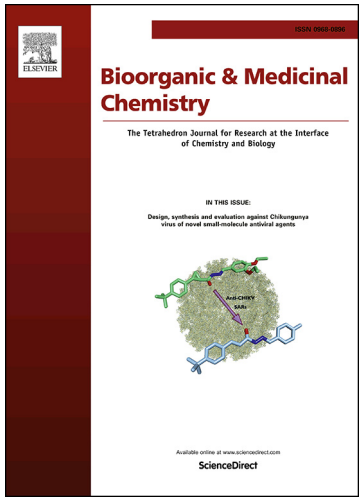
# Accepted Manuscript




Synthesis and SAR study of new hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines as selective topoisomerase IIα-targeting anti-cancer agents

Til Bahadur Thapa Magar, Seung Hee Seo, Tara Man Kadayat, Hyunji Jo, Aarajana Shrestha, Ganesh Bist, Pramila Katila, Youngjoo Kwon, Eung-Seok Lee

PII: S0968-0896(18)30113-5
DOI: https://doi.org/10.1016/j.bmc.2018.02.035
Reference: BMC 14221

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 18 January 2018
Revised Date: 19 February 2018
Accepted Date: 20 February 2018

Please cite this article as: Magar, T.B.T., Seo, S.H., Kadayat, T.M., Jo, H., Shrestha, A., Bist, G., Katila, P., Kwon, Y., Lee, E-S., Synthesis and SAR study of new hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines as selective topoisomerase IIα-targeting anticancer agents, *Bioorganic & Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.bmc.2018.02.035

# Synthesis and SAR study of new hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines as selective topoisomerase II*α*-targeting anticancer agents

Til Bahadur Thapa Magar[a,1], Seung Hee Seo[b,1], Tara Man Kadayat[a,1†], Hyunji Jo[b], Aarajana Shrestha[a], Ganesh Bist[a], Pramila Katila[a], Youngjoo Kwon[b,*], Eung-Seok Lee[a,*]

[a]*College of Pharmacy, Yeungnam University, Gyeongsan 38541, Republic of Korea*

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

*Corresponding Authors:

email: ykwon@ewha.ac.kr (Y.K.)

email: eslee@yu.ac.kr (E.-S.L.)

[1]Authors are equal contributors

†Present Address: New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu 41061, Republic of Korea

# Synthesis and SAR study of new hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines as selective topoisomerase IIα-targeting anticancer agents

Til Bahadur Thapa Magar[a,1], Seung Hee Seo[b,1], Tara Man Kadayat[a,1†], Hyunji Jo[b], Aarajana Shrestha[a], Ganesh Bist[a], Pramila Katila[a], Youngjoo Kwon[b,*], Eung-Seok Lee[a,*]

**Abstract**

As part of our effort to develop potential topoisomerase IIα (topo IIα) targeting anticancer agents, we systematically designed a new series of hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines. Total eighteen compouds were synthesized and tested for their ablity to inhibit the function of topo I and IIα, and proliferation of human breast (T47D), colorectal (HCT15), and cervix (HeLa) cancer cells. Except compound **11**, all of the tested compounds displayed selective topo IIα inhibitory activity. Compounds **8**-**18**, **22**, **24**, and **25** showed excellent topo IIα inhibitory activity than a positive control, etoposide. Most of the compounds appeared to be superior to reference compounds in their antiproliferative activity. Structure-activity relationship (SAR) study has shown that it is better to place the hydroxyphenyl group at the 4-position of the central pyridine for superior topo IIα inhibition and antiproliferative activity. Similarly, the 3′-, or 4′-hydroxyphenyl substitution at the 2- and 4-positon of pyridine ring is important for better activity than 2′-substitution.

**Keywords**: Anticancer agents; *5H*-chromeno[4,3-*b*]pyridines; hydroxyl and chlorine-substitution; Selective topoisomerase IIα inhibition; SAR study

## 1. Introduction

In all prokaryotic and eukaryotic life, cell division is the only means of multiplication and the survival. Cell multiplication proceeds by forming daughter DNA from parental DNA through important cellular processes such as DNA transcription and replication.[1] During these cellular processes, the DNA helix tangles and bends to form a positive or negative supercoil. Topoisomerases (topos) are enzymes that solve the topological problems of DNA by converting the supercoiled DNA to relaxed form allowing vital cellular processes to proceed further.[2] There are two types of topo, depending upon their capabilities: a) topo I that cleaves the single strand of DNA at a time; and b) topo II that simultaneously breaks the double strand of DNA.[3, 4] Since topo II plays major role during cell proliferation, many researchers are focused to develop safe compounds that inhibit topo II function. Among different phases of the cell cycle, the synthesis phase (S phase) is considered to be the most important at which DNA gets duplicated. S phase lies in between $G_1$ and $G_2$ phases followed by mitotic phase (M phase) when the mother cell divides into two daughter cells.[5] Several studies showed that the level of alpha isoform of topo II (topo IIα) increases significantly at S phase and reaches maximum at $G_2$/M phase while level of another isoform, topo IIβ, remains constant throughout the cell cycle.[6-8] Therefore, inhibition of the topo IIα function results in interruption at vital stage of cell cycle, consequently leading to cell death.

Chromene is considered an important moiety in medicinal chemistry. Several chromene-derived compounds have shown biological activities such as anticancer,[9-11] antifungal,[12] antimicrobial,[13] anti-inflammatory,[14] antiproliferative,[15] and antiangiogenic activity.[16] Chromenopyridine is a three-ring system containing heterocyclic moiety which is formed by the fusion of a chromene and a pyridine fraction. Recently, four different compounds having chromenopyridine moiety were isolated from a fungus *Phomopsis sp.* possessing moderate

nitric oxide inhibitory activity in LPS induced RAW 264.7 macrophage and good antioxidant activity.[17] However, to date the major sources of compounds with chromenopyridine scaffolds are synthetic approach and different types of reaction methods have been utilized for their easier synthesis. Chromenopyridine skeleton have attracted many researchers for the discovery of new drug candidate due to their ability to adjust activity and selectivity via altering substitution pattern at its different positions.

Previously, our group discovered chromenopyridine derivatives possessing aryl moieties such as thienyl, furyl, pyridyl and phenyl as new and potential anticancer agents.[18] Moreover, we reported hydroxylated chromenopyridines exhibiting selective topo IIα inhibitory activity as well as moderate antiproliferative activity (Figure 1A).[19] Several studies have reported that halogen and hydroxyl group containing compounds possess better biological activity due to their ability to form of halogen bond and hydrogen bond, respectively, with active site of receptor.[20-22] Recently, we reported 2-chlorophenly-4-phenol-benzofuro[3,2-*b*]pyridines (**A**-**I**) containing constrained five-membered heterocycle (furan), which displayed excellent dual topo I/IIα inhibition and effectively suppressed the proliferation of human colorectal adenocarcinoma (HCT15) cell line.[23] In our quest to search novel topo IIα-targeted anticancer agents, we decided to further design and synthesize a new series of hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines by ring expansion from 5-membered furan ring to the six-membered pyran ring (Figure 1B). In addition, we investigated their topo inhibitory activity as well as antiproliferative activity. Herein, in this study we report design, synthesis and structure-activity relationships of *5H*-chromeno[4,3-*b*]pyridine derivatives as novel topo IIα-targeted anticancer agents.

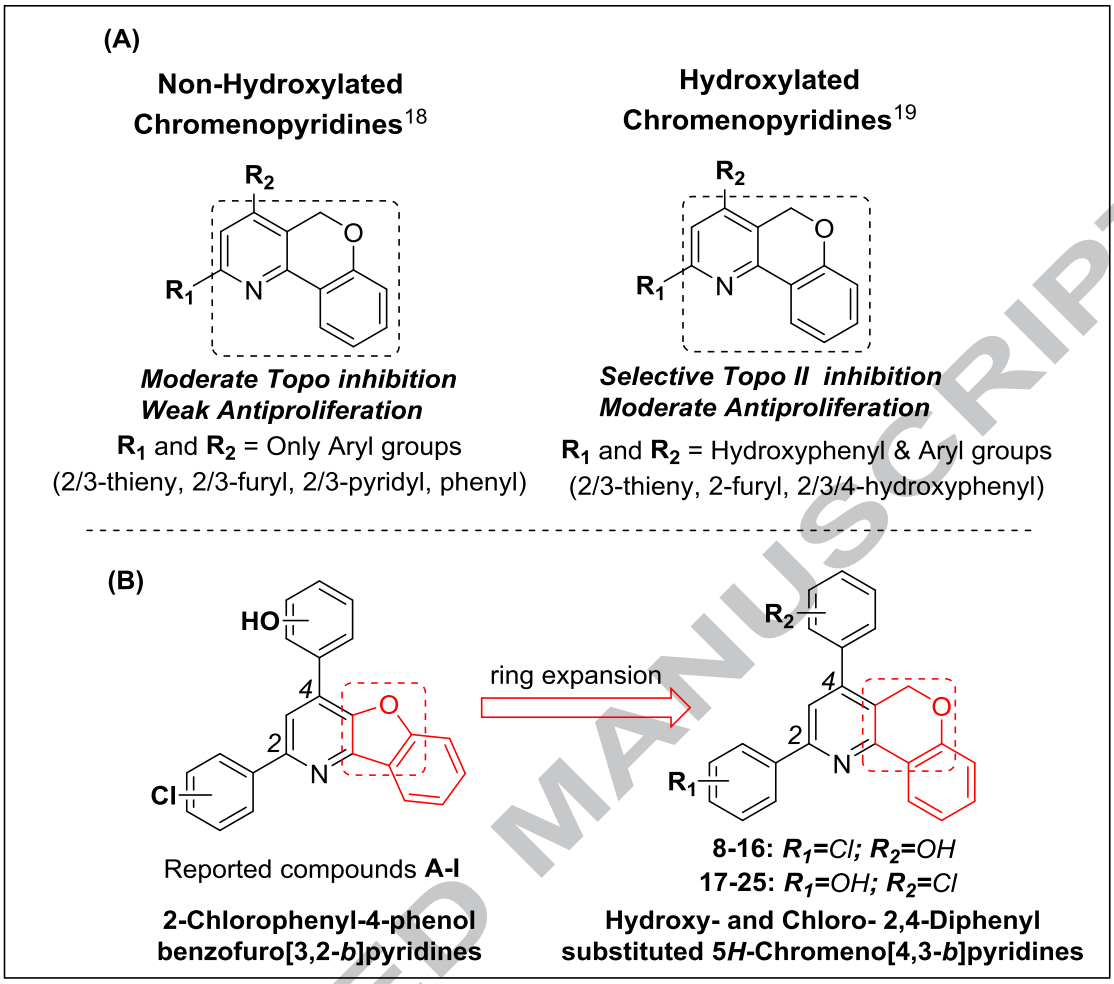
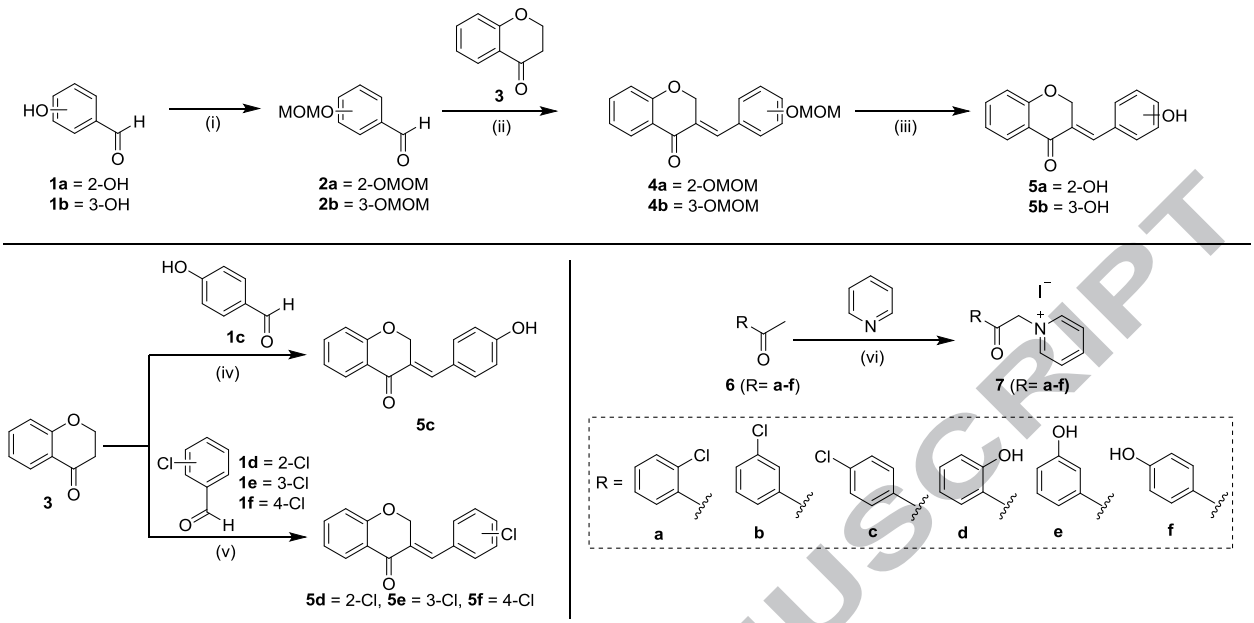



**Figure 1.** Chemical structure of (A) reported bioactive chromenopyridines, and (B) our designed compounds.

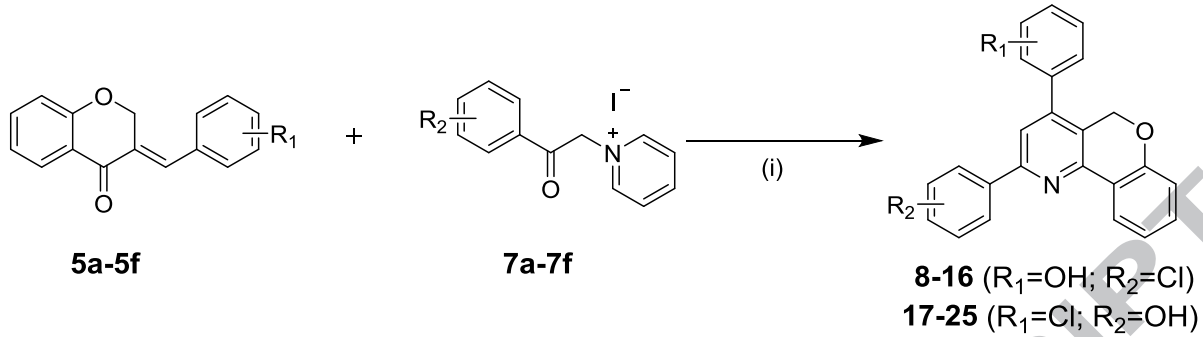
## 2. Results and discussion

### *2.1. Chemistry*

The target compounds **8-25** were synthesized from intermediates **5a**-**5f** and pyridinium iodide salts **7** (R = **a-f**). Scheme 1 illustrates the general synthetic route for the synthesis of 4-chromanone intermediates (**5a**-**5f**) and pyridinium iodide salts **7** (R = **a-f**). Intermediates **5a** and **5b** were synthesized by protecting hydroxyl moiety of corresponding aldehydes (**1a** and **1b**) with chloromethyl methyl ether (MOMCl) in the presence of *N*,*N*-diisopropylethylamine (DIPEA). Then, resulting hydroxyl protected aldehydes **2a** and **2b** were condensed with 4-chromanone (**3**) in basic condition to give products **4a** and **4b**. Deprotection was achieved by refluxing with aqueous 2 M HCl in ethanol to afford **5a** and **5b**. *Para*-hydroxyl containing intermediate (**5c**) was obtained by acid catalyzed condensation of 4-hydroxybenzaldehyde (**1c**) and 4-chromanone (**3**) in the presence of boron trifluoride etherate [$BF_3O(C_2H_5)_2$] in dioxane. Secondly, pyridinium iodide salts **7** (R = **a-f**) were prepared by refluxing equivalent amount of corresponding aryl methyl ketone **6** (R = **a-f**) with iodine in pyridine. Finally, modified Kröhnke pyridine synthetic method facilitated the reaction of pyridinium iodide salts **7** (R= **a-f**) and intermediates **5a**-**5f** in glacial acetic acid using $NH_4OAc$ as an ammonia source. The reaction was performed under reflux condition for 12-24 h to obtain total eighteen hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines as final products at the yield ranging from 22.1% to 74.3% (Scheme 2). Figure 2 shows the structures of target final compounds **8-25**. Physicochemical characterization data of all prepared compounds, such as, purity (%) as determined by HPLC, melting point (°C), and yield (%), are given in Supplementary Data.




**Scheme 1**. Synthesis of 4-chromanone intermediates **5a-5f** and aryl pyridinium iodide salts **7a-7f**. Reagents and conditions: (i) MOMCl (1.5 equiv.), DIPEA (3.0 equiv.), $CH_2Cl_2$, 3 h, room temperature, 86.3-90.1% yield; (ii) aq. NaOH, methanol, 24 h, room temperature, 74.0-95.6% yield; (iii) aq. 2 M HCl (1.5 equiv.), 2 h, 80 °C, 50.2-50.5% yield; (iv) $BF_3.Et_2O$ (1.0 equiv.), dioxane, 24 h, room temperature, 56.6% yield; (v) aq. NaOH, ethanol, 1-3 h, room temperature, 61.3-81.8% yield; (vi) iodine (1.0 equiv.), pyridine, 3 h, 140 °C, 52.2-93.0% yield.




**Scheme 2**. Synthesis of hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines **8-25**. Reagents and conditions: (i) $NH_4OAc$ (10.0 equiv.), glacial acetic acid, 12-24 h, 100 °C, 22.1-74.3% yield.

