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Effect of chlorine substituent on cytotoxic activities: Design and synthesis of systematically modified 2,4-diphenyl-5*H*-indeno[1,2-*b*] pyridines

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ABSTRACT

In continuation of our previous work, six hydroxylated 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridine analogs were modified by introducing one chlorine functionality at *ortho*, *meta* or *para* position of the 2- or 4-phenyl ring. Eighteen new chlorinated compounds were thus prepared and assessed for topoisomerase inhibitory activity and cytotoxicity against HCT15, T47D, and HeLa cancer cell lines. All of the chlorinated compounds displayed significant cytotoxic effect, revealing potent anticancer activity against T47D breast cancer cells. This functional group modification allowed us to explore the importance of chlorine group substitution for the cytotoxic properties. The information reported here provides valuable insight for further study to develop new anticancer agents using related scaffolds.

Functional group modification of biologically active compounds is one of the most efficient strategies in the field of medicinal chemistry. In the search of novel anticancer agents, our research group has been designing compounds containing indenopyridine skeleton which are reported to have anti-inflammatory activity and cytotoxicity against different human cancer cell lines.[1–4] We recently reported a series of analogs of 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridine (**1**) containing *ortho*, *meta* or *para* hydroxyl group at 2- or 4-phenyl ring (Fig. 1). The structure–activity relationship study revealed that introduction of hydroxyl group at *meta* or *para* positions of 2- or 4-phenyl ring is important for displaying significant topoisomerase inhibitory activity and cytotoxicity.[5] Topoisomerase (topo) plays an important role in solving various DNA topological problems occurred during DNA replication and other vital cellular processes, and consequently is considered one of the attractive molecular targets for the development of anticancer agents.[6,7]

Over the past few years, substitution of halogen group on the several compounds is attempted for modifying their chemical and pharmacological activities.[8–10] Furthermore, introduction of chlorine atom is reported to improve the stability, potency and specificity of the binding site due to its ability to accommodate in tight and deep hydrophobic pockets of the biological targets.[11,12] Therefore, with an aim to further explore structure–activity relationships, we decided to incorporate a chlorine functionality at *ortho*, *meta* or *para* positions of the 2- or 4-phenyl ring of hydroxylated 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridine compounds (**2**–**7**).

Herein, we report the systematic design (Fig. 2), synthesis, and topo inhibitory activity and cytotoxicity of novel 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridines containing hydroxyl and chlorine moiety at 2- or 4-phenyl ring of 5*H*-indeno[1,2-*b*]pyridine. Total eighteen modified 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridine compounds (**8**–**25**) were synthesized in six different series containing one phenolic ring and one chlorophenyl ring on 2- or 4-position of the central pyridine (Fig. 3).

The synthetic route with three different steps for the target compounds **8**–**25** is outlined in Scheme 1. In the first step, 1-indanone (**I**) was condensed with aryl aldehydes **II** (R^1 = **a**–**f**) to prepare indanone intermediates **III** (R^1 = **a**–**f**) in the presence of 5% aqueous NaOH in ethanol using *Claisen–Schmidt* condensation reaction.[13] Then, six pyridinium iodide salts **V** (R^2 = **a**–**f**) were synthesized by refluxing acetophenones **IV** (R^2 = **a**–**f**) with iodine in pyridine. Finally, using modified Kröhnke synthesis,[14,15] indanone intermediates **III**

