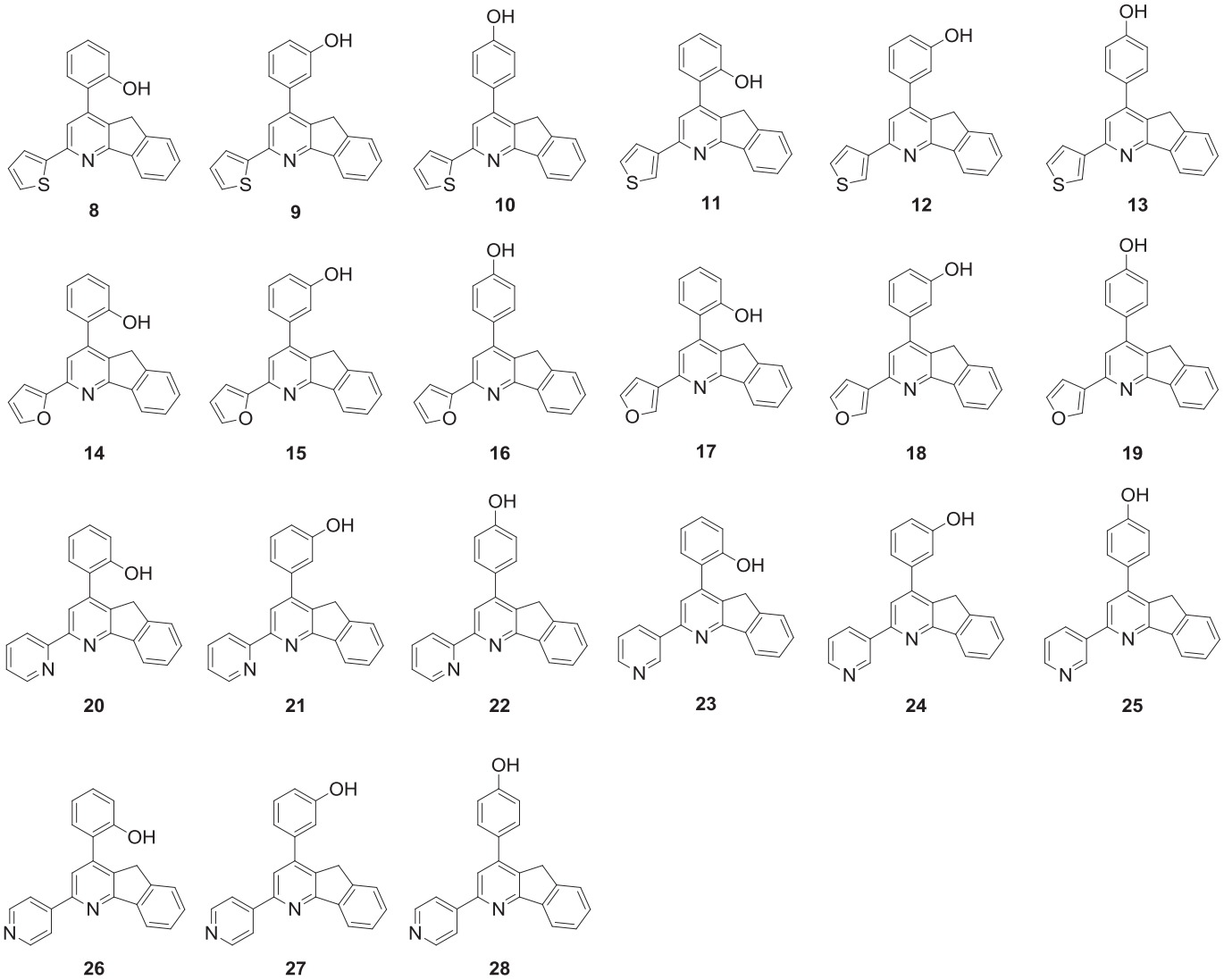
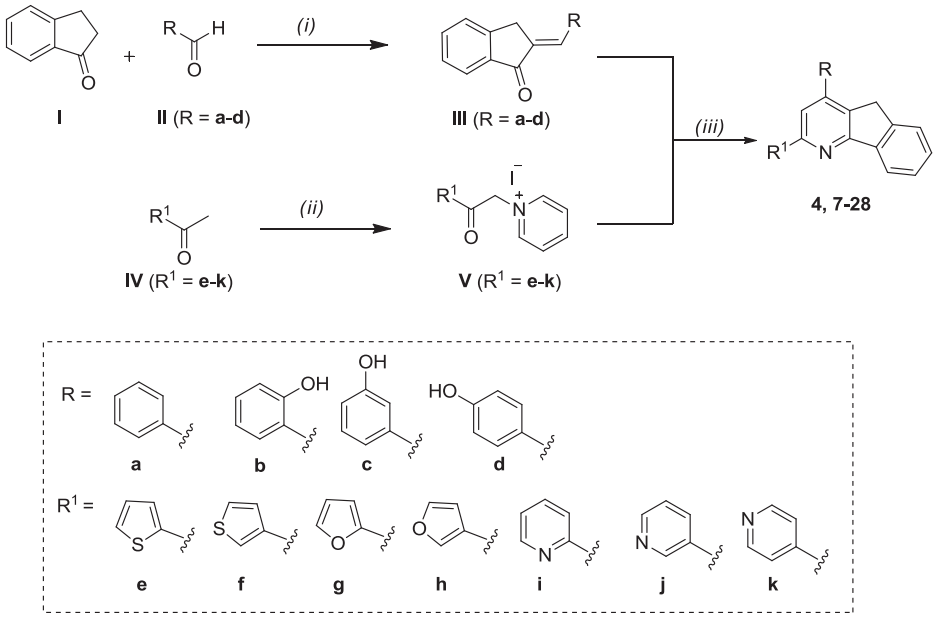


Fig. 4. Structures of prepared 2-aryl-4-(4′-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridines.



Scheme 1. General synthetic scheme of indanone intermediates **III** (R = **a**-**d**), pyridinium iodide salts **V** (R^1 = **e**-**k**), and 2-aryl-4-hydroxyphenyl-5*H*-indeno[1,2-*b*]pyridines (**4**, **7**–**28**); Reagents and conditions: i) aq. NaOH (0.5 g in 10 ml), EtOH, 1–12 h, room temperature, 62–97% yield; ii) iodine (1.0 equiv.), pyridine (15.0 equiv.), 3 h, 140 °C, 38–90% yield; iii) NH_4OAc (10.0 equiv.), glacial acetic acid or methanol, 24–36 h, 100–110 °C, 20–83% yield.

(compounds **8**–**19**). In addition, nine compounds **20**–**28** containing pyridyl moiety at the 2-position were additionally included for the evaluation of biological activity to perform the SAR study.

2.2. Biological evaluation

2.2.1. Topo I and topo IIα inhibitory activity

The effect of prepared compounds on recombinant topo I and topo IIα was evaluated by measuring the extent of those enzyme-mediated relaxed DNA remained after treatment of each compound (Figs. 5 and 6). Inhibitory activities were first evaluated at 100 μM and compound of which the percent inhibition was more than 30% at 100 μM was further tested at 20 μM. Camptothecin and etoposide were used as positive controls for topo I and II, respectively. After agarose gel electrophoresis of reaction product, the gel was stained in ethidium bromide containing solution then the extent of remained relaxed DNA was quantitated. As shown in Fig. 5 and Table 1, compounds **4** and **7** possessing no hydroxy in 4-phenyl ring had almost no inhibition on both topo I and IIα at 100 μM (less than 6.5%). Most of the hydroxylated compounds with furyl or thienyl moiety (**8**–**19**) at the 2-position, belonging to series 1–4 (Fig. 3), inhibited topo IIα much strongly than topo I except for compounds **14**, **16**, and **17**. Compounds **14** and **16** showed potent dual topo I and IIα inhibitory activity at both concentrations of 100 μM and 20 μM. Compound **14** inhibited topo I by 62.4% and 27.1% and inhibited topo IIα by 58.7% and 20.0% at 100 μM and 20 μM, respectively. While the percent inhibition of compound **16** against topo I are 69.7% and 53.5% at 100 μM and 20 μM,

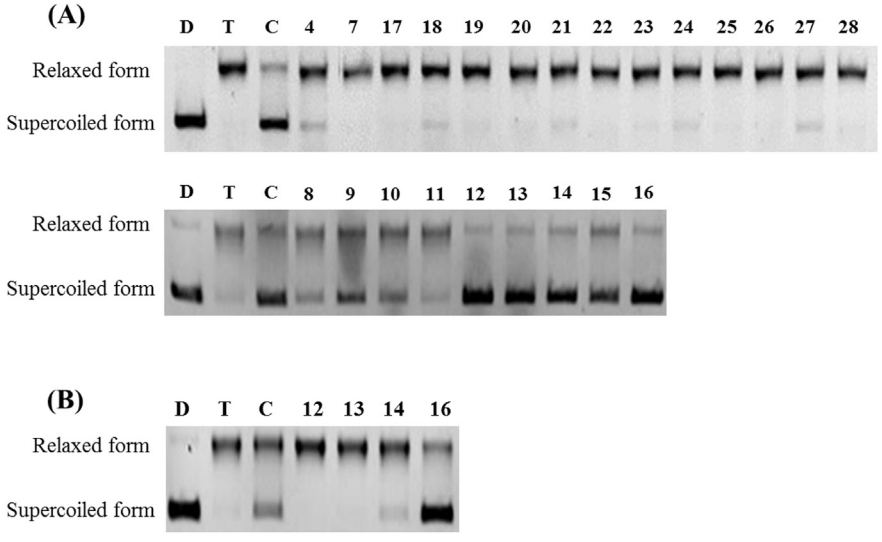


Fig. 5. Topoisomerase I inhibitory activities of prepared compounds **4, 7**–**28**. Lane D: pBR322 only, Lane T: pBR322 + Topo I, Lane C: pBR322 + Topo I + Camptothecin, and (A) Lane 4, **7**–**28**: pBR322 + Topo I + 100 μM of each of compounds **4**, **7**–**28**. (B) Lane 12–14, 16: pBR322 + Topo I + 20 μM of each of compounds **12**–**14**, **16**.

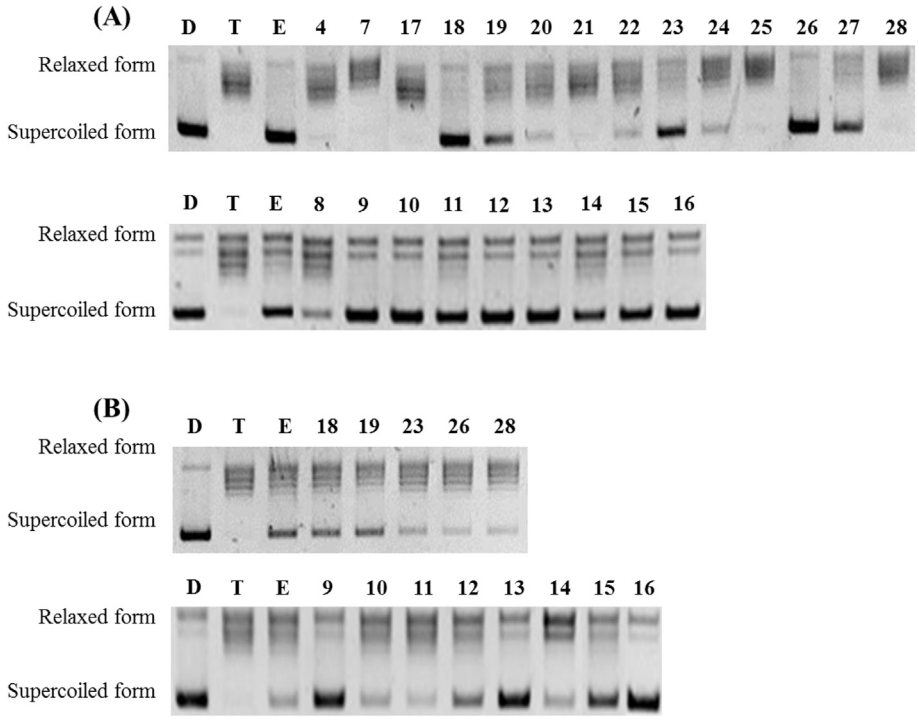


Fig. 6. Topoisomerase IIα inhibitory activities of prepared compounds **4, 7**–**28**. Lane D: pBR322 only, Lane T: pBR322 + Topo IIα, Lane E: pBR322 + Topo IIα + Etoposide, and (A) Lane 4, **7**–28: pBR322 + Topo IIα + 100 μM of each of compounds **4**, **7**–**28**. (B) Lane 9–16, 18, 19, 23, 26, 28: pBR322 + Topo IIα + 20 μM of each of compounds **9**–**16**, **18**, **19**, **23**, **26**, **28**.