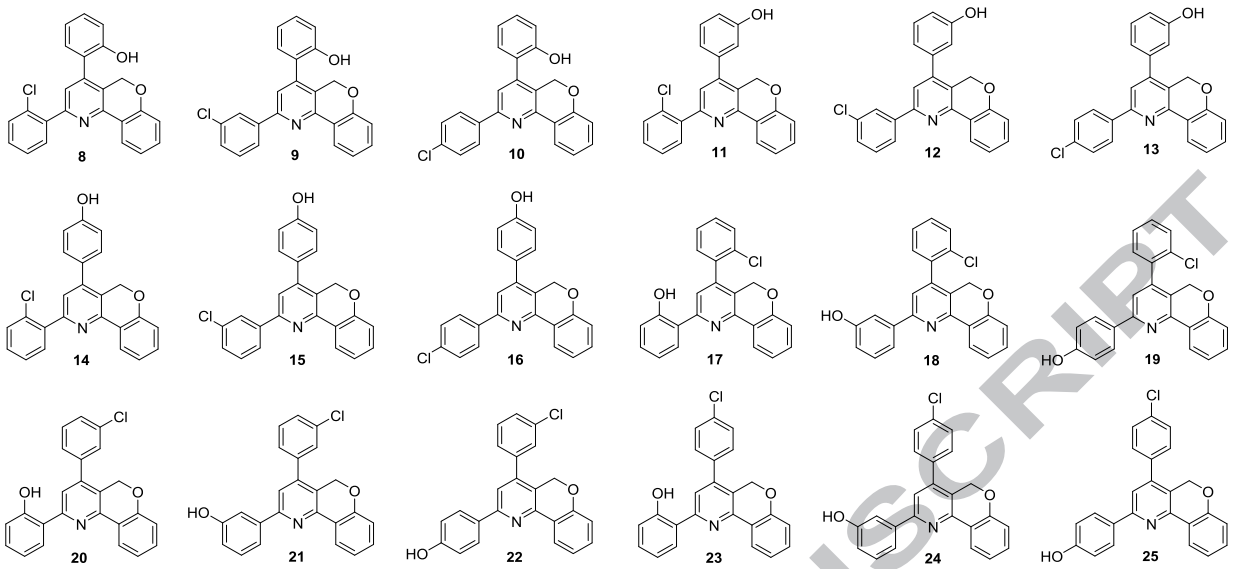



**Figure 2.** Structures of the synthesized hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines.

## 2.2. Topoisomerase I and IIα inhibitory activity

We investigated the ability of topo I and IIα to transform the supercoiled plasmid DNA to relaxed form under the influence of synthesized compounds (**8**-**25**). Two different concentrations, 100 μM and 20 μM of those compounds were used. Only those compounds displaying more than 30% inhibition at 100 μM were selected for evaluation in 20 μM concentration. Clinically available anticancer drugs, camptothecin as a selective topo I inhibitor and etoposide as a selective topo II inhibitor, were utilized as positive controls. The extent of topo I and IIα inhibition for the evaluated compounds is compiled in Table 1 and Figure 3.

### *2.2.1. Topoisomerase I inhibitory activity*

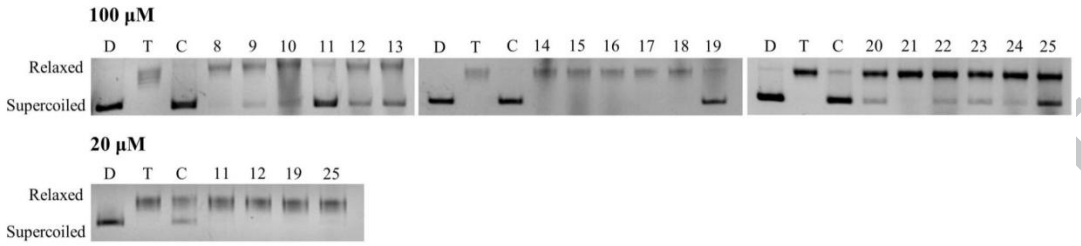
Majority of the tested compounds showed weak topo I inhibitory activity as compared to a reference compound, camptothecin, at both 100 μM and 20 μM with exception of compound **11**. Compound **11** possessing 2′-chlorophenyl at 2-position and 3′-hydroxyphenyl at 4-position of central pyridine ring displayed the most potent topo I inhibitory activity (79.1%) at 100 μM which is comparable to the positive control, camptothecin (79.9%). Compound **19** showed considerable topo I inhibition (56.1%) at 100 μM concentration (Table 1, Figure 3A).

### *2.2.2. Topoisomerase IIα inhibitory activity*

Table 1 and Figure 3B demonstrate the topo IIα inhibitory activities of the synthesized compounds **8**-**25**. Most of the evaluated compounds displayed comparable to significant topo IIα inhibition at both 100 μM and 20 μM concentrations. All the compounds **8**-**16**, containing 2′-, 3′-, or 4′-chlorophenyl moiety at 2-position and 2′-, 3′-, or 4′-hydroxyphenyl moiety at 4-position displayed better topo IIα inhibitory activity (71.3-95.9% at 100 μM and 41.9-74.5%

at 20 µM) than etoposide (66.5% at 100 µM and 35.5% at 20 µM), respectively. Among them, compound **15** significantly inhibited topo IIα activity at both 100 µM and 20 µM concentrations (95.9% at 100 µM and 57.3% at 20 µM). Similarly, compound **14** having 2′-chlorophenyl at 2-position and 4′-hydroxyphenyl at 4-position exhibited two-fold more potent as compared with reference compound, etoposide, at 20 µM. On the other hand, compounds **17**-**25** possessing 2′-, 3′-, or 4′-hydroxyphenyl moiety at 2-position, and 2′-, 3′-, or 4′-chlorophenyl moiety at 4-position showed comparable to strong topo IIα inhibition. Among them, compounds **17**, **18**, **22**, **24** and **25** displayed better activity (66.7-100%) than reference compound at 100 µM. Compound **25** showed 100% topo IIα inhibition at 100 µM.

**(A)** Topo I inhibition




**(B)** Topo IIα inhibition

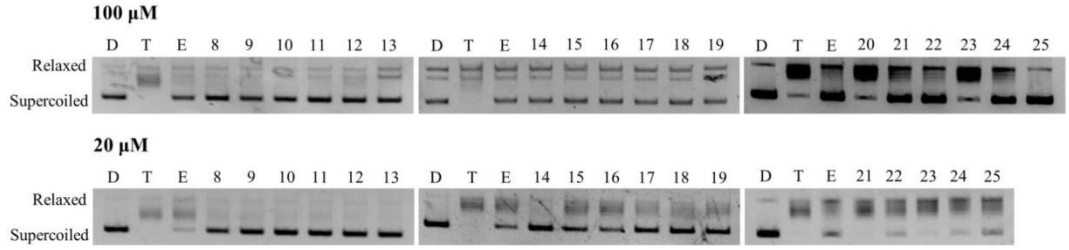



**Figure 3**. DNA topo I (A) and topo IIα (B) inhibitory activity of the synthesized compounds **8-25** at the concentrations of 100 μM and 20 μM. (A) Lane D: pBR322 only, Lane T: pBR322 + Topo I, Lane C: pBR322 + Topo I + Camptothecin, Lane 8-25: pBR322 + Topo I + compounds **8-25** at 100 μM or 20 μM. (B) Lane D: pBR322 only, Lane T: pBR322 + Topo IIα, Lane E: pBR322 + Topo IIα + Etoposide, Lane 8-25: pBR322 + Topo IIα + compounds **8-25** at 100 μM or 20 μM.

**Table 1**. Topo I and IIα inhibitory and antiproliferative activity of the prepared compounds **8-25.**

| Compounds | %Inhibition | | | | *$IC_{50}$ (μM) | | |
|---|---|---|---|---|---|---|---|
| | Topo IIα | | Topo I | | HCT15 | T47D | HeLa |
| | 100 μM | 20 μM | 100 μM | 20 μM | | | |
| **Camptothecin** | | | 79.9 | 34.3 | 0.02±0.00 | 0.49±0.02 | 0.13±0.00 |
| **Etoposide** | 66.5 | 35.5 | | | 2.45±0.08 | 0.32±0.01 | 10.03±0.33 |
| **Adriamycin** | | | | | 0.73±0.01 | 0.84±0.01 | 1.70±0.06 |
| **8** | 79.1 | 47.3 | 4.7 | NT | >50 | >50 | >50 |
| **9** | 75.1 | 51.9 | 4.4 | NT | 2.23±0.08 | 1.23±0.04 | 0.68±0.01 |
| **10** | 88.9 | 56.6 | 7.6 | NT | 1.66±0.03 | >50 | >50 |
| **11** | 90.8 | 57.3 | 79.1 | 1.7 | 0.87±0.01 | 0.70±0.01 | 2.50±0.10 |
| **12** | 84.0 | 63.7 | 30.0 | NA | 2.13±0.09 | 0.72±0.02 | 0.68±0.02 |
| **13** | 71.3 | 63.8 | 28.8 | NT | 2.30±0.39 | 1.18±0.01 | 0.76±0.00 |
| **14** | 82.7 | 74.5 | NA | NT | 1.74±0.05 | 0.75±0.01 | 2.11±0.01 |
| **15** | 95.9 | 57.3 | NA | NT | 1.77±0.02 | 0.88±0.08 | 0.89±0.01 |
| **16** | 87.8 | 41.9 | NA | NT | 1.83±0.06 | 1.72±0.14 | 0.86±0.03 |
| **17** | 78.1 | 45.5 | NA | NT | >50 | 1.66±0.05 | >50 |
| **18** | 91.7 | 57.0 | NA | NT | 1.52±0.05 | 0.86±0.01 | 0.74±0.03 |
| **19** | 63.5 | 48.1 | 56.1 | NA | 4.72±0.12 | 0.94±0.13 | 0.68±0.01 |
| **20** | 18.9 | NT | 21.1 | NT | >50 | >50 | >50 |
| **21** | 63.6 | NA | 12.0 | NT | 1.23±0.01 | 0.86±0.01 | 0.82±0.01 |
| **22** | 79.5 | 12.1 | 22.8 | NT | 3.41±0.55 | 1.27±0.01 | 0.66±0.01 |
| **23** | 34.7 | NA | 20.1 | NT | >50 | >50 | >50 |
| **24** | 66.7 | 4.7 | 20.2 | NT | 4.57±0.18 | 0.75±0.01 | 1.94±0.06 |
| **25** | 100.0 | 19.9 | 38.5 | 1.7 | 1.85±0.03 | 1.38±0.03 | 2.08±0.24 |

NT: not tested; NA; not active.

HCT15: human colorectal adenocarcinoma cell line; T47D: human breast cancer cell line; HeLa: human cervix tumor cell line. Camptothecin: positive control for topo I and antiproliferative activity; Etoposide: positive control for topo IIα and antiproliferative activity; Adriamycin: positive control for antiproliferative activity.

*****Results represent mean ± S.D. from three different experiments performed in triplicate.

### 2.3. Antiproliferative activity

Table 1 displays the antiproliferative activity ($IC_{50}$) of the synthesized compounds **8-25** against three different cancer cell lines: HCT15 (human colorectal adenocarcinoma cell line), T47D (human breast cancer cell line), and HeLa (human cervix tumor cell line). Except compounds **8**, **10**, **17**, **20**, and **23**, all other compounds displayed significantly better antiproliferative activity (0.66 µM to 2.08 µM) than etoposide (10.03 µM) against HeLa cancer cell line. Comparatively, compound **11** possessing 2′-chlorophenyl at 2-position and 3′-hydroxyphenyl at 4-position of central pyridine ring, which showed significant dual topo I and IIα inhibitory activity displayed the most potent antiproliferative activity against HCT15 (0.87 µM) and T47D (0.70 µM). Similarly, compound **22** possessing 4′-hydroxyphenyl moiety at 2-position and 3′-chlorophenyl moiety at 4-position which displayed a selective topo IIα inhibition, showed 15.2 and 2.6 fold more strong antiproliferative activity against HeLa cell lines (0.66 µM) than reference compounds, etoposide and adriamycin, respectively. Compounds **11**, **12**, **14**, **15**, **18**, **19**, **21**, and **24** have demonstrated comparable to stronger antiproliferative activity (0.70 µM to 0.94 µM) against T47D as compared with adriamycin (0.84 µM). It was observed that most of the compounds exhibiting significant topo IIα inhibition also displayed comparable to significant antiproliferative activity as compared with positive controls, etoposide and adriamycin, reflecting that there was positive correlation between selective topo IIα inhibition and antiproliferative activity.

### 2.4. Structure-activity relationship study (SAR)

In this study, most of the compounds with 2′-, 3′-, or 4′-hydroxyphenyl group at 4-position (**8**-**16**) exhibited better topo IIα inhibitory activity as well as antiproliferative activity than compounds containing 2′-, 3′-, or 4′-hydroxyphenyl group at 2-position (**17**-**25**) of central pyridine ring. This results indicated the importance of hydroxyphenyl moiety at 4-position and chlorophenyl moiety at 2-position for exhibiting selective topo IIα inhibitory and antiproliferative activity. Among **17**-**25**, in general compounds with 2′-hydroxyl group at 2-phenyl ring displayed weak topo IIα inhibition and antiproliferative activity, suggesting introduction of 3′- and 4′-hydroxyl group is more important than 2′-hydroxyl group at 2-phenyl ring for strongly inhibiting topo IIα activity and antiproliferative activity. However, selective topo IIα inhibitory activity and antiproliferative activity might not be correlated to the position of chlorine substitution on 4-phenyl ring.

Furthermore, to investigate the effect of ring expansion, in this study, we investigated structure-activity relationships of prepared hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines, and previously reported corresponding benzofuro[3,2-*b*]pyridines.[23] Table 2 displays the relative topo I and IIα inhibitory potencies of the synthesized compounds **8**-**16** and corresponding benzofuro[3,2-*b*]pyridines **A**-**I** at 100 μM and 20 μM concentrations. From comparison of relative topo inhibitory potency, it was evident that the replacement of five membered heterocyclic ring moiety with six membered heterocyclic ring moiety significantly influenced the topo I and IIα inhibitory properties of compounds. Compounds **A**-**I** having benzofuropyridine skeleton displayed dual topo I and IIα inhibitory activity, while currently modified compounds possessing chromenopyridine moiety have showed selective topo IIα inhibition. The overall SAR study is illustrated in Figure 4.

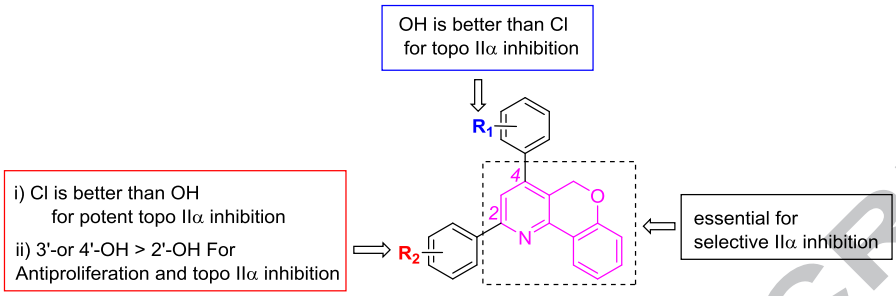
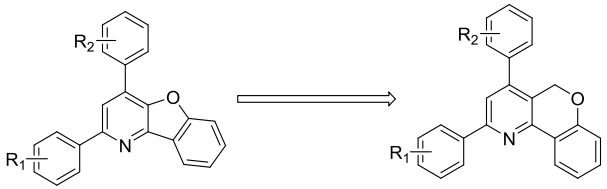



**Figure 4.** Overall structure-activity relationships of hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines.

**Table 2**. Relative topo I and IIα inhibitory potencies of synthesized compounds compared to camptothecin and etoposide.

Relative potency* for topo I and IIα (% inhibition) of compounds compared to positive controls, camptothecin (topo I) and etoposide (topo IIα)




**A-I** **8-16**

| Substitution | Reported Compound[a] | Topo IIα | | Topo I | | Newly Synthesized Compound[b] | Topo IIα | | Topo I | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 µM | 20 µM | 100 µM | 20 µM | | 100 µM | 20 µM | 100 µM | 20 µM |
| $R_1$= 2-Cl, $R_2$= 2-OH | **A** | 0.98 | 0.62 | 0.18 | NT | **8** | 1.19 | 1.33 | 0.06 | NT |
| $R_1$= 3-Cl, $R_2$= 2-OH | **B** | 0.97 | 0.91 | 1.01 | 0.38 | **9** | 1.13 | 1.46 | 0.06 | NT |
| $R_1$= 4-Cl, $R_2$= 2-OH | **C** | 0.99 | 0.63 | 1.39 | 0.93 | **10** | 1.34 | 1.59 | 0.10 | NT |
| $R_1$= 2-Cl, $R_2$= 3-OH | **D** | 0.95 | 0.42 | 1.22 | 0.98 | **11** | 1.37 | 1.61 | 0.99 | 0.05 |
| $R_1$= 3-Cl, $R_2$= 3-OH | **E** | 0.98 | 0.65 | 1.12 | 2.02 | **12** | 1.26 | 1.79 | 0.36 | NA |
| $R_1$= 4-Cl, $R_2$= 3-OH | **F** | 1.01 | 0.27 | 1.32 | 2.09 | **13** | 1.07 | 1.80 | 0.36 | NT |
| $R_1$= 2-Cl, $R_2$= 4-OH | **G** | 1.07 | 0.56 | 1.39 | 1.99 | **14** | 1.24 | 2.10 | NA | NT |
| $R_1$= 3-Cl, $R_2$= 4-OH | **H** | 1.01 | 0.77 | 1.56 | 1.29 | **15** | 1.44 | 1.61 | NA | NT |
| $R_1$= 4-Cl, $R_2$= 4-OH | **I** | 1.15 | 1.75 | 1.62 | 1.40 | **16** | 1.32 | 1.18 | NA | NT |

*Relative potency: % inhibition of compound / % inhibition of positive control, NT: not tested, NA: not active.
[a]Previously reported compounds **A-I,**[23]; [b]newly synthesized compounds **8-16.**

## 3. Conclusion

In conclusion, we synthesized a new series of hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines based on the strategy of ring expansion from constrained five membered heterocyclic ring to more stable six membered heterocycles. We evaluated the influence of this replacement on topo I and IIα inhibitory activity, and antiproliferative activity. All the synthesized compounds displayed selective topo IIα inhibitory activity in contrast to dual topo I and IIα inhibitory activity of previously reported benzofuro[3,2-*b*]pyridines. Most of the compounds showed comparatively better antiproliferative activity against human cervix tumor cell line (HeLa) than etoposide. Structure-activity relationships of compounds suggests the importance of hydroxyphenyl moiety at 4-position of central pyridine ring for selectively inhibiting topo IIα activity and antiproliferativ activity. Similarly, 3′- and 4′-hydroxyphenyl substitution was favorable for better activity as compared to 2′-hydroxyphenyl substitution at 2- position of central pyridine. Experiments to investigate mode of action of a compound **14,** which showed potent and selective topo IIα inhibition and strong antiproliferative activity, is underway and will be reported in somewhere else. The findings from this study could be useful for researcher to further design and develop new chromenopyridine analogues as safer and more efficacious selective topo IIα targeting anticancer agents.

## 4. Experimental

Commercially available starting materials and reagents were purchased from Sigma-Aldrich, TCI Chemicals, Alfa-Aesar, and Junsei, and were used without further purification. HPLC grade acetonitrile (ACN) and methanol were purchased from Burdick and Jackson, USA. Column chromatography was carried out using silica gel (Kieselgel 60, 230-400 mesh, Merck). Thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60 $F_{254,}$ Merck) having layer thickness of 0.25 mm. Melting points were recorded using open capillary tube on electrothermal 1A 9100 digital melting point apparatus and were uncorrected. $^{1}$H NMR (250 MHz) and $^{13}$C NMR (62.5 MHz) spectra were recorded in Bruker AMX 250 (250 MHz, FT) spectrometer using $CDCl_3$ and DMSO-$d_6$ as solvent, and tetramethylsilane (TMS) as internal standard. All the chemical shifts values ($\delta$) were recorded in parts per million (ppm) and coupling constants ($J$) in hertz (Hz).

