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Design, synthesis, and structure-activity relationships of new benzofuro[3,2-*b*]pyridin-7-ols as DNA topoisomerase II inhibitors

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ABSTRACT

Human DNA topoisomerases have become attractive targets for developing more effective anticancer drugs. In this study, a series of new benzofuro[3,2-*b*]pyridin-7-ols were designed and synthesized for the first time and screened for their topoisomerase I and II inhibitory and antiproliferative activity. Structure-activity relationships revealed the position of *ortho*- and *para*-hydroxyl group at 2-phenyl ring, and *meta*-hydroxyl group at 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol are important for potent and selective topo II inhibitory activity. Compound **11** showed the most selective and potent topo II inhibition (100% inhibition at 100 μM) and strongest antiproliferative activity (IC_{50} = 0.86 μM) than all the positive controls in HeLa cell line.

Topoisomerases (topos) are DNA binding nuclear protein that regulates DNA topology by releasing the torsional stress which occurs during cell-proliferation, transcription, recombination, mitosis and chromosomal segregation.[1] Because of their vital role, topos are key mechanistic targets of many anticancer agents whose inhibition impedes fundamental metabolic processes of cell growth and cell death.[2–4] In mammalian cells, based on the molecular mechanism of topo enzymes, they are divided into two forms: topo I that breaks only single strand, and topo II that cuts both strands of DNA.[5,6] Several studies based on natural and synthetic products as novel topo inhibitors is ongoing to overcome side effects and limitations of clinically used topo inhibitors such as adriamycin, etoposide, and camptothecin.[7,8]

In the recent years, bioactive compounds with fused heterocyclic rings containing nitrogen and oxygen atoms are gaining more popularity in drug discovery due to its diverse biological activities.[9] Moreover, many studies have reported that compounds containing benzofuran as an active pharmacophore exhibited wide range of pharmacological effects such as anti-inflammatory, anticancer, antiviral, and anti-hyperlipidemic activities.[10] Our research group has focused on study of topo inhibitory and antiproliferative activity of various fused heterocyclic structures containing

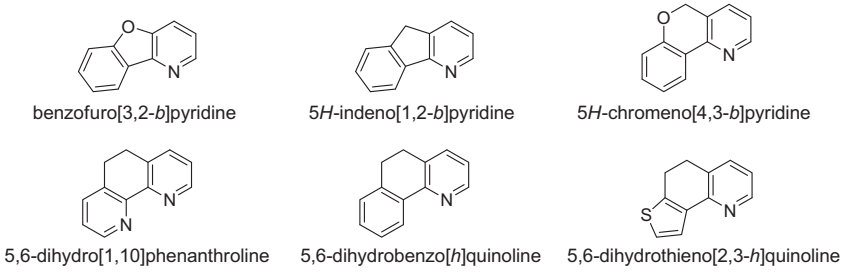

Fig. 1. Fused heterocyclic structures containing pyridine ring.

