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PII: S0223-5234(18)30961-9

DOI: https://doi.org/10.1016/j.ejmech.2018.11.007

Reference: EJMECH 10864

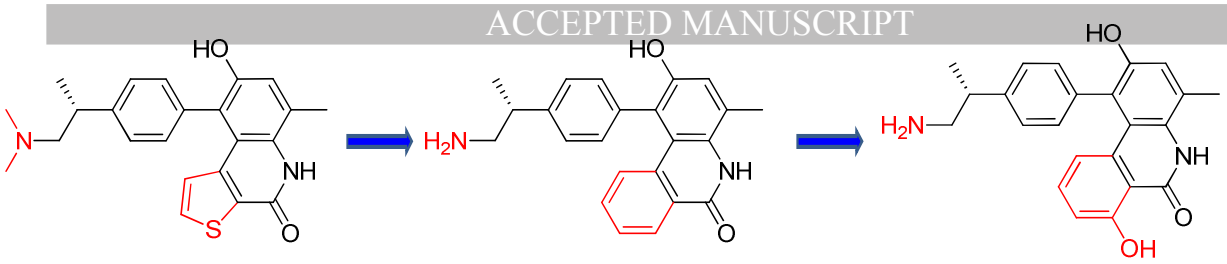
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 24 February 2018

Revised Date: 10 October 2018

Accepted Date: 4 November 2018

Please cite this article as: Q.-F. Hu, T.-T. Gao, Y.-J. Shi, Q. Lei, Z.-H. Liu, Q. Feng, Z.-J. Chen, L.-T. Yu, Design, synthesis and biological evaluation of novel 1-phenyl phenanthridin-6(5H)-one derivatives as anti-tumor agents targeting TOPK, *European Journal of Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.ejmech.2018.11.007.



OTS964

TOPK, IC_{50} = 353.7 nM

9b

TOPK, IC_{50} = 0.5 nM

9g

A549, IC_{50} = 5.4 nM
HCT116, IC_{50} = 7.8 nM; TGI 75.8% (15 mg/kg, po)
HCT-15, IC_{50} = 31.4 nM; TGI 78.8% (15 mg/kg, po)
SW620 TGI 79.7% (15 mg/kg, po)
F%~100%

Design, synthesis and biological evaluation of novel 1-Phenyl Phenanthridin-6(5H)-one derivatives as anti-tumor agents targeting TOPK

Quan-Fang Hu [a,1], Tian-Tao Gao [a,1], Yao-Jie Shi [a], Qian Lei [a], Zhi-Hao Liu [a], Qiang Feng [a], Zhen-Jia Chen [b], Luo-Ting Yu [a,*]

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Abstract

T–lymphokine-activated killer cell–originated protein kinase (TOPK) is a serine-threonine mitogen-activated protein kinase that is highly expressed in many types of human cancer. Due to its important role in cancer progression, TOPK is becoming an attractive target in chemotherapeutic drug design. In this study, a series of 1-phenyl phenanthridin-6(5H)-one derivatives have been identified as a novel chemical class of TOPK inhibitors. Some of them displayed very potent anti-cancer activity with IC_{50}s less than 100 nM, superior than reference compound OTS964. The most potent compound, **9g** suppressed the growth of cancer cells by apoptosis and specifically inhibited the activities of TOPK. Oral administration of **9g** effectively suppressed tumor growth with TGI >79.7% in colorectal cancer xenograft models, demonstrating superior efficacy compared to OTS964. Pharmacokinetic studies demonstrate its good oral bioavailability. Our findings therefore show that **9g** is a specific inhibitor of TOPK both *in vitro* and *in vivo* that may be further developed as a potential therapeutic agent against colorectal cancer.

Keywords: TOPK inhibitor**,** 1-Phenyl Phenanthridin-6(5H)-one, Structure activity relationship, Colorectal cancer, Pharmacokinetics

1. Introduction

TOPK (T–lymphokine-activated killer cell–originated protein kinase), also known as PBK or PDZ-binding kinase, is a member of the MAPKK protein family [1,2]. It is a serine-threonine mitogen-activated protein kinase that is highly expressed in many types of human cancer, such as lung cancer, breast cancer, colorectal cancer, lymphoma, leukemia, melanoma, cholangiocarcinoma and glioma [3-11]. However it is difficult to be detected in normal tissues except several fetal tissues and germ cells of the testis [3,11]. TOPK could be a promising molecular target for drug development which is involved in many cellular functions, including tumor development, cell growth, apoptosis, and inflammation [6,12-15]. Researchers have found that bidirectional signals transduced by TOPK-ERK (extracellular regulated protein kinase) interaction increases tumorigenesis of HCT116 colorectal cancer cells [6]. Also, studies have shown that TOPK could phosphorylate histone H3 (H3) at Ser10 and serves as a molecular marker in breast cancer [3]. Increased levels of PBK/TOPK may contribute to tumor cell development and progression through suppression of p53 function [13,16]. TOPK was also reported to increase cell migration by modulating a PI3K/ PTEN/AKT-dependent signaling pathway [17].

Despite so many reports have indicated that TOPK plays crucial roles in tumorigenesis, the three-dimensional structure of TOPK has not been reported. Only the crystal structure of an inactive dimer was reported by C. Dong et al., which has two phospho-mimicking mutations T9E and T198E. The structural data indicated that it exists in a conformational transition between dimers and monomers at different pH conditions [18]. However the active state of TOPK is a monomer and does not form a dimer. The unavailability of crystalline structure of TOPK makes the discovery of its inhibitors a little bit slow. There are just several PBK/TOPK inhibitors reported in pre-clinical research (Fig.1). HI-TOPK-032 [10,19] effectively suppresses colon cancer and glioma growth *in vivo* despite its inhibition *in vitro* is not so significant. Ginsenoside Rh2 (GRh2) is the only reported natural product targeting TOPK which inhibits colon cancer cell proliferation *in vivo* [20]. ADA-07 suppresses solar ultraviolet-induced skin carcinogenesis [21]. Ilaprazole [22] and Pantoprazole [23] are two proton pump inhibitors that suppress colorectal cancer growth. OTS514 and OTS964 [24-28] have great suppression in lung cancer [24,26], acute myeloid leukemia [27] and ovarian cancer [28]. Due to the toxicity of OTS514 with mild anemia and relatively severe leukocytopenia, whereas the increasing of peripheral platelets in a dose-dependent manner, it was developed as liposomal formulation which limited its application; On the contrary, OTS964 at some extend is better for oral administration,

despite that hematopoietic toxicity is still a concern [24].

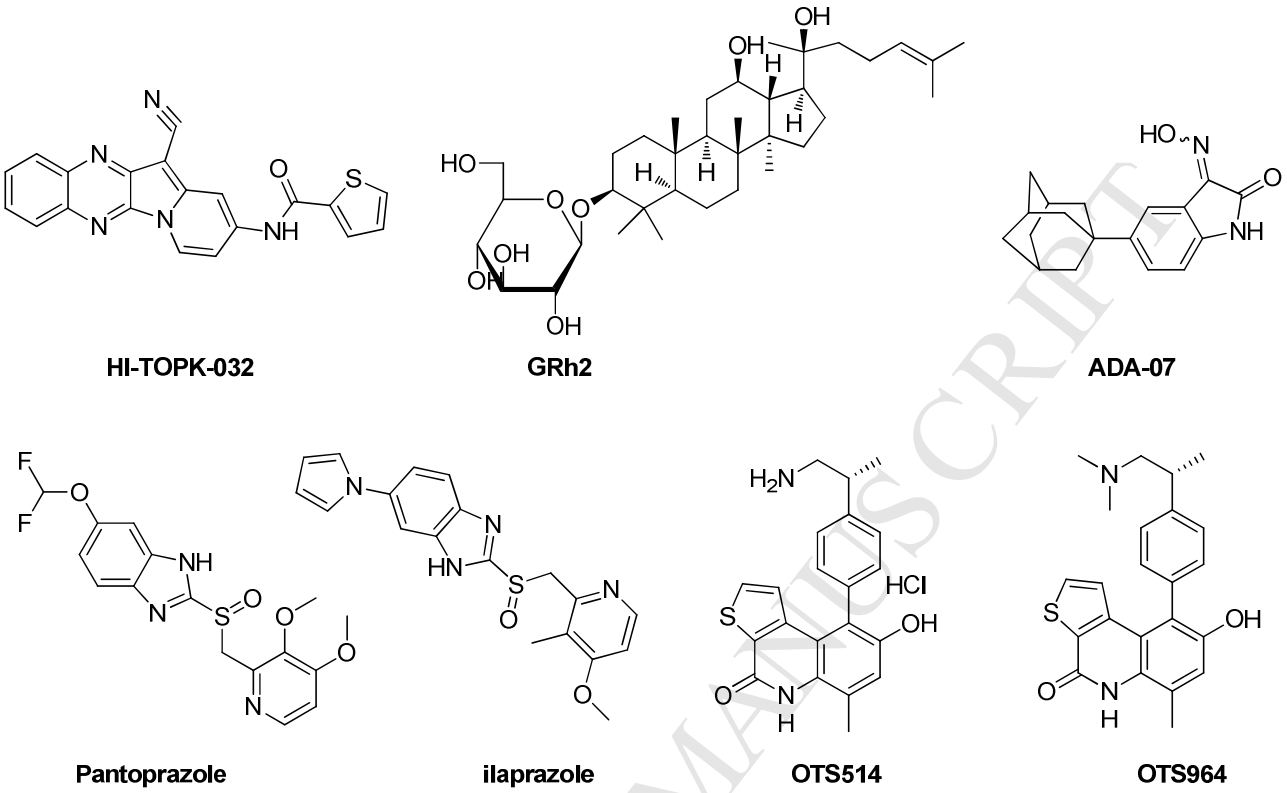


Fig. 1. Reported small molecular TOPK inhibitors

Although there have been significant advances in discovery of TOPK inhibitors as we discussed above, a systematic structure−activity relationship (SAR) study to investigate TOPK potency has not been reported. The potent TOPK inhibitor OTS964, which are promising therapeutic agents that may be applied to a wide range of human malignancies, served as a useful template to explore new TOPK inhibitors.