HPLC analyses were performed using two Shimadzu LC-10AT pumps gradient-controlled HPLC system equipped with Shimadzu system controller (SCL-10A VP) and photo diode array detector (SPD-M10A VP) utilizing Shimadzu LC Solution program. Sample volume of 10 µL, was run in Waters X- Terra® 5 µM reverse-phase $C_{18}$ column (4.6 x 250 mm) with a gradient elution of 85% to 100% of B in A for 10 min followed by 100% to 85% of B in A for 10 min at a flow rate of 0.7 mL/min at 254 nm UV detection, where mobile phase A was doubly distilled water with 50 mM ammonium formate (AF) and B was 100% ACN. Purity of compound is stated as percent (%) and retention time in minutes. Mass spectra of the compounds were recorded with Advion Expression CMS® ESI-MS spectrometer (Advion, Ithaca, NY, USA), using methanol: water: formic acid (80: 20: 0.1) as a mobile phase.

**4. 1. General method for preparation of 4-chromanone intermediate 5a-5f**

*4.1.1. Synthesis of 3-(2-hydroxybenzylidene)chroman-4-one (**5a**)*

To the mixture of 2-hydroxybenzaldehyde **1a** (1.6 mL, 15.0 mmol, 1.0 equiv.) and DIPEA (7.8 mL, 45.0 mmol, 3.0 equiv.) in $CH_2Cl_2$ (25 mL) was added MOM-Cl (1.7 mL, 22.5 mmol, 1.5 equiv.). The mixture was stirred for 3 h at room temperature. After the completion of reaction monitored by TLC, $H_2O$ (150 mL) was added and extracted with $CH_2Cl_2$ (50 mL, 3 times). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo at 60 °C and dried under high vacuum to give ayellowish brown oil. Silica gel column chromatography was used to purify the compound using ethyl acetate and *n*-hexane as eluents to yield **2a** (2.40 g, 96.5%, 14.5 mmol) as a light yellow oil. In a further step, to the solution of **2a** (2.4 g, 14.5 mmol, 1.0 equiv.) and 4-chromanone **3** (2.7 g, 16.2 mmol, 1.0 equiv.) in methanol (30 mL) was added aqueous NaOH (0.8 g in 11 mL $H_2O$) dropwise and stirred for 24 h at room temperature. The mixture was evaporated, extracted with ethyl acetate (200 mL), and washed with distilled water (50 mL, 5 times) followed by saturated NaCl solution (50 mL, 2 times). The organic layer was dried with anhydrous $MgSO_4$ and filtered off. Purification was performed by silica gel column chromatography using ethyl acetate and *n*-hexane as eluents to obtain **4a** (4.59 g, 95.6%, 15.5 mmol) as a yellow solid. Finally, deprotection of **4a** was achieved by refluxing **4a** (4.6 g, 15.5 mmol, 1.0 equiv.) with aqueous 2 M HCl (11.7 mL, 1.5 equiv.) in ethanol (60 mL) for 1 h at 80 °C. The mixture was evaporated and extracted with ethyl acetate (200 mL), washed with distilled water (50 mL, 4 times), and saturated NaCl solution (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered off. Further purification was performed using silica gel column chromatography by elution of ethyl acetate and *n*-hexane to give 4-chromanone intermediate **5a** (1.96 g, 50.2%, 7.8 mmol) as a yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:2) $R_f$ = 0.23, mp: 187.2-187.9 °C.

$^{1}$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 10.24 (s, 1H, phenyl 2-OH), 7.88-7.85 (m, 2H, chromanone H-5 and =C**H**-), 7.58 (td, $J$ = 6.90, 0.77 Hz, 1H, chromanone H-7), 7.28 (t, $J$ = 7.37 Hz, 1H, phenyl H-4), 7.14-7.08 (m, 2H, chromanone H-6 and phenyl H-6), 7.04 (d, $J$ = 8.30 Hz, 1H, chromanone H-8), 6.94 (d, $J$ = 8.17 Hz, 1H, phenyl H-3), 6.88 (t, $J$ = 7.47 Hz, 1H, phenyl H-5), 5.30 (s, 2H, -C**H**$_2$-). $^{13}$C NMR (62.5 MHz, DMSO-$d_6$) $\delta$ 181.46, 160.75, 156.89, 136.20, 133.17, 131.64, 130.63, 129.62, 127.33, 122.02, 121.70, 120.94, 119.11, 117.99, 115.83, 67.79.

*4.1.2. Synthesis of 3-(3-hydroxybenzylidene)chroman-4-one (**5b**)*

To the mixture of 3-hydroxybenzaldehyde **1b** (3.1 mL, 25.0 mmol, 1.0 equiv.) and DIPEA (13.1 mL, 75.0 mmol, 3.0 equiv.) in $CH_2Cl_2$ (40 mL) was added MOM-Cl (2.9 mL, 37.5 mmol, 1.5 equiv.). The mixture was stirred for 3 h at room temperature. After the completion of reaction monitored by TLC, $H_2O$ (150 mL) was added and extracted with $CH_2Cl_2$ (50 mL, 3 times). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo at 60 °C and dried under high vacuum to afford alight brown oil. Silica gel column chromatography was used to purify the compound using ethyl acetate and *n*-hexane as eluents to yield **2b** (3.59 g, 86.3%, 21.6 mmol) as a light yellow oil. In further step, to the solution of **2b** (3.6 g, 21.6 mmol, 1.0 equiv.) and 4-chromanone **3** (3.2 g, 21.6 mmol, 1.0 equiv.) in methanol (45 mL) was added aqueous NaOH (1.0 g in 15 mL $H_2O$) dropwise and stirred for 24 h at room temperature. The mixture was evaporated, extracted with ethyl acetate (200 mL), and washed with distilled water (50 mL, 6 times) followed by saturated NaCl solution (50 mL, 2 times). The organic layer was dried with anhydrous $MgSO_4$ and filtered off. Purification was performed by silica gel column chromatography using ethyl acetate and *n*-hexane as eluents to obtain **4b** (4.73 g, 74.0%, 15.9 mmol) as a yellow solid. Finally, deprotection of **4b** was achieved by refluxing **4b** (4.7 g, 15.9 mmol, 1.0 equiv.) with aqueous 2 M HCl (11.9 mL,

1.5 equiv.) in ethanol (70 mL) for 3.5 h at 80 °C. Then, the reaction mixture was evaporated and extracted with ethyl acetate (200 mL), washed with distilled water (50 mL, 4 times), and saturated NaCl solution (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered off. Further purification was performed using silica gel column chromatography by elution of ethyl acetate and *n*-hexane to give 4-chromanone intermediate **5b** (2.03 g, 50.5%, 8.1 mmol) as a yellow solid.

TLC (ethyl acetate/ *n* -hexane = 1:3) $R_f$ = 0.28, mp: 219.3-219.8 °C.

$^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 9.68 (s, 1H, phenyl 3-OH), 7.87 (dd, *J* = 7.85, 1.67 Hz, 1H, chromanone H-5), 7.65 (br s, 1H, -C**H**-), 7.58 (td, *J* = 7.05 1.77 Hz, 1H, chromanone H-7), 7.28 (t, *J* = 7.82 Hz, 1H, phenyl H-5), 7.12 (td, *J* = 7.95, 1.0 Hz, 1H, chromanone H-6), 7.04 (dd, *J* = 8.32, 0.55 Hz, 1H, chromanone H-8), 6.88-6.81 (m, 3H, phenyl H-2, H-4, and H-6), 5.38 (s, 2H, -C**H$_2$**-). $^{13}$C NMR (62.5 MHz, DMSO-$d_6$) $\delta$ 181.15, 160.60, 157.47, 136.69, 136.16, 134.93, 130.56, 129.81, 127.20, 121.90, 121.45, 121.05, 117.86, 116.85, 116.65, 67.33.

*4.1.3. Synthesis of 3-(4-hydroxybenzylidene)chroman-4-one (**5c**)*

To a solution of 4-chromanone **3** (1.5 g, 10.0 mmol, 1.0 equiv.) and 4-hydroxybenzaldehyde **1c** (1.2 g, 10.0 mmol, 1.0 equiv.) in dioxane (10 mL) was slowly added boron trifluoride diethyl etherate (2.1 mL, 15.0 mmol, 1.5 equiv.). The mixture was stirred for 24 h at room temperature. After the completion of reaction monitored by TLC, the mixture was diluted with ethyl acetate (100 mL) and washed with distilled water (40 mL, 4 times), and saturated NaCl (40 mL, 2 times). The organic layer was dried with anhydrous $MgSO_4$ and filtered off. Silica gel column chromatography was used to purify the compound using ethyl acetate and *n*-hexane as eluent to afford 4-chromaonne intermediate **5c** (1.43 g, 56.6%, 5.6 mmol) as a yellow solid.

TLC (ethyl acetate/ *n* -hexane = 1:2) $R_f$ = 0.23, mp: 238.9-239.6 °C.

$^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 10.18 (s, 1H, phenyl 4-OH), 7.85 (dd, *J* = 7.82, 1.62 Hz, 1H, chromanone H-5), 7.66 (br s, 1H, -C**H**-), 7.56 (td, *J* = 8.50, 1.67 Hz, 1H, chromanone H-7), 7.33 (d, *J* = 8.62 Hz, 2H, phenyl H-2 and H-6), 7.10 (t, *J* = 7.37 Hz, 1H, chromanone H-6), 7.03 (d, *J* = 8.25 Hz, 1H, chromanone H-8), 6.87 (d, *J* = 8.55 Hz, 2H, phenyl H-3 and H-5), 5.41 (s, 2H, -C**H$_2$**-). $^{13}$C NMR (62.5 MHz, DMSO-$d_6$) $\delta$ 181.13, 160.52, 159.40, 137.06, 136.06, 132.93 (2C), 127.65, 127.28, 124.87, 121.97, 121.71, 117.91, 115.88 (2C), 67.65.

*4.1.4. Synthesis of 3-(2-chlorobenzylidene)chroman-4-one* (**5d**)

To the solution of 4-chromanone **3** (0.9 g, 6.0 mmol, 1.0 equiv.) and 2-chlorobenzaldehyde **1d** (1.0 mL, 9.0 mmol, 1.5 equiv.) in ethanol (6 mL), 5% aqueous NaOH solution (4 mL) was added dropwise which resulted precipitation. The mixture was stirred for 1 h at room temperature. The precipitate was isolated by filtration and washed with cold ethanol to obtain 4-chromanone intermediate **5d** (1.27 g, 78.2%, 4.7 mmol) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:7 v/v) $R_f$ = 0.27; mp: 114.5-115.1 $^o$C

$^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.02 (dd, *J* = 7.87, 1.70 Hz, 1H, chromanone H-5), 7.94 (s, 1H, =C**H**-), 7.51-7.44 (m, 2H, chromanone H-6 and 2-phenyl H-6), 7.37-7.27 (m, 2H, 2-phenyl H-4 and H-5), 7.12-7.03 (m, 2H, chromanone H-7 and 2-phenyl H-3), 6.95 (dd, *J* = 8.35, 0.67 Hz, 1H, chromanone H-8), 5.16 (s, 2H, chromanone H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 182.35, 161.47, 136.28, 135.15, 134.77, 133.04, 132.59, 130.74, 130.54, 130.27, 128.21, 126.83, 122.22, 122.11, 118.19, 67.70.

*4.1.5. Synthesis of 3-(3-chlorobenzylidene)chroman-4-one* (**5e**)

The procedure described in Section 4.1.4 was employed with **3** (0.9 g, 6.0 mmol, 1.0 equiv.), **1e** (1.0 mL, 9.0 mmol, 1.5 equiv.) and aqueous NaOH solution (4 mL) to obtain intermediate **5e** (1.33 g, 81.8%, 4.9 mmol) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:10 v/v) $R_f$ = 0.26; mp: 138.9-139.7 $^oC$

$^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 8.00 (dd, $J$ = 7.90, 1.67 Hz, 1H, chromanone H-5), 7.76 (s, 1H, =C**H**-), 7.48 (td, $J$ = 7.12, 1.77 Hz, 1H, chromanone H-6), 7.37-7.32 (m, 2H, 2-phenyl H-5 and H-6), 7.26 (br, 1H, 2-phenyl H-2), 7.19-7.14 (m, 1H, 2-phenyl H-4), 7.06 (td, $J$ = 7.07, 1.02 Hz, 1H, chromanone H-7), 6.95 (dd, $J$ = 8.32, 0.65 Hz, 1H, chromanone H-8), 5.28 (s, 2H, chromanone H-2). $^{13}C$ NMR (62.5 MHz, $CDCl_3$) $\delta$ 182.13, 161.34, 136.29, 135.93, 134.93, 132.26, 130.20, 129.82, 129.62, 128.17, 128.15, 122.26, 122.04, 118.18, 67.57.

*4.1.6. Synthesis of 3-(4-chlorobenzylidene)chroman-4-one* (**5f**)

The procedure described in Section 4.1.4 was employed with **3** (0.9 g, 6.0 mmol, 1.0 equiv.), **1f** (1.3 g, 9.0 mmol, 1.5 equiv.) and aqueous NaOH solution (4 mL) to obtain intermediate **5f** (0.99 g, 61.3%, 3.7 mmol) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:10 v/v) $R_f$ = 0.33; mp: 128.8-129.3 $^oC$

$^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 7.99 (dd, $J$ = 7.87, 1.75 Hz, 1H, chromanone H-5), 7.78 (s, 1H, =C**H**-), 7.51-7.38 (m, 3H, chromanone H-6, 2-phenyl H-2 and H-6), 7.22 (d, $J$ = 8.65 Hz, 2H, 2-phenyl H-3 and H-5), 7.05 (td, $J$ = 7.80, 1.02 Hz, 1H, chromanone H-7), 6.95 (dd, $J$ = 8.35, 0.60 Hz, 1H, chromanone H-8), 5.29 (s, 2H, chromanone H-2). $^{13}C$ NMR (62.5 MHz, $CDCl_3$) $\delta$ 182.20, 161.26, 134.26, 136.24, 135.77, 132.95, 131.54, 131.40 (2C), 129.25 (2C), 128.15, 122.24, 122.07, 118.14, 67.62.

**4. 2.General method for preparation of aryl pyridinium iodide salts 7 (R =a-f)**

Aryl pyridinium iodide salts **7** (R = **a-f**) were synthesized by refluxing aryl methyl ketones **6** (R = **a-f**) with the equivalent amount of iodine in pyridine at 140 °C for 3 hours. The mixture was cooled to room temperature which resulted precipitation. The precipitate was filtered and washed with cold pyridine followed by drying overnight to yield 52.2-93.0% of **7a-7f**. Compounds were used without further purification.

**4. 3. General method for preparation of 8-25**

Modified Kröhnke pyridine synthetic method was used for the synthesis of chloro and hydroxy-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines (**8-25**). To the mixture of chromanone intermediates (**5a-5f**) and pyridinium iodide salts **7** (R = **a-f**) in glacial acetic acid was added anhydrous ammonium acetate (10.0 equiv.). The mixtue was refluxed at 100 °C for 12-24 h. After the completion of reaction monitored by TLC, the mixture was extracted with ethyl acetate then washed with distilled water and saturated NaCl solution. The organic layer was dried with anhydrous $MgSO_4$ and filtered off. Further purification was performed by silica gel column chromatography using ethyl acetate and *n*-hexane as eluents to afford 2,4-disubstituted *5H*-chromeno[4,3-*b*]pyridines (**8-25**) with the yields of 22.1-74.3%.

*4.3.1. Synthesis of 2-(2-(2-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**8**)

The procedure described in Section 4.3 was employed with **5a** (0.3 g, 1.0 mmol, 1.0 equiv.), **7a** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **8** (0.10 g, 26.8%, 0.3 mmol) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:3 v/v) $R_f$ = 0.27; mp: 208.1-208.9 $^oC$; HPLC: Retention time: 11.7 min, purity: 96.6%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.16.

$^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.35 (dd, $J$ = 7.72, 1.55 Hz, 1H, chromeno H-5), 7.77 (dd, $J$ = 7.20, 2.12 Hz, 1H, 2-phenyl H-6), 7.55 (s, 1H, pyridine H-3), 7.48 (dd, $J$ = 7.60, 1.50 Hz, 1H, 2-phenyl H-3), 7.41-7.27 (m, 4H, chromeno H-7, 2-phenyl H-4, H-5 and 4-phenyl H-5), 7.19 (dd, $J$ = 7.57, 1.60 Hz, 1H, 4-phenyl H-6), 7.11-7.01 (m, 2H, chromeno H-6 and 4-phenyl H-4), 6.95 (d, $J$ = 8.12 Hz, 2H, chromeno H-8 and 4-phenyl H-3), 5.13 (s, 2H, chromeno H-2), 4.98 (s, 1H, 4-phenyl 2-OH). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.56, 156.10, 152.36, 149.17, 141.74, 138.83, 132.30, 131.91, 131.38, 130.49, 130.30, 130.23, 129.70, 127.03, 125.36, 124.86, 123.81, 123.67, 123.47, 122.33, 121.23, 116.80, 116.14, 66.05.

*4.3.2. Synthesis of 2-(2-(3-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**9**)

The procedure described in Section 4.3 was employed with **5a** (0.3 g, 1.0 mmol, 1.0 equiv.), **7b** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **9** (0.09 g, 24.4%, 0.2 mmol) as a yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:3 v/v) $R_f$ = 0.29; mp: 181.7-182.3 $^o$C; HPLC: Retention time: 11.8 min, purity: 95.4%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.28. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.41 (dd, $J$ = 7.71, 1.65 Hz, 1H, chromeno H-5), 7.79 (dd, $J$ = 7.21, 2.22 Hz, 1H, 2-phenyl H-6), 7.58 (s, 1H, pyridine H-3), 7.48 (dd, $J$ = 7.65, 1.49 Hz, 1H, 2-phenyl H-3), 7.38-7.10 (m, 4H, chromeno H-7, 2-phenyl H-4, H-5 and 4-phenyl H-5), 7.13 (dd, $J$ = 7.57, 1.60 Hz, 1H, 4-phenyl H-6), 7.01-6.89 (m, 2H, chromeno H-6 and 4-phenyl H-4), 6.95 (d, $J$ = 8.12 Hz, 2H, chromeno H-8 and 4-phenyl H-3), 5.16 (s, 2H, chromeno H-2), 4.99 (s, 1H, 4-phenyl 2-OH). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.51, 156.11, 152.36, 149.25, 141.74, 138.73, 132.30, 131.88, 131.37, 130.52, 130.30, 130.19, 129.70, 127.13, 125.36, 124.76, 123.81, 123.60, 123.47, 122.29, 121.28, 116.73, 116.34, 66.07.