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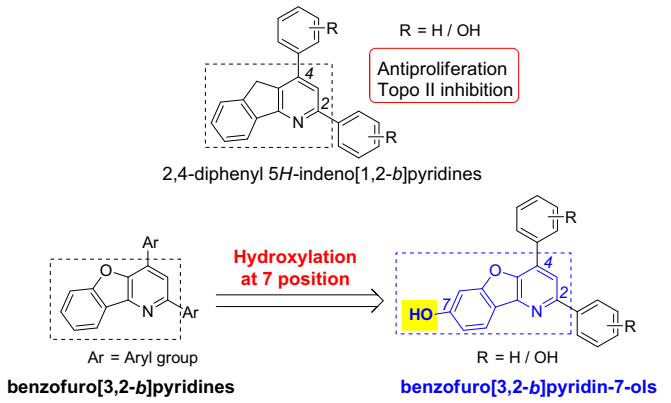
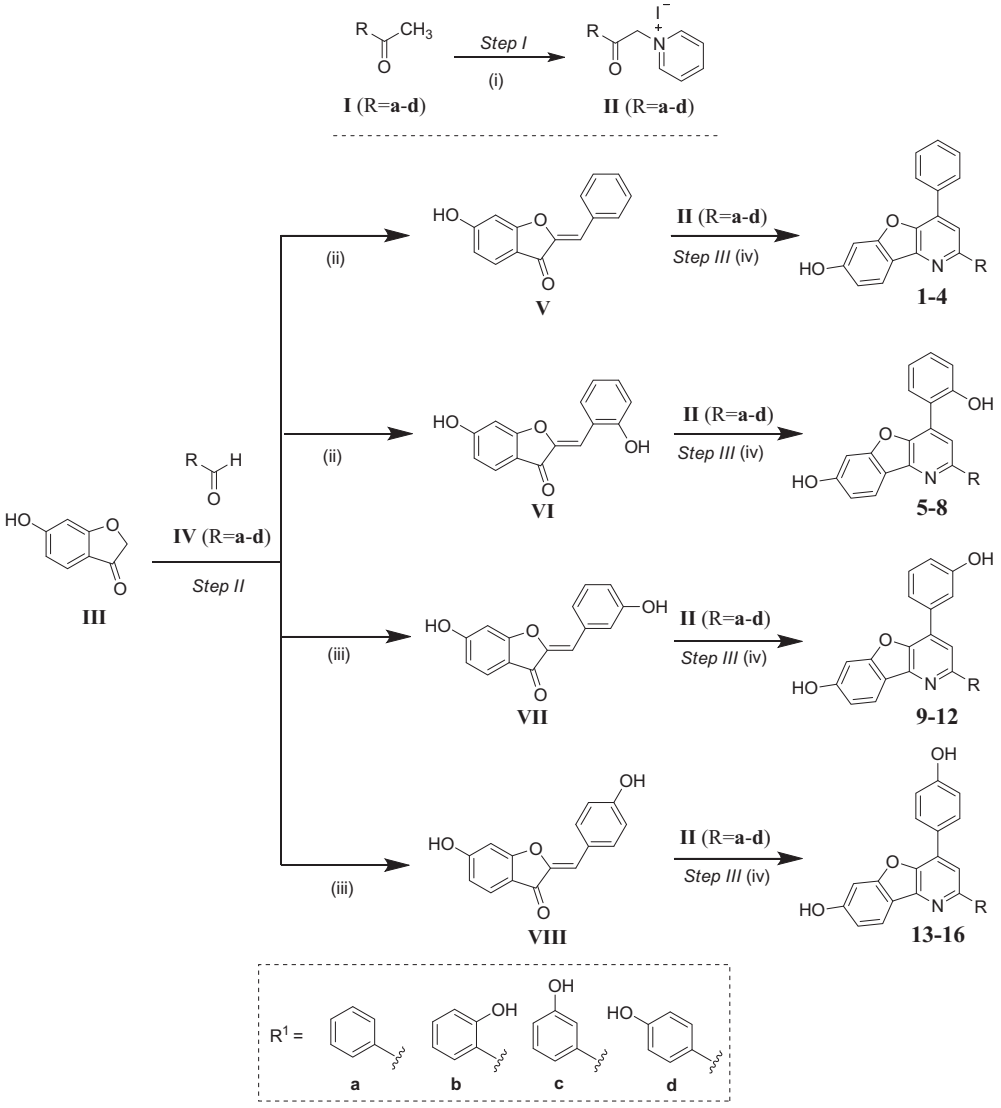


Fig. 2. Designed target compounds.

pyridine ring (Fig. 1).[11–15] Recently, we reported importance of hydroxyl group in 2- and/or 4-phenyl rings of benzofuro[3,2-*b*]pyridine and 5*H*-indeno[1,2-*b*]pyridine.[13,14] Inspired by these results, we were encouraged to further investigate the effect on topo inhibitory activity and antiproliferative activity by incorporating a hydroxyl substituent at 7-position of benzofuro[3,2-*b*]pyridine ring (Fig. 2). Therefore, in this report, we systematically designed and synthesized a novel series of sixteen benzofuro[3,2-*b*]pyridin-7-ol derivatives (**1**–**16**), and evaluated for antiproliferative activity and topo I/II inhibitory activity. In addition, presence of *ortho*/*meta*/*para*-hydroxyl group at 2- and/or 4-position of phenyl ring allowed us to explore structure-activity relationships of hydroxylated benzofuro[3,2-*b*]pyridin-7-ols. To the best of our knowledge, this is the first study of benzofuro[3,2-*b*]pyridin-7-ol as a novel scaffold for developing topo II-targeted antiproliferative agents.