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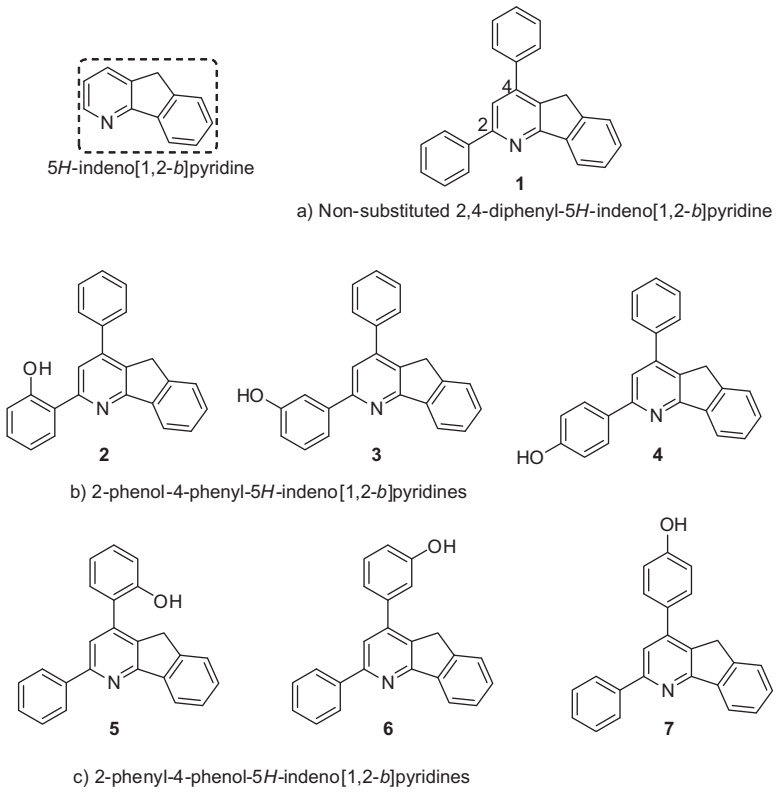


Figure 1. Structures of previously synthesized 5*H*-indeno[1,2-*b*]pyridine derivatives; (a) non-substituted, (b) 2-phenol-4-phenyl substituted, and (c) 4-phenol-2-phenyl substituted -5*H*-indeno[1,2-*b*]pyridines.

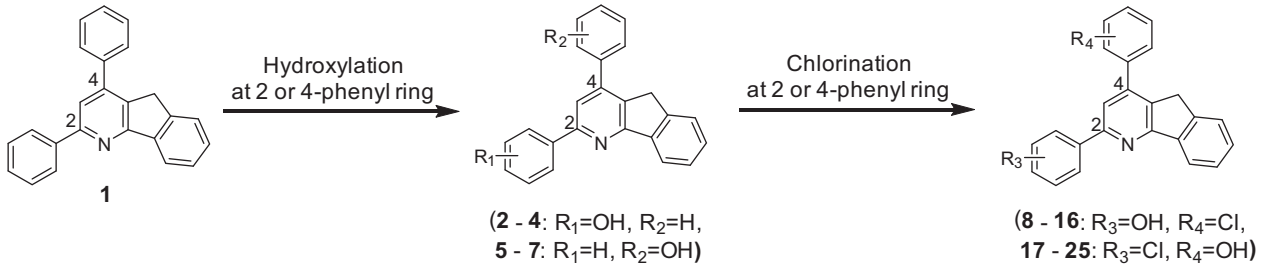


Figure 2. Strategy for the design of chloro- and hydroxy-substituted 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridines.

(R^1 = **a**–**f**) and pyridinium iodide salts **V** (R^2 = **a**–**f**) were reacted in the presence of dry ammonium acetate in methanol or acetic acid to give final compounds **8**–**25** in the yields of 23.3–69.0%. Although, protection of hydroxyl groups was not needed for the preparation of the final compounds, yields of the compounds **8**–**10** having hydroxyl groups at *ortho* position of 2-phenyl ring was relatively lower than *meta* or *para* hydroxyl substituted compounds. This may be due to the formation of intermolecular H-bonding, and the higher steric hindrance of compounds bearing *ortho* hydroxyl substitution at 2-phenyl ring. Structures, yields (%), HPLC purities (%), and melting point (°C) of the prepared compounds are shown in Table S1 of Supporting information.

All the prepared compounds were evaluated for topo I and II inhibitory activity and cytotoxicity against several human cancer cell lines. Figure 4A and Table 1 summarize the topo I inhibitory activity of the synthesized compounds (**8**–**25**). Except compounds **11** and **15**, all of the compounds containing one hydroxyl and one chlorine group at 2- or 4-phenyl ring of the central pyridine showed very weak topo I inhibitory activities (0.8–19.1% inhibition at 100 μM) as compared to the positive control, camptothecin (74.5% inhibition at 100 μM). Compounds **11** and **15** displayed moderate topo I inhibitory activity (29.3% and 35.5%, respectively) at 100 μM concentration. None of the compounds were active at 20 μM. Similarly, all of the compounds showed very weak topo II inhibitory activity as compared to the positive control, etoposide at 100 μM (Fig. 4B and Table 1).