*4.3.3. Synthesis of 2-(2-(4-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**10**)

The procedure described in Section 4.3 was employed with **5a** (0.2 g, 0.8 mmol, 1.0 equiv.), **7c** (0.4 g, 1.2 mmol, 1.5 equiv.), and dry ammonium acetate (0.6 g, 8.0 mmol, 10.0 equiv.) in glacial acetic acid (2 mL) to afford **10** (0.12 g, 30.9%, 0.3 mmol) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:3 v/v) $R_f$ = 0.27; mp: 199.6-200.4 $^{o}$C; HPLC: Retention time: 7.3 min, purity: 97.5%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.19. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.42 (dd, $J$ = 7.7, 1.8 Hz, 1H, chromeno H-5), 8.08 (d, $J$ = 8.6 Hz, 2H, 2-phenyl H-2 and H-6), 7.56 (s, 1H, pyridine H-3), 7.44 (d, $J$ = 8.6 Hz, 2H, 2-phenyl H-3 and H-5), 7.39-7.27 (m, 2H, chromeno H-7 and 4-phenyl H-5), 7.22-7.01 (m, 3H, chromeno H-6, 4-phenyl H-4 and H-6), 6.95 (dd, $J$ = 8.1, 1.1 Hz, 2H, chromeno H-8 and 4-phenyl H-3), 5.09 (s, 2H, chromeno H-2), 4.99 (s, 1H, 4-phenyl 2-OH). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.90, 155.51, 152.52, 149.26, 143.14, 137.62, 135.52, 131.68, 130.76, 130.45, 129.12 (2C), 128.44 (2C), 125.51, 124.14, 124.06, 123.70, 122.54, 121.56, 120.43, 117.11, 116.33, 66.29.

*4.3.4. Synthesis of 3-(2-(2-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**11**)

The procedure described in Section 4.3 was employed with **5b** (0.3 g, 1.0 mmol, 1.0 equiv.), **7a** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **11** (0.18 g, 46.7%, 0.5 mmol) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:5 v/v) $R_f$ = 0.19; mp: 217.8-218.4 $^{o}$C; HPLC: Retention time: 5.7 min, purity: 98.4%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.18. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.33 (dd, $J$ = 7.72, 1.62 Hz, 1H, chromeno H-5), 7.73 (dd, $J$ = 7.15, 2.22 Hz, 1H, 2-phenyl H-6), 7.51 (s, 1H, pyridine H-3), 7.47 (dd, $J$ = 7.25, 1.97 Hz, 1H, 2-phenyl H-3), 7.40-7.27 (m, 4H, chromeno H-7, 2-phenyl H-4, H-5 and 4-phenyl H-5), 7.09 (td, $J$ = 7.55, 1.10 Hz, 1H, chromeno H-6), 6.95 (dd, $J$ = 8.10, 0.90 Hz, 1H, chromeno H-8),

6.89-6.85 (m, 2H, 4-phenyl H-4 and H-6), 6.77-6.76 (m, 1H, 4-phenyl H-2), 5.40 (s, 1H, 4-phenyl 3-OH), 5.24 (s, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.56, 156.04, 156.01, 149.14, 146.29, 139.06, 138.87, 132.52, 132.06, 131.55, 130.43, 130.24, 129.92, 127.23, 125.58, 124.50, 123.47, 122.71, 122.46, 121.20, 116.99, 115.90, 115.71, 66.06.

*4.3.5. Synthesis of 3-(2-(3-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**12**)

The procedure described in Section 4.3 was employed with **5b** (0.3 g, 1.0 mmol, 1.0 equiv.), **7b** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **12** (0.29 g, 74.3%, 0.7 mmol) as a yellowish white solid.

TLC (ethyl acetate/*n*-hexane = 1:5 v/v) $R_f$ = 0.20; mp: 230.9-231.5 $^{o}$C; HPLC: Retention time: 9.4 min, purity: 97.4 %; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.21. $^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 9.71 (s, 1H, 4-phenyl 3-OH), 8.36-8.22 (m, 3H, chromeno H-5, 2-phenyl H-2 and H-6), 7.90 (s, 1H, pyridine H-3), 7.54-7.49 (m, 2H, 2-phenyl H-4 and H-5), 7.43-7.31 (m, 2H, chromeno H-7, 4-phenyl H-5), 7.19 (td, $J$ = 7.52, 0.95 Hz, 1H, chromeno H-6), 7.01 (d, $J$ = 7.37 Hz, 1H, chromeno H-8), 6.93-6.88 (m, 3H, 4-phenyl H-2, H-4 and H-6), 5.27 (s, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, DMSO-$d_6$) $\delta$ 157.53, 156.01, 153.47, 147.83, 147.50, 140.36, 137.65, 133.75, 131.54, 130.58, 129.76, 129.01, 126.32, 125.31, 124.85, 123.03, 122.79, 122.32, 119.98, 119.27, 116.71, 115.76, 115.47, 65.36.

*4.3.6. Synthesis of 3-(2-(4-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**13**)

The procedure described in Section 4.3 was employed with **5b** (0.3 g, 1.0 mmol, 1.0 equiv.), **7c** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.)

in glacial acetic acid (3 mL) to afford **13** (0.24 g, 62.8%, 0.6 mmol) as a yellowish white solid.

TLC (ethyl acetate/*n*-hexane = 1:5 v/v) $R_f$ = 0.21; mp: 178.8-179.6 $^{o}$C; HPLC: Retention time: 8.7 min, purity: 97.7%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.26. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.42 (d, *J* = 7.67 Hz, 1H, chromeno H-5), 8.07 (d, *J* = 8.52 Hz, 2H, 2-phenyl H-2 and H-6), 7.53 (s, 1H, pyridine H-3), 7.43 (d, *J* = 8.50 Hz, 2H, 2-phenyl H-3 and H-5), 7.36-7.30 (m, 2H, chromeno H-7 and 4-phenyl H-5), 7.14 (t, *J* = 7.57 Hz, 1H, chromeno H-6), 6.97-6.83 (m, 3H, chromeno H-8, 4-phenyl H-4 and H-6), 6.75 (br s, 1H, 4-phenyl H-2), 5.21 (br s, 3H, 4-phenyl 3-OH and chromeno H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.61, 156.01, 155.12, 149.08, 147.30, 139.06, 137.56, 135.44, 131.63, 130.32, 129.09 (2C), 128.37 (2C), 125.48, 123.73, 122.60, 121.07, 119.76, 117.05, 115.9, 115.52, 66.05.

*4.3.7. Synthesis of 4-(2-(2-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**14**)

The procedure described in Section 4.3 was employed with **5c** (0.2 g, 0.8 mmol, 1.0 equiv.), **7a** (0.4 g, 1.2 mmol, 1.5 equiv.), and dry ammonium acetate (0.6 g, 8.0 mmol, 10.0 equiv.) in glacial acetic acid (2 mL) to afford **14** (0.14 g, 37.1%, 0.4 mmol) as a yellowish white solid.

TLC (ethyl acetate/*n*-hexane = 1:3 v/v) $R_f$ = 0.26; mp: 231.4-232.2 $^{o}$C; HPLC: Retention time: 6.5 min, purity: 96.6%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.31. $^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 9.90 (s, 1H, 4-phenyl 4-OH), 8.17 (dd, *J* = 7.70, 1.40 Hz, 1H, chromeno H-5), 7.76-7.72 (m, 1H, 2-phenyl H-6), 7.62-7.58 (m, 1H, 2-phenyl H-3), 7.51-7.46 (m, 3H, pyridine H-3, chromeno H-7 and 2-phenyl H-4), 7.41-7.29 (m, 3H, 2-phenyl H-5, 4-phenyl H-2 and H-6), 7.12 (t, *J* = 7.60 Hz, 1H, chromeno H-6), 7.01 (d, *J* = 8.05 Hz, 1H, chromeno H-8), 6.91 (d, *J* = 8.42 Hz, 2H, 4-phenyl H-3 and H-5), 5.34 (s, 2H,

chromeno H-2). $^{13}$C NMR (62.5 MHz, DMSO-$d_6$) $\delta$ 157.50, 156.78, 154.93, 149.73, 147.61, 141.84, 135.51, 131.77, 130.49 (2C), 129.92, 129.30, 127.29, 125.94, 125.26, 123.84, 123.09, 122.69, 120.55, 116.87, 115.96 (2C), 66.41

*4.3.8. Synthesis of 4-(2-(3-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**15**)

The procedure described in Section 4.3 was employed with **5c** (0.1 g, 0.5 mmol, 1.0 equiv.), **7b** (0.3 g, 0.8 mmol, 1.5 equiv.), and dry ammonium acetate (0.4 g, 5.0 mmol, 10.0 equiv.) in glacial acetic acid (1.5 mL) to afford **15** (0.04 g, 22.1%, 0.1 mmol) as a yellowish white solid. TLC (ethyl acetate/*n*-hexane = 1:3 v/v) $R_f$ = 0.28; mp: 188.6-189.2 $^{o}$C; HPLC: Retention time: 8.7 min, purity: 96.9%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.13. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.43 (dd, $J$ = 7.72, 1.62 Hz, 1H, chromeno H-5), 8.14 (br s, 1H, 2-phenyl H-2), 8.04-7.95 (m, 1H, 2-phenyl H-6), 7.54 (s, 1H, pyridine H-3), 7.43-7.29 (m, 3H, chromeno H-7, 2-phenyl H-4 and H-5), 7.22-7.11 (m, 3H, chromeno H-6, 4-phenyl H-2 and H-6), 6.97-6.90 (m, 3H, chromeno H-8, 4-phenyl H-3 and H-5), 5.24-5.21 (m, 3H, chromeno H-2 and 4-phenyl 4-OH). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.59, 156.28, 154.81, 149.13, 147.37, 141.14, 135.01, 131.57, 130.18 (2C), 129.89, 129.22, 127.29, 125.56, 125.16, 123.84, 122.99, 122.62, 120.23, 116.99, 115.94 (2C), 66.12.

*4.3.9. Synthesis of 4-(2-(4-chlorophenyl)-5H-chromeno[4,3-b]pyridin-4-yl)phenol* (**16**)

The procedure described in Section 4.3 was employed with **5c** (0.1 g, 0.5 mmol, 1.0 equiv.), **7c** (0.3 g, 0.8 mmol, 1.5 equiv.), and dry ammonium acetate (0.4 g, 5.0 mmol, 10.0 equiv.) in glacial acetic acid (1.5 mL) to afford **16** (0.06 g, 32.4%, 0.2 mmol) as a yellowish white solid. TLC (ethyl acetate/*n*-hexane = 1:3 v/v) $R_f$ = 0.26; mp: 223.5-224.3 $^{o}$C; HPLC: Retention time: 9.6 min, purity: 99.3%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.45.

$^{1}$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 9.89 (s, 1H, 4-phenyl 4-OH), 8.36-8.28 (m, 3H, chromeno H-5, 2-phenyl H-2 and H-6), 7.84 (s, 1H, pyridine H-3), 7.56 (d, $J$ = 8.57 Hz, 2H, 2-phenyl H-3 and H-5), 7.42-7.32 (m, 3H, chromeno H-7, 2-phenyl H-2 and H-6), 7.71 (t, $J$ = 7.40 Hz, 1H, chromeno H-6), 7.00 (d, $J$ = 7.85 Hz, 1H, chromeno H-8), 6.92 (d, $J$ = 8.50 Hz, 2H, 4-phenyl H-3 and H-5), 5.30 (s, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, DMSO-$d_6$) $\delta$ 158.22, 056.02, 153.76, 147.82, 147.45, 137.25, 134.11, 131.54, 130.30, 128.82, 128.55, 126.99, 124.93, 123.37, 122.57, 122.38, 119.78, 116.80, 115.59, 65.57.

*4.3.10. Synthesis of 2-(4-(2-chlorophenyl)-5H-chromeno[4,3-b]pyridin-2-yl)phenol* (**17**)

The procedure described in Section 4.3 was employed with **5d** (0.3 g, 1.0 mmol, 1.0 equiv.), **7d** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **17** (0.11 g, 27.6%, 0.3 mmol) as a yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:7 v/v) $R_f$ = 0.29; mp: 202.1-202.9 $^{o}$C; HPLC: Retention time: 9.3 min, purity: 97.8%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.39.

$^{1}$H NMR (250 MHz, $CDCl_3$) $\delta$ 14.47 (s, 1H, 2-phenyl 2-OH), 8.10 (dd, $J$ = 7.75, 1.60 Hz, 1H, chromeno H-5), 7.79 (dd, $J$ = 8.03, 1.40 Hz, 1H, 2-phenyl H-6), 7.68 (s, 1H, pyridine H-3), 7.55-7.50 (m, 1H, 4-phenyl H-6), 7.47-7.28 (m, 5H, 2-phenyl H-4, 4-phenyl H-3, H-4, H-5 and chromeno H-7), 7.15 (td, $J$ = 7.62, 1.05 Hz, 1H, chromeno H-6), 7.06 (dd, $J$ = 8.27, 1.12 Hz, 1H, chromeno H-8), 6.98 (dd, $J$ = 8.15, 0.80 Hz, 1H, 2-phenyl H-3), 6.89 (td, $J$ = 7.07, 1.17 Hz, 1H, 2-phenyl H-5), 5.52 (dd, $J$ = 43.95, 13.97 Hz, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 160.07, 157.04, 156.81, 146.30, 146.05, 135.93, 132.86, 132.28, 131.93, 130.61, 130.47, 130.16, 127.51, 126.61, 124.79, 123.05, 122.95, 122.04, 119.22, 119.18, 118.91, 119.74, 117.51, 65.84.

*4.3.11. Synthesis of 3-(4-(2-chlorophenyl)-5H-chromeno[4,3-b]pyridin-2-yl)phenol* (**18**)

The procedure described in Section 4.3 was employed with **5d** (0.3 g, 1.0 mmol, 1.0 equiv.), **7e** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **18** (0.11 g, 27.9%, 0.3 mmol) as a light yellow solid. TLC (ethyl acetate/*n*-hexane = 1:5 v/v) $R_f$ = 0.20; mp: 169.8-170.6 $^{o}$C; HPLC: Retention time: 6.8 min, purity: 96.7%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.33. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.45 (dd, *J* = 7.70, 1.65 Hz, 1H, chromeno H-5), 7.71 (br s, 1H, 2-phenyl H-2), 7.63 (d, *J* = 7.82 Hz, 1H, 2-phenyl H-6), 7.53-7.49 (m, 2H, pyridine H-3 and 4-phenyl H-6), 7.42-7.26 (m, 5H, 2-phenyl H-5, 4-phenyl H-3, H-4, H-5 and chromeno H-7), 7.12 (td, *J* = 7.47, 0.97 Hz, 1H, chromeno H-6), 6.96-6.87 (m, 2H, chromeno H-8 and 2-phenyl H-4), 5.20-4.92 (m, 3H, 2-phenyl 3-OH and chromeno H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.68, 156.22, 155.78, 148.73, 144.77, 140.78, 136.36, 132.98, 131.59, 130.62, 130.29, 130.17, 130.06, 127.36, 125.46, 123.68, 123.40, 122.54, 120.35, 119.52, 117.07, 116.41, 114.09, 66.03.

*4.3.12. Synthesis of 4-(4-(2-chlorophenyl)-5H-chromeno[4,3-b]pyridin-2-yl)phenol* (**19**)

The procedure described in Section 4.3 was employed with **5d** (0.3 g, 1.0 mmol, 1.0 equiv.), **7f** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **19** (0.14 g, 35.4%, 0.4 mmol) as a yellow solid. TLC (ethyl acetate/*n*-hexane = 1:3 v/v) $R_f$ = 0.24; mp: 187.5-188.1 $^{o}$C; HPLC: Retention time: 8.3 min, purity: 98.3%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.35. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.45 (dd, *J* = 7.70, 1.70 Hz, 1H, chromeno H-5), 8.05 (d, *J* = 8.7 Hz, 2H, 2-phenyl H-2 and H-6), 7.53-7.26 (m, 6H, pyridine H-3, 4-phenyl H-3, H-4, H-5, H-6 and chromeno H-7), 7.14 (td, *J* = 7.32, 1.10 Hz, 1H, chromeno H-6), 6.95-6.90 (m, 3H, chromeno H-8 and 2-phenyl H-3 and H-5), 5.25-4.90 (m, 3H, 2-phenyl 4-OH and chromeno

H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.93, 156.68, 155.98, 148.55, 144.70, 136.56, 133.01, 131.98, 131.46, 130.65, 130.21, 130.04, 128.71 (2C), 127.34, 125.42, 123.83, 122.48, 122.44, 119.43, 117.03, 115.79 (2C), 66.06.

*4.3.13. Synthesis of 2-(4-(3-chlorophenyl)-5H-chromeno[4,3-b]pyridin-2-yl)phenol* (**20**)

The procedure described in Section 4.3 was employed with **5e** (0.3 g, 1.0 mmol, 1.0 equiv.), **7d** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **20** (0.21 g, 51.3%, 0.5 mmol) as a light yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:10 v/v) $R_f$ = 0.30; mp: 188.1-188.9 $^{o}$C; HPLC: Retention time: 10.2 min, purity: 98.7%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.17.

$^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 14.40 (s, 1H, 2-phenyl 2-OH), 8.07 (dd, *J* = 7.75, 1.50 Hz, 1H, chromeno H-5), 7.79 (dd, *J* = 8.05, 2.32 Hz, 1H, 2-phenyl H-6), 7.71 (s, 1H, pyridine H-3), 7.56-7.19 (m, 6H, 2-phenyl H-4, 4-phenyl H-2, H-4, H-5, H-6 and chromeno H-7), 7.13 (td, *J* = 7.50, 1.12 Hz, 1H, chromeno H-6), 7.05 (dd, *J* = 8.25, 1.12 Hz, 1H, chromeno H-8), 6.98 (dd, *J* = 8.15, 0.92 Hz, 1H, 2-phenyl H-3), 6.90 (td, *J* = 8.30, 1.25 Hz, 1H, 2-phenyl H-5), 5.18 (s, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 160.07, 157.09, 156.69, 147.29, 146.68, 138.89, 135.19, 132.32, 132.01, 130.43, 129.38, 128.46, 126.47, 126.56, 124.81, 123.03, 122.07, 119.32, 118.77, 117.47, 65.74.

*4.3.14. Synthesis of 3-(4-(3-chlorophenyl)-5H-chromeno[4,3-b]pyridin-2-yl)phenol* (**21**)

The procedure described in Section 4.3 was employed with **5e** (0.3 g, 1.0 mmol, 1.0 equiv.), **7e** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **21** (0.09 g, 23.4%, 0.2 mmol) as a white solid.