Taking the preclinical inhibitor OTS964 as lead structures, here we attempted to design and synthesize a novel series of anti-TOPK compounds and found candidates suitable for oral administration. Based on the principle of bioisosteres, thiophene core in left side was substituted with phenyl core which proved to be an effective pharmacophore for inhibitory activity, giving rise to the novel series of 1-phenyl phenanthridin-6(5H)-one compounds. Preliminary binding study and biological evaluation had proved this modified structure successfully maintained potent inhibitory activity. More than forty compounds were prepared and afforded a systematic biological analysis. The preliminary structure-activity relationship (SAR) was also summarized. And candidate was identified by high *in vivo* activity against colon cancer, as well as

good oral bioavailability that may be further developed as potential therapeutic.

2. Results and discussion

2.1. Chemistry

We investigated the following four regions of the chemical series represented by OTS964 (Fig. 2): The crucial thiophene core (green box), which we attempted to replace with phenyl core; the crucial aminoalkyl group (light blue box), the alternative substituents of methyl (red box), the substitution on hydroxyl group (dark blue box).

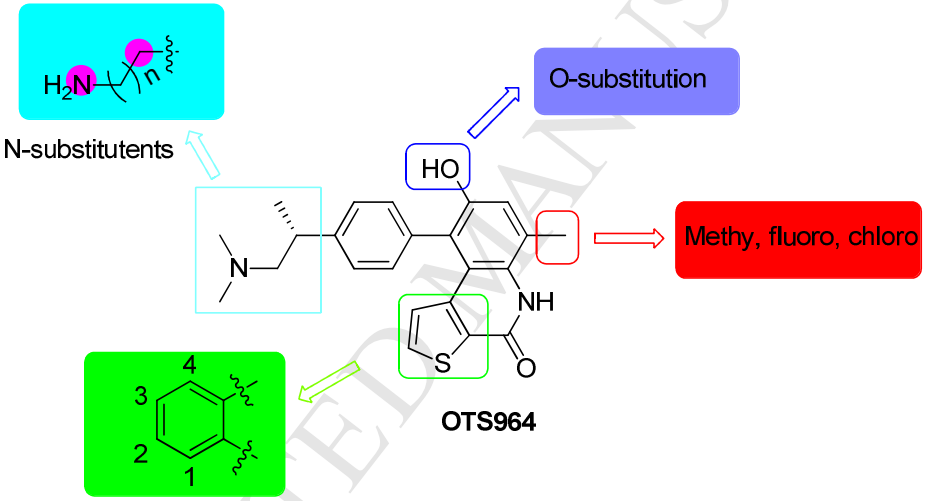
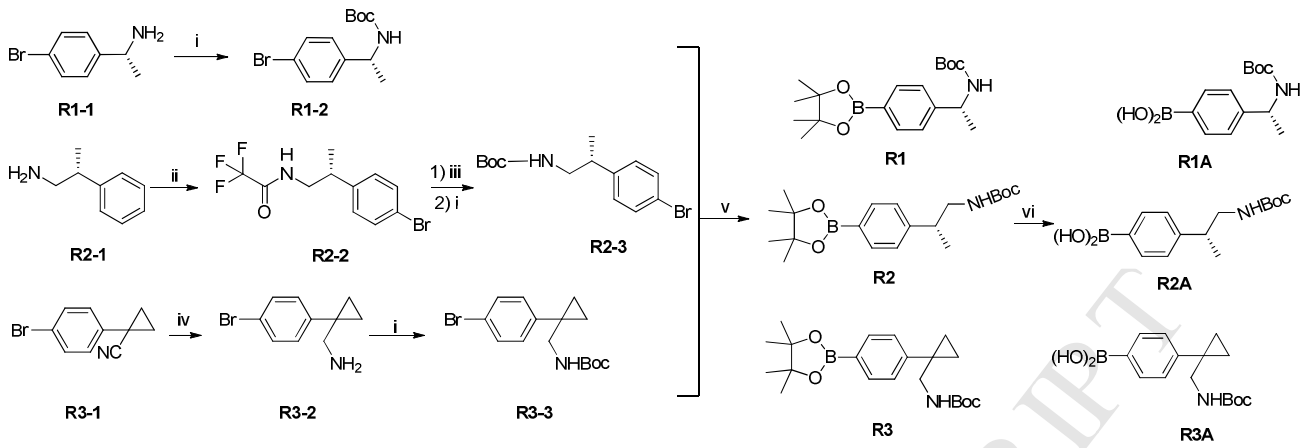


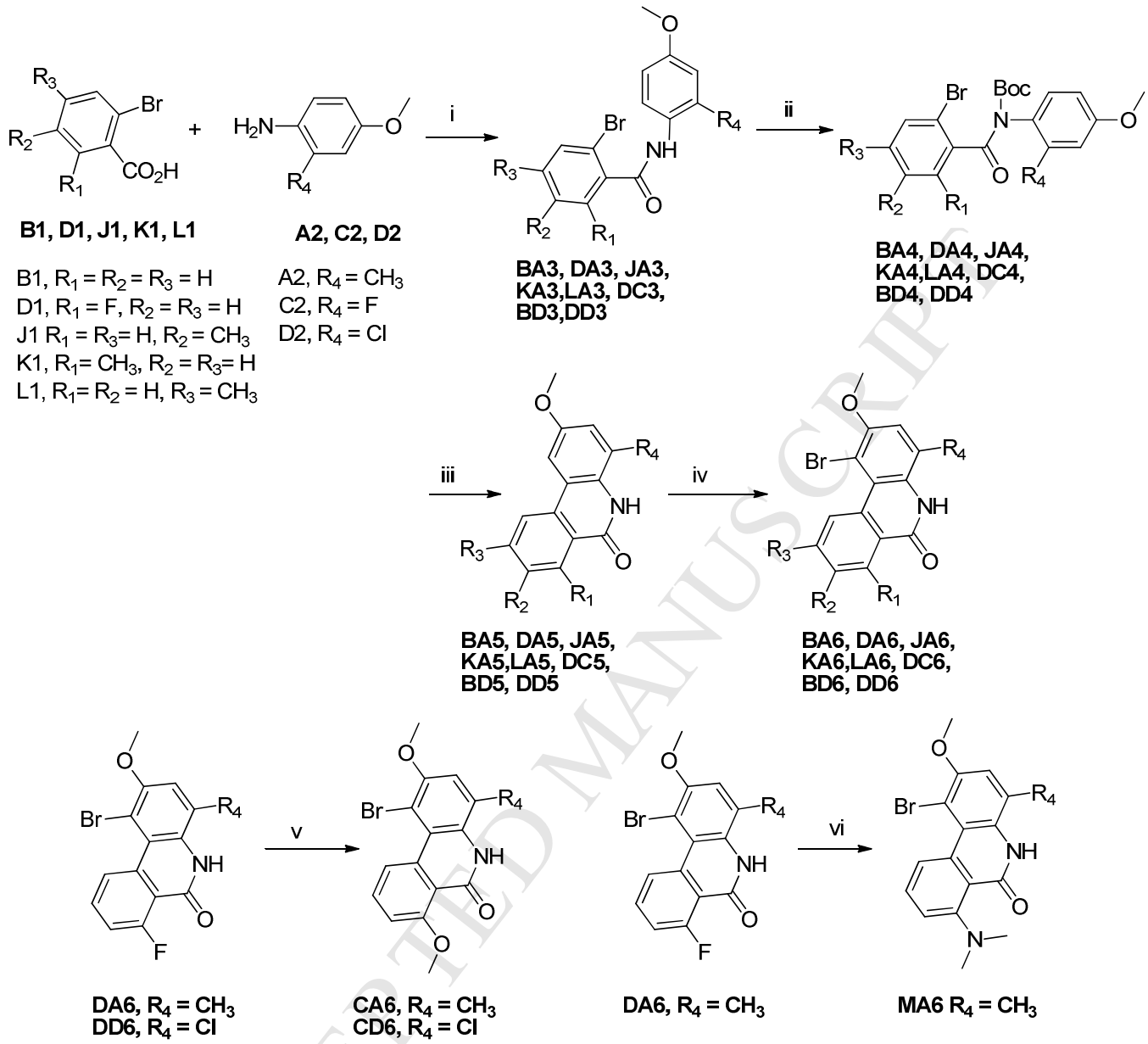
Fig. 2. Regions of OTS964 investigated for TOPK potency

The general synthesis of key intermediates boronic acids **R1A**~**R3A** are depicted in Scheme 1. Different from the reference procedures [24], we synthesized the aryl boronic acids instead of corresponding pinacol esters, which were easily purified by crystallization and gave all products as good off-white solids. **R1A** was synthesized from the commercial available chiral amine **R1-1** by three steps: protecting amino group with Boc, converting to boronic ester via Suzuki-Miyaura reaction and then hydrolyzing under $NaIO_4$ to yield **R1A** [29]; **R2A** was synthesized from **R2-1** by bromination with DBDMH (1,3-dibromo-5,5-dimethylhydantoin) in TFAA [24]; **R3A** was synthesized from corresponding nitrile **R3-1**, which was reduced to primary amine with BH_3•THF.



Scheme 1. Synthesis of aryl boronic acid. i) Boc_2O, DCM, rt; ii) 1) TFAA, DCM; 2) $MeSO_3H$, DBDMH; iii) LiOH, H_2O; iv) BH_3•THF, THF, rt; v) Bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, dioxane, 90 °C, 18 h; vi) $NaIO_4$, acetone/H_2O, 40 °C.