Evaluation of anticancer activity for the prepared compounds was performed against three different human cancer cell lines: human colorectal adenocarcinoma cell line (HCT15), human ductal breast epithelial tumor cell line (T47D), and human cervix tumor cell line (HeLa) with adriamycin, etoposide, and camptothecin as positive controls. The inhibitory activity (IC_{50}) of the synthesized compounds (**8**–**25**) is expressed in micromolar concentration as summarized in Table 1. Except compounds **8**–**10**, **20**, and **23**, all the compounds showed stronger or moderate cytotoxicity (IC_{50} < 7.01 μM) than positive controls. Among the compounds tested against HCT15 colon cancer cells, compounds **11**, **14**–**16**, **18**, **22**, and **25** displayed stronger cytotoxicity (IC_{50} < 1.17 μM) than etoposide (6.28 μM) and camptothecin (1.29 μM), but weaker than adriamycin (0.09 μM). Similarly, among the compounds tested

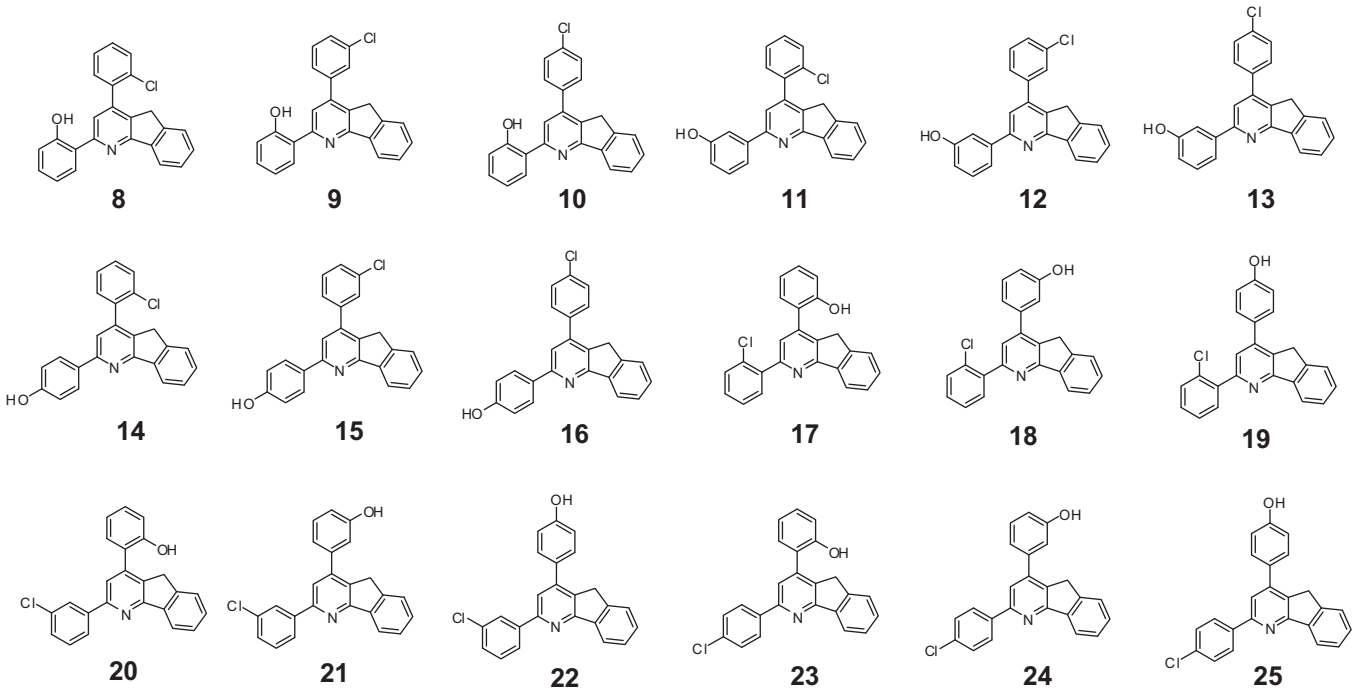
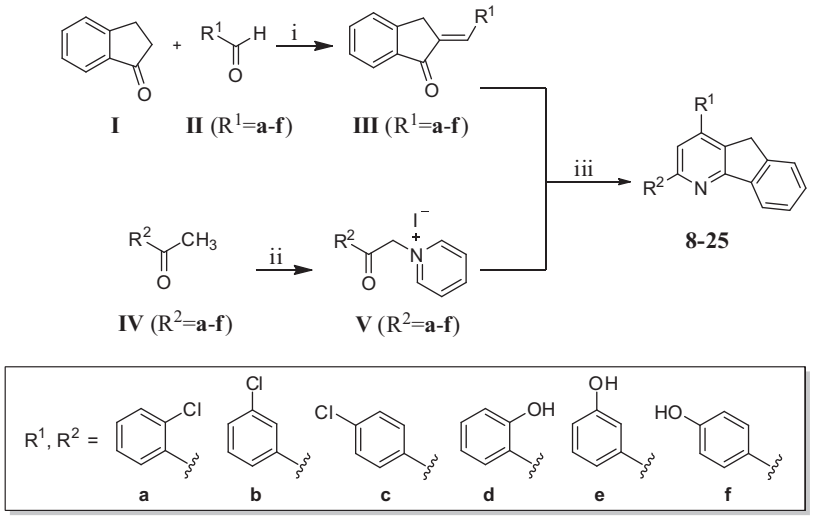