Table 1
Topo I and IIα inhibitory activities, and antiproliferative activity of the prepared compounds **4**, **7**–**28**.

Compounds	Topo I (% inhibition)		Topo IIα (% inhibition)		IC_{50} (μM)[b]			
	100 μM	20 μM	100 μM	20 μM	HCT15	T47D	DU145	HeLa
Adriamycin	–	–	–	–	1.23 ± 0.001	1.34 ± 0.03	0.52 ± 0.28	0.88 ± 0.08
Etoposide	–	–	61.5/80.1[a]	20.1/32.3[a]	2.82 ± 0.24	1.84 ± 0.44	0.02 ± 0.001	0.18 ± 0.02
Camptothecin	58.3/67.5[a]	21.3	–	–	18.87 ± 0.34	13.7 ± 0.81	2.09 ± 0.11	7.32 ± 0.15
4	6.4	–[c]	3.9	–	7.05 ± 0.11	17.41 ± 0.55	1.09 ± 0.02	10.83 ± 0.74
7	0.0	–	2.3	–	16.35 ± 0.27	>50	>50	>50
8	24.3	–	22.6	–	>50	>50	>50	>50
9	22.3	–	75.6	49.1	2.81 ± 0.14	1.43 ± 0.05	4.49 ± 0.02	4.10 ± 0.10
10	15.7	–	71.9	15.1	5.19 ± 0.15	6.53 ± 0.05	4.23 ± 0.02	3.94 ± 0.04
11	12.8	–	50.2	4.9	>50	>50	>50	>50
12	33.1	0.0	75.5	22.0	2.49 ± 0.05	1.52 ± 0.07	3.12 ± 0.09	1.74 ± 0.43
13	45.5	2.5	74.8	65.9	2.45 ± 0.03	4.52 ± 0.05	3.35 ± 0.02	4.90 ± 0.12
14	62.4	27.1	58.7	20.0	>50	>50	>50	>50
15	28.9	–	77.5	36.9	1.58 ± 0.07	1.07 ± 0.03	3.75 ± 0.06	6.27 ± 0.07
16	69.7	53.5	95.1	92.2	3.12 ± 0.04	0.95 ± 0.03	3.74 ± 0.01	3.96 ± 0.19
17	0.4	–	1.8	–	>50	>50	>50	>50
18	4.5	–	82.2	29.6	1.24 ± 0.06	2.91 ± 0.06	1.51 ± 0.01	4.48 ± 0.02
19	1.4	–	61.4	28.0	1.83 ± 0.02	2.60 ± 0.01	6.01 ± 0.02	5.21 ± 0.16
20	0.5	–	15.2	–	>50	29.62 ± 2.96	>50	>50
21	2.4	–	3.5	–	>50	>50	>50	>50
22	0.0	–	14.8	–	>50	>50	>50	>50
23	0.0	–	65.5	10.5	3.52 ± 0.05	5.15 ± 0.04	7.46 ± 0.21	7.80 ± 0.12
24	1.5	–	18.5	–	1.87 ± 0.09	1.17 ± 0	2.80 ± 0.02	5.19 ± 0.01
25	0.0	–	4.0	–	>50	>50	>50	>50
26	0.0	–	75.2	0.9	2.18 ± 0.05	2.44 ± 0.01	4.70 ± 0.07	5.78 ± 0.02
27	1.3	–	49.5	–	1.94 ± 0.06	1.05 ± 0.03	4.36 ± 0.02	2.41 ± 0.16
28	0.0	–	1.14	–	>50	>50	>50	>50

HCT15: human colorectal adenocarcinoma; T47D: Human breast ductal carcinoma; DU145: human prostate tumor; HeLa: human cervix adenocarcinoma cell line; Adriamycin: positive control for antiproliferative activity; Etoposide: positive control for topo IIα and antiproliferative activity; Camptothecin: positive control for topo I and antiproliferative activity.
For compounds **8**–**10**, topo I and IIα inhibitory and antiproliferative activity results reported earlier [26].
[a] Control value for compounds **4**, **7**, and **17**–**28**.
[b] Each data represents mean ± S.D. from three different experiments performed in triplicate.
[c] Not determined.

respectively and those against topo IIα are 95.1% and 92.2%. Compound **16** showed the strongest topo IIα inhibitory activity at both concentrations of 100 μM and 20 μM among all the prepared compounds. Compound **17** showed no inhibitory activity for both enzymes. All of the compounds **20**–**28** possessing pyridyl moiety at the 2-position, belonging to series 5–7 (Fig. 3), did not inhibit topo I nor IIα except for compounds **23**, **26**, and **27**. Compounds **9, 10, 12, 13, 15, 16, 18, 19** possessing 2-thienyl or 2-furyl ring in combination with *meta*- or *para*-hydroxy-4-phenyl ring showed greater or comparable topo IIα inhibitory activity at both 100 μM and 20 μM than etoposide (61.5% at 100 μM and 20.1% at 20 μM) (Fig. 6 and Table 2). Compounds **23** (65.5%) and **26** (75.2%) possessing 2-pyridyl ring in combination with *ortho*-hydroxy-4-phenyl ring showed stronger topo IIα inhibitory activity at 100 μM than etoposide without topo I inhibition.

2.2.2. Antiproliferative activity of compounds

Antiproliferative potency of all the synthesized compounds was evaluated using HCT15 (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). The antiproliferative activities of compounds **4** and **7**–**28** against used cancer cells are listed as the IC_{50} values (μM) in Table 2. Compounds **9**, **10**, **12**, **13**, **15**, **16**, **18**, **19**, **23**, **26**, and **27** possessing significant topo IIα inhibitory activity showed similar or stronger antiproliferative activities compared to adriamycin, etoposide, and camptothecin. This result demonstrates the propensity that the effective inhibition on topo IIα mediated by compound is positively correlated with its antiproliferative activities. Only compounds **24** showed certain cytotoxicity against used cancer cells but no inhibitory activity against topo I and IIα.

2.2.3. Study on mechanism of action of compound *16*

Compound **16** exhibited strongest potency as dual topo I and IIα inhibitor with significant tumor cell growth inhibition as listed in Table 2. First of all, the cytotoxicity against a healthy mammalian cell lines of the most active compound **16** was checked using positive controls, HEK293 (derived from human healthy embryonic kidney cell grown in tissue culture) and HFL-1 (human embryonic lung cell). As shown in Table 3, compound **16** inhibited HEK293 cell with less potency than etoposide and camptothecin. While the cytotoxic efficacy against HFL-1 cell of compound **16** was much stronger than that of etoposide, and weaker than those of adriamycin and camptothecin. Especially at low concentration, compound **16** has the selectivity for topo IIα over topo I. Since topo IIα is overexpressed in highly proliferative cancer cells and the expression level of topo IIα is in accordance with cell cycle of cancer cells; it is highest in the late S and G2/M phase of cell cycle [23,24]. In addition, topo IIα enables to transiently cleave both strands of a DNA duplex resulted in decatenation which is a requirement process for progression toward mitosis [25]. There are two types of topo IIα inhibitors; one is to stabilize transiently formed topo IIα-DNA complex leading to blockage of the DNA double strand breaks from being religated. Then undesired truncated DNAs are accumulated, which causes a severe genetic toxicity. By this reason, the topo IIα inhibitors stabilizing the cleavable DNA-topo IIα complex named topo IIα poison. The other, called a topo IIα catalytic inhibitor, is to inhibit any another step of topo IIα catalytic cycle besides blocking the religation step. Thus, the current trend is more focusing on developing topo IIα catalytic inhibitor as an anticancer agent [17,26–29]. Therefore, the mechanism of action of compound **16** was inspected whether to act as a topo IIα poison or topo IIα catalytic inhibitor. First, topo IIα-DNA cleavable complex assay was performed. As shown in Fig. 7A etoposide, a well-known topo IIα poison, induced the linear truncated DNA but compound **16** did not generate linear form even at much higher concentration (500 μM). It is evident that the compound **16** functioned as a topo IIα catalytic inhibitor not a topo IIα poison. Second, DNA intercalating character of compound **16** was tested with DNA unwinding assay. Compound **16** did not unwind DNA in the presence of excess amount of topo I. While amsacrine, an eminent DNA intercalating topo II poison, inhibited topo I-mediated DNA unwinding in dose dependent manner, which is consistent with the previous report (Fig. 7B) [28,29]. The high concentration treatment of compound **16** (500 μM) in the last lane of Fig. 7A showed the supercoiled DNA unlike low concentration treatment (10 or 50 μM). It might be explained that compound **16** blocked enzymatic activity of topo IIα at high concentration by interfering DNA binding to topo IIα through either induction of tertiary conformational change in enzyme or DNA leading to blockage of the relaxation process before starting of catalytic cycle of topo IIα. Compound **16** can be further analogized as a topo I catalytic inhibitor not a topo I poison based on its functions such as not inducing linear DNA and no intercalating. Third, since compound **16** catalytically inhibited topo IIα

Table 3
The cytotoxicity of compound **16** against normal mammalian cells.

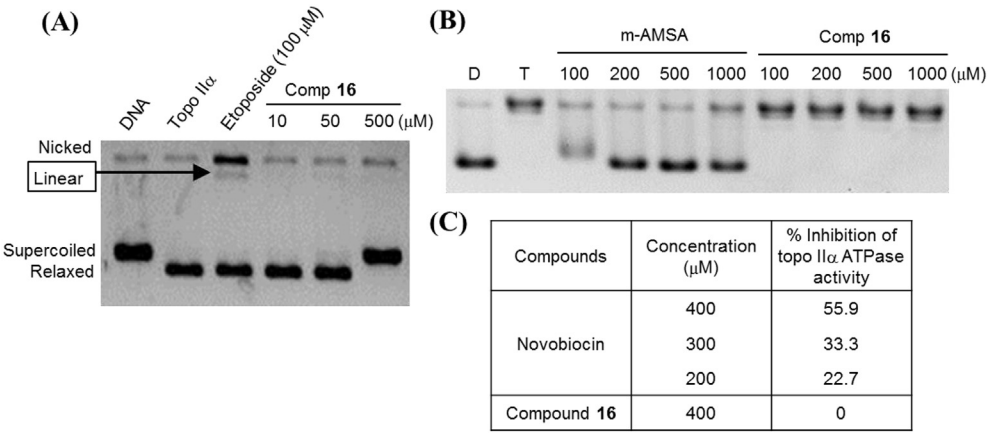
Compounds	IC50 (μM)	
	HEK293	HFL-1
Adriamycin	4.96 ± 0.001	2.31 ± 0.01
Etoposide	1.74 ± 0.05	87.63 ± 9.29
Camptothecin	0.83 ± 0.02	1.06 ± 0.001
16	3.47 ± 0.06	7.99 ± 0.14

HEK293: Derived from human healthy embryonic kidney cell grown in tissue culture; HFL-1: Human normal embryonic lung cell.

Table 2
Topo I and IIα inhibitory activities, and antiproliferative activity of previously synthesized compounds **1**–**3, 5, 6** [25].