TLC (ethyl acetate/*n*-hexane = 1:5 v/v) $R_f$ = 0.25; mp: 210.8-211.6 $^{o}$C; HPLC: Retention time: 7.5 min, purity: 99.6%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.49. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 9.61 (s, 1H, 2-phenyl 3-OH), 8.32 (dd, *J* = 7.72, 1.57 Hz, 1H, chromeno H-5), 7.80 (s, 1H, pyridine H-3), 7.71 (br s, 1H, 2-phenyl H-2), 7.68-7.36 (m, 6H, 2-phenyl H-6, 4-phenyl H-2, H-4, H-5, H-6 and chromeno H-7), 7.30 (t, *J* = 7.92 Hz, 1H, 2-phenyl H-5), 7.19 (td, *J* = 7.52, 1.10 Hz, 1H, chromeno H-6), 7.01 (dd, *J* = 8.10, 0.85 Hz, 1H, chromeno H-8), 6.86 (dd, *J* = 8.00, 2.37 Hz, 1H, 2-phenyl H-4), 5.28 (s, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 157.84, 156.07, 155.30, 147.82, 156.64, 139.54, 138.73, 133.64, 131.64, 130.60, 129.84, 128.65. 128.53, 127.56, 124.80, 123.21, 122.39, 122.34. 119.93, 117.63, 116.87, 116.46, 113.55, 65.32.

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

The procedure described in Section 4.3 was employed with **5e** (0.3 g, 1.0 mmol, 1.0 equiv.), **7f** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **22** (0.15 g, 38.7%, 0.4 mmol) as a light yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:5 v/v) $R_f$ = 0.27; mp: 197.7-198.5 $^{o}$C; HPLC: Retention time: 7.4 min, purity: 97.6%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.42. $^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 9.77 (s, 1H, 2-phenyl 4-OH), 8.33 (dd, *J* = 7.67, 1.57 Hz, 1H, chromeno H-5), 8.13 (d, *J* = 8.67 Hz, 2H, 2-phenyl H-2 and H-6), 7.75 (s, 1H, pyridine H-3), 7.63-7.38 (m, 5H, 4-phenyl H-2, H-4, H-5, H-6 and chromeno H-7), 7.17 (t, *J* = 7.47 Hz, 1H, chromeno H-6), 6.99 (d, *J* = 8.10 Hz, 1H, chromeno H-8), 6.89 (d, *J* = 8.67 Hz, 2H, 2-phenyl H-3 and H-5), 5.24 (s, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, DMSO-$d_6$) $\delta$ 158.83, 155.99, 155.39, 147.45, 145.49, 138.88, 133.55, 131.39, 130.50, 129.06, 128.66, 128.41, 128.25 (2C), 127.44, 124.80, 123.28, 122.26, 121.02, 118.67, 116.70, 115.49 (2C), 65.25.

*4.3.16. Synthesis of 2-(4-(4-chlorophenyl)-5H-chromeno[4,3-b]pyridin-2-yl)phenol* (**23**)

The procedure described in Section 4.3 was employed with **5f** (0.3 g, 1.0 mmol, 1.0 equiv.), **7d** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **23** (0.12 g, 31.5%, 0.3 mmol) as a light yellow solid. TLC (ethyl acetate/*n*-hexane = 1:10 v/v) $R_f$ = 0.39; mp: 217.1-217.9 $^{o}$C; HPLC: Retention time: 10.6 min, purity: 97.1%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.36.

$^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 14.45 (s, 1H, 2-phenyl 2-OH), 8.06 (dd, *J* = 7.75, 1.52 Hz, 1H, chromeno H-5), 7.78 (dd, *J* = 8.05, 1.35 Hz, 1H, 2-phenyl H-6), 7.69 (s, 1H, pyridine H-3), 7.49 (d, *J* = 8.37 Hz, 2H, 4-phenyl H-2 and H-6), 7.39-7.25 (m, 4H, 2-phenyl H-4, 4-phenyl H-3, H-6 and chromeno H-7), 7.13 (td, *J* = 7.55, 0.95 Hz, 1H, chromeno H-6), 7.05 (dd, *J* = 8.25, 1.00 Hz, 1H, chromeno H-8), 6.98 (dd, *J* = 8.12, 0.62 Hz, 1H, 2-phenyl H-3), 6.89 (t, *J* = 6.97 Hz, 1H, 2-phenyl H-5), 5.17 (s, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 160.04, 156.97, 156.62, 147.54, 146.57, 135.49, 132.27, 131.95, 129.90 (2C), 129.39 (2C), 126.50, 124.81, 123.01, 122.08, 122.04, 119.19, 118.78, 118.74, 117.44, 65.74.

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

The procedure described in Section 4.3 was employed with **5f** (0.3 g, 1.0 mmol, 1.0 equiv.), **7e** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **24** (0.16 g, 40.5%, 0.4 mmol) as a light yellow solid. TLC (ethyl acetate/*n*-hexane = 1:5 v/v) $R_f$ = 0.21; mp: 154.3-155.1 $^{o}$C; HPLC: Retention time: 7.8 min, purity: 96.9%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.11.

$^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 9.56 (s, 1H, 2-phenyl 3-OH), 8.33 (dd, *J* = 7.67, 1.55 Hz, 1H, chromeno H-5), 7.76 (s, 1H, pyridine H-3), 7.70-7.53 (m, 6H, 2-phenyl H-2, H-6, 4-

phenyl H-2, H-3, H-5 and H-6), 7.40 (td, *J* = 7.62, 1.67 Hz, 1H, chromeno H-7), 7.30 (t, *J* = 7.90 Hz, 1H, 2-phenyl H-5), 7.18 (t, *J* = 7.52 Hz, 1H, chromeno H-6), 7.01 (d, *J* = 7.97 Hz, 1H, chromeno H-8), 6.86 (dd, *J* = 7.87, 2.02 Hz, 1H, 2-phenyl H-4), 5.28 (s, 2H, chromeno H-2). $^{13}$C NMR (62.5 MHz, DMSO-$d_6$) $\delta$ 157.77, 156.01, 155.24, 147.66, 145.83, 139.52, 135.43, 133.75, 131.52, 130.59 (2C), 129.76, 128.76 (2C), 124.73, 123.17, 122.29, 122.23, 119.76, 117.49, 116.76, 116.37, 113.84, 65.29.

*4.3.18. Synthesis of 4-(4-(4-chlorophenyl)-5H-chromeno[4,3-b]pyridin-2-yl)phenol* (**25**)

The procedure described in Section 4.3 was employed with **5f** (0.3 g, 1.0 mmol, 1.0 equiv.), **7f** (0.5 g, 1.5 mmol, 1.5 equiv.), and dry ammonium acetate (0.8 g, 10.0 mmol, 10.0 equiv.) in glacial acetic acid (3 mL) to afford **25** (0.15 g, 38.6%, 0.4 mmol) as a light yellow solid.

TLC (ethyl acetate/*n*-hexane = 1:3 v/v) $R_f$ = 0.28; mp: 189.7-190.2 $^{o}$C; HPLC: Retention time: 7.9 min, purity: 98.3%; ESI-MS: *m/z* calcd for $C_{24}H_{16}ClNO_2$ $[M+H]^+$: 386.09; found: 386.25. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.43 (dd, *J* = 7.72, 1.62 Hz, 1H, chromeno H-5), 8.05 (d, *J* = 8.75 Hz, 2H, 2-phenyl H-2 and H-6), 7.53-7.44 (m, 3H, pyridine H-3, 4-phenyl H-2 and H-6), 7.35-7.25 (m, 3H, 4-phenyl H-3, H-5 and chromeno H-7), 7.13 (td, *J* = 7.55, 1.15 Hz, 1H, chromeno H-6), 6.96-6.91 (m, 3H, 2-phenyl H-3, H-5 and chromeno H-8), 5.18 (s, 2H, chromeno H-2), 4.95 (br s, 1H, 2-phenyl 4-OH). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ 156.94, 156.56, 156.15, 148.92, 146.25, 136.18, 135.01, 131.92, 131.49, 129.97 (2C), 129.21 (2C), 128.68 (2C), 125.53, 123.87, 122.57, 121.53, 119.15, 116.97, 115.84 (2C), 65.96.

### 4.4. Pharmacology

#### 4.4.1. DNA topoisomerase I and IIα relaxation assay

DNA topoisomerase I and IIα relaxation assay was determined following the previously reported method.[24] All the test compounds were dissolved in DMSO at a concentration of 20

mM as a stock solution and stored under -20 °C until needed. The DNA topoisomerase I and IIα inhibitory activity of each compound was measured as follows according to the manufacturer's protocol. A mixture comprising of 100 ng supercoiled pBR322 plasmid DNA (Thermo Scientific, USA) and 1 unit of recombinant human DNA topo I (TopoGEN INC., USA) or topo IIα (Usb Corp., USA) was incubated with and without the prepared compounds in the assay buffer (For topo I, 10 mM Tris-HCl (pH 7.9) containing 150 mM NaCl, 0.1% bovine serum albumin (BSA), 0.1 mM spermidine and 5% glycerol; For topo IIα, 10 mM Tris-HCl (pH 7.9) containing 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP and 15 μg/mL BSA) for 30 min at 37°C. The reaction in a final volume of 10 μL was quenched by adding 1 μL of the stop solution (For topo I, 10% SDS solution containing 0.2% bromophenol blue, 0.2% xylene cyanol and 30% glycerol; for topo IIα, 7 mM EDTA). The reaction products were analyzed on 0.8% agarose gel at 50 V for 1 h with TAEas the running buffer. The gels were stained in an EtBr solution (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and were quantitated using Alpha Tech Imager (Alpha Innotech Corporation).

**4.4.2. Antiproliferative Assay**

Cancer cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of 2-4 x $10^4$ cells per well with incubation for overnight in 0.1 mL of media supplied with 10% fetal bovine serum (FBS, Hyclone, USA) in 5% $CO_2$ incubator at 37 °C. 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 additional incubation for 72 h. Then each well was added with 5 μL of the cell counting kit-8 solution (Dojindo, Japan) followed by additional incubation for 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 using Table Curve 2D (SPSS Inc., USA). The reference compounds such as adriamycin, etoposide and camptothecin were purchased from Sigma (USA).

**Acknowledgements**

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2017R1A2B2003944 and NRF-2017R1D1A1B03027846).

**References**

1. Salazar-Roa M, Malumbres M. Fueling the cell division cycle. *Trends Cell Biol.* 2017;27: 69-81.

2. Cortés F, Pastor N, Mateos S, Domínguez I. Roles of DNA topoisomerases in chromosome segregation and mitosis. *Mutat Res Rev Mutat Res.* 2003;543: 59-66.

3. Wang JC. DNA topoisomerases. *Annu Rev Biochem.* 1996;65: 635-692.

4. Janočková J, Plšíková J, Koval' J, et al. Tacrine derivatives as dual topoisomerase I and II catalytic inhibitors. *Bioorg Chem.* 2015;59: 168-176.

5. Williams GH, Stoeber K. The cell cycle and cancer. *J Pathol.* 2012;226: 352-364.

6. Isaacs RJ, Davies SL, Sandri MI, Redwood C, Wells NJ, Hickson ID. Physiological regulation of eukaryotic topoisomerase II. *BBA-Gene Struct Expr.* 1998;1400: 121-137.

7. Woessner RD, Mattern MR, Mirabelli C, Johnson R, Drake F. Proliferation-and cell cycle-dependent differences in expression of the 170 kilodalton and 180 kilodalton forms of topoisomerase II in NIH-3T3 cells. *Cell Growth Differ.* 1991;2: 209-214.

8. Nicholas DA, Brian G. Topoisomerase II inhibitors and poisons, and the influence of cell cycle checkpoints. *Curr Med Chem.* 2017;24: 1504-1519.

9. Aridoss G, Zhou B, Hermanson DL, Bleeker NP, Xing C. Structure–activity relationship (SAR) study of ethyl 2-amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-*4H*-chromene-3-carboxylate (CXL017) and the potential of the lead against multidrug resistance in cancer treatment. *J Med Chem.* 2012;55: 5566-5581.

10. Puppala M, Zhao X, Casemore D, et al. *4H*-Chromene-based anticancer agents towards multi-drug resistant HL60/MX2 human leukemia: SAR at the $4^{th}$ and $6^{th}$ positions. *Bioorg Med Chem.* 2016;24: 1292-1297.

11. Das SG, Srinivasan B, Hermanson DL, et al. Structure–activity relationship and molecular mechanisms of ethyl 2-amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-*4H*-chromene-3-carboxylate (CXL017) and its analogues. *J Med Chem.* 2011;54: 5937-5948.

12. Rode M, Gupta R, Karale B, Rindhe S. Synthesis and characterization of some substituted chromones as an anti-infective and antioxidant agents. *J Heterocyclic Chem.* 2008;45: 1597-1602.

13. Deng Y, Lee JP, Tianasoa-Ramamonjy M, et al. New antimicrobial flavanones from *Physena madagascariensis*. *J Nat Prod.* 2000;63: 1082-1089.

14. Fujimoto H, Nozawa M, Okuyama E, Ishibashi M. Five new chromones possessing monoamine oxidase inhibitory activity from an ascomycete, *Chaetomium quadrangulatum*. *Chem Pharm Bull.* 2002;50: 330-336.

15. Conrad Jr, Förster-Fromme B, Constantin M-A, et al. Flavonoid glucuronides and a chromone from the aquatic macrophyte *Stratiotes aloides*. *J Nat Prod.* 2009;72: 835-840.

16. Wu CP, Calcagno AM, Hladky SB, Ambudkar SV, Barrand MA. Modulatory effects of plant phenols on human multidrug-resistance proteins 1, 4 and 5 (ABCC1, 4 and 5). *FEBS J.* 2005;272: 4725-4740.

17. Chen H, Huang M, Li X, et al. Phochrodines A–D, first naturally occurring new chromenopyridines from mangrove entophytic fungus *Phomopsis sp.* 33#. *Fitoterapia.* 2018;124: 103-107.

18. Thapa U, Thapa P, Karki R, et al. Synthesis of 2, 4-diaryl chromenopyridines and evaluation of their topoisomerase I and II inhibitory activity, cytotoxicity, and structure–activity relationship. *Eur J Med Chem.* 2011;46: 3201-3209.

19. Thapa P, Jun K-Y, Kadayat TM, et al. Design and synthesis of conformationally constrained hydroxylated 4-phenyl-2-aryl chromenopyridines as novel and selective

topoisomerase II-targeted antiproliferative agents. *Bioorg Med Chem.* 2015;23: 6454-6466.

20. Hernandes MZ, Cavalcanti SMT, Moreira DRM, de Azevedo Junior WF, Leite ACL. Halogen atoms in the modern medicinal chemistry: Hints for the drug design. *Curr Drug Targets.* 2010;11: 303-314.

21. Lin F-Y, MacKerell AD. Do halogen–hydrogen bond donor interactions dominate the favorable contribution of halogens to ligand–protein binding? *J Phys Chem B.* 2017;121: 6813-6821.

22. Thapa Magar TB, Kadayat TM, Lee H-J, et al. 2-Chlorophenyl-substituted benzofuro[3,2-*b*]pyridines with enhanced topoisomerase inhibitory activity: The role of the chlorine substituent. *Bioorg Med Chem Lett.* 2017;27: 3279-3283.

23. Park S, Thapa Magar TB, Kadayat TM, et al. Rational design, synthesis, and evaluation of novel 2,4-chloro- and hydroxy-substituted diphenyl benzofuro[3,2-*b*]pyridines: Non-intercalative catalytic topoisomerase I and II dual inhibitor. *Eur J Med Chem.* 2017;127: 318-333.

24. Riou J-F, Fossé P, Nguyen CH, et al. Intoplicine (RP 60475) and its derivatives, a new class of antitumor agents inhibiting both topoisomerase I and II activities. *Cancer Res.* 1993;53: 5987-5993.

# Revised Supplementary Data

## Synthesis and SAR study of new hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines as selective topoisomerase II*α*-targeting anticancer agents

Til Bahadur Thapa Magar[a,1], Seung Hee Seo[b,1], Tara Man Kadayat[a,1†], Hyunji Jo[b], Aarajana Shrestha[a], Ganesh Bist[a], Pramila Katila[a], Youngjoo Kwon[b,*], Eung-Seok Lee[a,*]

[a]*College of Pharmacy, Yeungnam University, Gyeongsan 38541, Republic of Korea*

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

*Corresponding Authors:

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

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

[1]Authors are equal contributors

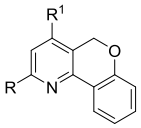
† Present Address: New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu 41061, Republic of Korea

# Contents

1. Table **S1**: prepared compounds with yields, purities measured by HPLC, retention times, and melting points

2. $^{1}$H NMR and $^{13}$C NMR spectra of representative compounds

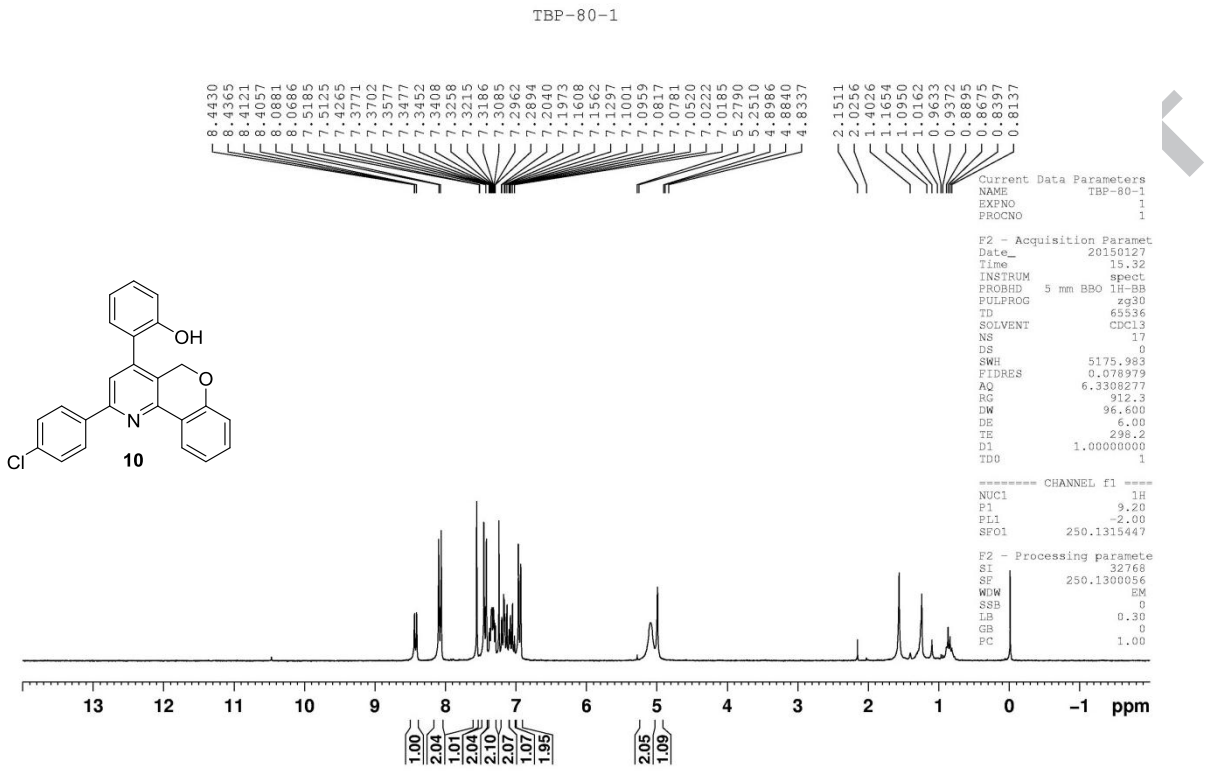
**1.** Table S1. Prepared compounds with yields, purity and retention time by HPLC and melting points