The synthesis of target compounds (benzofuro[3,2-*b*]pyridin-7-ols) **1**–**16** was in three steps (Scheme 1) using modified *Kröhnke pyridine synthesis* based on previously reported methods.[16,17] At first, four different non/mono-hydroxylated phenyl pyridinium iodine salts **II** (**R = a**–**d**) were synthesized in 80–94% yields by refluxing non/mono-hydroxy acetophenone **I** (**R = a**–**d**) and iodine in pyridine at 140 °C for 3 h. In parallel, a series of four hydroxylated benzofuranone intermediates (**V**, **VI**, **VII**, and **VIII**) were synthesized in 89–96% yields using commercially available 6-hydroxy-3-coumaranone (**III**) and benzaldehyde or



Scheme 1. Synthetic route of compounds **1**–**16**. Reagents and conditions: (i) iodine (1.0 equiv.), pyridine, 3 h, 140 °C, 80–94% yield; (ii) aq. 5 M NaOH, Ethanol, 2–3 h, 0 °C, 89–96% yield; (iii) $SOCl_2$, Ethanol, 1–2 h, 0 °C, 90–95% yield; (iv) NH_4OAc in methanol, 12–30 h, 110–125 °C, 7–37% yield.

mono-hydroxy benzaldehyde **IV** (**R = a–d**) in the presence of aqueous sodium hydroxide (5 M NaOH) or thienyl chloride ($SOCl_2$) in ethanol (Scheme 1 Step II).[18] Finally, both the hydroxylated benzofuranone intermediates (**V–VIII**) and pyridinium salts, **II** (**R = a–d**) were refluxed in methanol at 110–125 °C for 12–30 h in the presence of ammonium acetate (NH_4OAc) to synthesize corresponding target compounds **1–16** in 7–37% yields. Structures of synthesized compounds are shown in Fig. 3. Though the protection of hydroxyl moiety was not required, yield of compounds with *ortho*-phenol ring at 2-position of pyridine (**2**, **6**, **10**, and **14**) were relatively low, which may be due to intermolecular hydrogen bonding as well as steric hindrance. Physical properties including yield (%), melting point (°C), retention time (min) and percentage purity obtained by HPLC are listed in Table S1 (Supporting Information).