Compounds	Topo I (% inhibition)		Topo IIα (% inhibition)		IC_{50} (μM)[c]		
	100 μM	20 μM	100 μM	20 μM	HCT15	DU145	HeLa
Adriamycin					1.08 ± 0.10[a]/1.21 ± 0.02[b]	0.95 ± 1.05[a]/1.0 ± 0.14[b]	1.20 ± 0.20[a]/1.00 ± 0.07[b]
Etoposide	–	–	87.8[a]/32.4[b]	46.7[a]/20.2[b]	2.6 ± 1.681[a]/1.10 ± 0.02[b]	13.1 ± 1.71[a]/0.50 ± 0.03[b]	1.26 ± 0.03[a]/1.40 ± 0.15[b]
Camptothecin	62.9[a]/45.2[b]	19.6[a]/25.9[b]	–	–	0.31 ± 0.09[a]/0.50 ± 0.06[b]	0.11 ± 0.05[a]/0.42 ± 0.16[b]	1.30 ± 0.14[a]/0.40 ± 0.13[b]
1	56.0	0.0	5.8	–	>50	>50	>50
2	72.5	0.0	0.5	–	>50	>50	>50
3	18.7	-[d]	41.3	5.7	>50	7.74 ± 0.85	16.60 ± 1.22
5	12.3	–	0.0	–	–	–	–
6	ND	–	ND	–	–	–	–

[a] Control value for previously reported compounds **1**–**2**.
[b] Control value for previously reported compounds **3, 5, 6**.
[c] Each data represents mean ± S.D. from three different experiments performed in triplicate.
[d] Not determined.



Compounds	Concentration (μM)	% Inhibition of topo IIα ATPase activity
Novobiocin	400	55.9
	300	33.3
	200	22.7
Compound **16**	400	0

Fig. 7. Mechanism of action of compound **16** was determined by systematic assays including (A) DNA-topo IIα cleavable complex assay, (B) topo I-mediated DNA unwinding assay and (C) topo IIα ATPase assay. Compound **16** functions as a DNA non-intercalative topo catalytic inhibitor.

without intercalation into DNA, it was necessitated to evaluate whether to inhibit ATPase activity of topo IIα. As detected in Fig. 7C, compound **16** did not inhibit ATPase activity and ATP hydrolysis of topo IIα unlike novobiocin which is reported to hinder topo II catalytic activity through inhibiting ATPase activity of topo II [30]. Taken together, compound **16** may work on the other catalytic steps of topo IIα reaction cycle besides its religation step as a topo IIα catalytic inhibitor.

2.2.4. Effect of compound *16* on endogenous topoisomerase

The effect of compound **16** was finally evaluated against endogenous topo in T47D human breast tumor cells. After T47D cells were treated with 20 and 50 μM of compound **16** followed by nuclear extraction, the each of prepared nuclear extract was reacted with the supercoiled pBR322 DNA. Compound **16** attenuated the intracellular topo activity by 34.3% and 97.0% inhibition at 20 and 50 μM treatments, respectively. While etoposide almost did not inhibit the intracellular topo at 20 μM and inhibited it by 61.1% at 50 μM which are much lower than compound **16** (Fig. 8). This result is consistent what observed in cell free relaxation assay system compound **16** inhibited both recombinant topo I and IIα with much stronger activity than camptothecin and etoposide.

2.2.5. Structure-activity relationship (SAR) studies

Most of all the synthesized compounds inhibited recombinant topo IIα with stronger intensity than topo I, thus SAR of compounds **1**–**28** was studied based on the extent of inhibition on both topo IIα and cell proliferation. The non-hydroxylated compounds (**1**–**7**) showed weak topo IIα inhibitory activity and less cytotoxic than the hydroxylated compounds as shown in Tables 1 and 2. Among the hydroxylated compounds, 2- or 3-thienyl compounds (**9**, **10**, **12**, and **13**) and 2- or 3-furyl compounds (**15**, **16**, **18**, and **19**) have a preference with *meta*- or *para*-hydroxy-4-phenyl ring for strong topo IIα inhibition and noticeable cytotoxicity over *ortho*-hydroxy-4-phenyl ring (**8**, **11**, **14** and **17**). However, 2-, 3- or 4-pyridyl compounds **20**–**28** showed the different trend from compounds **8**–**19**. 2-Pyridyl compounds **20**–**22** are almost inactive. 3- or 4-Pyridyl compounds with *ortho*-hydroxy-4-phenyl ring (**23** and **26**) showed better potency than compounds **24**, **25**, **27**, and **28** containing *meta*- or *para*-hydroxy-4-phenyl ring. The preference for positioning of hydroxy group in 4-phenyl rings of thienyl (**9**, **10**, **12**, and **13**) and furyl compounds (**15**, **16**, **18**, and **19**) differs from that of pyridyl compounds (**23** and **26**). However, introduction of hydroxy to 4-phenyl ring of 5*H*-indeno[1,2-*b*]pyridines enhanced topo IIα inhibitory activity and cytotoxicity which is similar tendency to previous results [31].

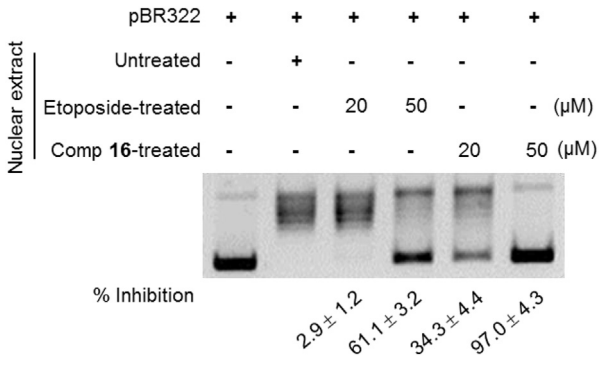


Fig. 8. DNA non-intercalative topo catalytic inhibition of compound **16** attenuated endogenouse topo-mediated DNA relaxation with better potency than etoposide.

2.3. Molecular docking study

The biological studies clarified that compound **16** potently inhibited both topo I and topo IIα (Figs. 5 and 6 and Table 2) but it did not block DNA from re-ligation (Fig. 7A). In addition, the compound **16** neither intercalated into DNA (Fig. 7B) nor inhibited topo II ATP hydrolysis (Fig. 7C). Therefore, docking studies were carried out to inspect the mechanism of action of compound **16** with topo I and topo IIα. Fig. 9 shows the compound **16** binding site of topo I (Fig. 9A) and topo IIα (Fig. 9B). First, analysis of interaction between topo I and compound **16** showed that it interacts with single-strand cleavage site. Especially, the oxygen of furyl group of compound **16** has hydrogen bonding interaction with Arg364. This is an important residue where it also has interactions with other inhibitors such as camptothecin, indolocarbazole, and indenoisoquinoline [32]. Compound **16** also bound to the topo IIα DNA cleavage site where etoposide is positioned. It had interaction with Lys456 and Asp479 with the hydroxyl group of the phenyl ring. The hydrogen bonding interaction of Asp479 is also observed in the hydroxyl group of E ring of etoposide [33]. The 5*H*-indeno [1,2-*b*]pyridine moiety of compound **16** overlaps with the polycyclic aglycone core of etoposide. The total Surflex-Dock scores of compound **16** for topo I and topo IIα were 8.9012 and 6.7948, respectively while that of camptothecin was 10.3861 and that of etoposide 11.7508. The much higher Surflex-Dock scores meant that camptothecin or etoposide interacted very strongly with the nicked DNA, leading to its

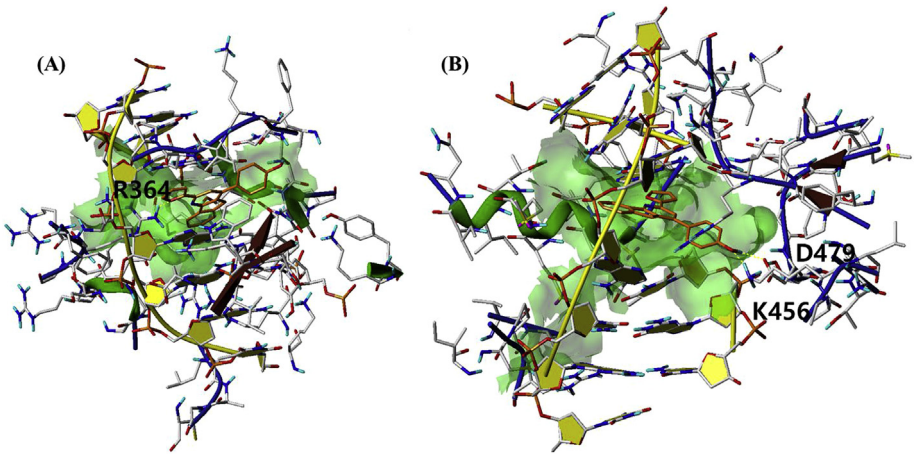


Fig. 9. Binding mode of compound **16**. (A) Binding of compound **16** with topo I and (B) topo IIα. Compound **16** and the interacting residues of the receptor are shown in sticks colored by atom types except for carbon atoms of compound **16** are colored in orange. The DNA phosphate backbone and bases are shown in cartoons in yellow. The hydrogen bonding are represented in yellow dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

function as a topo I or topo II poison. Merbarone under clinical trial is a non-intercalative topo II catalytic inhibitor through binding to the topo II active cleavage site including Tyr805 which resulted in inhibition of DNA cleavage. The Tyr805 residue of topo II is required for DNA cutting and religation. Etoposide also interacts with Tyr805. Merbarone induces neither changes on DNA-topo II complex nor binding to DNA [10,34]. From these binding modes and diverse assay results, it can be suggested that similar to merbarone, compound **16** could not intercalate into regularly stacked base-pairs of DNA duplex but could interact with topo-bound DNA without causing changes on topo-DNA complex and then could inhibit catalytic activity of both topo I and topo IIα without blocking DNA religation.

3. Conclusion

A total of twenty-eight new rigid analogs of 5*H*-indeno[1,2-*b*] pyridine substituted with thienyl, furyl, or pyridyl at the 2-position and phenyl or phenolic moiety at the 4-position were prepared by modified Kröhnke synthesis. The biological activity of these newly synthesized compounds was verified using recombinant human DNA topo I and IIα, and human cancer cell lines (HCT15, T47D, DU145, and HeLa). Among tested compounds, compound **16** inhibited most strongly both topo I and IIα with comparable or stronger cytotoxicity in compared with positive controls. The structure-activity relationship study showed that compounds **9**, **10**, **12**, **13**, **15**, **16**, **18** and **19** containing 2- or 3-thienyl and 2- or 3-furyl moiety at 2-position with and hydroxyl group at *meta* or *para* positions of the 4-phenyl ring are important for exhibiting potent topo IIα inhibitory activity and significant antiproliferative activity. The mechanism of action of compound **16**, the most potent topo IIα inhibitor with significant antiproliferative activity, was further evaluated with various assays such as topo IIα-DNA cleavable complex, topo IIα-mediated ATPase assays, topo I-mediated DNA unwinding, *in vitro* topo I and topo IIα relaxation, and *ex vivo* topo relaxation assays. Compound **16** was determined as a non-intercalative topo catalytic inhibitor with better potency than etoposide in T47D cells. Molecular docking study of compound **16** with topo I and topo IIα revealed that the binding sites of compound **16** partially overlapped with those of camptothecin and etoposide, respectively. However the total binding scores of compound **16** were much lower than those of camptothecin and etoposide. The new information obtained from these efforts provide a basis for designing novel effective anticancer agents by substituting other functionalities at rigid 5*H*-indeno[1,2-*b*]pyridine skeleton.

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 (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) using Shimadzu Class VP program. Sample volume of 10 μL was injected in Waters X-Terra® 5 μM reverse-phase C_{18} column (4.6x250 mm) with a gradient elution of 70%–100% of B in A for 10 min followed by 100%–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 (%). 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 an 8 μL injection volume on a Waters Atlantis® T_3 reverse-phase C_{18} column (2.1x 50 mm, 3 μm) with a gradient elution from; (A) 50%–90% of B in A for 4 min and remained linear 90% of B in A for 1 min followed by 90%–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%–90% of B in A for 5 min followed by 90%–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 AF in distilled water and mobile phase B was ACN. MS ionization

conditions were: Sheath gas flow rate: 40 arb, aux gas flow rate: 0 arb, I spray voltage: 5.3 KV, capillary temperature: 275 °C, capillary voltage: 27 V, tube lens offset: 45 V. Retention time was given in minutes.