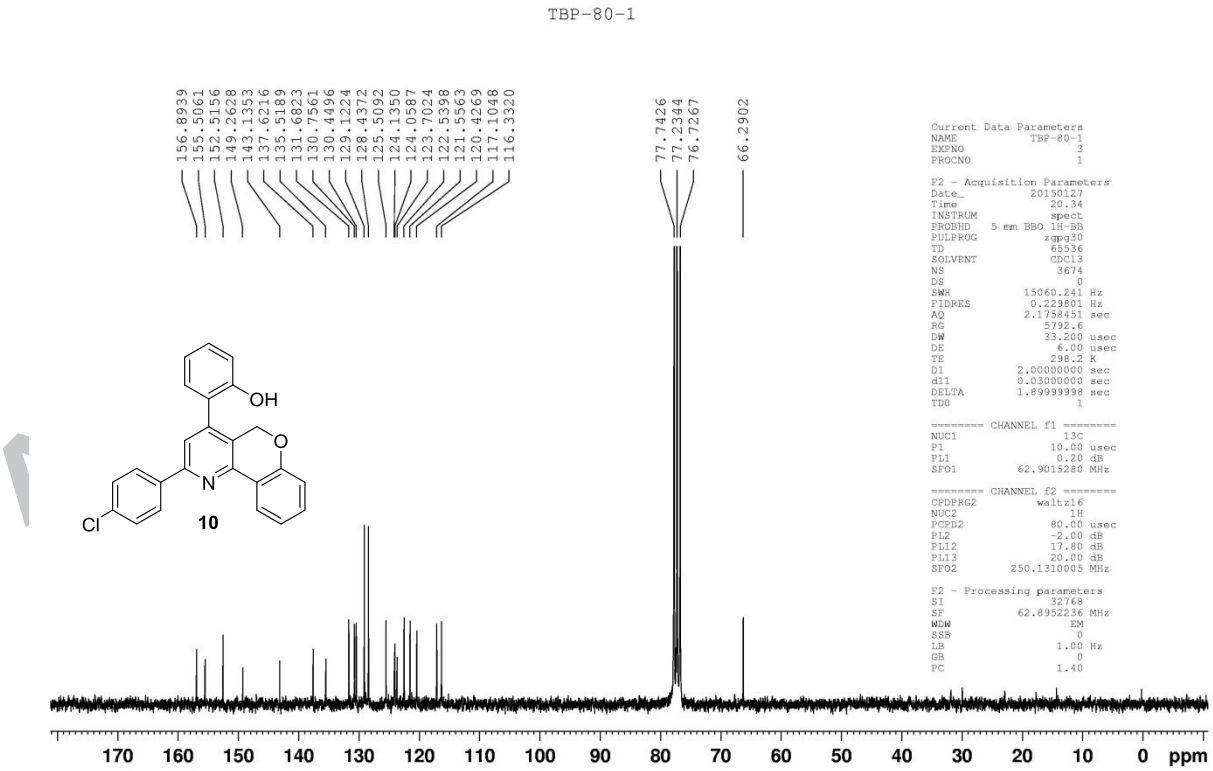

| Compounds | R | $R^1$ | Yield (%) | Purity (%) | Ret. time (Min) | Melting point (°C) |
|---|---|---|---|---|---|---|
| **8** | 2′-Cl phenyl | 2′-OH phenyl | 26.8 | 96.6 | 11.7 | 208.1-208.9 |
| **9** | 3′-Cl phenyl | 2′-OH phenyl | 24.4 | 95.4 | 11.8 | 181.7-182.3 |
| **10** | 4′-Cl phenyl | 2′-OH phenyl | 30.9 | 97.5 | 7.3 | 199.6-200.4 |
| **11** | 2′-Cl phenyl | 3′-OH phenyl | 46.7 | 98.4 | 5.7 | 217.8-218.4 |
| **12** | 3′-Cl phenyl | 3′-OH phenyl | 74.3 | 97.4 | 9.4 | 230.9-231.5 |
| **13** | 4′-Cl phenyl | 3′-OH phenyl | 62.8 | 97.7 | 8.7 | 178.8-179.6 |
| **14** | 2′-Cl phenyl | 4′-OH phenyl | 37.1 | 96.6 | 6.5 | 131.4-132.2 |
| **15** | 3′-Cl phenyl | 4′-OH phenyl | 22.1 | 96.9 | 8.7 | 188.6-189.2 |
| **16** | 4′-Cl phenyl | 4′-OH phenyl | 32.4 | 99.3 | 9.6 | 223.5-224.3 |
| **17** | 2′-OH phenyl | 2′-Cl phenyl | 27.6 | 97.8 | 9.3 | 202.1-202.9 |
| **18** | 3′-OH phenyl | 2′-Cl phenyl | 27.9 | 96.7 | 6.8 | 169.8-170.6 |
| **19** | 4′-OH phenyl | 2′-Cl phenyl | 35.4 | 98.3 | 8.3 | 187.5-188.1 |
| **20** | 2′-OH phenyl | 3′-Cl phenyl | 51.3 | 98.7 | 10.2 | 188.1-188.9 |
| **21** | 3′-OH phenyl | 3′-Cl phenyl | 23.4 | 99.6 | 7.5 | 210.8-211.6 |
| **22** | 4′-OH phenyl | 3′-Cl phenyl | 38.7 | 97.6 | 7.4 | 197.7-198.5 |
| **23** | 2′-OH phenyl | 4′-Cl phenyl | 31.5 | 97.1 | 10.6 | 217.1-217.9 |
| **24** | 3′-OH phenyl | 4′-Cl phenyl | 40.5 | 96.9 | 7.8 | 154.3-155.1 |
| **25** | 4′-OH phenyl | 4′-Cl phenyl | 38.6 | 98.3 | 7.9 | 189.7-190.2 |

Purity of compound is given as percent (%) and retention time in minutes as recorded in High Performance Liquid Chromatography (HPLC) system. Two Shimadzu LC-10AT pumps gradient-controlled HPLC system equipped with Shimadzu system controller (SCL-10A VP) and photo diode array detector (SPD-M10A VP) utilizing Shimadzu LC Solution program. Sample volume of 10 µL, was run in Waters X- Terra® 5 µM reverse-phase $C_{18}$ column (4.6 x 250 mm) with a gradient elution of 85% to 100% of B in A for 10 min followed by 100% to 85% of B in A for 10 min at a flow rate of 0.7 mL/min at 254 nm UV detection, where mobile phase A was doubly distilled water with 50 mM ammonium formate (AF) and B was 100% ACN. Melting points were recorded using open capillary tube on electrothermal 1A 9100 digital melting point apparatus and were uncorrected.

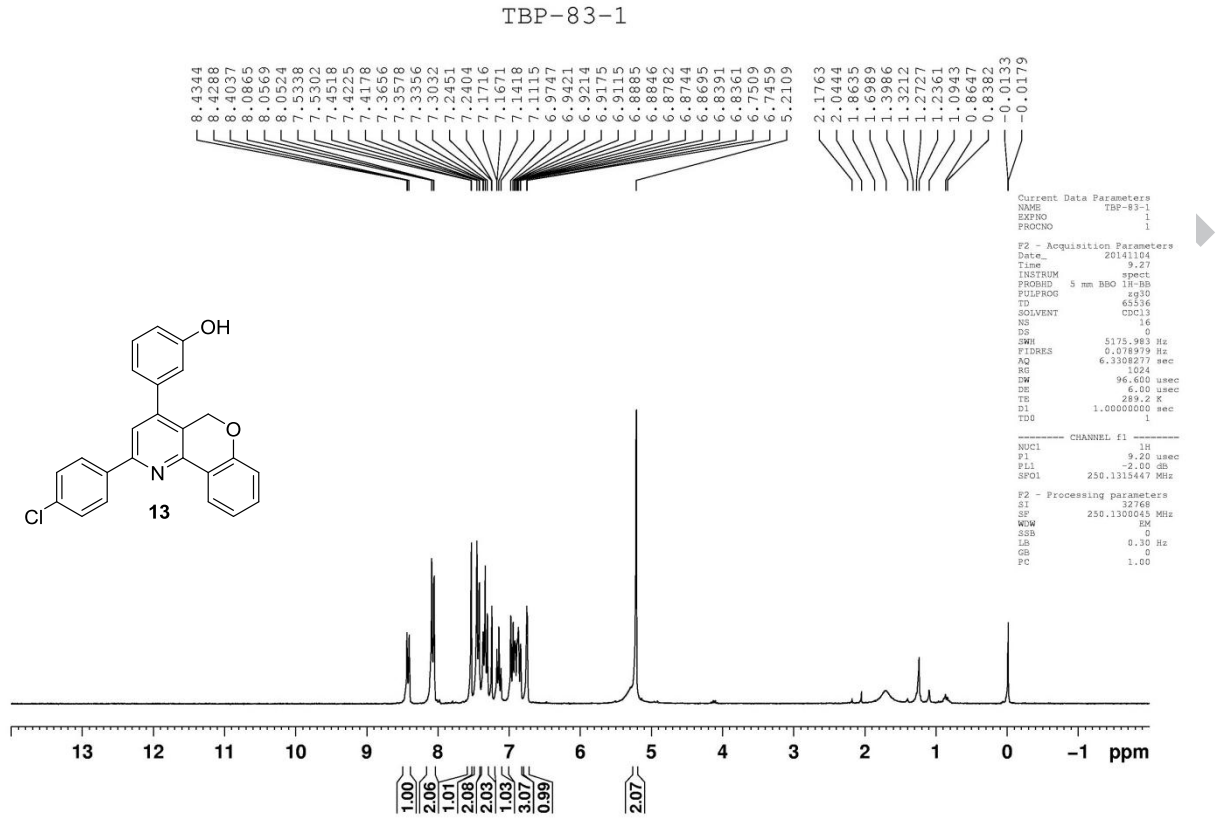
**2.** $^{1}$H NMR and $^{13}$C NMR Spectra of representative compounds