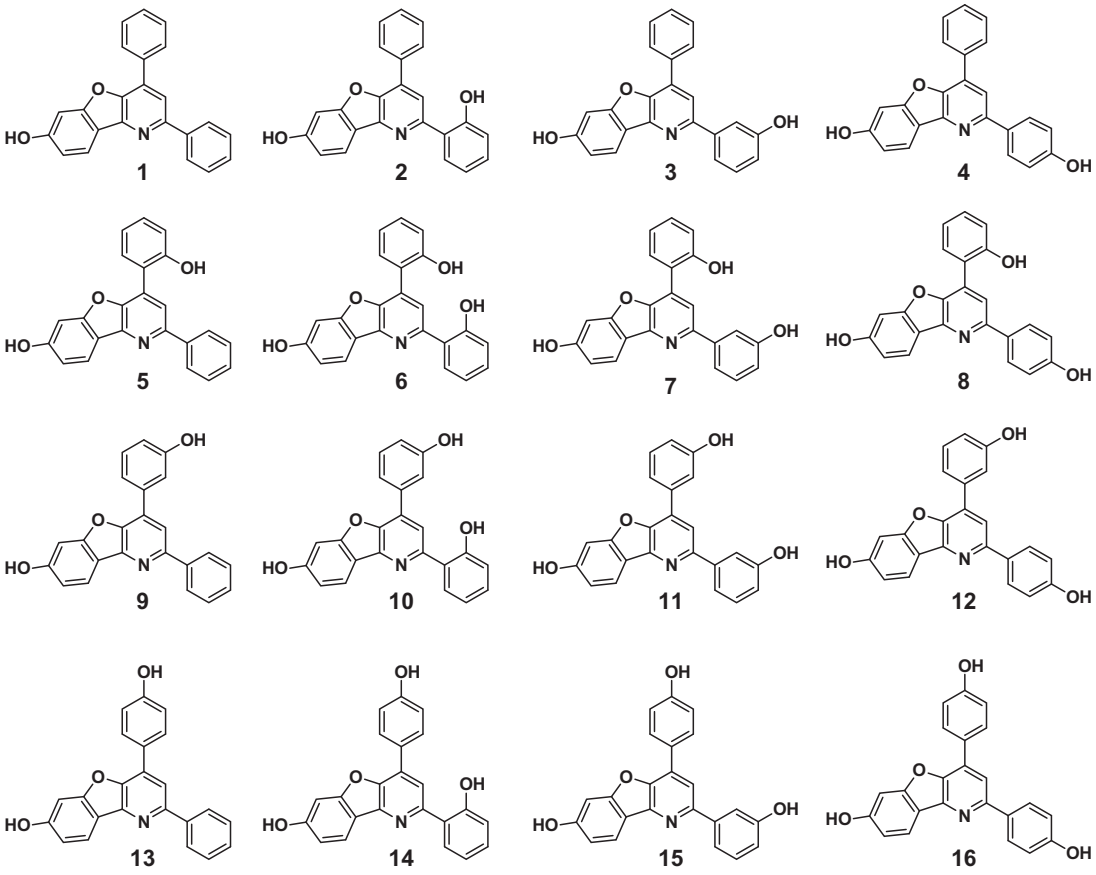


Fig. 3. Structures of synthesized benzofuro[3,2-*b*]pyridin-7-ol derivatives.

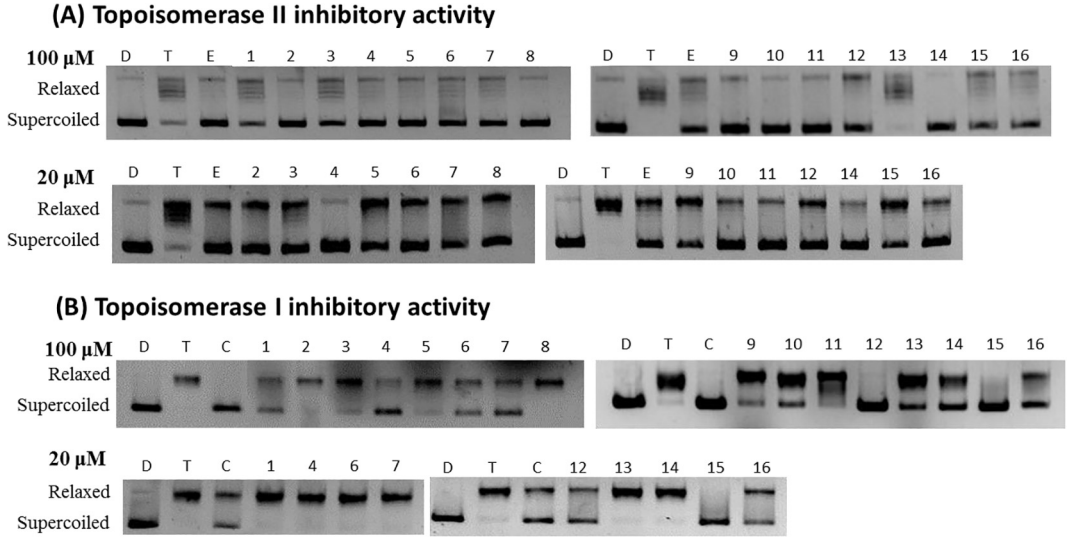


Fig. 4. Topoisomerase I and IIα inhibitory activities of compounds. (A) Lane D: pBR322 DNA only; Lane T: pBR322 DNA + topoisomerase I; Lane C: pBR322 DNA + topoisomerase I + camptothecin; Lane 1–27: pBR322 DNA + topoisomerase I + compound **1–16**; (B) Lane D: pBR322 DNA only; Lane T: pBR322 DNA + topoisomerase IIα; Lane E: pBR322 DNA + topoisomerase IIα + etoposide; Lane 1–27: pBR322 DNA + topoisomerase IIα + compound **1–16**.