4.1. General method for preparation of **III (R = a-d)**

1-Indanone (**I**) was added in ethanol followed by the addition of equivalent amount of aryl aldehydes **II** (R = **a**-**d**). The 5% aqueous solution of NaOH was added drop wise to the mixture at room temperature which resulted in precipitation. The mixture was then cooled for 30 min, filtered, washed with cold methanol, and dried to yield 62–97% solid compounds. In those reactions where no precipitation occurred, the reaction mixtures were extracted with ethyl acetate and washed with water and saturated solution of NaCl (brine). Crude products were further purified by column chromatography. Four different indanone intermediates **III** (R = **a-d**) were synthesized following the same method.

4.1.1. Synthesis of 2-Benzylidene-2,3-dihydro-1H-inden-1-one (**IIIa**)

The procedure described in Section 4.1 was employed with 1-indanone (**I**, 2.64 g, 20 mmol) and benzaldehyde (**IIa**, 2.03 mL, 20 mmol) to yield 4.29 g (19.46 mmol, 97%) as an off-white solid.

TLC (ethyl acetate/*n*-hexane = 1:10) R_f = 0.27, mp 132.1–133.2 °C.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.9 (d, J = 7.6 Hz, 1H, indeno H-7), 7.68–7.64 (m, 3H, phenyl H-2, H-6, indeno H-5), 7.61–7.53 (m, 2H, indeno H-4, =CH-), 7.49–7.36 (m, 4H, indeno H-6, phenyl H-3, H-4, H-5), 4.04 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, $CDCl_3$) δ 194.23, 145.61, 138.04, 135.43, 134.76, 134.57, 133.88, 130.68, 129.61, 128.90, 127.65, 126.14, 124.43, 32.42.

4.1.2. Synthesis of 2-(2-hydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (**IIIb**)

The procedure described in Section 4.1 was employed with 1-indanone (**I**, 3.96 g, 30 mmol) and salicylaldehyde (**IIb**, 3.14 mL, 30 mmol) to yield 6.85 g (28.99 mmol, 97%) as a dark-red solid.

TLC (ethyl acetate/*n*-hexane = 1:3) R_f = 0.23, mp 203.4–204.9 °C.

^{1}H NMR (250 MHz, DMSO-d_6) δ 8.27 (s, 1H, =CH-), 7.69 (d, J = 7.55 Hz, 1H, indeno H-7), 7.61–7.56 (m, 2H, indeno H-4, H-5), 7.48 (d, J = 7.82 Hz, 1H, phenyl H-6), 7.41 (t, J = 5.4 Hz, 1H, indeno H-6), 6.93 (t, J = 7.35 Hz, 1H, phenyl H-4), 6.45 (d, J = 8.5 Hz, 1H, phenyl H-3), 6.20 (t, J = 7.25 Hz, 1H, phenyl H-5), 3.93 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, DMSO-d_6) δ 193.72, 172.46, 150.09, 139.72, 134.23, 134.01, 132.80, 130.65, 127.93, 127.29, 127.14, 123.60, 123.47, 121.66, 111.56, 33.30.

4.1.3. Synthesis of 2-(3-hydroxybenzylidene)-2, 3-dihydro-1H-inden-1-one (**IIIc**)

The procedure described in Section 4.1 was employed with 1-indanone (**I**, 3.96 g, 30 mmol) and 3-hydroxybenzaldehyde (**IIc**, 3.66 g, 30 mmol) to yield 4.81 g (20.33 mmol, 68%) as an orange solid.

TLC (ethyl acetate/*n*-hexane = 1:3) R_f = 0.21, mp 264.8–265.4 °C.

^{1}H NMR (250 MHz, DMSO-d_6) δ 7.72 (d, J = 7.55 Hz, 1H, indeno H-7), 7.63 (t, J = 7.50 Hz, 1H, indeno H-5), 7.56 (d, J = 7.75 Hz, 1H, indeno H-4) 7.42 (t, J = 7.32 Hz, 1H, indeno H-6), 7.35 (s, 1H, =CH-), 7.12 (t, J = 7.72 Hz, 1H, phenyl H-5), 7.01 (s, 1H, phenyl H-2), 6.82 (d, J = 7.45 Hz, 1H, phenyl H-4), 6.69 (d, J = 7.85 Hz, 1H, phenyl H-6), 3.88 (s, 2H, indeno H-3).

^{13}C NMR (62.5 MHz, DMSO-d_6) δ 193.78, 164.81, 150.25, 137.67, 135.73, 135.18, 134.91, 133.92, 129.81, 127.88, 126.93, 123.75, 119.91, 119.11, 117.93. 32.25.

4.1.4. Synthesis of 2-(4-hydroxybenzylidene)-2, 3-dihydro-1H-inden-1-one (**IIId**)

The procedure described in Section 4.1 was employed with 1-indanone (**I**, 2.64 g, 20 mmol) and 4-hydroxybenzaldehyde (**IId**, 2.44 g, 20 mmol) to yield 2.93 g (12.39 mmol, 62%) as a light greenish solid.

TLC (ethyl acetate/*n*-hexane = 1:2) R_f = 0.3, mp 235.4–236.0 °C.

^{1}H NMR (250 MHz, DMSO-d_6) δ 10.13 (s, 1*H*, phenyl 4-OH), 7.75 (d, J = 7.27 Hz, 1*H*, indeno H-7), 7.67–7.60 (m, 4*H*, phenyl H-2, H-6, indeno H-4, H-5), 7.46–7.42 (m, 2*H*, =CH–, indeno H-6), 6.88 (d, J = 8.35 Hz, 2*H*, phenyl H-3, H-5), 4.04 (s, 2*H*, indeno H-3).

^{13}C NMR (62.5 MHz, DMSO-d_6) δ 194.11, 160.30, 150.66, 138.45, 135.36, 134.22, 133.87 (2C), 132.40, 128.45, 127.47, 126.85, 124.28, 116.90 (2C), 32.83.

4.2. General method for the preparation of **V (R^1 = e-k)**

A mixture of equivalent amounts of acetophenones and iodine in pyridine (15.0 equiv) was refluxed at 140 °C 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 38–90% of **V (R^1 = e-k).**

4.3. General method for the preparation of **4, 7–28**

Anhydrous ammonium acetate (10.0 equiv.) was mixed with glacial acetic acid or methanol followed by addition of the indanone intermediates (1.0 equiv.), **III** (**R** = **a-d**) and pyridinium iodide salts **V** (**R^1** = **e-k**) (1.5 equiv.). The reaction mixture was then refluxed at 100–110 °C for 24–36 h followed by extraction with ethyl acetate and washing with water and brine solution. The organic layer was dried with magnesium sulfate and filtered. The filtrate was evaporated at reduced pressure, which was purified with silica gel column chromatography with the gradient elution of ethyl acetate/*n*-hexane to afford solid compounds **4, 7–28** in 20–83% yields.

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

The same procedure described in section 4.3 was employed with **IIIa** (0.33 g, 1.5 mmol), anhydrous ammonium acetate (1.15 g, 15 mmol), **Vh** (0.63 g, 2 mmol) and methanol (8 mL) at 100 °C for 24 h to yield 196 mg (0.63 mmol, 42%) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:5) R_f = 0.3, mp: 144.4–145.9 °C, HPLC: Retention time: 7.52 min, purity: 96.5%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NO$ $[MH]^+$ 310.12; found 310.28. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.49 (s, 1H, 2-furyl H-2), 8.06 (d, J = 8.35 Hz, 1H, indenopyridine H-9), 7.85–7.80 (m, 3H, 4-phenyl H-2, H-6, 2-furyl H-4), 7.73 (s, 1H, indenopyridine H-3), 7.66–7.45 (m, 6H, indenopyridine H-6, H-7, H-8, 4-phenyl H-3, H-4, H-5), 7.24 (br, 1H, 2-furyl H-5), 4.04 (s, 2H, indneopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.19, 150.82, 145.93, 144.45, 144.40, 142.01, 140.32, 137.91, 132.45, 128.98 (2C), 128.89 (2C), 128.52 (2C), 127.20, 127.16, 125.53, 120.65, 117.46, 109.22, 34.19.

4.3.2. Synthesis of 4-phenyl-2-(pyridin-4-yl)-5H-indeno[1,2-b] pyridine (**7**)

The same procedure described in section 4.3 was employed with **IIIa** (0.33 g, 1.5 mmol), anhydrous ammonium acetate (1.15 g, 15 mmol), **Vk** (0.81 g, 2.5 mmol) and methanol (8 mL) at 100 °C for 24 h to yield 156 mg (0.48 mmol, 33%) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:1) R_f = 0.26, mp:

182.7–183.5 °C, HPLC: Retention time: 7.68 min, purity: 99.3%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2$ $[MH]^+$ 321.14; found 321.40. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.73 (d, J = 6.02 Hz, 2H, 2-pyrisyl H-2, H-6), 8.28 (d, J = 6.05 Hz, 2H, 2-pryridyl H-3, H-5), 8.14 (d, J = 8.45 Hz, 1H, indneopyridine H-9), 8.09 (s, 1H, indenopyridine H-3), 7.91 (d, J = 6.65 Hz, 2H, 4-phenyl H-2, H-6), 7.67 (d, J = 8.02 Hz, 1H, indenopyridine H-6), 7.63–7.49 (m, 5H, indneopyridine H-7, H-8, 4-phenyl H-3, H-4, H-5), 4.19 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.77, 153.15, 150.34 (2C), 146.27, 145.85, 144.58, 139.97, 137.62, 134.91, 129.26, 129.04, 128.98 (2C), 128.62 (2C), 127.32, 125.59, 121.08 (2C), 120.79, 118.44, 34.29.

4.3.3. Synthesis of 2-(2-(thiophen-2-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**8**)

The same procedure described in section 4.3 was employed with **IIIb** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Ve** (1.49 g, 4.5 mmol) and methanol (15 mL) at 100 °C for 24 h to yield 378 mg (1.12 mmol, 37%) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:7) R_f = 0.29, mp: 292.6–293.9 °C, HPLC: Retention time: 10.12 min, purity: 98.5%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.09; found 342.41. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.86 (s, 1H, 4-phenyl 2-OH), 8.01 (d, J = 6.27 Hz, 1H, indenopyridine H-9), 7.87 (d, J = 3.5 Hz, 1H, 2-thiophene H-3), 7.76 (s,1H, indenopyridine H-3), 7.64–7.60 (m, 2H, 2-thiophene H-5 and indenopyridine H-6), 7.51–7.44 (m, 2H, indenopyridine H-7, H-8), 7.39 (d, J = 7.55 Hz, 1H, 4-phenyl H-6), 7.31 (t, J = 7.25 Hz, 1H, 4-phenyl H-4), 7.16 (d, J = 3.97 Hz, 1H, 2-thiophene H-4), 7.03 (d, J = 8.1 Hz, 1H, 4-phenyl H-3), 6.96 (t, J = 7.4 Hz, 1H, 4-phenyl H-5), 3.87 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.93, 155.15, 151.75, 145.81, 145.46, 145.12, 141.01, 135.47, 131.28, 130.79, 129.64, 129.23, 128.67, 127.95, 126.33, 125.96, 125.74, 121.32, 120.21, 118.58, 116.90, 34.98.

4.3.4. Synthesis of 3-(2-(thiophen-2-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**9**)

The same procedure described in section 4.3 was employed with **IIIc** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Ve** (1.49 g, 4.5 mmol) and methanol (15 mL) at 100 °C for 30 h to yield 473 mg (1.39 mmol, 46%) as a light yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:5) R_f = 0.22, mp: 211.4–212.8 °C, HPLC: Retention time: 10.29 min, purity: 97.9%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.09; found 342.42. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.71 (s, 1H, 4-phenyl 3-OH), 8.03 (m, 1H, indenopyridine H-9), 7.96 (dd, J = 3.6, 0.97 Hz, 1H, 2-thiophene H-3), 7.85 (s,1H, indenopyridine H-3), 7.66–7.46 (m, 2H, 2-thiophene H-5 and indenopyridine H-6), 7.50–7.46 (m, 2H, indenopyridine H-7, H-8), 7.36 (t, J = 7.77 Hz, 1H, 4-phenyl H-5), 7.25 (t, J = 7.82 Hz, 1H, 4-phenyl H-6), 7.20–7.16 (m, 2H, 2-thiophene H-4 and 4-phenyl H-2), 6.89 (d, J = 7.95 Hz, 1H, 4-phenyl H-4), 4.07 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.85, 158.62, 152.47, 147.07, 145.66, 145.29, 140.82, 139.87, 133.62, 130.86, 129.82, 129.29, 128.91, 128.08, 126.34 (2C), 121.42, 119.97, 116.68, 116.63, 116.07, 35.16.