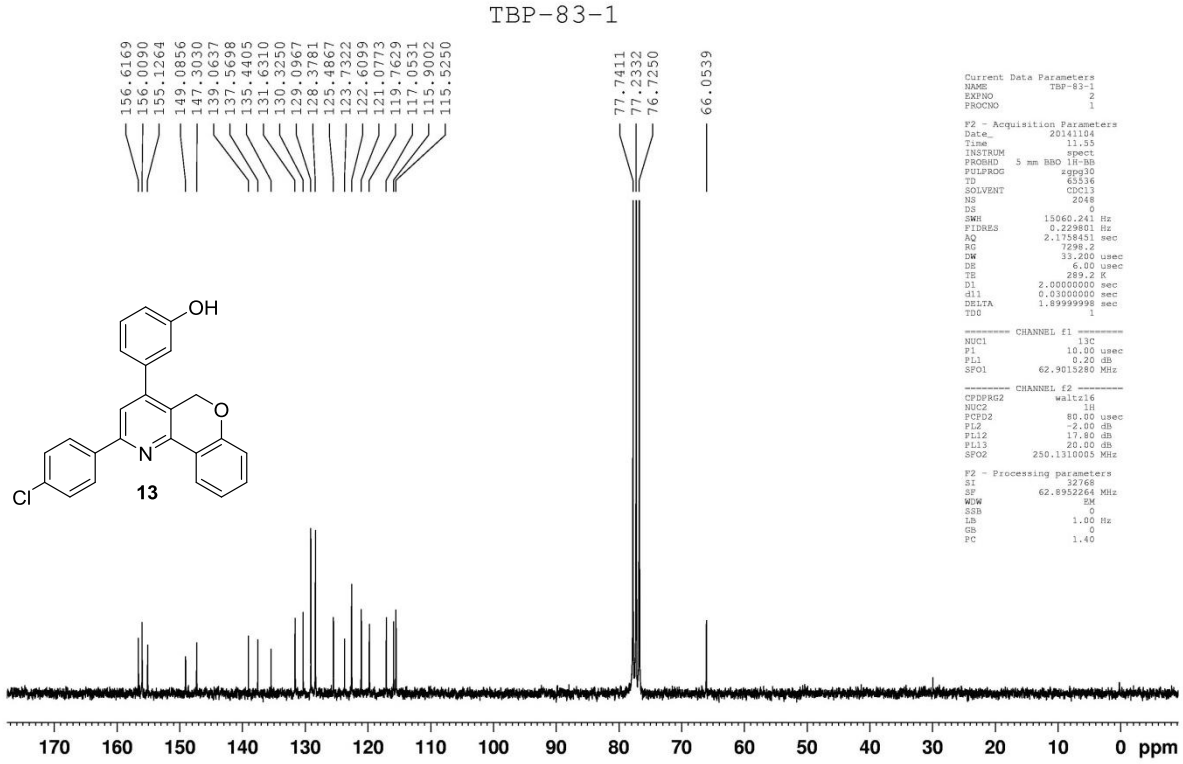

$^{1}$H NMR spectrum of compound **10**



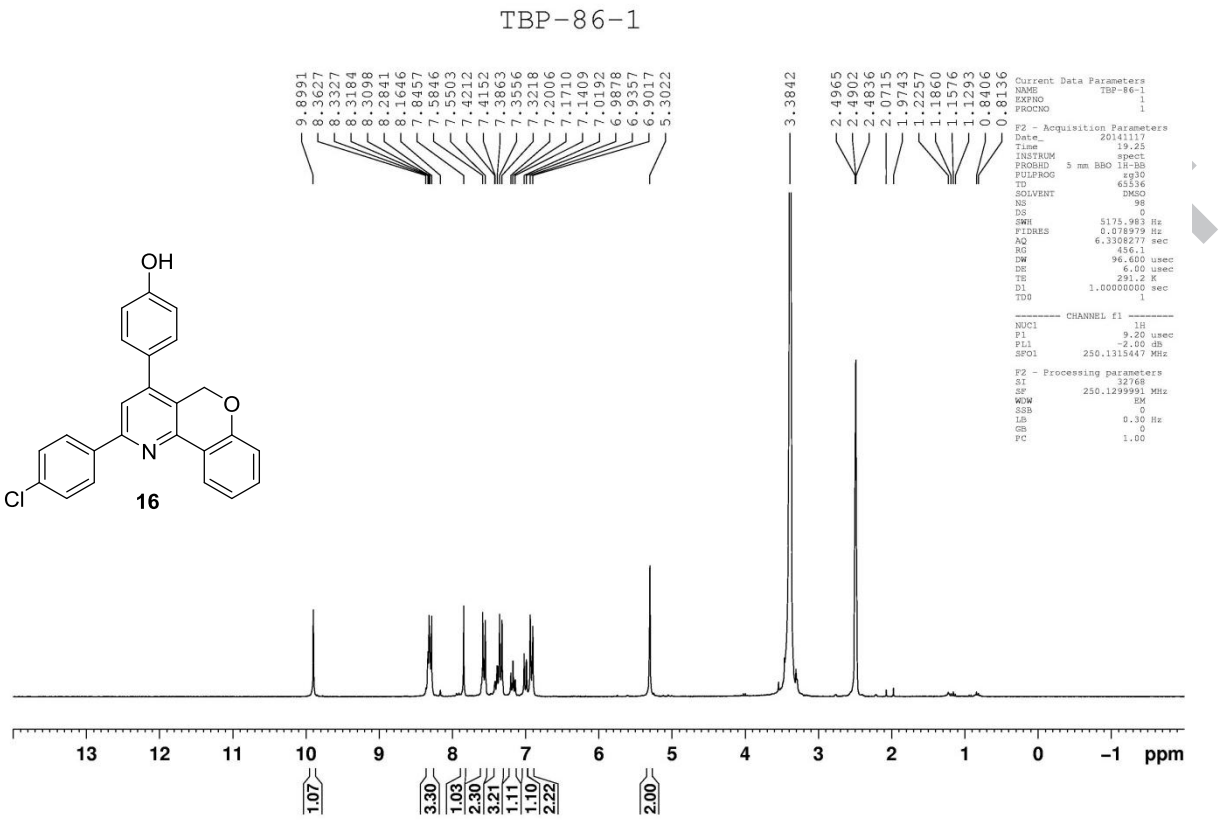

$^{13}$C NMR spectrum of compound **10**



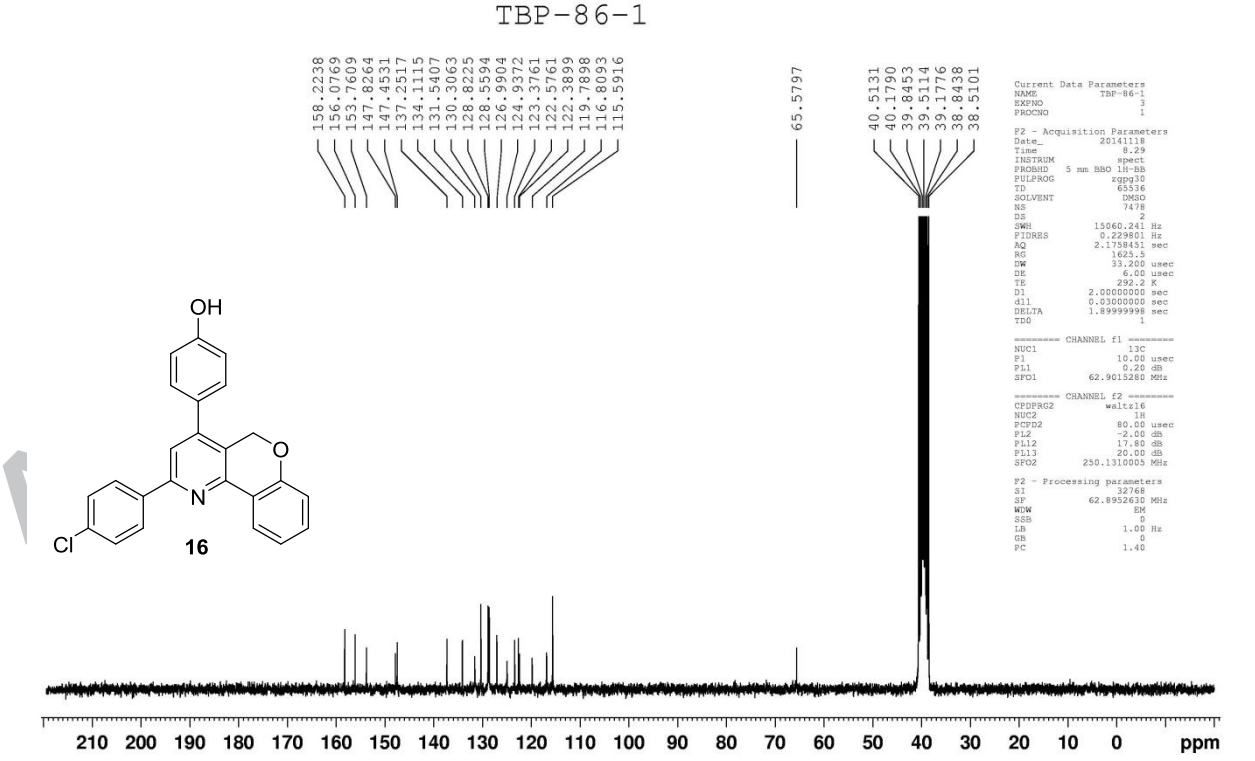

$^{1}$H NMR spectrum of compound **13**



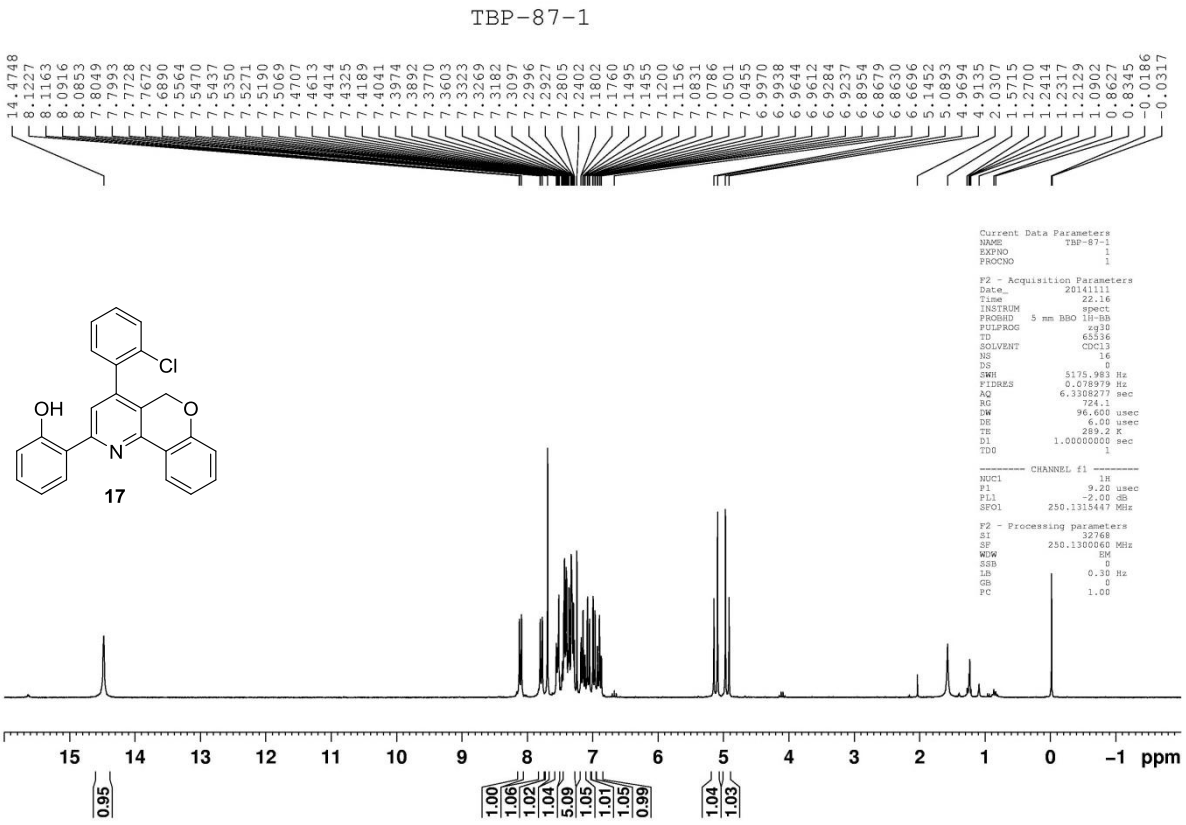

$^{13}$C NMR spectrum of compound **13**



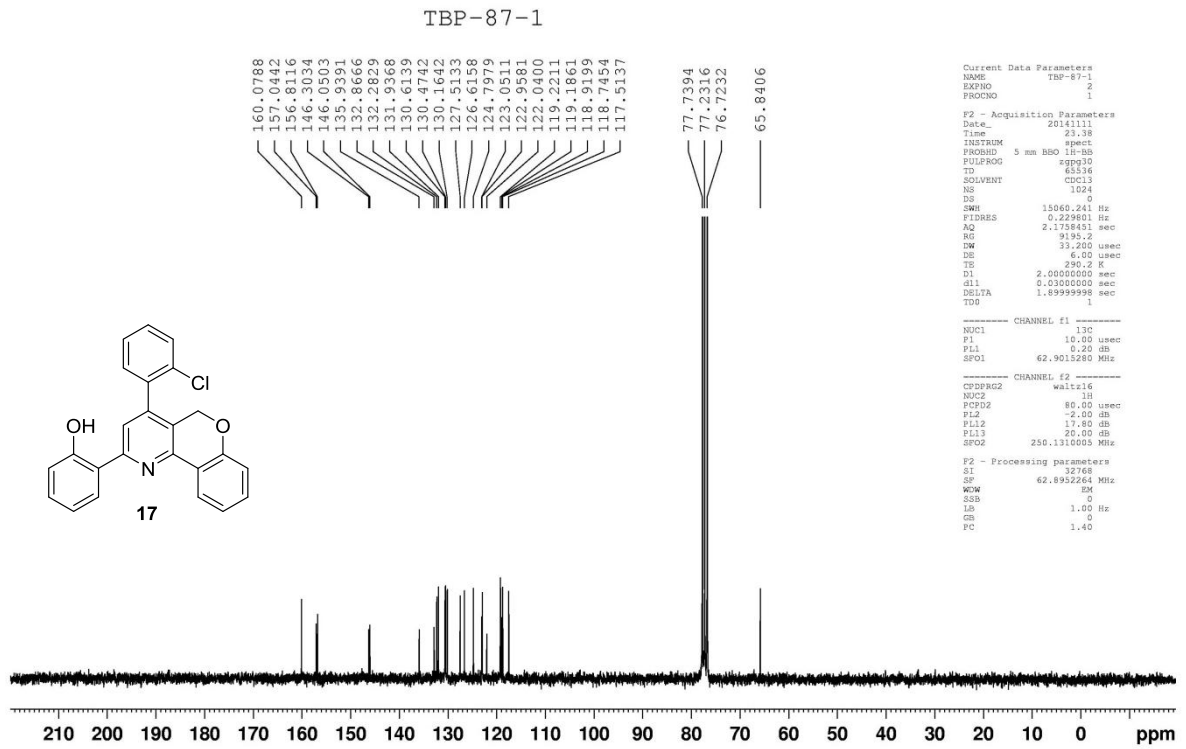

$^{1}$H NMR spectrum of compound **16**



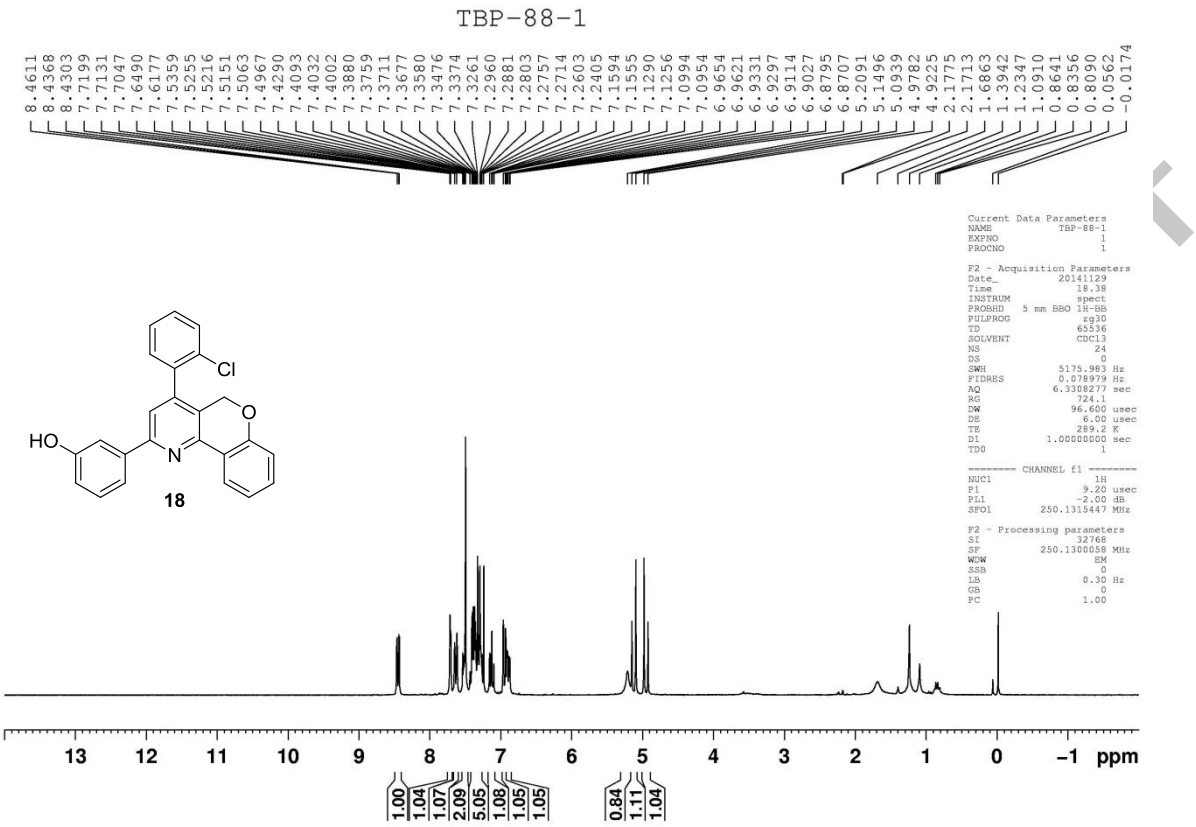

$^{13}$C NMR spectrum of compound **16**



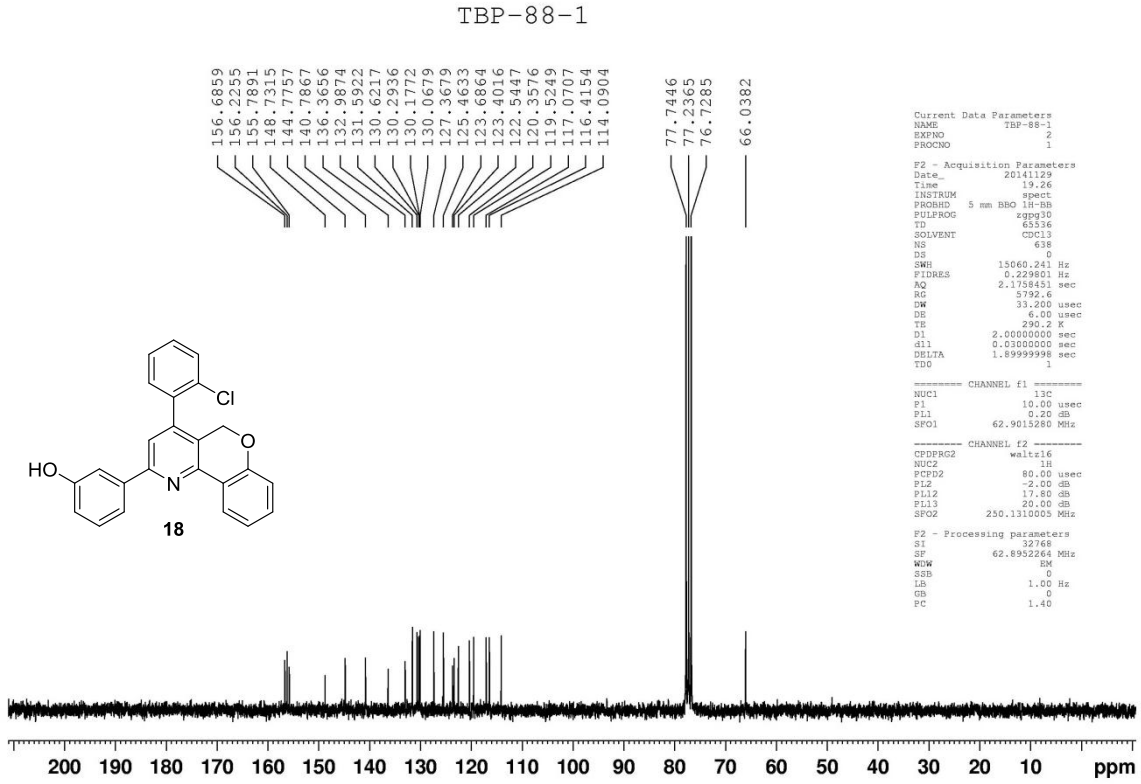

$^{1}$H NMR spectrum of compound **17**



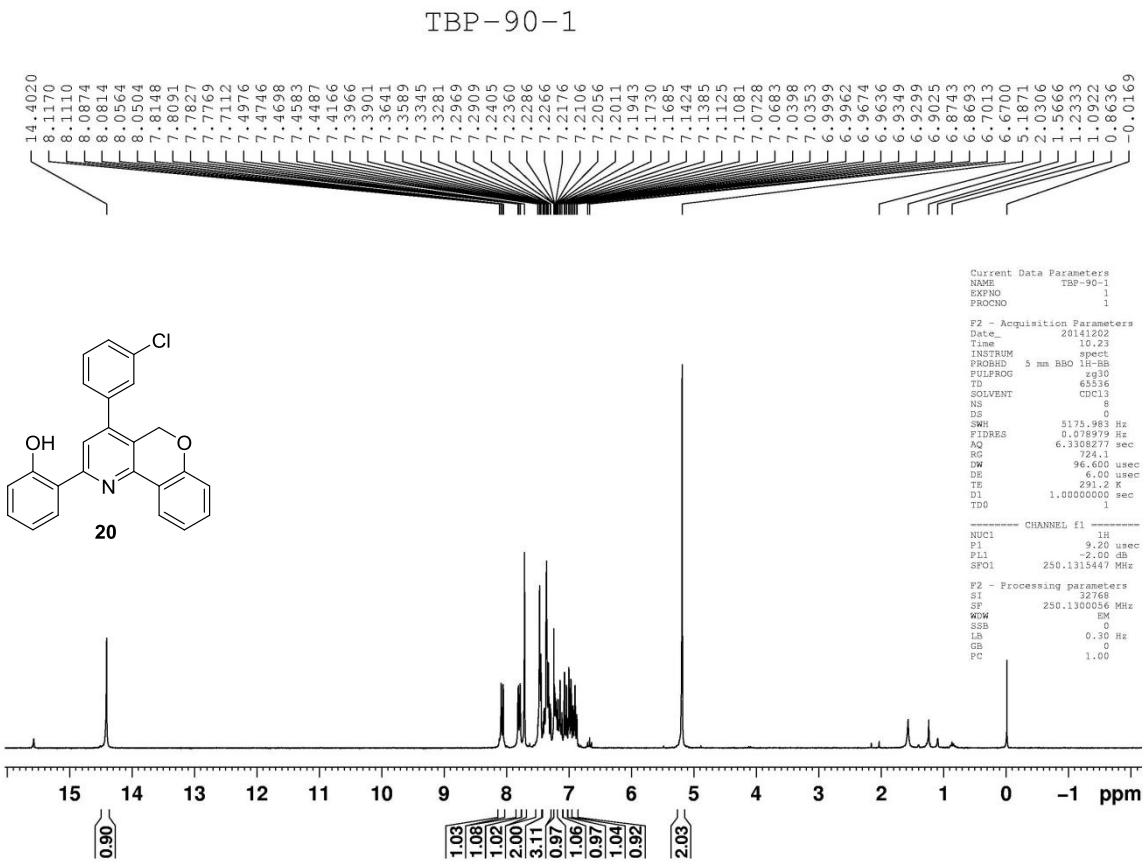

$^{13}$C NMR spectrum of compound **17**



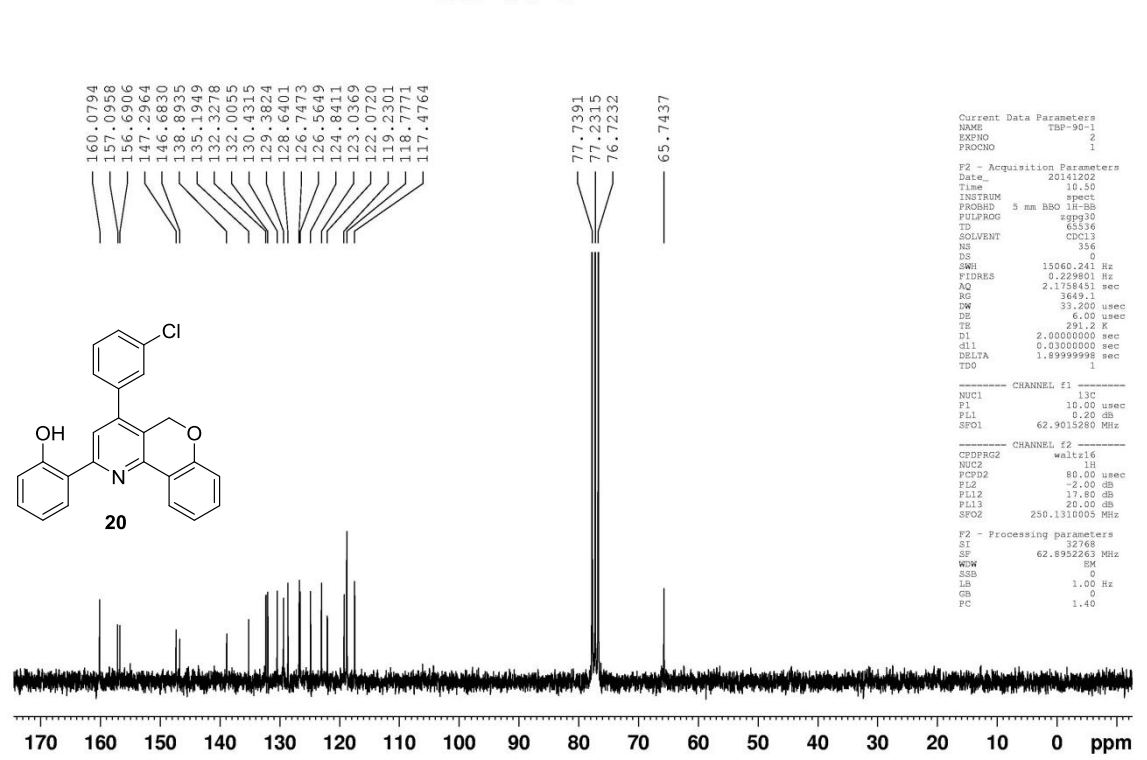

$^{1}$H NMR spectrum of compound **18**



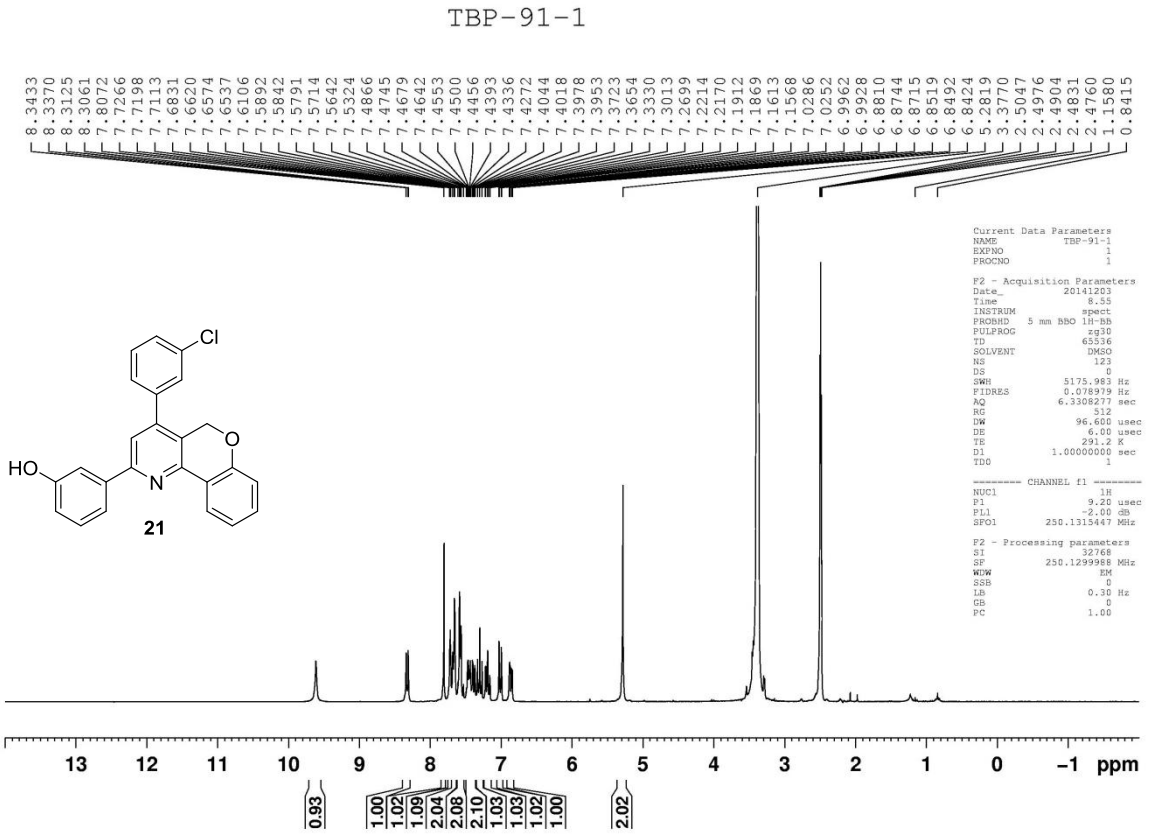

$^{13}$C NMR spectrum of compound **18**



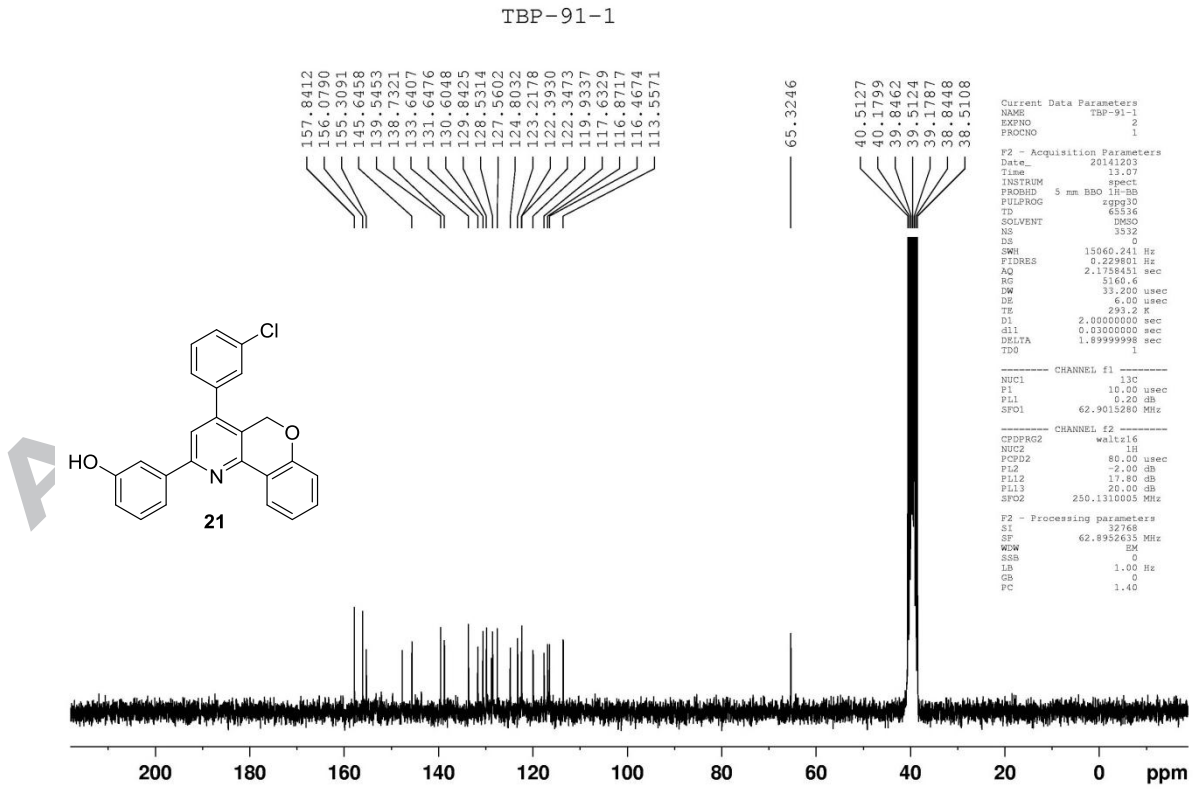