Figure 3. Structures of the prepared chloro- and hydroxy-substituted 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridines.



Scheme 1. General synthetic method of chloro- and hydroxy-substituted 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridines. Reagents and conditions: (i) aq NaOH (5%), EtOH, 1–12 h, room temperature, 61.9–99.7% yield; (ii) pyridine (15 equiv), iodine, 3 h, 140 °C, 64.7–93.2% yield; (iii) NH_4OAc (10 equiv), methanol or glacial acetic acid, 24–36 h, 100 °C, 23.3–69.0% yield.

against T47D breast cancer cells, compounds **11**–**19**, **21**, **22**, **24**, and **25** displayed stronger cytotoxicity (IC_{50} < 6.64 μM) than etoposide (57.9 μM) and camptothecin (11.2 μM), and similar or moderate cytotoxicity compared to adriamycin (1.47 μM). Likewise, for the HeLa cervix cancer cells, most of the compounds displayed significant cytotoxicity than etoposide but moderate as compared to adriamycin and camptothecin.

Compounds **11**–**16** with *meta*- or *para*-phenolic ring at 2-position and chlorophenyl moiety at 4-position of central pyridine showed the significant cytotoxicity with a range of IC_{50} values of 0.81–3.68 μM against HCT15, T47D and HeLa cells. Similarly, compounds **18**, **19**, **21**, **22**, **24**, and **25** with *meta* or *para*-phenolic ring at 4-position and chlorophenyl moiety at 2-position of central pyridine showed significant cytotoxicity with a range of IC_{50} values of 0.31–4.59 μM against HCT15, T47D and HeLa cells. Compounds **8**–**10**, **17**, **20**, and **23** displayed weak cytotoxicity against all the tested cancer cells. Interestingly, it was observed that combination of *meta*- or *para*-phenolic group with chlorophenyl ring is favorable for the cytotoxic activity.