4.3.5. Synthesis of 4-(2-(thiophen-2-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**10**)

The same procedure described in section 4.3 was employed with **IIId** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Ve** (1.49 g, 4.5 mmol) and methanol (15 mL) at 100 °C for 24 h to yield 314 mg (0.92 mmol, 31%) as a light yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:5) R_f = 0.2, mp: 268.9–269.9 °C, HPLC: Retention time: 10.08 min, purity: 96.1%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.09; found 342.42. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.88 (s, 1H, 4-phenyl 4-OH), 8.01–7.98 (m, 1H, indenopyridine H-9), 7.93 (d, J = 3.0 Hz, 1H, 2-thiophene H-3), 7.83 (s,1H, indenopyridine H-3), 7.71 (d, J = 8.52 Hz, 2H, 4-phenyl H-2, H-6), 7.63–7.62 (m, 2H, 2-thiophene H-5 and indenopyridine H-6), 7.47–7.45 (m, 2H, indenopyridine H-7, H-8), 7.17 (t, J = 3.77 Hz, 1H, 2-thiophene H-4), 6.95 (d, J = 8.55 Hz, 2H, 4-phenyl H-3, H-5), 4.09 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.83, 159.14, 152.32, 146.81, 145.82, 145.31, 140.90, 137.53, 133.21, 130.77 (2C), 129.74, 129.26, 129.05, 128.04, 126.30, 126.19, 121.40, 116.60 (2C), 116.27, 35.6.

4.3.6. Synthesis of 2-(2-(thiophen-3-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**11**)

The same procedure described in section 4.3 was employed with **IIIb** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Vf** (1.49 g, 4.5 mmol) and methanol (20 mL) at 100 °C for 24 h to yield 669 mg (1.96 mmol, 66%) as a light brown solid.

TLC (ethyl acetate/*n*-hexane = 1:5) R_f = 0.19, mp: 297.0–298.7 °C, HPLC: Retention time: 9.98 min, purity: 100%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.09; found 342.41. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.85 (s, 1H, 4-phenyl 2-OH), 8.29–8.28 (m, 1H, 2-thiophene H-2), 8.08–8.05 (m, 1H, indenopyridine H-9), 7.92 (dd, J = 4.92, 0.9 Hz, 1H, 2-thiophene H-4), 7.72 (s, 1H, indenopyridine H-3), 7.67–7.60 (m, 2H, 2-thiophene H-5 and indenopyridine H-6), 7.51–7.43 (m, 2H, indenopyridine H-7, H-8), 7.34 (d, J = 7.57 Hz, 1H, 4-phenyl H-6), 7.30 (t, J = 8.05 Hz, 1H, 4-phenyl H-4), 7.02 (d, J = 8.0 Hz, 1H, 4-phenyl H-3), 6.95 (t, J = 7.37 Hz, 1H, 4-phenyl H-5), 3.87 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.96, 155.11, 152.60, 145.37, 145.03, 143.07, 141.40, 135.07, 131.29, 130.65, 129.45, 127.85, 127.63, 127.47, 126.26, 125.93, 124.51, 121.26, 120.13 (2C), 116.83, 34.88.

4.3.7. Synthesis of 2-(2-(thiophen-3-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**12**)

The same procedure described in section 4.3 was employed with **IIIc** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Vf** (1.49 g, 4.5 mmol) and methanol (15 mL) at 100 °C for 36 h to yield 586 mg (1.7 mmol, 57%) as a light brown solid.

TLC (ethyl acetate/*n*-hexane = 1:4) R_f = 0.21, mp: 225.9–226.6 °C, HPLC: Retention time: 9.47 min, purity: 100%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.09; found 342.42. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.71 (s, 1H, 4-phenyl 3-OH), 8.35 (s, 1H, 2-thiophene H-2), 8.07 (d, J = 6.45 Hz, 1H, indenopyridine H-9), 7.95 (d, J = 4.85 H, 1H, 2-thiophene H-4), 7.82 (s,1H, indenopyridine H-3), 7.66 (br, 2H, 2-thiophene H-5 and indenopyridine H-6), 7.48 (br, 2H, indenopyridine H-7, H-8), 7.36 (t, J = 7.8 Hz, 1H, 4-phenyl H-5), 7.25 (d, J = 7.17 Hz, 1H, 4-phenyl H-6), 7.19 (s, 1H, 4-phenyl H-2), 6.90 (d, J = 7.6 Hz, 1H, 4-phenyl H-4), 4.07 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.93, 158.58, 153.32, 146.97, 145.81, 142.99, 141.21, 140.07, 133.24, 190.80, 129.65, 127.98, 127.70, 127.58, 125.30, 124.36, 121.39, 119.95, 118.21, 116.56, 116.36, 35.07.

4.3.8. Synthesis of 2-(2-(thiophen-3-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**13**)

The same procedure described in section 4.3 was employed with **IIId** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Vf** (1.49 g, 4.5 mmol) and methanol (15 mL) at 100 °C for 30 h to yield 470 mg (1.37 mmol, 46%) as a light brown solid.

TLC (ethyl acetate/*n*-hexane = 1:4) R_f = 0.25, mp: 291.2–292.4 °C, HPLC: Retention time: 9.47 min, purity: 96.1%; ESI LC/MS (condition A): *m/z* calcd for $C_{22}H_{15}NOS$ $[MH]^+$ 342.09; found 342.42. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.84 (s, 1H, 4-phenyl 4-OH), 8.33 (br, 1H, 2-thiophene H-2), 8.05 (br, 1H, indenopyridine H-9), 7.94 (d, J = 6.7 Hz, 1H, 2-thiophene H-4), 7.81 (s,1H, indenopyridine H-3), 7.74–7.66 (m, 4H, 4-phenyl H-2, H-6, 2-thiophene H-5 and

indenopyridine H-6), 7.47 (br, 2H, indenopyridine H-7, H-8), 6.95 (d, J = 7.47 Hz, 2H, 4-phenyl H-3, H-5), 4.10 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.88, 159.02, 153.24, 146.69, 145.23, 143.14, 141.30, 132.82, 130.71 (2C), 129.53, 129.23, 127.93, 127.62 (2C), 126.23, 124.69, 121.35, 117.87, 116.51 (2 C), 35.26.

4.3.9. Synthesis of 2-(2-(furan-2-yl)-5H-indeno[1,2-b]pyridin-4-yl) phenol (**14**)

The same procedure described in section 4.3 was employed with **IIIb** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Vg** (1.41 g, 4.5 mmol) and methanol (15 mL) at 100 °C for 24 h to yield 391 mg (1.2 mmol, 40%) as a brown solid.

TLC (ethyl acetate/n-hexane = 1:5) R_f = 0.19, mp: 313.8–314.4 °C, HPLC: Retention time: 8.37 min, purity: 97.6%; ESI LC/MS (condition A): m/z calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.36. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.87 (s, 1H, 4-phenyl 2-OH), 8.05–8.02 (m, 1H, indenopyridine H-9), 7.84 (s,1H, indenopyridine H-3), 7.60 (br, 2H, 2-furan H-5 and indenopyridine H-6), 7.50–7.44 (m, 2H, indenopyridine H-7, H-8), 7.40 (dd, J = 7.47, 1.47 Hz, 1H, 4-phenyl H-6), 7.30 (t, J = 8.05 Hz, 1H, 4-phenyl H-4), 7.23 (d, J = 3.25 Hz, 1H, 2-furan H-3), 7.02 (d, J = 7.97 Hz, 1H, 4-phenyl H-3), 6.95 (t, J = 7.37 Hz, 1H, 4-phenyl H-5), 6.67 (br, 1H, 2-furan H-4), 3.89 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.35, 155.14, 154.36, 148.42, 145.24, 145.12, 145.74, 141.10, 135.36, 131.18, 130.84, 129.69, 127.95, 126.32, 125.63, 121.40, 120.23, 118.26, 116.97, 113.13, 109.46, 34.99.

4.3.10. Synthesis of 3-(2-(furan-2-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**15**)

The same procedure described in section 4.3 was employed with **IIIc** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Vg** (1.41 g, 4.5 mmol) and methanol (15 mL) at 100 °C for 24 h to yield 416 mg (1.28 mmol, 43%) as a light yellow solid.

TLC (ethyl acetate/n-hexane = 1:4) R_f = 0.29, mp: 225.1–226.3 °C, HPLC: Retention time: 8.32 min, purity: 99.6%; ESI LC/MS (condition A): m/z calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.36. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.72 (s, 1H, 4-phenyl 3-OH), 8.04 (m, 1H, indenopyridine H-9), 7.86 (s,1H, indenopyridine H-3), 7.66 (br, 2H, 2-furan H-5 and indenopyridine H-6), 7.49–7.46 (m, 2H, indenopyridine H-7, H-8), 7.36 (t, J = 7.85 Hz, 1H, 4-phenyl H-5), 7.26 (br, 2H, 2-furan H-3 and 4-phenyl H-6), 7.17 (s, 1H, 4-phenyl H-2), 6.89 (d, J = 7.25 Hz, 1H, 4-phenyl H-4), 6.69 (br, 1H, 2-furan H-4), 4.09 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 161.22, 158.64, 154.18, 149.13, 146.83, 145.27, 144.91, 140.86, 136.81, 133.56, 130.92, 129.87, 128.05, 126.31, 121.50, 119.82, 116.75, 116.17, 115.87, 113.19, 109.78, 35.13.

4.3.11. Synthesis of 4-(2-(furan-2-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**16**)

The same procedure described in section 4.3 was employed with **IIId** (0.71 g, 3 mmol), anhydrous ammonium acetate (2.31 g, 30 mmol), **Vg** (1.41 g, 4.5 mmol) and methanol (15 mL) at 100 °C for 24 h to yield 312 mg (0.96 mmol, 32%) as a light yellow solid.

TLC (ethyl acetate/n-hexane = 1:4) R_f = 0.19, mp: 282.2–283.7 °C, HPLC: Retention time: 8.29 min, purity: 98.5%; ESI LC/MS (condition A): m/z calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.27. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.82 (s, 1H, 4-phenyl 4-OH), 8.04 (m, 1H, indenopyridine H-9), 7.84 (s,1H, indenopyridine H-3), 7.70–7.66 (m, 4H, 4-phenyl H-2, H-6, 2-furan H-5 and indenopyridine H-6), 7.48–7.45 (m, 2H, indenopyridine H-7, H-8), 7.24 (d, J = 3.03 Hz, 1H, 2-furan H-3), 6.95 (d, J = 8.42 Hz, 2H, 4-phenyl H-3, H-5),6.68 (br, 1H, 2-furan H-4), 4.11 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 161.08, 159.08, 154.33, 149.02, 146.58, 145.23, 144.69, 140.945, 133.09, 130.52 (2C), 129.65, 128.97, 127.92, 126.18, 121.40, 116.58 (2C), 115.80, 113.06, 109.51, 35.26.

4.3.12. Synthesis of 2-(2-(furan-3-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**17**)

The same procedure described in section 4.3 was employed with **IIIb** (0.35 g, 1.5 mmol), anhydrous ammonium acetate (1.15 g, 15 mmol), **Vh** (0.63 g, 2 mmol) and methanol (8 mL) at 100 °C for 24 h to yield 403 mg (1.24 mmol, 83%) as a white solid.