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Table 1
Topo I and II inhibitory activity and antiproliferative activity of the prepared compounds **1**–**16**.

Compounds	% Inhibition				IC_{50} (μM)		
	Topo II		Topo I		HCT15	T47D	HeLa
	100 μM	20 μM	100 μM	20 μM			
Camptothecin			85.1[a]/83.9[b]	45.6[a]/47.8[b]	0.05 ± 0.01	0.07 ± 0.02	1.37 ± 0.14
Etoposide	76.6[a]/61.9[b]	39.5[a]/49.7[b]			2.94 ± 0.10	0.40 ± 0.03	9.21 ± 0.16
Adriamycin					0.51 ± 0.09	0.31 ± 0.04	1.50 ± 0.01
1	18.3	NT	30.8	0	>50	>50	>50
2	83.1	52.6	18.2	NT	1.60 ± 0.06	0.71 ± 0.02	4.11 ± 0.11
3	46.3	32.5	15.9	NT	>50	2.79 ± 0.14	>50
4	78.4	72.2	43.9	0	>50	5.30 ± 0.39	>50
5	52.5	19.1	14.2	NT	5.74 ± 0.10	0.74 ± 0.02	6.93 ± 0.01
6	46.0	38.0	30.1	0	>50	1.03 ± 0.04	>50
7	63.0	12.0	33.9	0	2.5 ± 0.1	0.64 ± 0.06	3.63 ± 0.18
8	87.3	29.0	3.3	NT	1.26 ± 0.09	0.62 ± 0.01	2.86 ± 0.19
9	98.0	41.9	27.4	NT	4.11 ± 0.01	0.34 ± 0.03	3.27 ± 0.30
10	99.1	76.0	25.6	NT	6.96 ± 0.70	1.26 ± 0.19	3.39 ± 1.98
11	100.0	74.1	22.4	NT	1.22 ± 0.07	0.59 ± 0.01	0.86 ± 0.02
12	66.1	55.1	66.3	38.1	3.73 ± 0.23	0.19 ± 0.02	2.16 ± 0.10
13	0.4	NT	30.3	0.0	>50	>50	>50
14	100.0	70.8	39.9	0.0	9.34 ± 0.38	1.97 ± 0.29	3.62 ± 1.26
15	58.4	45.1	71.7	60.9	23.88 ± 0.82	5.25 ± 0.31	29.11 ± 2.60
16	71.0	65.9	33.8	18.6	4.21 ± 0.06	0.17 ± 0.02	3.32 ± 0.10

Each datum represents mean ± S.D. from three different experiments performed in triplicate.
NT: Not tested, [a]control value for compounds **1**–**8**; [b]value for compounds **9**–**16.**
HCT15: human colorectal adenocarcinoma cell line; T47D: human ductal breast cancer cell line; HeLa: human cervix tumor cell line.
Camptothecin: positive control for topo I inhibition and antiproliferative activity; Etoposide: positive control for topo II inhibition and antiproliferative activity; Adriamycin: positive control for antiproliferative activity.