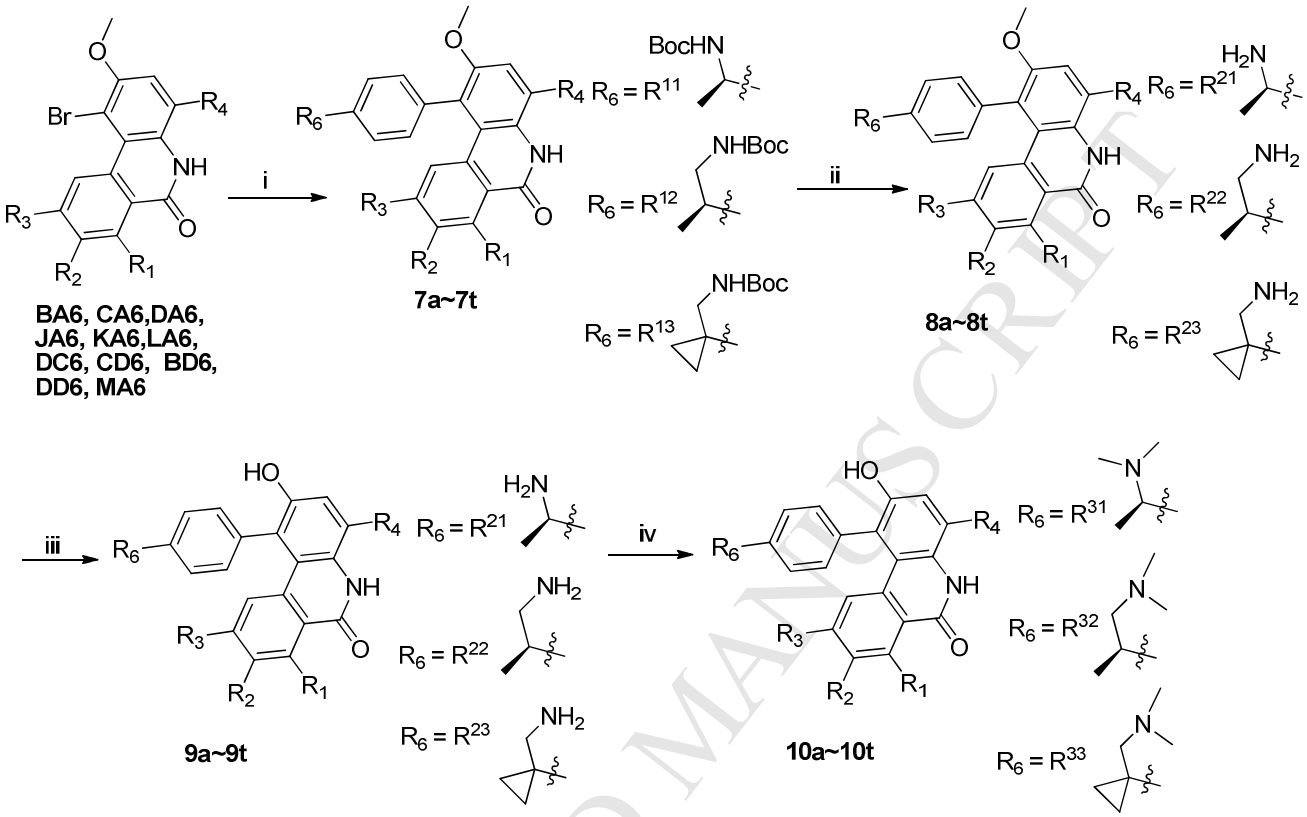
The syntheses of another series of building blocks, 1-bromo-phenanthridin-6(5H)-ones are shown in scheme 2. The bromo-benzoic acid was converted to bromo-benzoyl chloride, then reacted with aniline to give amide, followed by protection amide with Boc [30, 31]. Then the protected amide was cyclized under catalytic $Pd(t\text{-}Bu_3P)_2$ to give phenanthridin-6(5H)-one through C-H direct activation [32-34]. 1-bromo-phenanthridin-6(5H)-one was obtained by bromination with NBS in HOAc/DCM. DA6 and DD6 were converted to CA6 and CD6 in DMA by substitution of sodium methoxide. DA6 was converted to MA6 by substitution of aqueous dimethlyamine.



Scheme 2. Synthesis of aryl bromide. i) 1) aryl acid in $SOCl_2$/cat. DMF, reflux; 2) aryl amine/Et_3N, DCM, rt; ii) DMAP, Boc_2O, DCM, rt; iii) Pd(t-$Bu_3P)_2$, KOAc, DMA, 120~130 ^oC, argon; iv) NBS, HOAc/DCM, rt; v) MeONa, DMA, 50 ~60 ^oC; vi) aq. dimethylamine, DMA, 110 ^oC.

The syntheses of final TOPK inhibitors are outlined in scheme 3. The above 1-bromo-phenanthridin-6(5H)-one was reacted with aryl boronic acid **R1A~R3A** under classical Suzuki coupling condition to give **7**. The weak base, $NaHCO_3$ was best for the reaction which yielded lower de-bromination by-product compared with other stronger bases. Inhibitors **8a~8t** were obtained by direct decarboxylation in HCl/MeOH. **9a~9t** were obtained by de-methylation with BBr_3 in DCM. They were further

developed to **10a**~**10t** by reductive amination. Compound **1**, OTS514 and OTS964 were obtained by known procedures [24,25].



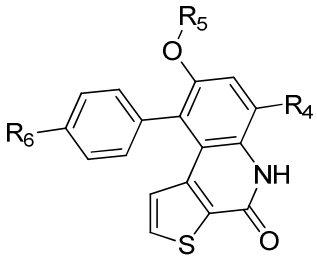
Scheme 3. Synthesis of TOPK inhibitors. i) **R1A**~**R3A**, $Pd(dppf)Cl_2$, dioxane/H_2O, $NaHCO_3$, 85 ^oC; ii) HCl/MeOH, rt; iii) BBr_3/DCM, rt; iv) paraformaldehyde, $NaBH_3CN$, HOAc, MeOH, rt.

2.2. Structure -activity relationships

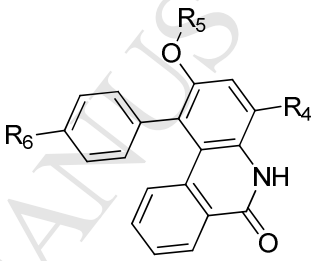
We synthesized compound **1** [25] which is an analog of OTS514/OTS964 and all of the prepared compounds were first screened with a binding affinity assay against TOPK kinase [35,36] (Table 1). Inhibition of cell proliferation was measured using the MTT assay with A549, HCT116 and HCT-15 cell lines which observed high expression of TOPK [24]. Compound **1** has comparable binding effect with OTS514 although less potent in cell-based assay. On the contrary, OTS964 is 30-fold lower than OTS514 in kinase activity but almost as potent as the later at cell level. When exploring the SARs of OTS514 derivatives, firstly we investigated the impact of replacing the thiophene rings with phenyl rings, which yielded nine 1-phenyl phenanthridin-6(5H)-one inhibitors and the results are shown in table 1. SAR analysis revealed that most of them were potent again TOPK. **9a** is 48-fold more potent in the biochemical assay than **8a**, and **9b** is almost

10-fold more potent than **8b**. This confirms that the phenyl hydroxy function group is very important for interaction with TOPK, presumably by the formation of hydrogen bonds. To our delight, **9a**, **9b** and **9c** have potency of less than 30 nM IC_{50} in the biochemical assay, and the potency of **9b** , **9c** was well translated into a benefit of A549, HCT116 and HCT-15 cellular context, although **9a** is much less active in cell-based assay. When those compounds were methylated to give **10a**, **10b**, **10c**, more than 5-fold binding potency reduced, just similar to OTS964. The best substitution out of six R6 groups turned out to be R^{22}. Encouragingly **9b** was almost 21-fold more potent than OTS514 at kinase level and has comparable cellular potency, which leaded us to further optimization.

Table 1. Structure−activity relationship of substitutions to phenyl core



cpd1, OTs514, OTS964



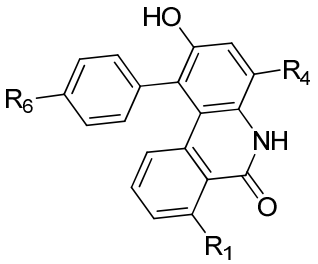
8a~8c, 9a~9c, 10a~10c

compound	R_4	R_5	R_6	IC_{50} (nM) Kinase [a]	IC_{50} (nM) [b]		
					A549	HCT116	HCT-15
Cpd 1	Me	H	R^{21}	23.00	360 ± 2.21	468 ± 11.03	256.9 ± 4.01
OTS514	Me	H	R^{22}	10.69	19.52 ± 0.23	18.89 ± 4.30	187.1 ± 2.31
OTS964	Me	H	R^{32}	353.7	20.41 ± 0.34	45 ± 1.22	73.08 ± 0.88
8a	Me	Me	R^{21}	142.5	>1000	>1000	NT
9a	Me	H	R^{21}	2.94	920 ± 6.52	>1000	>1000
10a	Me	H	R^{31}	44.89	250.3 ± 9.32	314.1 ± 1.05	NT
8b	Me	Me	R^{22}	5.44	360 ± 2.09	357 ± 2.48	277.7 ± 2.86
9b	Me	H	R^{22}	0.50	78.7 ± 7.54	114.2 ± 7.56	365 ± 4.09
10b	Me	H	R^{32}	63.83	263.5 ± 4.90	299.3 ± 0.48	599.5 ± 5.39
8c	Me	Me	R^{23}	NT	894.3 ± 10.32	>1000	NT
9c	Me	H	R^{23}	25.1	210.9 ± 2.78	231.2 ± 0.79	398.2 ± 6.34
10c	Me	H	R^{33}	143.9	>1000	949.9 ± 2.94	NT

[a] [ATP] = 10 μM; Compounds were tested by 10 dose singlet assay; [b] Each compound was tested in triplicate assay, IC_{50} values are presented as the mean ± SD; NT: not tested.