TLC (ethyl acetate/n-hexane = 1:5) R_f = 0.21, mp: 288.2–288.8 °C, HPLC: Retention time: 5.83 min, purity: 98.6%; ESI LC/MS (condition A): m/z calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.28. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.79 (s, 1H, 4-phenyl 2-OH), 8.42 (s, 1H, 2-furyl H-2), 8.05 (d, J = 7.32 Hz, 1H, indenopyridine H-9), 7.77 (br, 1H, 2-furyl H-4), 7.61 (d, J = 6.55 Hz, 1H, indenopyridine H-6), 7.57 (s, 1H, indenopyridine H-3), 7.50–7.39 (m, 2H, indenopyridine H-7, H-8), 7.34 (dd, J = 7.52, 1.25 Hz, 1H, 4-phenyl H-6), 7.29 (t, J = 7.95 Hz, 1H, 4-phenyl H-4), 7.18 (br, 1H, 2-furyl H-5), 7.02 (d, J = 8.1 Hz, 1H, 4-phenyl H-3), 6.95 (t, J = 7.43 Hz, 1H, 4-phenyl H-5), 3.85 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.15, 154.24, 149.97, 144.46, 144.20, 144.14, 141.60, 140.52, 134.13, 130.39, 129.79, 128.59, 127.18, 126.99, 125.40, 125.08, 120.43, 119.31, 119.11, 116.02, 109.07, 34.05.

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

The same procedure described in section 4.3 was employed with **IIIc** (0.23 g, 1 mmol), anhydrous ammonium acetate (0.77 g, 10 mmol), **Vh** (0.63 g, 2 mmol) and methanol (8 mL) at 110 °C for 24 h to yield 126 mg (0.39 mmol, 39%) as a white solid.

TLC (ethyl acetate/n-hexane = 1:3) R_f = 0.24, mp: 243.9–244.7 °C, HPLC: Retention time: 6.03 min, purity: 97.3%; ESI LC/MS (condition B): m/z calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.28. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.66 (s, 1H, 4-phenyl 3-OH), 8.48 (s, 1H, 2-furyl H-2), 8.05 (d, J = 7.37 Hz, 1H, indenopyridine H-9), 7.79 (br, 1H, 2-furyl H-4), 7.68 (s, 1H, indenopyridine H-3), 7.66–7.64 (m, 1H, indenopyridine H-6), 7.51–7.42 (m, 2H, indenopyridine H-7, H-8), 7.35 (t, J = 7.95 Hz, 1H, 4-phenyl H-5), 7.23–7.21 (m, 2H, 4-phenyl H-6, 2-furyl H-5), 7.16 (br, 1H, 4-phenyl H-2), 6.90 (dd, J = 7.05, 2.27 Hz, 1H, 4-phenyl H-4), 4.06 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.22, 157.86, 150.85, 146.16, 144.45 (2C), 142.03, 140.46, 139.29, 132.39, 130.07, 128.92, 127.26 (2C), 125.58, 120.67, 119.21, 117.34, 115.84, 115.33, 109.26, 34.37.

4.3.14. Synthesis of 4-(2-(furan-3-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**19**)

The same procedure described in section 4.3 was employed with **IIId** (0.35 g, 1.5 mmol), anhydrous ammonium acetate (1.15 g, 15 mmol), **Vh** (0.63 g, 2 mmol) and methanol (10 mL) at 100 °C for 24 h to yield 138 mg (0.42 mmol, 28%) as a white solid.

TLC (ethyl acetate/n-hexane = 1:3) R_f = 0.21, mp: 266.8–267.3 °C, HPLC: Retention time: 6.42 min, purity: 97.6%; ESI LC/MS (condition B): m/z calcd for $C_{22}H_{15}NO_2$ $[MH]^+$ 326.12; found 326.26. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.79 (s, 1H, 4-phenyl 4-OH), 8.46 (s, 1H, 2-furyl H-2), 8.04 (d, J = 7.17 Hz, 1H, indenopyridine H-9), 7.78 (br, 1H, 2-furyl H-4), 7.69 (d, J = 8.7 Hz, 2H, 4-phenyl H-2, H-6), 7.67 (s, 1H, indenopyridine H-3), 7.63 (br, 1H, indenopyridine H-6), 7.50–7.44 (m, 2H, indenopyridine H-7, H-8), 7.22 (br, 1H, 2-furyl H-5), 6.93 (d, J = 8.75 Hz, 2H, 4-phenyl H-3, H-5), 4.09 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.17, 158.32, 150.74, 145.86, 144.49, 144.37, 141.91, 140.56, 131.99, 129.97 (2C), 128.79, 128.46, 127.36, 127.19, 125.51, 120.64, 116.99, 115.80 (2C), 109.30, 34.58.

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

The same procedure described in section 4.3 was employed with

IIIb (0.47 g, 2 mmol), anhydrous ammonium acetate (1.54 g, 20 mmol), **Vi** (0.97 g, 3 mmol) and methanol (10 mL) at 110 °C for 24 h to yield 242 mg (0.72 mmol, 36%) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:2) R_f = 0.26, mp: 263.2–263.8 °C, HPLC: Retention time: 7.72 min, purity: 98.5%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.14; found 337.46. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.89 (s, 1H, 4-phenyl 2-OH), 8.67 (d, J = 4.73 Hz, 1H, 2-pyridyl H-3), 8.62 (d, J = 7.95 Hz, 1H, indenopyridine H-9), 8.32 (s, 1H, indenopyridine H-3), 8.12 (d, J = 6.85 Hz, 1H, 2-pyridyl H-6), 7.99 (td, J = 7.85, 1.62 Hz, 1H, 2-pyridyl H-4), 7.64 (d, J = 7.1 Hz, 1H, 4-phenyl H-6), 7.53–7.40 (m, 4H, 2-pyridyl H-5, indenopyridine H-6, H-7, H-8), 7.32 (td, J = 7.47, 1.37 Hz, 1H, 4-phenyl H-4), 7.03 (d, J = 8.17 Hz, 1H, 4-phenyl H-3), 6.96 (t, J = 7.42 Hz, 1H, 4-phenyl H-5), 3.95 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.62, 155.83, 154.52, 154.34, 149.51, 144.72, 144.53, 140.57, 137.58, 136.60, 130.57, 130.23, 129.10, 127.38, 125.78, 125.22, 124.25, 120.74 (2C), 119.90, 119.65, 116.35, 34.43.

4.3.16. Synthesis of 3-(2-(pyridin-2-yl)-5H-indeno[1,2-b]pyridin-4-yl)phenol (**21**)

The same procedure described in section 4.3 was employed with **IIIc** (0.47 g, 2 mmol), anhydrous ammonium acetate (1.54 g, 20 mmol), **Vi** (0.97 g, 3 mmol) and methanol (10 mL) at 100 °C for 24 h to yield 196 mg (0.58 mmol, 29%) as a creamy white solid.

TLC (ethyl acetate/*n*-hexane = 1:2) R_f = 0.18, mp: 272.8–273.6 °C, HPLC: Retention time: 7.94 min, purity: 98.4%; 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, 4-phenyl 3-OH), 8.71 (d, J = 4.73 Hz, 1H, 2-pyridyl H-3), 8.62 (d, J = 7.95 Hz, 1H, indenopyridine H-9), 8.39 (s, 1H, indenopyridine H-3), 8.14 (d, J = 5.55 Hz, 1H, 2-pyridyl H-6), 8.01 (t, J = 7.67 Hz, 1H, 2-pyridyl H-4), 7.68 (br, 1H, indenopyridine H-6), 7.54–7.45 (m, 3H, 2-pyridyl H-5, indenopyridine H-7, H-8), 7.37 (t, J = 7.75 Hz, 1H, 4-phenyl H-5), 7.26 (d, J = 7.62 Hz, 1H, 4-phenyl H-6), 7.20 (s, 1H, 4-phenyl H-2), 6.91 (d, J = 7.9 Hz, 1H, 4-phenyl H-4), 4.15 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.60, 158.12, 155.65, 155.15, 149.64, 146.28, 144.76, 140.37, 139.46, 137.75, 134.92, 130.49, 129.37, 127.57, 125.86, 124.53, 120.95, 120.87, 119.27, 117.79, 116.24, 115.23, 34.64.

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

The same procedure described in section 4.3 was employed with **IIId** (0.47 g, 2 mmol), anhydrous ammonium acetate (1.54 g, 20 mmol), **Vi** (0.97 g, 3 mmol) and methanol (10 mL) at 110 °C for 24 h to yield 272 mg (0.81 mmol, 41%) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:2) R_f = 0.13, mp: 278.3–279.0 °C, HPLC: Retention time: 8.02 min, purity: 99.6%; 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 9.93 (s, 1H, 4-phenyl 4-OH), 8.70 (d, J = 4.72 Hz, 1H, 2-pyridyl H-3), 8.61 (d, J = 7.9 Hz, 1H, indenopyridine H-9), 8.37 (s, 1H, indenopyridine H-3), 8.12 (d, J = 5.42 Hz, 1H, 2-pyridyl H-6), 8.0 (td, J = 7.7, 1.72 Hz, 1H, 2-pyridyl H-4), 7.71 (d, J = 8.64 Hz, 2H, 4-phenyl H-2, H-6), 7.66 (br, 1H, indenopyridine H-6), 7.53–7.44 (m, 3H, 2-pyridyl H-5, indenopyridine H-7, H-8), 6.95 (d, J = 8.6 Hz, 2H, 4-phenyl H-3, H-5), 4.16 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.46, 158.54, 155.81, 155.02, 149.57, 146.10, 144.76, 140.47, 137.66, 134.45, 130.02 (2C), 129.19, 128.65, 127.47, 125.76, 124.40, 120.88, 120.83, 117.44, 116.12 (2C), 34.77.

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

The same procedure described in section 4.3 was employed with **IIIb** (0.47 g, 2 mmol), anhydrous ammonium acetate (1.54 g, 20 mmol), **Vj** (0.97 g, 3 mmol) and methanol (10 mL) at 100 °C for 24 h to yield 279 mg (0.82 mmol, 42%) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:1) R_f = 0.23, mp: 238.5–239.2 °C, HPLC: Retention time: 6.26 min, purity: 98.8%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.14; found 337.36. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.94 (s, 1H, 4-phenyl 2-OH), 9.41 (s, 1H, 2-pyridyl H-2), 8.64 (d, J = 4.7 Hz, 1H, 2-pyridyl H-4), 8.59(dd, J = 8.02, 1.82 Hz, 1H, 2-pyridyl H-6), 8.12 (d, J = 6.72 Hz, 1H, indenopyridine H-9), 7.88 (s, 1H, indenopyridine H-3), 7.63 (d, J = 7.65 Hz, 1H, 4-phenyl H-6), 7.56–7.43 (m, 4H, 2-pyridyl H-5, indenopyridine H-6, H-7, H-8), 7.32 (td, J = 8.25, 1.37 Hz, 1H, 4-phenyl H-4),7.03 (d, J = 8.07 Hz, 1H, 4-phenyl H-3), 6.96 (t, J = 7.42 Hz, 1H, 4-phenyl H-5), 3.93 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.96, 154.58, 152.98, 149.91, 148.24, 145.07, 144.55, 140.58, 135.68, 134.74, 134.42, 130.88, 130.29, 129.22, 127.43, 125.80, 125.15, 124.09, 120.89, 119.96, 119.66, 116.31, 34.43.

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

The same procedure described in section 4.3 was employed with **IIIc** (0.23 g, 1 mmol), anhydrous ammonium acetate (0.77 g, 10 mmol), **Vj** (0.65 g, 2 mmol) and methanol (10 mL) at 110 °C for 36 h to yield 136 mg (0.41 mmol, 41%) as a creamy white solid.

TLC (ethyl acetate/*n*-hexane = 2:1) R_f = 0.24, mp: 263.5–264.1 °C, HPLC: Retention time: 6.81 min, purity: 97.1%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.14; found 337.36. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.65 (s, 1H, 4-phenyl 3-OH), 9.45 (s, 1H, 2-pyridyl H-2), 8.66–8.61 (m, 2H, 2-pyridyl H-4, H-6), 8.13 (d, J = 8.67 Hz, 1H, indenopyridine H-9), 7.96 (s, 1H, indenopyridine H-3), 7.67 (d, J = 7.95 Hz, 1H, indenopyridine H-6), 7.57–7.45 (m, 3H, 2-pyridyl H-5, indenopyridine H-7, H-8), 7.37 (t, J = 7.65 Hz, 1H, 4-phenyl H-5), 7.29 (d, J = 7.75 Hz, 1H, 4-phenyl H-6), 7.23 (s, 1H, 4-phenyl H-2), 6.91 (d, J = 7.8 Hz, 1H, 4-phenyl H-4), 4.13 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.71, 157.87, 153.61, 149.85, 148.20, 146.46, 144.53, 140.27, 139.13, 134.49, 134.31, 133.66, 130.06, 129.16, 127.34, 125.60, 123.85, 120.80, 119.31, 117.88, 115.96, 115.42, 34.42.