$^{1}$H NMR spectrum of compound **20**



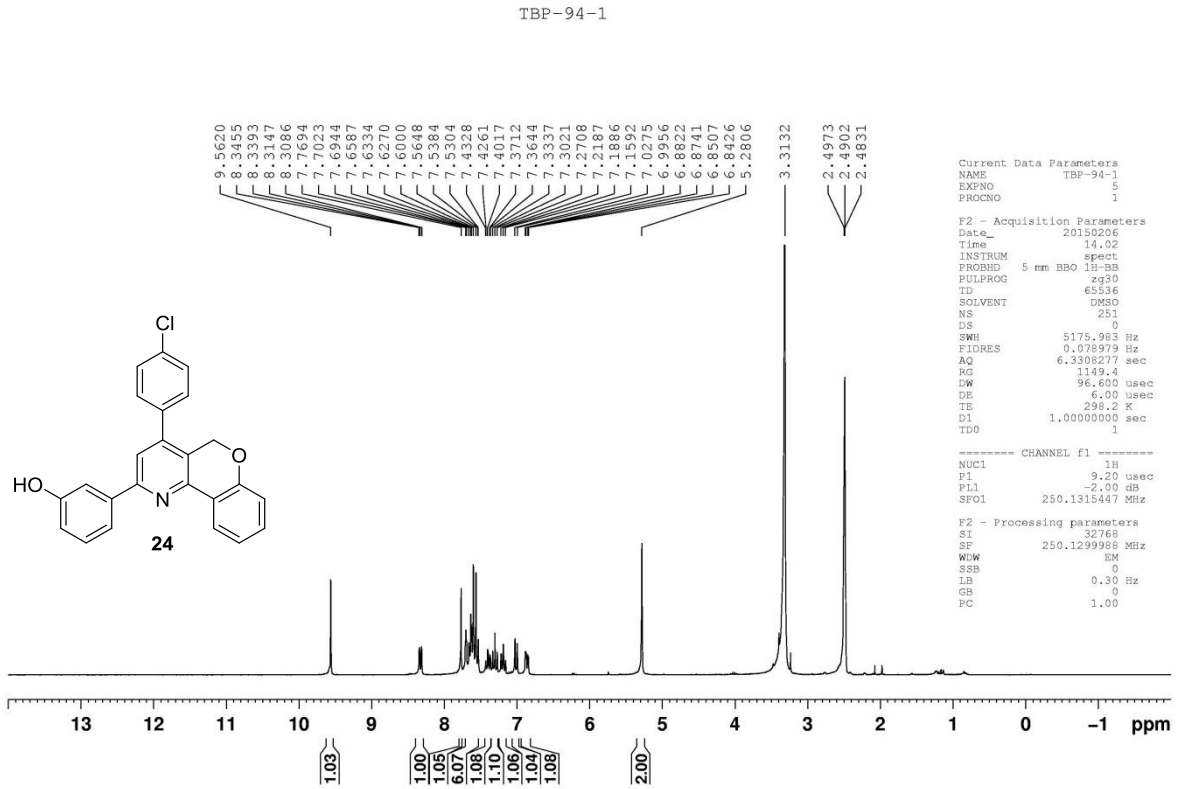

$^{13}$C NMR spectrum of compound **20**



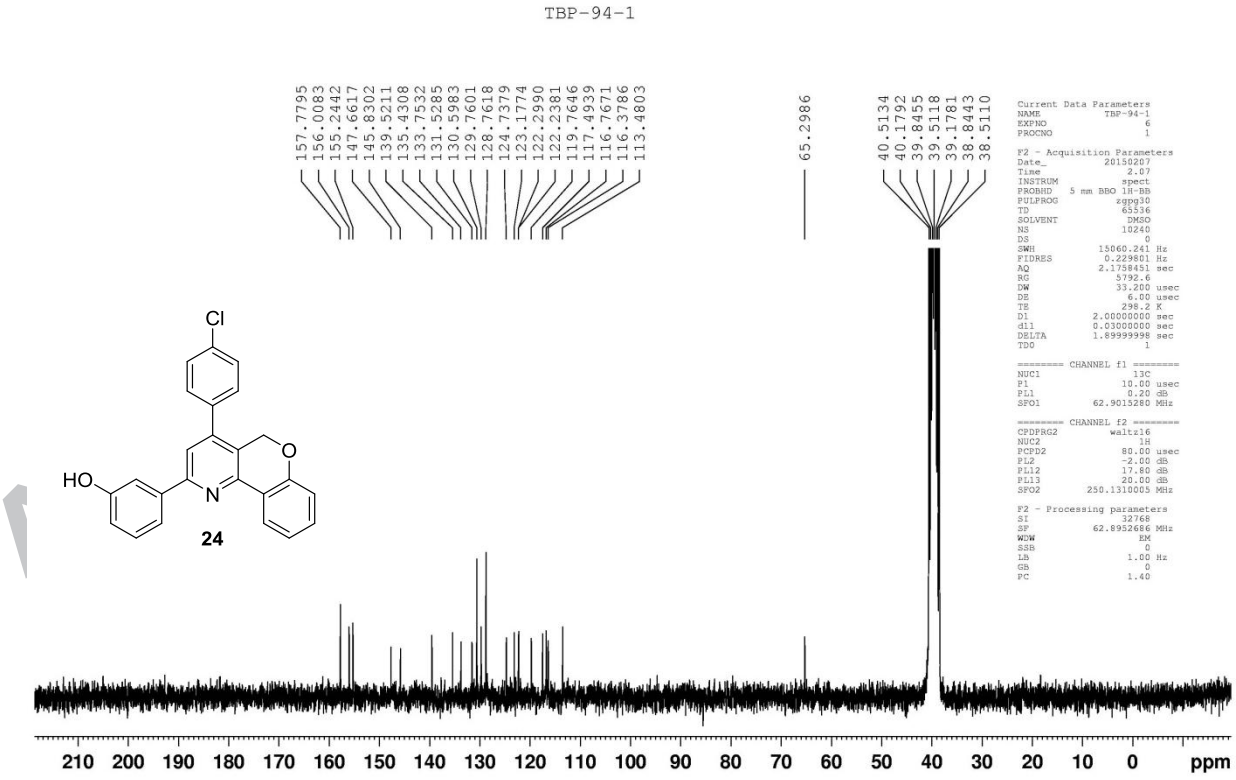

$^{1}$H NMR spectrum of compound **21**



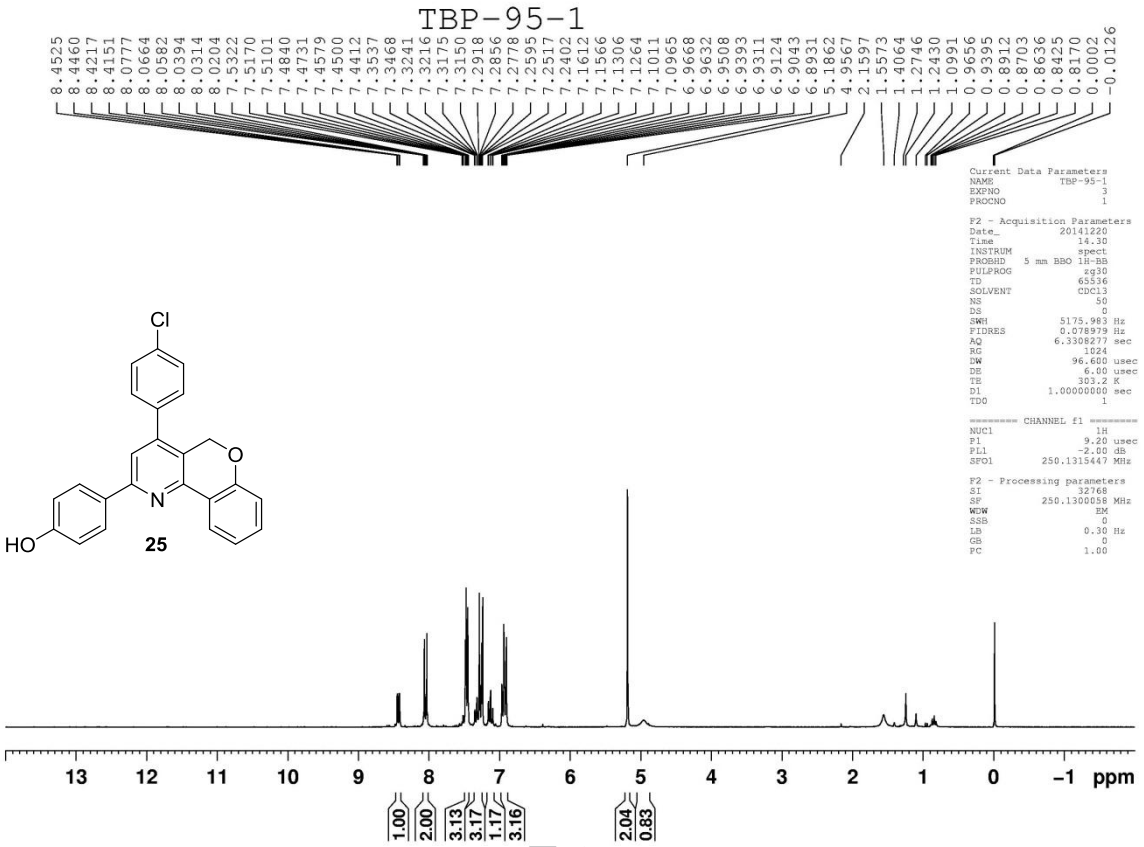

$^{13}$C NMR spectrum of compound **21**



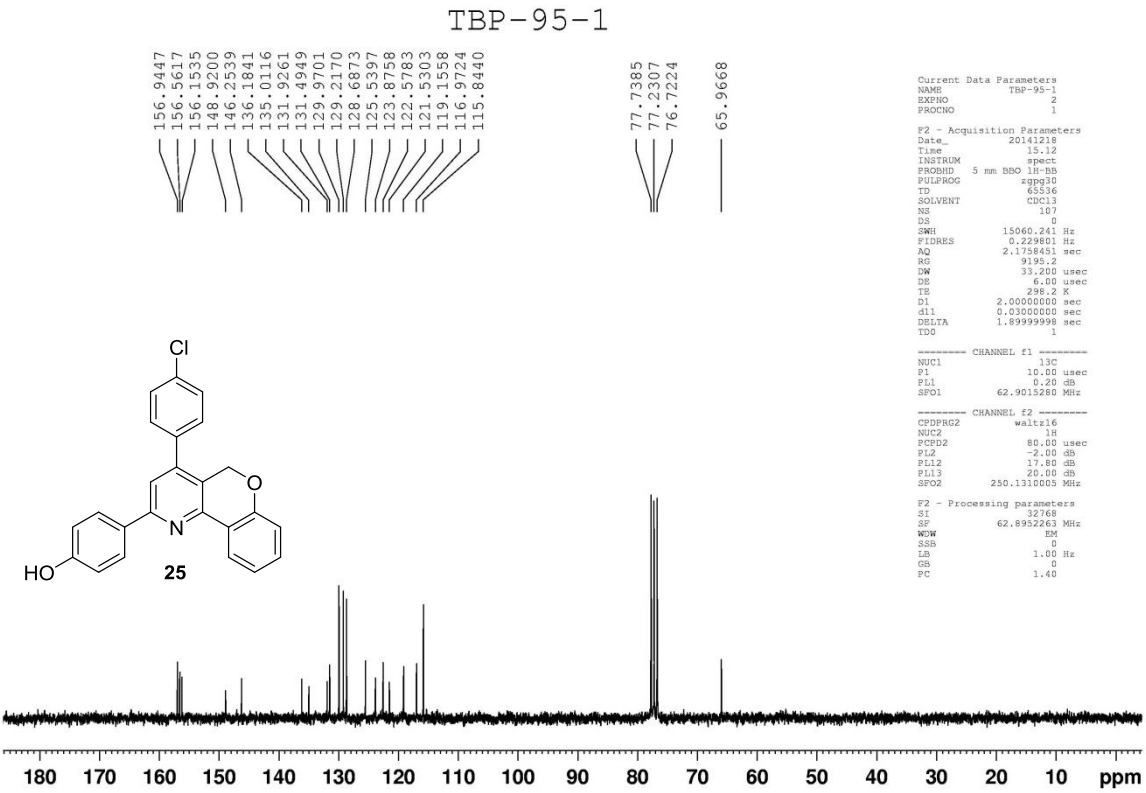

$^{1}$H NMR spectrum of compound **24**




$^{13}$C NMR spectrum of compound **24**




$^{1}$H NMR spectrum of compound **25**




$^{13}$C NMR spectrum of compound **25**

**Highlights**

- A new series of hydroxy and chloro-substituted 2,4-diphenyl *5H*-chromeno[4,3-*b*]pyridines were synthesized.
- Evaluated for topo I and IIα inhibitory activity, and antiproliferative activity.
- Compounds showed selective topo IIα inhibitory activity.
- SAR study indicated the importance of hydroxyphenyl-substitution at 4-position.

## Graphical abstract