Our next effort was to introduce multiple substituents of R1, R4 as well as R6 with the goal to identify the best combination (Table 2, compounds **9d**−**10n**). Small fluoro atom was substituted as R1 (**9d**, **9e**) but it was not well tolerated. Interestingly, when R1 was substituted by hydroxyl group, the kinase activity (**9f**, **9g** and **9h**) was reduced with 60~660-fold (compared with **9a**, **9b** and **9c** accordingly), but all the cytotoxic activities were enhanced. The best cellular inhibition is **9g**, its IC_{50} is as low as 5.4 nM to A549 and 7.8 nM to HCT116, less than 10 nM with more than 30-fold potency positive shift from kinase level inhibition (IC_{50} 0.33 μM). The high cellular potency encouraged us for its further optimization. When **9f**, **9g** and **9h** were methylated to their derivatives: 1**0f**, **10g** and **10h**, the cellular inhibitions were slightly reduced just like our results above (**10a**, **10b**, **10c**). After that, we tested **9i** and **9j**, chloro substitution compounds at R4. Both compounds were almost as active as **9a**/**9b** in the biochemical assay with IC_{50} less than 10 nM, but the cytotoxic activities were not potent, presumably due to lower PKa value resulted from the chloro atom [37-39]. Next we investigated the substitution of R4 with chloro atom and R1 with hydroxyl group, which yielded three compounds **9k** , **9l** and **9m**. However, all compounds just gave moderate enzyme and cytotoxic activities, indicating the negative effect of combination hydroxyl group and chloro atom. Considering that both hydroxyl group and halogen group are acidic substituents, we introduced a basic substitution group dimethyl amine at the R1 position and obtained the compound **9n**, **10n**. But that substitution abolished the potency completely. Comparing all those 20 compounds with different R6 substitutents, R^{22} still turned out to be the best.

Table 2. Structure−activity relationship of further substituents R_1, R_4, R_6



compound	R_1	R_4	R_6	IC_{50} (nM) Kinase [a]	IC_{50} (nM) [b]		
					A549	HCT116	HCT-15
9d	F	Me	R^{21}	61.41	>1000	>1000	>1000
9e	F	Me	R^{22}	64.46	110 ± 4.70	438 ± 0.69	>1000
9f	OH	Me	R^{21}	977.5	82.52 ± 3.88	102.4 ± 1.06	96.69 ± 11.34

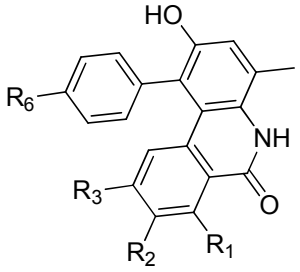
10f	OH	Me	R^{31}	4790	128.5 ± 1.99	396.5 ± 1.39	162.4 ± 2.11
9g	OH	Me	R^{22}	335.7	5.405 ± 0.66	7.867 ± 5.42	31.42 ± 0.46
10g	OH	Me	R^{32}	>10000	113.3 ± 0.21	215 ± 0.56	75.97 ± 3.56
9h	OH	Me	R^{23}	1505	135.9 ± 2.45	44.73 ± 0.95	NT
10h	OH	Me	R^{33}	ND	405.6 ± 0.39	141.2 ± 6.33	NT
9i	H	Cl	R^{21}	2.71	600 ± 8.34	>1000	800.7 ± 7.80
10i	H	Cl	R^{31}	26.13	>1000	>1000	>1000
9j	H	Cl	R^{22}	4.59	>1000	636 ± 1.26	308.9 ± 5.08
10j	H	Cl	R^{32}	65	>1000	>1000	> 1000
9k	OH	Cl	R^{21}	642.9	554.1 ± 3.88	719.9 ± 9.53	NT
10k	OH	Cl	R^{31}	2440	515.6 ± 4.25	>1000	NT
9l	OH	Cl	R^{22}	321.3	102.7 ± 1.22	371.7 ± 3.05	NT
10l	OH	Cl	R^{32}	2663	554.1 ± 5.23	534.3 ± 2.60	NT
9m	OH	Cl	R^{23}	1266	437.4 ± 5.11	563.7 ± 4.31	NT
10m	OH	Cl	R^{33}	5238	897.6 ± 7.60	>1000	NT
9n	Dimethyl amine	Me	R^{22}	ND	>1000	>1000	NT
10n	Dimethyl amine	Me	R^{32}	ND	>1000	>1000	NT

[a] [ATP] = 10 μM; Compounds were tested by 10 dose singlet assay; [b] Each compound was tested in triplicate assay, IC_{50} values are presented as the mean ± SD; NT: not tested; ND: not determined.

To explore the effect of alkyl substitution at phenyl core, we incorporated methyl substitution at different position and the results are shown on table 3. However it seemed that those substitution groups had no obvious positive effect on potency. When R1 was substituted by methyl, the potency of kinase activity was not improved but the cytotoxic activity was much better (**9r**, IC_{50} against A549 is 25.8 nM, IC_{50} against HCT116 is 75.8 nM). When R2 was methyl (**9o**, **10o**, **9p**, **10p**), the compounds turned out to be moderate active in kinase assay (**9o**, **9p**) but inactive in cell-based assay. When R3 was substituted by methyl, the potency of kinase activity was improved to nanomole (**9s**, **9t**) and had comparable inhibition with **9b** in cell-based assay. Generally methyl substitution of R1, R2, R3 obtained negligible potency optimization.

Here we summarize the structure−activity relationships of the investigated compounds as inhibitors of the human TOPK: The crucial thiophene core was successfully substituted with phenyl core, the best substituent of crucial aminoalkyl group is (R)-1-aminopropan-2-yl (R^{22}), the alkyl substitution of phenyl core is not necessary, the substitution on hydroxyl group completely abolish the potency.

Table 3. Structure−activity relationship of methyl substitution at phenyl core



compound	R_1	R_2	R_3	R_6	IC_{50} (nM) Kinase [a]	IC_{50} (nM) [b]		
						A549	HCT116	HCT-15
9q	Me	H	H	R^{21}	721.4	187.3 ± 0.79	242.2 ± 2.59	505.4 ± 3.08
10q	Me	H	H	R^{31}	3013	469.7 ± 0.94	469 ± 4.68	397.3 ± 5.41
9r	Me	H	H	R^{22}	586.1	25.86 ± 4.80	75.85 ± 1.30	300.6 ± 0.61
10r	Me	H	H	R^{32}	>10000	286.7 ± 3.59	419.2 ± 4.77	337.3 ± 0.48
9o	H	Me	H	R^{21}	894.3	>1000	571 ± 1.25	NT
10o	H	Me	H	R^{31}	6415	>1000	>1000	NT
9p	H	Me	H	R^{22}	223.7	>1000	962 ± 4.38	NT
10p	H	Me	H	R^{32}	7055	>1000	830 ± 5.90	NT
9s	H	H	Me	R^{21}	1.56	119.7 ± 2.78	384.8 ± 3.42	216.7 ± 2.06
10s	H	H	Me	R^{31}	20.88	316.9 ± 1.65	273.7 ± 0.95	467.3 ± 0.94
9t	H	H	Me	R^{22}	6.44	60.27 ± 3.42	156.1 ± 2.13	326.8 ± 2.09
10t	H	H	Me	R^{32}	19.06	466.2 ± 4.11	385.2 ± 1.49	501.3 ± 4.32

[a] [ATP] = 10 μM; Compounds were tested by 10 dose singlet assay; [b] Each compound was tested in triplicate assay, IC_{50} values are presented as the mean ± SD; NT: not tested.

2.3. Western blot analysis and colorectal cancer cell lines growth inhibition of **9g**

Considering **9g** has higher cell level activity (IC_{50}<50 nM), we selected it for further western blot analysis. Immunoblot were performed with the lysates of colorectal cancer cell lines SW620, HCT-15, HCT116 and lung cancer cell line A549 after treated with **9g** for 48 h (Fig. 3). The results undoubtedly showed that TOPK was expressed in all four cell lines and **9g** inhibited TOPK activity by directly binding with TOPK. Treatment with **9g** significantly decreased autophosphorylation of TOPK (Thr9), as well as phosphorylation of histone H3 (Ser10) and ERK1/2 which is consistent with reported western blot results after knockdown of TOPK [5,6,9]. It indicated that cell growth was significantly decreased in a manner of reducing

TOPK expression. Next, we found that compound **9g** inhibited the proliferation of three colorectal cells in a time and concentration depended manner (Fig.4).

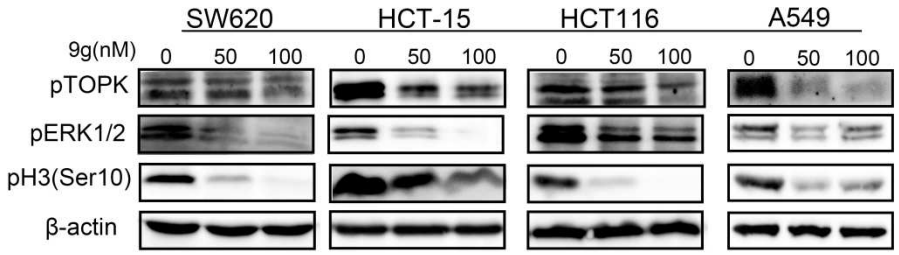


Fig. 3. 9g inhibited TOPK activity *in vitro* by directly binding with TOPK**.** Treatment with **9g** (50 and 100 nM, 48 h) reduced phosphorylation of TOPK together with its substrate, ERK1/2 and histone H3 (Ser10) in SW620, HCT-15, HCT116 cells.

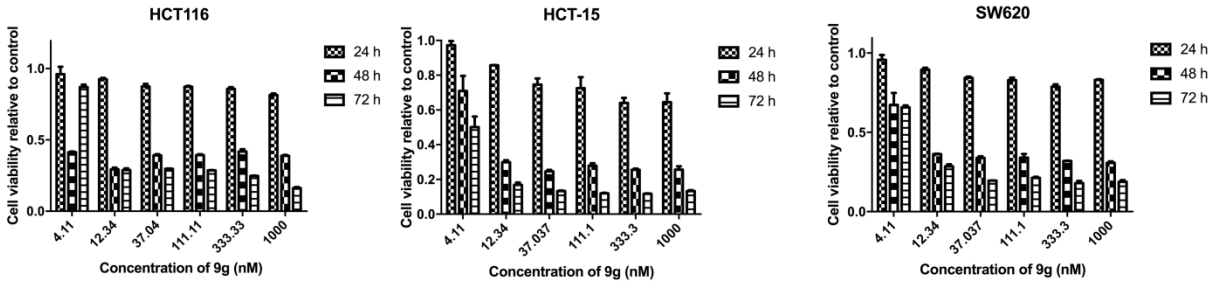


Fig.4. 9g inhibited the proliferation of SW620, HCT-15, HCT116 cells. Tumor cells were treated with **9g** with different concentrations after treatment for 24 h, 48 h and 72 h. MTT assays were performed to measure the proliferation of the cells.