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

The same procedure described in section 4.3 was employed with **IIId** (0.23 g, 1 mmol), anhydrous ammonium acetate (0.77 g, 10 mmol), **Vj** (0.65 g, 2 mmol) and methanol (10 mL) at 110 °C for 36 h to yield 114 mg (0.33 mmol, 34%) as a light yellow solid.

TLC (ethyl acetate/*n*-hexane = 2:1) R_f = 0.29, mp: 290.8–291.7 °C, HPLC: Retention time: 6.67 min, purity: 95.3%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.14; found 337.46. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.88 (s, 1H, 4-phenyl 4-OH), 9.6 (s, 1H, 2-pyridyl H-2), 8.65–8.61 (m, 2H, 2-pyridyl H-4, H-6), 8.11 (d, J = 8. 7 Hz, 1H, indenopyridine H-9), 7.97 (s, 1H, indenopyridine H-3), 7.78 (d, J = 8.57 Hz, 2H, 4-phenyl H-2, H-6), 7.67, (d, J = 8.02 Hz, 1H, indenopyridine H-6), 7.58–7.48 (m, 3H, 2-pyridyl H-5, indenopyridine H-7, H-8), 6.95 (d, J = 8.57 Hz, 2H, 4-phenyl H-3, H-5), 4.17 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.64, 158.51, 155.63, 155.09, 150.57, 146.12, 144.71, 139.64, 136.92, 135.42, 129.89 (2C), 129.11, 128.92, 127.32, 124.73, 124.12, 121.32, 120.83, 117.96, 116.32 (2C), 34.09.

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

The same procedure described in section 4.3 was employed with **IIIb** (0.23 g, 1 mmol), anhydrous ammonium acetate (0.77 g, 10 mmol), **Vk** (0.65 g, 2 mmol) and methanol (10 mL) at 110 °C for 36 h to yield 78 mg (0.24 mmol, 24%) as a light yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:1) R_f = 0.12, mp: 282.6–283.3 °C, HPLC: Retention time: 6.73 min, purity: 96.8%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.14; found 337.47. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.86 (s, 1H, 4-phenyl 2-OH), 8.71 (d, J = 5.82 Hz, 2H, 2-pyridyl H-2, H-6), 8.21 (d, J = 6.02 Hz, 2H, 2-pyridyl H-3, H-5), 8.13 (d, J = 7.77 Hz, 1H, indenopyridine H-9), 7.53–7.42 (m, 3H, indenopyridine H-6, H-7, H-8), 7.32 (td, J = 8.12, 1.47 Hz, 1H, 4-phenyl H-4),7.05 (d, J = 8.07 Hz, 1H, 4-phenyl H-3), 6.97 (t, J = 7.4 Hz, 1H, 4-phenyl H-5), 3.95 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.02, 154.51, 152.50, 150.46 (2C), 146.14, 145.02, 144.49, 140.35, 136.70, 130.71, 130.21, 129.22, 127.35, 125.71, 124.93, 121.09 (2C), 120.80, 120.26, 119.53, 116.27, 34.39.

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

The same procedure described in section 4.3 was employed with **IIIc** (0.23 g, 1 mmol), anhydrous ammonium acetate (0.77 g, 10 mmol), **Vk** (0.65 g, 2 mmol) and methanol (10 mL) at 100 °C for 36 h to yield 108 mg (0.32 mmol, 32%) as a creamy white solid.

TLC (ethyl acetate/*n*-hexane = 2:1) R_f = 0.24, mp: 285.8–286.5 °C, HPLC: Retention time: 8.51 min, purity: 99.4%; ESI LC/MS (condition A): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.14; found 337.47. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.72 (s, 1H, 4-phenyl 3-OH), 8.72 (d, J = 5.55 Hz, 2H, 2-yridyl H-2, H-6), 8.26 (d, J = 5.65 Hz, 2H, 2-pyridyl H-3, H-5), 8.13 (d, J = 8.05 Hz, 1H, indenopyridine H-9), 8.04 (s, 1H, indenopyridine H-3), 7.68 (d, J = 7.8 Hz, 1H, indenopyridine H-6), 7.55–7.47 (m, 2H, indenopyridine H-7, H-8), 7.37 (t, J = 7.7 Hz, 1H, 4-phenyl H-5), 7.29 (d, J = 7.77 Hz, 1H, 4-phenyl H-6), 7.23 (s, 1H, 4-phenyl H-2), 6.93 (d, J = 8.0 Hz, 1H, 4-phenyl H-4), 4.15 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.89, 157.93, 157.22, 153.29, 150.46 (2C), 146.56, 146.04, 144.66, 140.14, 139.09, 134.97, 130.15, 129.44, 127.49, 125.76, 121.17 (2C), 120.89, 119.37, 118.34, 115.52, 34.46.

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

The same procedure described in section 4.3 was employed with **IIId** (0.23 g, 1 mmol), anhydrous ammonium acetate (0.77 g, 10 mmol), **Vk** (0.65 g, 2 mmol) and methanol (10 mL) at 100 °C for 36 h to yield 68 mg (0.19 mmol, 20%) as a light yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:1) R_f = 0.23, mp: 301.2–302.7 °C, HPLC: Retention time: 8.64 min, purity: 95.6%; ESI LC/MS (condition B): *m/z* calcd for $C_{23}H_{16}N_2O$ $[MH]^+$ 337.14; found 337.47. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.82 (s, 1H, 4-phenyl 4-OH), 8.76 (d, J = 6.05 Hz, 2H, 2-pyridyl H-2, H-6), 8.27 (d, J = 6.03 Hz, 2H, 2-pyridyl H-3, H-5), 8.15 (d, J = 7.62 Hz, 1H, indenopyridine H-9), 7.99 (s, 1H, indenopyridine H-3), 7.73 (d, J = 7.92 Hz, 2H, 4-phenyl H-2, H-6), 7.62 (d, J = 46 Hz, 1H, indenopyridine H-6), 7.54–7.46 (m, 2H, indenopyridine H-7, H-8), 6.94 (d, J = 8.43 Hz, 2H, 4-phenyl H-3, H-5), 4.14 (s, 2H, indenopyridine H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.46, 157.92, 144.51 (2C), 143.34, 140.98 (2C), 133.57 (2C), 130.29 (2C), 128.50 (2C), 127.32 (2C), 127.14, 125.66 (2C), 120.53 (2C), 115.74 (2C), 34.11.

4.4. Pharmacology

4.4.1. DNA topoisomerase I and IIα inhibition assay in vitro

DNA topo I inhibition assay was determined following the previously reported method [35]. The test compounds were dissolved in DMSO at 20 mM as a stock solution. The activities of DNA topo I and IIα were checked periodically by assessing the relaxation of supercoiled pBR322 DNA according to each reaction protocol described below because both enzymes are very labile during storage. Whenever both enzymes were used, the unit of each enzyme was re-determined and set as one unit when supercoiled pBR322 DNA was fully relaxed by reaction. The mixture of 100 ng of plasmid pBR322 DNA and 1 unit of recombinant human DNA topo I (TopoGEN INC., USA) was incubated without and with the prepared each compound 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 topo IIα inhibitory activity of compounds were measured as follows [36]. The mixture of 200 ng of supercoiled pBR322 plasmid DNA and 1 unit of human DNA topo 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 after each of topo I and IIα relaxation assay were analyzed on 1% agarose gel at 15 V for 7 h for topo I and 25 V for 4 h for topo IIα, respectively, 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).

4.4.2. Antiproliferative activity assay

Cancer and normal cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of 2–4 × 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 the next day, 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 followed by incubation for 72 h. Each well was then 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. Adriamycin, etoposide, and camptothecin were purchased from Sigma and used as positive controls.

4.4.3. Topo IIα-DNA cleavable complex assay

Cleavage complex assay was carried out by the previously reported methods with minor modification [37]. 100 ng supercoiled DNA pBR322 (Fermentas, USA) was reacted with 3 units of topo IIα (Usb Corp., USA) for 10 min before the addition of test compounds. The reaction mixture was incubated at 37 °C for 20 min and the reaction was stopped by addition of 10% SDS and 7 M EDTA followed by digestion with proteinase K at 45 °C for 30 min. After addition of loading buffer, the reaction mixture was heated for 2 min at 70 °C and electrophoresed with a 0.8% agarose gel in TAE buffer containing 0.5 μL/mL ethidium bromide, followed by destaining the gel with water for 20 min. The gel was visualized using an AlphaImager™ (Alpha Innotech Corporation).

4.4.4. Topo IIα ATPase activity assay

ATPase assays were performed using ATP dehydrogenase reaction with topo IIα. Reaction was performed in a 100 μL of mixture containing reaction buffer (10 mM tris-HCl, pH 7.9, 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 15 μg/mL BSA), supercoiled DNA pBR322 100 ng, ATP 250 μM, Topo IIα (Usb Corp., USA) 4 unit per well in the 96-well plate and incubated at 37 °C for 1 h. After reaction mixture was incubated for 30 min at room temperature with 20 μL of working reagent of malachite green phosphate assay kit (BioAssay Systems, USA) per well, and then

analyzed by ELISA Microplate reader (VERSAmax, Molecular Devices) at a wavelength of 620 nm.

4.4.5. DNA unwinding assay

The DNA unwinding capacity of compound **16** was analyzed using a DNA unwinding kit (TopoGEN, Port Orange, FL, USA) according to the manufacturer's instructions. Briefly, 100 ng pHOT1 plasmid DNA was treated with 3 units of topo I (TopoGEN, Port Orange, FL, USA) in 20 μL of reaction buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) for 30 min at 37 °C. Relaxed plasmids were then incubated in the presence of compound **16** at 37 °C for an additional 30 min. Amsacrine (100, 200, 500, and 1000 μM) was used as a positive control. The reaction was terminated by adding 1% SDS and loading dye at the end of the incubation and reaction product was resolved on 1% agarose gels at 15 V/cm for 12–15 h. After electrophoresis, gels were stained in TAE buffer with ethidium bromide for 30 min and visualized using an AlphaImager™ (Alpha Innotech Corporation).

4.4.6. Endogenous topo inhibitory activity assay in T47D cells

To analyze effect of compound **16** on intracellular topo-mediated DNA relaxation in cells, we prepared nuclear fractionations of compound-treated and untreated cells. Cells were seeded in 60 mm dishes at a density of 5×10^5 cells per dish and incubated until cells reached a confluence of 80%. Cells were washed with FBS-free medium and treated with compound for 24 h at the concentration indicated in figure legend. After treatment, cells were washed twice with PBS, and then centrifuged at 3200 rpm for 3 min at 4 °C. Nucleus was extracted from cell pellets using the buffer A (pH 7.9) containing 1 M HEPES, 1 M $MgCl_2$, 1 M KCl, 1 M DTT and 0.05% NP40 and the buffer B (pH 7.9) containing 1 M HEPES, 1 M $MgCl_2$, 0.5 M EDTA, 1 M DTT and 26% NP40 and 4.6 M NaCl as follows: addition of 500 μL of buffer A with 10 μL cocktail inhibitor to the pellet followed by ice incubation for 10 min and then centrifugation at 3200 rpm for 10 min at 4 °C. The pellet was only taken and followed by addition of 374 μL of buffer B with 26 μL of 4.6 M NaCl, ice incubation for 30 min and then centrifugation at 12,000 rpm for 20 min at 4 °C. The activity unit of DNA relaxation of prepared nuclear extract was defined through the determination of the amount of each nuclear extraction products that could completely relax 100 ng pBR322 DNA (Fermentas, USA) by incubating at 37 °C for 20 min. The DNA reacted with nuclear extract was separated via electrophoresis on a 1.0% agarose gel at 50 V for 50 min with TAE as the running buffer. The gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). The DNA bands were visualized via transillumination with a UV light and quantified using an AlphaImager™ (Alpha Innotech Corporation).