We investigated the structure–activity relationship study by assessing the role of substituted chlorine group in the biological activity of modified 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridines. Introduction of hydroxyl moiety at 2- or 4-phenyl ring (compounds **2**–**7**) of the 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridine (**1**) significantly increased topo II inhibitory activity. However, further substitution of a chlorine moiety at 2- or 4-phenyl ring (compounds **8**–**25**) of the corresponding hydroxylated 2,4-diphenyl-5*H*-indeno [1,2-*b*]pyridines (**2**–**7**) resulted decrease in the topo II inhibitory

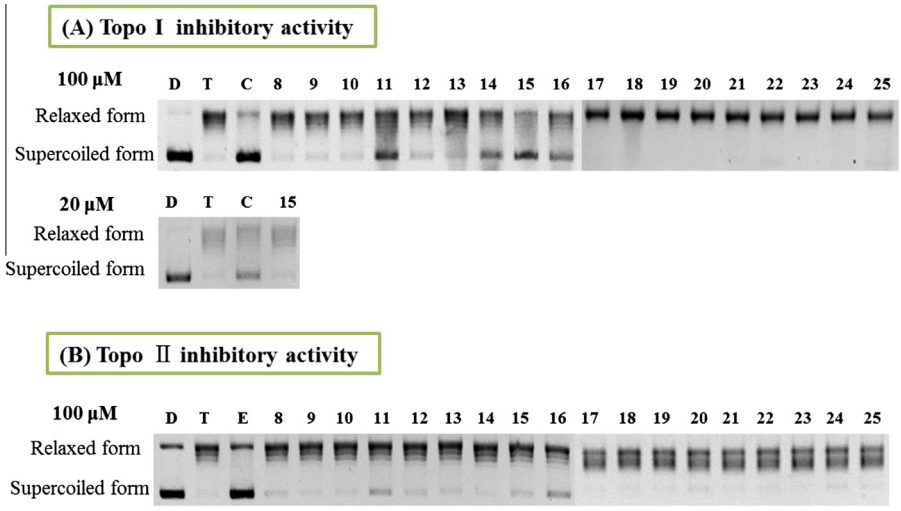


Figure 4. Human DNA topo I (A) and topo II (B) inhibitory activity of the prepared compounds **8**–**25** at the concentration of 100 μM and 20 μM, respectively. (A) Lane D: pBR322 DNA only; lane T: pBR322 DNA + topo I; lane C: pBR322 DNA + topo I + camptothecin; lanes 8–25: pBR322 DNA + topo I + each of compounds **8**–**25**. (B) Lane D: pBR322 DNA only; lane T: pBR322 DNA + topo II; lane E: pBR322 DNA + topo II + etoposide; lanes 8–25: pBR322 DNA + topo II + each of compounds **8**–**25**.

Table 1
Topo I and II inhibitory activity and cytotoxicity of the prepared compounds

Compounds	Topo I (% inhibition)		Topo II (% inhibition)	IC_{50}* (μM)		
	100 μM	20 μM	100 μM	HCT15	T47D	HeLa
Adriamycin				1.23 ± 0.00[a]/0.09 ± 0.01[b]	1.34 ± 0.03[a]/1.47 ± 0.02[b]	0.88 ± 0.08[a]/0.82 ± 0.00[b]
Etoposide			58.6[a]/81.5[b]	2.82 ± 0.24[a]/6.28 ± 0.19[b]	1.84 ± 0.44[a]/57.98 ± 0.25[b]	0.18 ± 0.02[a]/15.03 ± 0.17[b]
Camptothecin	58.7[a]/74.5[b]	27.6[a]/33.5[b]		18.87 ± 0.34[a]/1.29 ± 0.01[b]	13.7 ± 0.81[a]/11.17 ± 0.58[b]	7.32 ± 0.15[a]/0.59 ± 0.02[b]
1	0.0	ND	17.8	>50	26.8 ± 0.26	17.3 ± 0.56
2	1.3	ND	36.4	>50	>50	>50
3	2.3	ND	76.0	2.71 ± 0.12	3.93 ± 0.17	2.99 ± 0.31
4	5.2	ND	80.6	2.01 ± 0.18	2.10 ± 0.04	3.93 ± 0.07
5	15.1	ND	69.6	>50	>50	>50
6	20.6	ND	70.5	1.35 ± 0.03	1.55 ± 0.04	4.00 ± 0.15
7	25.9	ND	65.9	2.15 ± 0.12	1.36 ± 0.04	4.84 ± 0.05
8	0.0	ND	4.8	>50	>50	>50
9	1.1	ND	2.8	>50	>50	>50
10	8.3	ND	2.8	>50	>50	>50
11	29.3	ND	6.7	1.17 ± 0.02	2.19 ± 0.02	2.65 ± 0.16
12	7.5	ND	4.4	1.59 ± 0.04	1.85 ± 0.31	1.91 ± 0.01
13	5.8	ND	3.8	2.09 ± 0.01	1.49 ± 0.04	2.27 ± 0.02
14	15.5	ND	1.6	0.84 ± 0.00	1.63 ± 0.01	3.41 ± 0.12
15	35.5	2.8	4.9	0.81 ± 0.00	1.63 ± 0.03	3.68 ± 0.05
16	19.1	ND	8.5	0.93 ± 0.0	0.88 ± 0.02	2.45 ± 0.01
17	0.8	ND	1.9	6.23 ± 0.13	6.64 ± 0.04	7.01 ± 0.09
18	0.0	ND	3.1	0.40 ± 0.09	0.31 ± 0.04	2.01 ± 0.26
19	0.0	ND	3.5	1.82 ± 0.02	0.79 ± 0.05	2.70 ± 0.06
20	0.0	ND	5.4	30.26 ± 0.97	>50	17.07 ± 0.56
21	0.0	ND	3.2	3.09 ± 0.03	1.32 ± 0.03	4.59 ± 0.02
22	0.0	ND	3.0	0.31 ± 0.01	1.42 ± 0.09	5.04 ± 0.14
23	0.0	ND	2.7	>50	>50	>50
24	0.0	ND	3.9	1.88 ± 0.01	1.36 ± 0.02	6.58 ± 0.06
25	1.4	ND	3.2	0.87 ± 0.01	1.27 ± 0.06	4.34 ± 0.16