2.4. *In vivo* antitumor efficacy in human xenografts of **9g**

HCT116 colon cancer is the most widely discussed xenograft model for TOPK efficacy evaluation *in vivo* [19,20,22,23]. To compare the antitumor activity of **9g** with reference compound OTS964, HCT116 colon cancer cells were engrafted subcutaneously into nude mice. **9g** and OTS964 were orally administered at

10 or 15 mg/kg once daily over a period of three weeks after the tumor size reached about 100 mm^3. The tumor size and body weight were measured every 3 days, and the percentage of tumor growth inhibition (TGI) was calculated according to the formula $[1 - (T - T_0)/(C - C_0)] \times 100$, where T and T_0 are the mean tumor volumes at day 21 and day 0 for the experimental group, and C and C_0 are those for the vehicle control group. Treatment of mice with 10 mg/kg of **9g** significantly inhibited HCT116 tumor growth relative to the vehicle group with TGI of 53.7%, better than OTS964 (TGI 49.4%). To our delight, when treated with **9g** at 15 mg/kg, TGI increased to 75.8%. In addition, mice seemed to tolerate treatment with **9g** without adverse toxicity or significant loss of body weight, similar to the vehicle group (Fig. 5).

Since **9g** demonstrated superior efficacy compared to OTS964, we further evaluated its efficacy in HCT-15 and SW620 colorectal cancer xenograft models. Oral administration of **9g** at 10 and 15 mg/kg once daily for 3 weeks resulted in final TGIs of 42.1% and 78.8% for HCT-15, 72.1% and 79.7% for SW620 respectively (Fig. 5a), with slightly body weight loss (Fig.5d). Those results indicated that the compound was potent to substantially inhibit tumor growth at tolerated doses in mouse xenograft model, and supported TOPK inhibition as a therapeutically relevant target.

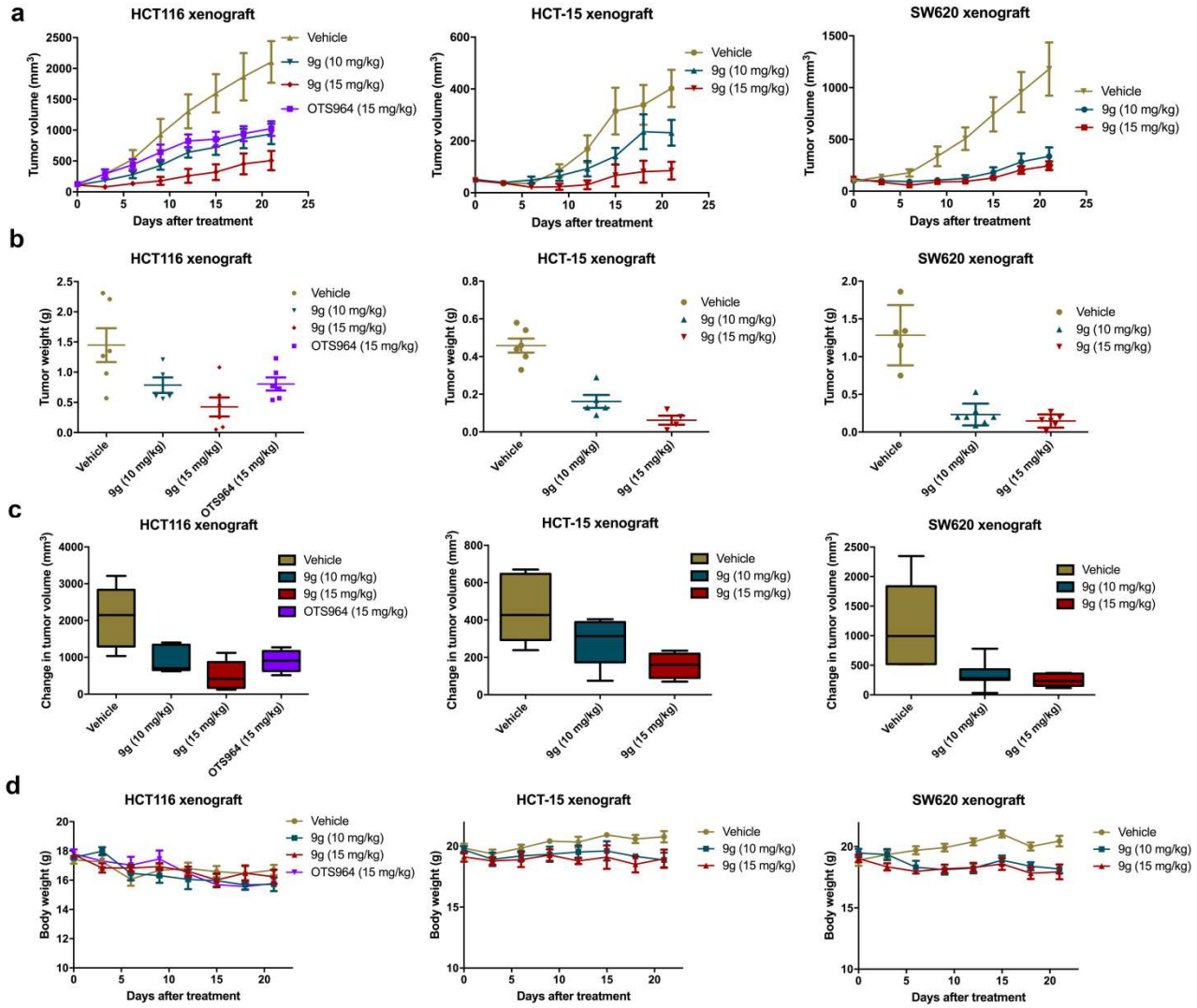


Fig. 5. *In vivo* efficacy of **9g** in colorectal cancer xenograft models (n = 6). Nude mice bearing HCT116/HCT-15/SW620 were orally treated with vehicle control or **9g**/OTS964 once daily for 3 weeks. (a) Effect of **9g**/OTS964 on tumor growth in mouse xenograft. (b) Tumor weight at day 21. (c) Tumor volume at day 21. (d) Corresponding body weight change.

2.5. Pharmacokinetic study of **9g**

To support further pharmacological and toxicological study, a pharmacokinetic study of **9g** was also performed. **9g** was administrated to rats via po and iv dose separately. Blood samples were collected at 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after dosing. The blood drug level was then determined with LC-MS/MS. The mean **9g** concentration in plasma versus time profile was presented in Fig. 6. The pharmacokinetic parameters are listed in Table 4. **9g** showed moderate systemic plasma clearance (2037.19 ±

295.19 mL/h/kg), a high volume of distribution (20.25 ± 7.97 L/kg), and a long mean residence time via oral administration (10.54 ± 0.29 h), suggesting the compound has good drug like characters. This together with a good oral bioavailability (100.11 ± 24.39%) would predict further drug development of the small molecule as anti-cancer agents.

Table 4. Pharmacokinetic properties of **9g** in rats[a]

Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{(0-t)}$ (ng·h/mL)	Cl (mL/h/kg)	Vss (L/kg)	$t_{1/2}$ (h)	$MRT_{(0-t)}$ (h)	F (%)
2 mg iv	188.03 ± 45.43	0.08 ± 0.00	563.69 ± 131.54	2037.19 ± 295.19	20.25 ± 7.97	7.02 ± 2.74	3.24 ± 0.14	
15 mg po	250.83 ± 38.26	6.67 ± 1.15	4232.35 ± 1031.31[b]				10.54 ± 0.29	100.11 ± 24.39

[a] WinNonlin® software version 7.0 was used to estimate pharmacokinetic parameters; [b]$AUC_{(0-24h)}$.

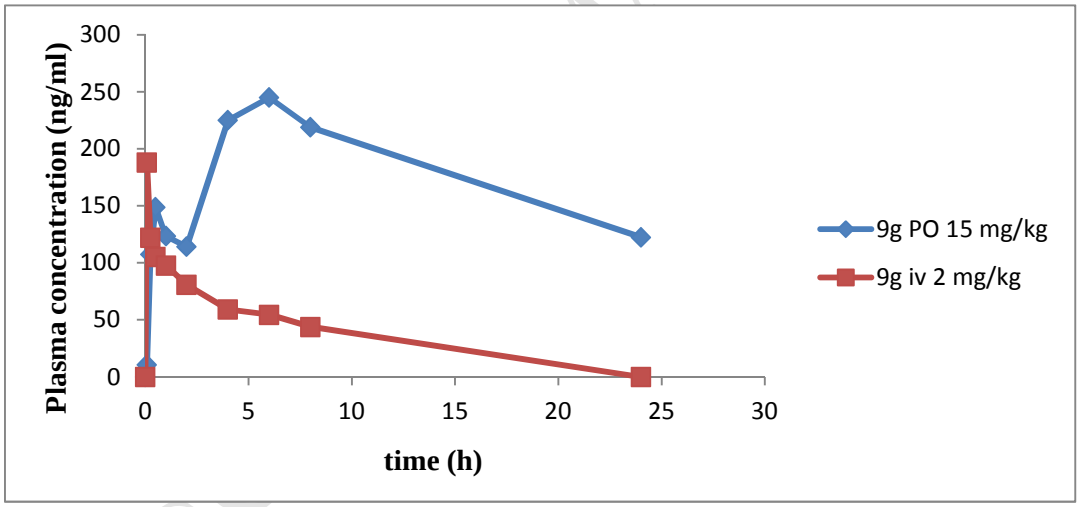


Fig. 6. Drug concentration time curve of **9g** in plasma of SD rats after single oral/ intravenous administration. **9g** was administrated to rats (n = 3) iv 2mg/kg and po 15 mg/kg separately. Blood samples of 0.2 mL each were collected from the jugular veins into heparized tubes at 0 h (before drug administration) and at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after dosing. The blood drug concentration was then determined with LC-MS/MS.