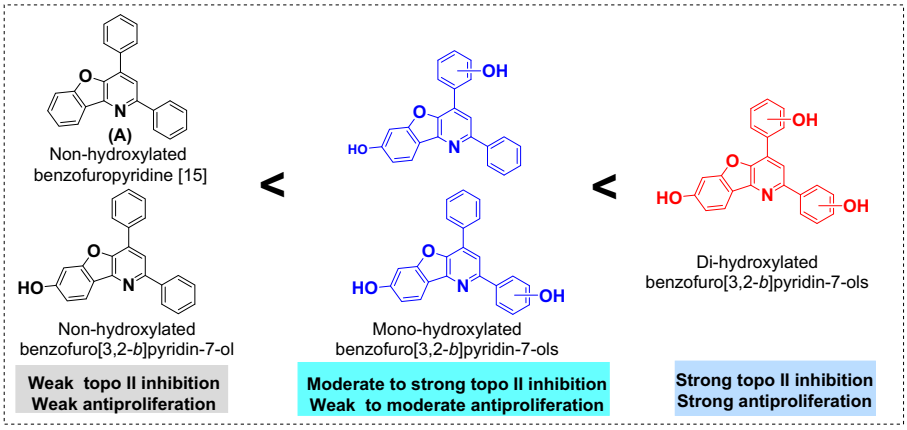
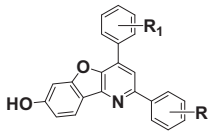


Fig. 5. SAR study based on importance of hydroxyl group at 2- and 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol.

The newly synthesized benzofuro[3,2-*b*]pyridin-7-ols **1**–**16** were evaluated for both topo I and II inhibitory activity, and *in vitro* antiproliferative activity against three different human cancer cell lines: HCT15 (colorectal adenocarcinoma cell line), T47D (ductal breast cancer cell line), and HeLa (cervix tumor cell line). Camptothecin, etoposide, and adriamycin were used as positive control to compare the biological results and study structure-activity relationship of the synthesized compounds. Fig. 4 and Table 1 summarize the topo I and II inhibitory activity of compounds **1**–**16** at 100 and 20 μM, and antiproliferative activity measuring IC_{50} value.

Interestingly, all the new compounds showed weaker topo I inhibitory activity than positive control, camptothecin, both at 100 and 20 μM (Table 1) with the exception of **15**. Compound **1** without hydroxyl group at 2- and 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol showed very weak topo I/II inhibition and antiproliferative activity in all tested cell lines. Except **13**, compounds **2**–**5**, and **9** with an additional hydroxyl group either in 2- or 4-phenyl ring exhibited comparable or stronger topo II inhibitory activity (46.3–98.0%) at 100 μM. Particularly, compound **2** and **4** containing *ortho*- and *para*-hydroxyl group at 2-phenyl ring, respectively, showed stronger topo II inhibitory activity than etoposide both at 100 and 20 μM. Among the compounds containing hydroxyl group both at 2- and 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol, compounds **8**, **10**–**12**, **14**, and **16** exhibited strong topo II inhibitory activity with moderate to strong antiproliferative activity in all cancer cell lines. Compounds **10**–**12**, **14**, and **16** displayed selective and potent topo II inhibitory activity with the range from 55.1 to 76.0% as compared to etoposide (49.7%) at 20 μM. Compounds **11** and **14** exhibited 100% topo II inhibition at 100 μM. It is interesting to note that all the compounds (**9**–**12**) containing *meta*-hydroxyl group at 4-phenyl of benzofuro[3,2-*b*]pyridin-7-ol showed potent topo II inhibitory activity and strong antiproliferative activity (IC_{50} = 0.19–6.96 μM) in HCT15 and T47D cell lines. Furthermore, compound **11** with *meta*-hydroxyl group at both 2- and 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol showed highly selective and potent topo II inhibition along with strongest antiproliferative activity (IC_{50} = 0.86 μM) than all the positive controls in HeLa cell line. In addition, results from Table 1 also indicated that T47D cancer cell lines are very sensitive (IC_{50} =

Table 2
Relative topo II inhibitory potencies of synthesized compounds compared to etoposide.