4.5. Molecular docking study

The structures used as templates for the structure-based docking study was prepared by using the X-ray structures (PDB code 1T8I [32] and 3QX3 [33] for human topo I and topo IIα, respectively. Since the structure of topo IIβ complexed with etoposide and DNA is the only structure available for drug bound topo II, homology model of topo IIα was generated by Modeller (ver. 9.13) [38] using 3QX3 as the template. All the water molecules and ligands were removed and the hydrogen atoms were added. The coordinate file for the structure of compound **16** was constructed in Sybyl X-2.1.1 and energetically minimized using a Tripos force field with Gasteiger-Huckel charges. The receptor and ligand file were prepared according to the original publication protocols [39, 40]. Docking was carried out with both programs of AutoDock using the Lamarckian Genetic Algorithm and Sybyl X-2.1.1. Default search parameters were used except for 270,000 population size and 50 docking runs. The ligand was redocked to the receptor and analyzed with the original ligand bound to the receptor to validate the method.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.09.019.

References

[1] B.A. Chabner, T.G. Roberts, Chemotherapy and the war on cancer, Nat. Rev. Cancer 5 (2005) 65–72.
[2] P. Forterre, S. Gribaldo, D. Gadelle, M.-C. Serre, Origin and evolution of DNA topoisomerases, Biochimie 89 (2007) 427–446.
[3] C. Bailly, Contemporary challenges in the design of topoisomerase II inhibitors for cancer chemotherapy, Chem. Rev. 112 (2012) 3611–3640.
[4] O. Sundin, A. Varshavsky, Arrest of segregation leads to accumulation of highly intertwined catenated dimers: dissection of the final stages of SV40 DNA replication, Cell 25 (1981) 659–669.
[5] A.L. Bodley, L.F. Liu, Topoisomerases as novel targets for cancer chemotherapy, Nat. Biotech. 6 (1988) 1315–1319.
[6] R. Miri, K. Javidnia, B. Hemmateenejad, A. Azarpira, Z. Amirghofran, Synthesis, cytotoxicity, QSAR, and intercalation study of new diindenopyridine derivatives, Bioorg. Med. Chem. 12 (2004) 2529–2536.
[7] M.M. Ghorab, M.S. Al-Said, Anticancer activity of novel indenopyridine derivatives, Arch. Pharm. Res. 35 (2012) 987–994.
[8] T. Utsugi, K. Aoyagi, T. Asao, S. Okazaki, Y. Aoyagi, M. Sano, K. Wierzba, Y. Yamada, Antitumor activity of a novel quinoline derivative, TAS-103, with inhibitory effects on topoisomerases I and II, Jpn. J. Cancer Res. 88 (1997) 992–1002.
[9] M. Manpadi, P.Y. Uglinskii, S.K. Rastogi, K.M. Cotter, Y.S. Wong, L.A. Anderson, A.J. Ortega, S. Van Slambrouck, W.F. Steelant, S. Rogelj, P. Tongwa, M.Y. Antipin, I.V. Magedov, A. Kornienko, Three-component synthesis and anticancer evaluation of polycyclic indenopyridines lead to the discovery of a novel indenoheterocycle with potent apoptosis inducing properties, Org. Biomol. Chem. 5 (2007) 3865–3872.
[10] A.T. Baviskar, S.M. Amrutkar, N. Trivedi, V. Chaudhary, A. Nayak, S.K. Guchhait, U.C. Banerjee, P.V. Bharatam, C.N. Kundu, Switch in site of inhibition: a strategy for structure-based discovery of human topoisomerase IIα catalytic inhibitors, ACS Med. Chem. Lett. 6 (2015) 481–485.
[11] A.T. Baviskar, C. Madaan, R. Preet, P. Mohapatra, V. Jain, A. Agarwal, S.K. Guchhait, C.N. Kundu, U.C. Banerjee, P.V. Bharatam, N-fused imidazoles as novel anticancer agents that inhibit catalytic activity of topoisomerase IIα and induce apoptosis in G1/S phase, J. Med. Chem. 54 (2011) 5013–5030.
[12] D.B. Khadka, G.H. Tran, S. Shin, H.T. Nguyen, H.T. Cao, C. Zhao, Y. Jin, H.T. Van, M.V. Chau, Y. Kwon, T.N. Le, W.J. Cho, Substituted 2-arylquinazolinones: design, synthesis, and evaluation of cytotoxicity and inhibition of topoisomerases, Eur. J. Med. Chem. 103 (2015) 69–79.
[13] P. Majumdar, C. Bathula, S.M. Basu, S.K. Das, R. Agarwal, S. Hati, A. Singh, S. Sen, B.B. Das, Design, synthesis and evaluation of thiohydantoin derivatives as potent topoisomerase I (Top1) inhibitors with anticancer activity, Eur. J. Med. Chem. 102 (2015) 540–551.
[14] H. Hao, W. Chen, J. Zhu, C. Lu, Y. Shen, Design and synthesis of 2-phenylnaphthalenoids and 2-phenylbenzofuranoids as DNA topoisomerase inhibitors and antitumor agents, Eur. J. Med. Chem. 102 (2015) 277–287.
[15] T.M. Kadayat, C. Song, S. Shin, T.B.T. Magar, G. Bist, A. Shrestha, P. Thapa, Y. Na, Y. Kwon, E.-S. Lee, Synthesis, topoisomerase I and II inhibitory activity, cytotoxicity, and structure–activity relationship study of 2-phenyl- or hydroxylated 2-phenyl-4-aryl-5H-indeno[1,2-b]pyridines, Bioorg. Med. Chem. 23 (2015) 3499–3512.
[16] R. Karki, C. Park, K.-Y. Jun, T.M. Kadayat, E.-S. Lee, Y. Kwon, Synthesis and biological activity of 2,4-di-p-phenolyl-6-2-furanyl-pyridine as a potent topoisomerase II poison, Eur. J. Med. Chem. 90 (2015) 360–378.
[17] K.-Y. Jun, H. Kwon, S.-E. Park, E. Lee, R. Karki, P. Thapa, J.-H. Lee, E.-S. Lee, Y. Kwon, Discovery of dihydroxylated 2,4-diphenyl-6-thiophen-2-yl-pyridine as a non-intercalative DNA-binding topoisomerase II-specific catalytic inhibitor, Eur. J. Med. Chem. 80 (2014) 428–438.
[18] T.M. Kadayat, C. Park, K.-Y. Jun, T.B.T. Magar, G. Bist, H.Y. Yoo, Y. Kwon, E.-S. Lee, Design and synthesis of novel 2,4-diaryl-5H-indeno[1,2-b]pyridine derivatives, and their evaluation of topoisomerase inhibitory activity and cytotoxicity, Bioorg. Med. Chem. 23 (2015) 160–173.

[19] T.M. Kadayat, C. Song, Y. Kwon, E.-S. Lee, Modified 2,4-diaryl-5H-indeno[1,2-b]pyridines with hydroxyl and chlorine moiety: synthesis, anticancer activity, and structure–activity relationship study, Bioorg. Chem. 62 (2015) 30–40.
[20] D. Batovska, S. Parushev, A. Slavova, V. Bankova, I. Tsvetkova, M. Ninova, H. Najdenski, Study on the substituents' effects of a series of synthetic chalcones against the yeast Candida albicans, Eur. J. Med. Chem. 42 (2007) 87–92.
[21] F. Kröhnke, Syntheses using pyridinium salts (IV), Angew. Chem. Int. Ed. 2 (1963) 380–393.
[22] F. Krohnke, Synthesis using pyridinium salts. 5. Specific synthesis of pyridines and oligopyridines, Synthesis 1 (1976) 1–24.
[23] T.A. Järvinen, J. Kononen, M. Pelto-Huikko, J. Isola, Expression of topoisomerase IIalpha is associated with rapid cell proliferation, aneuploidy, and c-erbB2 overexpression in breast cancer, Am. J. Pathol. 148 (1996) 2073–2082.
[24] C.S. Downes, D.J. Clarke, A.M. Mullinger, J.F. Giménez-Abián, A.M. Creighton, R.T. Johnson, A topoisomerase II-dependent G2 cycle checkpoint in mammalian cells, Nature 372 (1994) 467–470.
[25] C.S. Downes 1, A.M. Mullinger, R.T. Johnson, Inhibitors of DNA topoisomerase II prevent chromatid separation in mammalian cells but do not prevent exit from mitosis, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 8895–8899.
[26] P. Furet, J. Schoepfer, T. Radimerski, P. Chène, Discovery of a new class of catalytic topoisomerase II inhibitors targeting the ATP-binding site by structure based design. Part I, Bioorg. Med. Chem. Lett. 19 (2009) 4014–4017.
[27] J. Qiu, B. Zhao, W. Zhong, Y. Shen, H. Lin, Synthesis, biological evaluation and modeling studies of terphenyl topoisomerase IIα inhibitors as anticancer agents, Eur. J. Med. Chem. 94 (2015) 427–435.
[28] J.L. Nitiss, DNA topoisomerase II and its growing repertoire of biological functions, Nat. Rev. Cancer 9 (2009) 327–337.
[29] J.L. Nitiss, Targeting DNA topoisomerase II in cancer chemotherapy, Nat. Rev. Cancer 9 (2009) 338–350.
[30] A.K. Larsen, A.E. Escargueil, A. Skladanowski, Catalytic topoisomerase II inhibitors in cancer therapy, Pharmacol. Ther. 99 (2003) 167–181.
[31] R. Karki, C. Park, K.-Y. Jun, T.M. Kadayat, E.-S. Lee, Y. Kwon, Synthesis and biological activity of 2,4-di-p-phenolyl-6-2-furanyl-pyridine as a potent topoisomerase II poison, Eur. J. Med. Chem. 90 (2015) 360–378.
[32] B.L. Staker, M.D. Feese, M. Cushman, Y. Pommier, D. Zembower, L. Stewart, A.B. Burgin, Structures of three classes of anticancer agents bound to the human topoisomerase I-DNA covalent complex, J. Med. Chem. 48 (2005) 2336–2345.
[33] C.-C. Wu, T.-K. Li, L. Farh, L.-Y. Lin, T.-S. Lin, Y.-J. Yu, T.-J. Yen, C.-W. Chiang, N.-L. Chan, Structural basis of type II topoisomerase inhibition by the anticancer drug etoposide, Science 333 (2011) 459–462.
[34] J.M. Fortune, N. Osheroff, Merbarone inhibits the catalytic activity of human topoisomerase IIα by blocking DNA cleavage, J. Biol. Chem. 273 (1998) 17643–17650.
[35] J.-F. Riou, P. Fossé, C.H. Nguyen, A.K. Larsen, M.-C. Bissery, L. Grondard, J.-M. Saucier, E. Bisagni, F. Lavelle, Intoplicine (RP 60475) and its derivatives, a new class of antitumor agents inhibiting both topoisomerase I and II activities, Cancer Res. 53 (1993) 5987–5993.
[36] D.-H. Kang, J.-S. Kim, M.-J. Jung, E.-S. Lee, Y. Jahng, Y. Kwon, Y. Na, New insight for fluoroquinophenoxazine derivatives as possibly new potent topoisomerase I inhibitor, Bioorg. Med. Chem. Lett. 18 (2008) 1520–1524.
[37] A.S. Krishna, G. Panda, A.K. Kondapi, Mechanism of action of ferrocene derivatives on the catalytic activity of topoisomerase IIα and β—Distinct mode of action of two derivatives, Arch. Biochem. Biophys. 438 (2005) 206–216.
[38] A. Sali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815.
[39] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, Automated docking using a lamarckian genetic algorithm and empirical binding free energy function, J. Comput. Chem. 19 (1998) 1639–1662.
[40] M.F. Sanner, Python: a programming language for software integration and development, J. Mol. Graph. Mod. 17 (1999) 57–61.