ND: not determined; HCT15: human colorectal adenocarcinoma; T47D: human breast ductal carcinoma; HeLa: human cervix adenocarcinoma cell line; Adriamycin: positive control for cytotoxicity; Etoposide: positive control for topo II and cytotoxicity; Camptothecin: positive control for topo I and cytotoxicity.

[a] Control value for previously reported compounds **1**–**7**.[5]

[b] Control value for the compounds **8**–**25**.

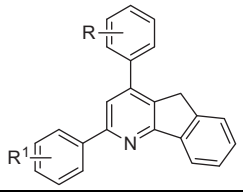
* Each data represents mean ± SD from three different experiments performed in triplicate.

activity. From the results, reported in Table 1, it is evident that chlorine moiety substitution in 2- or 4-phenyl ring of 5*H*-indeno [1,2-*b*]pyridine skeleton is not favorable for displaying topo II inhibitory activity.

As expected from the result of previously reported compound (**2** and **5**), compounds **8**–**10**, **17**, **20**, and **23** that contain *ortho*-hydroxyl moiety at 2- or 4-phenyl ring were less active against tested cancer cells. However, compounds **11**–**16**, **18**, **19**, **21**, **22**, **24**, and **25** containing *meta* or *para*-phenolic ring on 2 or 4-position displayed significant cytotoxicity. It is interesting to note that introduction of chlorine group on *meta*- or *para*-hydroxylated 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridine resulted in significant cytotoxicity against all the tested cells as compared to etoposide, and moderate activity than that of adriamycin and camptothecin.

Table 2
Relative potency of representative compounds showing effect of position of *meta* or *para*-phenolic moiety at 2 or 4-position of 5*H*-indeno[1,2-*b*]pyridines

Relative potency* for cytotoxicity compared to positive control (adriamycin)