3. Conclusion

We have demonstrated the development of series of novel 1-phenyl phenanthridin-6(5H)-one TOPK

inhibitors. This phenanthridinone scaffold exhibited more potent anti-TOPK activity compared to reference compound OTS964. Based on the results of binding affinity and cell level screening, the preliminary SARs were summarized. In the *in vitro* assays, **9b** showed >700 fold stronger binding affinity at subnanomolar level than OTS964 and **9g** showed 2~6 fold superior activity against a panel of cancer cell lines at nanomolar level. For the *in vivo* evaluation, compound **9g** was shown to be effective in treating three mouse xenograft models of colorectal cancer (>75% TGI) by oral administration without severe adverse events, superior than OTS964 (49% TGI for HCT116) at the same dose (15 mg/kg, po). Its potency may be further developed and may be applied to a wide range of human malignancies. In the pharmacokinetic study, compound **9g** also displayed favorable plasma stability and oral bioavailability. The *in-vivo* tumor suppression activity and pharmacokinetic results of **9g** suggest that the drug candidate has great clinical application potential.

In summary, the novel 1-phenyl phenanthridin-6(5H)-one derivatives exhibited potent TOPK inhibitory activity both *in vitro* and *in vivo*, and showed excellent oral bioavailability. Developing inhibitors targeting TOPK provides an exciting strategy for treatment of tumor.

4. Experimental

4.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 25 °C using DMSO-d_6, CD_3OD or $CDCl_3$ as the solvent. Chemical shifts (δ) are reported in ppm relative to Me_4Si (internal standard), coupling constants (*J*) are reported in hertz, and peak multiplicity are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). High resolution mass analysis is performed on a Waters Q-TOF Premier mass spectrometer with electron spray ionization (ESI). Thin layer chromatography (TLC) was performed on 0.20 mm silica gel F-254 plates (Qingdao Haiyang Chemical, China). Visualization of TLC was accomplished with UV light and/or aqueous potassium permanganate or I_2 in silica gel. Column chromatography was performed using silica gel 60 of 300−400 mesh (Qingdao Haiyang Chemical, China).

*4.1.1. Synthesis of (R)-(4-(1-((tert-butoxycarbonyl)amino)ethyl)phenyl)boronic acid (**R1A**)*

Step1: (R)-tert-butyl (1-(4-bromophenyl)ethyl)carbamate (**R1-2**). (R)-1-(4-bromophenyl)ethanamine (14.4 g, 72.0 mmol) was dissolved in 60 ml dichloromethane (DCM), a solution of Boc_2O (17.3 g, 79.2 mmol, 1.1 eq) in 60 ml DCM was added dropwise at room temperature. The resulting solution was stirred at room temperature for further 2 h after LCMS indicating completion. The mixture was concentrated and suspended in hexane. The solid was collected and dried under vacuum to give 20.0 g white solid, yield: 92.6%.

Step2: (R)-tert-butyl (1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethyl)carbamate (**R1**).To a suspension of product of step1 (20 g, 66.6 mmol) in dioxane (120 ml) was added Bis(pinacolato)diboron (20.3 g, 80.0 mmol, 1.2 eq), KOAc (13.1 g, 133.2 mmol, 2 eq) and $PdCl_2$(dppf) (1.95 g, 2.66 mmol, 0.04 eq). The mixture was heated under nitrogen overnight, then filtered and concentrated. The residue was dissolved in a mixture of hexane and ethylacetate (EA) (4:1) and filtered through silica gel pad, then concentrated and directly used for hydrolysis. ESI-MS m/z 292.3 $[M-56+H]^+$.

Step3: To the residue above was added acetone/H_2O (200ml/100ml), $NaIO_4$ (61.2 g, 286 mmol), NH_4OAc (14.7 g, 190.6 mmol). The suspension was stirred at 40 °C for 5 h, then filtered and the solid was washed with acetone. The filtrate was concentrated and re-dissolved in DCM/MeOH (5/1), washed with water, concentrated and suspended in hexane/EA (1:1), filtered to give 14.5 g **R1A** as off-white solid, yield: 82.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (s, 2H), 7.71 (d, J = 7.6 Hz, 2H), 7.35 (d, J = 7.2 Hz, 1H), 7.24 (d, J = 7.6 Hz, 2H), 4.67-4.51 (m, 1H), 1.36 (s, 9H), 1.29 (d, J = 6.8 Hz, 3H). ESI-MS m/z 210.2 $[M-56+H]^+$.

4.1.2. Synthesis of (R)-(4-(1-((tert-butoxycarbonyl)amino)propan-2-yl)phenyl)boronic acid (**R2A**)

Step1: (R)-tert-butyl (2-(4-bromophenyl)propyl)carbamate (**R2-3**). A solution of trifluoro acetic anhydride (23.0 mL, 164.4 mmol) in anhydrous DCM (150 mL) was cooled to 0 °C, and (R)-2-phenylpropan-1-amine (20.2 g, 149.3 mmol) in anhydrous DCM (40 mL) was added dropwise. The mixture was stirred at room temperature for 2 h. The flask was again cooled to 0 °C and methane sulfonic acid (24.2 mL) was added, followed by 1,3-dibromo-5,5-dimethylhydantoin (21.3 g, 74.5 mmol) in one portion. The mixture was stirred overnight, quenched with water (80 mL), and diluted with DCM (250 mL). The

organic layers were separated and concentrated. The residue was diluted with water/EtOH (200ml/200ml), LiOH•H_2O (8 g, 190.5 mmol) was added and stirred at room temperature for 1 h. Then Boc_2O (29.3 g, 134.4 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was diluted with H_2O (200 ml) and extracted with DCM. DCM layer was concentrated to give yellow residue and the residue was purified by column chromatography (silica, 0-30% ethyl acetate/heptane) to afford the desired product (45.9 g, 98.0%) as yellow oil. ESI-MS m/z 260.1 $[M-56+H]^+$.

Step2: Following procedure of step2 and step3 of **R1A** with above 45.9 g intermediate, **R2A** 20.8 g (yield: 51%) was obtained as off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 3.08-3.01 (m, 2H), 2.87-2.82 (m, 1H), 1.35 (s, 9H), 1.14 (d, *J* = 6.8 Hz, 3H). ESI-MS m/z 224.1 $[M-56+H]^+$.

4.1.3. Synthesis of (4-(1-(((tert-butoxycarbonyl)amino)methyl)cyclopropyl)phenyl)boronic acid (**R3A**)

Step1: tert-butyl ((1-(4-bromophenyl)cyclopropyl)methyl)carbamate (**R3-3**). 1-(4-bromophenyl)cyclopropanecarbonitrile (4.45 g, 20 mmol) was added to 1M Borane-THF complex (100 ml) and the mixture was stirred at room temperature overnight and then heated at 65 °C for 5 h. The mixture was concentrated, diluted with MeOH, 1N HCl was added dropwise until PH<1. The mixture was heated at 65 °C while keeping the PH~1 by addition of 1N HCl intermittently, then cooled down and basified with solid $NaHCO_3$. The suspension was concentrated and re-dissolved in acetonitrile and filtered to give the acetonitrile solution of free amine. Boc_2O (4.1g, 18.7 mmol) in acetonitrile was added dropwise. The mixture was stirred at room temperature for 3 h and then concentrated to give 6.1 g yellow oil, yield: 93.3%. ESI-MS m/z 272.0 $[M-100+H]^+$.

Step2: Following procedure of step2 and step3 of **R1A** with above 6.1 g intermediate, **R3A** 3.9 g was obtained as off-white solid, yield: 71.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (s, 2H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.21 (d, *J* = 7.6 Hz, 2H), 6.82 (t, *J* = 5.6 Hz, 1H), 3.22 (d, *J* = 5.6 Hz, 2H), 1.34 (s, 9H), 0.85 (s, 2H), 0.71 (s, 2H). ESI-MS m/z 236.1 $[M-56+H]^+$.

*4.1.4. The representative procedure for the preparation of intermediate **5***

Step1: 2-bromo-N-(4-methoxy-2-methylphenyl)benzamide (**BA3**): 1 drop of DMF was added to a suspension of 2-bromobenzoic acid (2 g, 9.95 mmol) in 5 ml $SOCl_2$, the mixture was heated under reflux for 2 h, then concentrated and diluted with DCM 20 ml, concentrated again and re-diluted with DCM 20 ml and concentrated. The residue was dissolved in 20 ml DCM, added dropwise to a solution of 4-methoxy-2-methylaniline (1.4 g, 9.95 mmol) and triethylamine (3.43 ml, 24.9 mmol) in 10 ml DCM, and then stirred for 2 h. The mixture was concentrated to give brown solid, which was treated with EtOH/H_2O (10ml/20ml) to give off-white solid 2.5 g, yield: 78.4%. ESI-MS m/z 320.1 $[M+H]^+$.

Step 2: tert-butyl (2-bromobenzoyl)(4-methoxy-2-methylphenyl)carbamate (**BA4**): BOC_2O (2.0 g, 9.4 mmol) was added dropwise to a suspension of **BA3** (2.5 g, 7.81 mmol) and 4-dimethylaminopyridine (DMAP) (0.39 mmol, 47.7 mg, 0.05 eq) in 30 ml dry DCM, then stirred at room temperature overnight. It was concentrated and treated with hexane/EA/H_2O (20 ml/2 ml/20 ml) to precipitate the solid, the solid was filtered and washed with hexane, dried under vacuum to give off-white solid 2.9 g, yield: 90%. ESI-MS m/z 366.1$[M-56+H]^+$.

Step3: *2-methoxy-4-methylphenanthridin-6(5H)-one* (**BA5**): A suspension o f **BA3** (1.8 g, 4.28 mmol), KOAc (1.68 g, 17.13 mmol) and Pd(t-$Bu_3P)_2$ (131 mg, 0.26 mmol) in dry N,N-Dimethylacetamide (DMA) (5 ml) was heated at 130~140 °C for 2 h under argon. The mixture was cooled down and quenched with cold water, the precipitated solid was collected and washed with H_2O, EtOH successively, then dried under vacuum to yield **BA4** as gray-white solid. Weight: 770 mg, yield: 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.53 (d, *J* = 8.0 Hz, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 7.82 (t, *J* = 7.4 Hz, 1H), 7.72 (s, 1H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.00 (s, 1H), 3.84 (s, 3H), 2.43 (s, 3H). ESI-MS m/z 240.2 $[M+H]^+$.