Mono-hydroxylated benzofuro[3,2-*b*]pyridin-7-ols	Topo II		Dihydroxylated benzofuro[3,2-*b*]pyridin-7-ols	Topo II	
	100 μM	20 μM		100 μM	20 μM
2: R = *ortho*-OH, R_1 = H	1.08	1.33	**6:** R = *ortho*-OH, R_1 = *ortho*-OH	0.60	0.96
5: R = H, R_1 = *ortho*-OH	0.69	0.48	**10:** R = *ortho*-OH, R_1 = *meta*-OH	1.60	1.53
			14: R = *ortho*-OH, R_1 = *para*-OH	1.62	1.42
3: R = *meta*-OH, R_1 = H	0.60	0.82	**7:** R = *meta*-OH, R_1 = *ortho*-OH	0.82	0.31
9: R = H, R_1 = *meta*-OH	1.58	0.84	**11:** R = *meta*-OH, R_1 = *meta*-OH	1.62	1.49
			15: R = *meta*-OH, R_1 = *para*-OH	0.94	0.91
4: R = *para*-OH, R_1 = H	1.02	1.83	**8:** R = *para*-OH, R_1 = *ortho*-OH	1.12	0.73
13: R = H, R_1 = *para*-OH	NA	NT	**12:** R = *para*-OH, R_1 = *meta*-OH	1.07	1.11
			16: R = *para*-OH, R_1 = *para*-OH	1.15	1.33

*Relative potency (RP):% inhibition of compound/% inhibition of positive control, NT: Not Tested, NA: Not Active.

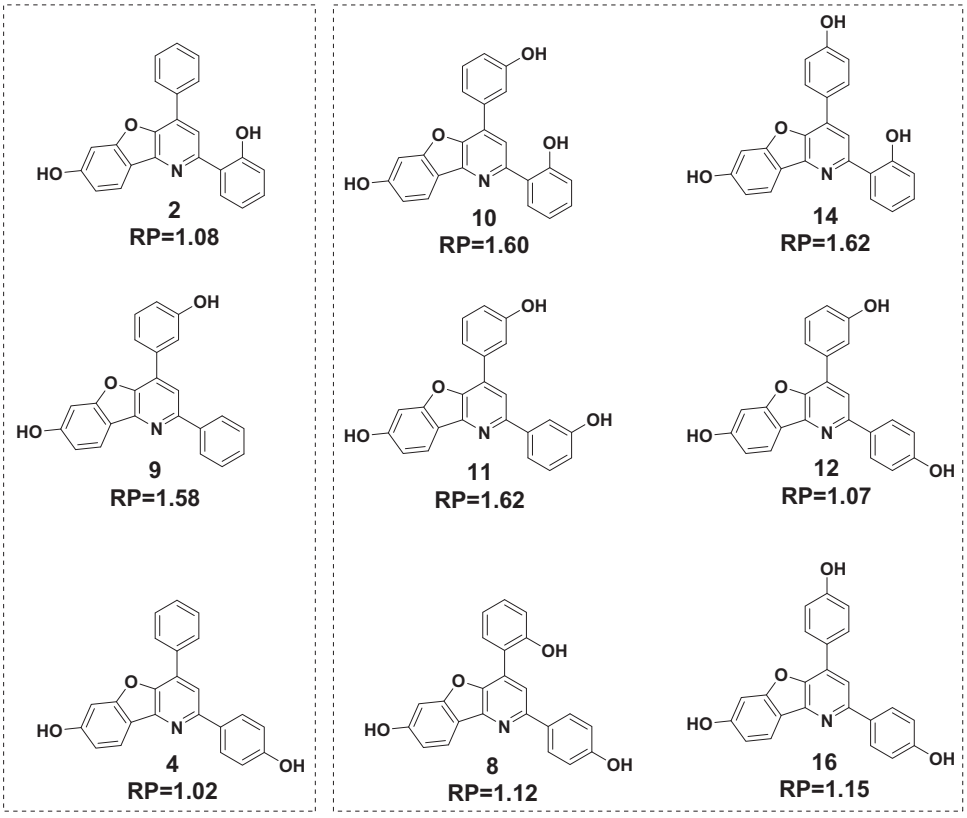


Fig. 6. Structure of compounds with stronger topo II inhibitory activity than positive control, etoposide, at 100 μM.

0.17–5.25 μM) to all the compounds (**6**–**8**, **10**–**12**, **14**–**16)** with 2,4-diphenol moieties at benzofuro[3,2-*b*]pyridin-7-ol ring.