Compound	IC_{50} (μM)			Compound	IC_{50} (μM)		
	HCT15	T47D	HeLa		HCT15	T47D	HeLa
Adriamycin[a] (IC_{50})	1.23 ± 0.0	1.34 ± 0.3	0.88 ± 0.1	Adriamycin[b] (IC_{50})	0.09 ± 0.0	1.47 ± 0.0	0.82 ± 0.0
3: R = H, R^1 = *meta*-OH	0.45	0.34	0.29	**11**: R = *ortho*-Cl, R^1 = *meta*-OH	0.08	0.67	0.31
				12: R = *meta*-Cl, R^1 = *meta*-OH	0.06	0.79	0.43
				13: R = *para*-Cl, R^1 = *meta*-OH	0.05	0.99	0.36
4: R = H, R^1 = *para*-OH	0.61	0.63	0.22	**14**: R = *ortho*-Cl, R^1 = *para*-OH	0.12	0.90	0.24
				15: R = *meta*-Cl, R^1 = *para*-OH	0.12	0.90	0.22
				16: R = *para*-Cl, R^1 = *para*-OH	0.11	1.67	0.33
6: R = *meta*-OH, R^1 = H	0.91	0.86	0.22	**18**: R = *meta*-OH, R^1 = *ortho*-Cl	0.25	4.74	0.41
				19: R = *para*-OH, R^1 = *ortho*-Cl	0.05	1.86	0.30
				21: R = *meta*-OH, R^1 = *meta*-Cl	0.03	1.11	0.18
7: R = *para*-OH, R^1 = H	0.57	0.98	0.18	**22**: R = *para*-OH, R^1 = *meta*-Cl	0.32	1.03	0.16
				24: R = *meta*-OH, R^1 = *para*-Cl	0.05	1.08	0.12
				25: R = *para*-OH, R^1 = *para*-Cl	0.11	1.16	0.19

* Relative potency: IC_{50} of positive control adriamycin/IC_{50} of the compound.
[a] IC_{50} control value of adriamycin for the previously reported compounds **3**, **4**, **6** and **7**.
[b] IC_{50} control value of adriamycin for newly synthesized compounds **11**–**16**, **18**, **19**, **21**, **22**, **24**, and **25**.

From this result, it can be implied that introduction of *meta*- or *para*-phenolic ring and chlorophenyl ring at the 2- or 4-position is important for cytotoxic activity. Chlorinated compounds are generally more active against T47D breast cancer cells among all the tested cancer cells.

Table 2 illustrates relative potencies for cytotoxicity (against HCT15, T47D, and HeLa cell lines) of the representative compounds **11**–**16**, **18**, **19**, **21**, **22**, **24**, and **25** compared to adriamycin, a widely used anticancer drug as positive control, for easier comparison with previously synthesized compounds **3**, **4**, **6** and **7**. Interestingly, compared with the corresponding *meta*- or *para*-hydroxylated 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridines (**3**, **4**, **6** and **7**), the presence of chlorine moiety enhanced the cytotoxicity against T47D breast cancer cells. Chlorinated compounds were less active against HCT15 cells. However, these chlorinated compounds had similar or greater cytotoxic activity than their non-chlorinated analogs against HeLa cell lines. Moreover, compounds **16**, **18**, **19**, **21**, **22**, **24**, and **25** exhibited stronger activities (Relative Potency > 1) compared to positive control, adriamycin against T47D breast cancer cells. This result suggests that introduction of chlorine group at 2-phenyl ring and *meta* or *para*-hydroxyl group at 4-phenyl ring of 5*H*-indeno[1,2-*b*]pyridine skeleton is important for potent cytotoxic effect.

In conclusion, we systematically designed and prepared a new series of eighteen chloro- and hydroxy-substituted 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridines using modified Kröhnke synthesis, and evaluated their topo I and II inhibitory activity and cytotoxicity against HCT15, T47D, and HeLa cancer cell lines. Introduction of chlorine group on 2- or 4-phenyl ring of their corresponding hydroxylated analogs resulted in decrease of topo I and II inhibitory activity. However, these chlorinated compounds displayed significant cytotoxic activity against tested cancer cell lines. All of the chlorinated compounds exhibited stronger cytotoxic effect compared to corresponding non-chlorinated compounds against T47D breast cancer cells. Particularly, compounds **16**, **18**, **19**, **21**, **22**, **24**, and **25** were found to be more cytotoxic than adriamycin against T47D cells. These results suggest that introduction of chlorine and hydroxyl moiety on 2,4-diphenyl-5*H*-indeno[1,2-*b*]pyridine skeleton is important for displaying the significant cytotoxic activity. Thus, this study may provide valuable information to medicinal chemist working with indenopyridines containing chlorine or hydroxyl functionalities. Experiment to investigate the mechanistic pathway of these chlorinated compounds is a future scope of our research that seeks to develop novel and potent anticancer agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.053.

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