4.1.4.1. 7-fluoro-2-methoxy-4-methylphenanthridin-6(5H)-one (**DA5**). Yield (three steps): 35.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.76-7.70 (m, 1H), 7.49 (d, *J* = 2.4 Hz, 1H), 7.28-7.23 (m, 1H), 6.99 (d, *J* = 2.0 Hz, 1H), 3.91 (s, 3H), 2.46 (s, 3H). ESI-MS m/z 258.3 $[M+H]^+$.

4.1.4.2. 2-methoxy-4,8-dimethylphenanthridin-6(5H)-one (**JA5**). Yield (three steps): 43.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.32 (d, *J* = 0.4 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.60 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.51 (d, *J* = 2.8 Hz, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 3.91 (s, 3H), 2.53 (s, 3H), 2.48 (s, 3H). ESI-MS m/z 254.3

$[M+H]^+$.

4.1.4.3. 2-methoxy-4,7-dimethylphenanthridin-6(5H)-one (**KA5**). Yield (three steps): 42.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 2.4 Hz, 1H), 7.36 (*d*, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 3.90 (s, 3H), 2.98 (s, 3H), 2.47 (s, 3H). ESI-MS m/z 254.1 $[M+H]^+$.

4.1.4.4. 2-methoxy-4,9-dimethylphenanthridin-6(5H)-one (**LA5**). Yield (three steps): 43.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1H), 8.40 (d, *J* = 8.0 Hz, 1H), 7.98 (s, 1H), 7.51 (d, *J* = 1.6 Hz, 1H), 7.41 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 1.6 Hz, 1H), 3.91 (s, 3H), 2.57 (s, 3H), 2.49 (s, 3H). ESI-MS m/z 254.1 $[M+H]^+$.

4.1.4.5. 4,7-difluoro-2-methoxyphenanthridin-6(5H)-one (**DC5**). Yield (three steps): 33.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.78-7.73 (m, 1H), 7.41 (d, *J* = 1.6 Hz, 1H), 7.33-7.28 (m, 1H), 6.93 (dd, *J* = 11.6, 2.8 Hz, 1H), 3.91 (s, 3H). ESI-MS m/z 262.3 $[M+H]^+$.

4.1.4.6. 4-chloro-2-methoxyphenanthridin-6(5H)-one (**BD5**). Yield (three steps): 35.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.52 (d, *J* = 7.6 Hz, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 7.80 (t, *J* = 7.4 Hz, 1H), 7.64 (t, *J* = 7.4 Hz, 1H), 7.59 (d, *J* = 2.0 Hz, 1H), 7.16 (d, *J* = 2.4 Hz, 1H), 3.91 (s, 3H). ESI-MS m/z 260.1$[M+H]^+$.

*4.1.4.7. 4-chloro-7-fluoro-2-methoxyphenanthridin-6(5H)-one (***DD5***)*. Yield (three steps): 38.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.78-7.73 (m, 1H), 7.56 (d, *J* = 2.4 Hz, 1H), 7.33-7.28 (m, 1H), 7.19 (d, *J* = 2.4 Hz, 1H), 3.91 (s, 3H). ESI-MS m/z 278.1 $[M+H]^+$.

4.1.5. The representative procedure for the preparation of intermediate **6**

1-bromo-2-methoxy-4-methylphenanthridin-6(5H)-one (**BA6**): **BA5** (433 mg, 1.81 mmol) was added to a mixture of DCM/HOAc (1.5 ml/1.5 ml) and stirred to give a solution, followed by addition of N-bromosuccinimide (NBS) (322 mg, 1.81 mmol) by one portion. The mixture was stirred at room temperature overnight. LCMS showed the reaction was not finished. Another portion of NBS (32 mg) was added and then stirring was continued for 2 h. The reaction mixture was diluted with water, extracted with DCM. DCM layer was separated and concentrated to give red-brown solid. The solid was suspended in

EA/hexane (1:1) and heated under reflux for 30 min, cooled down and filtered, the solid was dried under vacuum to give 460 mg yellow solid, yield: 79.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (d, *J* = 8.4 Hz, 1H), 8.66 (s, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.01 (s, 1H), 3.95 (s, 3H), 2.50 (s, 3H). ESI-MS m/z 320.0 $[M+H]^+$.

4.1.5.1. 1-bromo-7-fluoro-2-methoxy-4-methylphenanthridin-6(5H)-one **(DA6).** Yield 88.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (d, *J* = 8.8 Hz, 1H), 8.36 (s, 1H), 7.76-7.71 (m, 1H), 7.34-7.29 (m, 1H), 7.03 (s, 1H), 3.96 (s, 3H), 2.47 (s, 3H). ESI-MS m/z 336.0 $[M+H]^+$.

4.1.5.2. 1-bromo-2-methoxy-4,8-dimethylphenanthridin-6(5H)-one (**JA6**). Yield: 89.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (d, *J* = 8.8 Hz, 1H), 8.72 (s, 1H), 8.37 (s, 1H), 7.61 (dd, *J* = 8.6, 1.8 Hz, 1H), 6.99 (s, 1H), 3.96 (s, 3H), 2.54 (s, 3H), 2.50 (s, 3H). ESI-MS m/z 334.1 $[M+H]^+$.

4.1.5.3. 1-bromo-2-methoxy-4,7-dimethylphenanthridin-6(5H)-one (**KA6**). Yield: 96.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (d, *J* = 8.0 Hz, 1H), 8.81 (s, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 7.6 Hz, 1H), 6.98 (s, 1H), 3.95 (s, 3H), 2.97 (s, 3H), 2.50 (s, 3H). ESI-MS m/z 332.0 $[M+H]^+$.

4.1.5.4. 1-bromo-2-methoxy-4,9-dimethylphenanthridin-6(5H)-one (**LA6**). Yield: 93.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.52 (s, 1H), 8.57 (s, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.01 (s, 1H), 3.96 (s, 3H), 2.59 (s, 3H), 2.49 (s, 3H). ESI-MS m/z 334.0 $[M+H]^+$.

4.1.5.5. 1-bromo-4,7-difluoro-2-methoxyphenanthridin-6(5H)-one (**DC6**). Yield: 90.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (d, *J* = 8.4 Hz, 1H), 7.74-7.68 (m, 1H), 7.32-7.27 (m, 1H), 6.97 (d, *J* = 11.2 Hz, 1H), 3.89 (s, 3H). ESI-MS m/z 340.1 $[M+H]^+$.

4.1.5.6. 1-bromo-4-chloro-2-methoxyphenanthridin-6(5H)-one (**BD6**). Yield: 88.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.68 (d, *J* = 8.8 Hz, 1H), 8.90 (s, 1H), 8.57 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.84-7.80 (m, 1H), 7.71-7.67 (m, 1H), 7.21 (s, 1H), 3.97 (s, 3H). ESI-MS m/z 338.0 $[M+H]^+$.

4.1.5.7. 1-bromo-4-chloro-7-fluoro-2-methoxyphenanthridin-6(5H)-one (**DD6**) Yield: 91.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (d, *J* = 8.4 Hz, 1H), 8.76 (s, 1H), 7.77-7.71 (m, 1H), 7.37-7.32 (m, 1H), 7.19 (s, 1H),

3.96 (s, 3H). ESI-MS m/z 356.0 $[M+H]^+$.

4.1.5.8. 1-bromo-2,7-dimethoxy-4-methylphenanthridin-6(5H)-one (**CA6**). To a 150 ml two necked flask was added **D6** (5.4 g, 16.06 mmol), MeONa (4.3 g, 80.32 mmol), DMF (54 ml). The mixture was stirred at 50~60 °C for 1 h, cooled to room temperature and quenched with 200 ml water. The precipitated solid was collected and washed with water and ethanol, dried under vacuum to give 4.5 g gray white solid. Yield: 80.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (d, *J* = 8.4 Hz, 1H), 8.18 (s, 1H), 7.69 (t, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.99 (s, 1H), 4.05 (s, 3H), 3.95 (s, 3H), 2.44 (s, 3H). ESI-MS m/z 350.2 $[M+H]^+$.

4.1.5.9. 1-bromo-4-chloro-2,7-dimethoxyphenanthridin-6(5H)-one (**CD6**). Yield: 88.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (d, *J* = 8.4 Hz, 1H), 8.59 (s, 1H), 7.71 (t, *J* = 8.4 Hz, 1H), 7.18 (s, 1H), 7.17 (d, *J* = 7.2 Hz, 1H), 4.06 (s, 3H), 3.96 (s, 3H). ESI-MS m/z 370.0 $[M+H]^+$.

4.1.5.10. 1-bromo-7-(dimethylamino)-2-methoxy-4-methylphenanthridin-6(5H)-one (**MA6**): **DA6** (530 mg, 1.58 mmol), 33% dimethlyamine aqueous solution (3 ml), DMF (10 ml) were added to a 30 ml sealed tube and the mixture was heated at 110 ^oC overnight. It was cooled to room temperature and sold precipitated out. The solid was collected and washed with water and EtOH, dried under vacuum to give 381 mg gray white solid, yield: 66.9%. ESI-MS m/z 363.1 $[M+H]^+$.

*4.1.6. The representative procedure for the preparation of intermediate **7***

(R)-tert-butyl (1-(4-(2-methoxy-4-methyl-6-oxo-5,6-dihydrophenanthridin-1-yl)phenyl)ethyl)carbamate (**7a**): **BA6** (210 mg, 0.66 mmol), **R1A** (228 mg, 0.86 mmol), $NaHCO_3$ (166 mg, 1.98 mmol) and $PdCl_2$(dppf) (24 mg, 0.033 mmol) were added to 25 ml two necked flask equipped with a thermometer, followed by DME/H_2O (10 ml/0.5 ml). The mixture was heated at 85 °C overnight. Then it was concentrated, diluted with water and extracted with DCM. The DCM layer was washed with water, concentrated and crystallized from mixture of EA and hexane to give 160 mg desired target as grey solid, yield: 52.9%.