Structure-activity relationships (SAR) from the preliminary biological results of compounds (**2**–**16)**, in comparison with **1** and previously synthesized non-hydroxylated benzofuropyridine (**A**),[15] revealed the importance of hydroxyl group in 2- and 4-position of benzofuro[3,2-*b*]pyridin-7-ol structure. Introduction of hydroxyl group at 2- and 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol scaffold enhances the selectivity and potency of topo II inhibitory activity with stronger antiproliferative activity in T47D cancer cell line (Fig. 5). Moreover, to determine the effect of position of hydroxyl group, relative topo II inhibitory potencies of the synthesized compounds compared to the positive control, etoposide, are listed in Table 2.

Results from Table 2 and Fig. 6 indicated that compounds **2**, **10**, and **14** with *ortho*- hydroxyl group at 2-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol with the exception of **6** showed stronger topo II inhibitory activity (Relative potency > 1) than positive control, etoposide, at both 100 and 20 μM. Similarly, compounds **4**, **8**, **12**, and **16** with *para*-hydroxyl group at 2-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol with the exception of **8** at 20 μM showed stronger topo II inhibitory activity (Relative potency > 1) at both 100 and

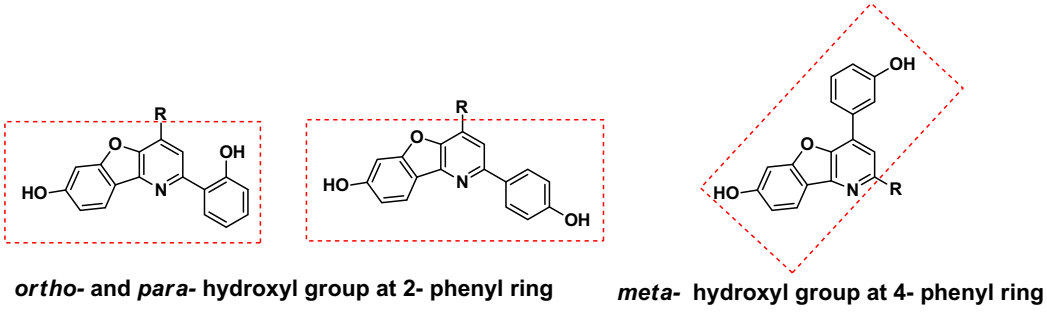


Fig. 7. Summary of SAR based on importance of position of hydroxyl group at 2- and 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol.

20 μM. Likewise, it is interesting to note that, all compounds with *meta*-hydroxyl group at 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol showed stronger topo II inhibitory activity (Relative potency > 1) than positive control, etoposide. As shown in Fig. 7, the overall structure-activity relationships indicated that *ortho*- and *para*-hydroxyl group at 2-phenyl ring, and *meta*-hydroxyl group at 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol is vital for potent topo II inhibitory activity.

In conclusion, novel series of benzofuro[3,2-*b*]pyridin-7-ols incorporating hydroxyl moiety were designed, synthesized, and evaluated for topo I/II inhibitory activity and antiproliferative activity. Results of topo II inhibitory and anti-proliferative activity indicated importance of addition of hydroxyl moiety in 2- and/or 4-phenyl position of benzofuro[3,2-*b*]pyridin-7-ols for the activity. Moreover, comparison of relative topo II inhibition potency based on position of hydroxyl moiety at 2- and 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol revealed that *ortho*- and *para*-hydroxyl group at 2-phenyl ring, and *meta*-hydroxyl group at 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol are crucial for potent and selective topo II inhibitory activity.

Among all the benzofuro[3,2-*b*]pyridin-7-ol derivatives, compound **11** showed the most potent and selective topo II inhibition as well as stronger antiproliferative activity than all the positive controls in HeLa cell line. Based on these findings, further optimization and mechanistic study on compound **11** is underway, and the results will be reported elsewhere. We believe that the present study provides useful information for designing a new strategy to develop topo II-targeted anticancer drugs.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.01.048.

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