*4.1.7. The representative procedure for the preparation of **8***

*(R)-1-(4-(1-aminoethyl)phenyl)-2-methoxy-4-methylphenanthridin-6(5H)-one hydrochloride (**8a**)*: **7a** (160 mg) was stirred in 5 ml 4N HCl/dioxane overnight, concentrated and the residue was washed with DCM and recrystallized from isopropanol to give 103 mg off-white solid, yield: 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.67 (s, 3H), 8.32 (d, *J* = 6.4 Hz, 1H), 7.64-7.60 (m, 2H), 7.50-7.40 (m, 1H), 7.29-7.23 (m, 4H), 6.87 (d, *J* = 8.8 Hz, 1H), 4.51-4.50 (m, 1H), 3.68 (s, 3H), 2.56 (s, 3H), 1.62 (d, *J* = 5.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.48, 151.54, 138.56, 138.50, 134.50, 131.13, 130.31, 129.70, 127.54, 127.36, 127.32, 127.18, 126.88, 124.84, 124.80, 117.10, 115.90, 56.33, 49.85, 20.88, 18.27. ESI-MS m/z 359.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{23}N_2O_2$ $[M+H]^+$ m/z 359.1754, found 359.1758.

*4.1.7.1. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-2-methoxy-4-methylphenanthridin-6(5H)-one hydrochloride (**8b**).* White solid, two step yield: 58.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.32-8.30 (m, 1H), 7.94 (s, 3H), 7.45-7.38 (m, 3H), 7.27 (s, 1H), 7.22-7.17 (m, 3H), 6.89 (d, *J* = 8.4 Hz, 1H), 3.68 (s, 3H), 3.16-3.06 (m, 3H), 2.51 (s, 3H), 1.35 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.50, 151.59, 142.05, 137.03, 134.63, 130.91, 130.43, 129.77, 127.71, 127.45, 127.33, 127.13, 126.97, 125.07, 124.66, 117.23, 115.93, 56.33, 44.79, 37.30, 19.49, 18.26. ESI-MS m/z 373.3 $[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{25}N_2O_2$ $[M+H]^+$ m/z 373.1911, found 373.1913.

*4.1.7.2. 1-(4-(1-(aminomethyl)cyclopropyl)phenyl)-2-methoxy-4-methylphenanthridin-6(5H)-one hydrochloride (**8c**).* White solid, two step yield: 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.32-8.29 (m, 1H), 8.09 (s, 3H), 7.46-7.42 (m, 3H), 7.27-7.17 (m, 4H), 6.94 (d, *J* = 8.4 Hz, 1H), 3.67 (s, 3H), 3.19 (s, 2H), 2.56 (s, 3H), 1.15-1.12 (m, 2H), 1.04-1.01 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.50, 151.58, 140.17, 136.70, 134.58, 131.12, 130.27, 129.76, 128.38, 127.61, 127.27, 127.10, 126.91, 124.95, 124.66, 117.22, 115.86, 56.29, 46.66, 23.04, 18.24, 13.88. ESI-MS m/z 385.3 $[M+H]^+$.

*4.1.8. The representative procedure for the preparation of compound **9***

*(R)-1-(4-(1-aminoethyl)phenyl)-2-hydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**9a**).* **8a** (136 mg) was suspended in 15% BBr_3 in DCM (5ml) and stirred overnight. The mixture was concentrated and treated with DCM 20 ml and concentrated, diluted with ice-water, neutralized with aq. ammonia until PH>9, the precipitated solid was collected and dissolved with 3N HCl/MeOH, concentrated and crystallized with

isopropanol to give 110 mg off-white solid, yield: 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 9.27 (s, 1H), 8.64 (s, 3H), 8.31 (d, *J* = 8.0 Hz, 1H), 7.62-7.59 (m, 2H), 7.43 (t, *J* = 7.4 Hz, 1H), 7.29-7.27 (m, 2H), 7.24-7.20 (m, 1H), 7.07 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 4.50 (t, *J* = 5.4 Hz, 1H), 2.47 (s, 3H), 1.62 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.37, 149.79, 139.32, 137.99, 134.78, 130.84, 130.54, 128.70, 127.34, 127.29, 127.26, 126.88, 126.82, 124.53, 122.40, 119.38, 117.02, 49.85, 20.78, 18.03. ESI-MS: m/z 345.3 $[M+H]^+$. HRMS (ESI) Calcd for $C_{22}H_{21}N_2O_2$ $[M+H]^+$ m/z 345.1598, found 345.1558.

*4.1.8.1. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-2-hydroxy-4-methylphenanthridin-6(5H)-one hydrochloride **(9b)**.* White solid, yield 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 9.16 (s, 1H), 8.30 (d, *J* = 7.6 Hz, 1H), 8.13 (s, 3H), 7.44-7.38 (m, 3H), 7.22-7.18 (m, 3H), 7.06 (s, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 3.18-3.08 (m, 3H), 2.51(s, 3H), 1.36 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.38, 149.79, 141.75, 137.56, 134.88, 130.77, 130.56, 128.71, 127.69, 127.55, 127.40, 127.25, 126.82, 126.80, 124.35, 122.64, 119.38, 117.13, 44.87, 37.27, 19.48, 18.00. ESI-MS m/z 359.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{23}N_2O_2$ $[M+H]^+$ m/z 359.1754, found 359.1758.

*4.1.8.2. 1-(4-(1-(aminomethyl)cyclopropyl)phenyl)-2-hydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**9c**).* White solid, yield: 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H), 9.12 (s, 1H), 8.30 (dd, *J* = 8.0, 2.1 Hz, 1H), 8.04 (s, 3H), 7.46-7.40 (m, 3H), 7.27-7.19 (m, 3H), 7.05 (s, 1H), 7.04 (d, *J* = 8.4 Hz, 1H), 3.18 (d, *J* = 4.0 Hz, 2H), 2.47 (s, 3H), 1.13-1.10 (m, 2H), 1.06-1.00 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.40, 149.80, 139.79, 137.34, 134.84, 130.98, 130.43, 128.71, 128.46, 127.56, 127.23, 126.81, 126.75, 124.37, 122.53, 119.34, 117.13, 46.83, 25.46, 23.14, 17.99, 13.65. ESI-MS m/z 371.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{23}N_2O_2$ $[M+H]^+$ m/z 371.1754, found 371.1754.

*4.1.8.3. (R)-1-(4-(1-aminoethyl)phenyl)-7-fluoro-2-hydroxy-4-methylphenanthridin-6(5H)-one (**9d**).* Brown solid, yield: 71.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 9.28 (s, 1H), 8.48 (s, 3H), 7.57-7.54 (m, 2H), 7.30-7.26 (m, 2H), 7.19-7.16 (m, 2H), 7.05 (s, 1H), 6.87-6.85 (m, 1H), 4.49 (br s, 1H), 2.44 (s, 3H), 1.59 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.93, 160.33, 157.97, 157.93, 149.79, 139.05, 137.94, 137.70, 131.78, 131.67, 130.62, 129.25, 127.25, 127.20, 124.29, 123.83, 123.79, 122.30, 120.07, 116.30, 115.73, 115.70, 114.28, 114.07, 49.82, 20.72, 17.86. ESI-MS m/z 363.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{22}H_{20}FN_2O_2$ $[M+H]^+$ m/z 363.1503, found 363.1498.

*4.1.8.4. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-7-fluoro-2-hydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**9e**).* Brown solid, yield: 78.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 9.22 (br s, 1H), 8.10 (s, 3H), 7.38-7.35 (m, 2H), 7.21-7.11 (m, 4H), 7.07 (s, 1H), 6.89 (d, *J* = 7.6 Hz, 1H), 3.17-3.03 (m, 3H), 2.44 (s, 3H), 1.34 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.88, 160.29, 157.95, 149.81, 141.69, 137.81, 137.30, 131.72, 131.63, 130.64, 129.24, 127.55, 124.06, 123.94, 122.58, 120.12, 116.40, 115.70, 115.66, 114.21, 113.99, 44.86, 37.23, 19.46, 17.79. ESI-MS m/z 377.3 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{22}FN_2O_2$ $[M+H]^+$ m/z 377.1660, found 377.1658.

*4.1.8.5. (R)-1-(4-(1-aminoethyl)phenyl)-2,7-dihydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**9f**).* Brown solid, yield: 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.86 (s, 1H), 10.93 (s, 1H), 9.35 (s, 1H), 8.44 (s, 3H), 7.57-7.54 (m, 2H), 7.28-7.25 (m, 2H), 7.09-7.7.05 (m, 2H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.43 (d, *J* = 8.4 Hz, 1H), 4.52-4.49 (m, 1H), 2.50 (s, 3H), 1.59 (d, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.09, 161.51, 150.65, 139.19, 137.93, 135.98, 133.19, 130.48, 127.68, 127.22, 127.15, 125.29, 122.61, 119.71, 117.86, 117.82, 113.48, 110.72, 49.83, 20.74, 18.09. ESI-MS m/z 361.3 $[M+H]^+$. HRMS (ESI) Calcd for $C_{22}H_{21}N_2O_3$ $[M+H]^+$ m/z 361.1547, found 361.1548.

*4.1.8.6. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-2,7-dihydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**9g**).* Off-white solid, yield: 81.4%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.41 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.32 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.26 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.07-7.03 (m, 2H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 3.33-3.19 (m, 3H), 2.53 (s, 3H), 1.49 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6) δ 165.09, 161.45, 150.62, 141.68, 137.43, 136.06, 133.16, 130.50, 127.71, 127.56, 127.46, 125.12, 122.87, 119.70, 117.97, 117.93, 113.42, 110.72, 44.84, 37.28, 19.46, 18.05. ESI-MS m/z 375.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{23}N_2O_3$ $[M+H]^+$ m/z 375.1703, found 375.1707.

*4.1.8.7. 1-(4-(1-(aminomethyl)cyclopropyl)phenyl)-2,7-dihydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**9h**).* White solid, yield: 71.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.84 (s, 1H), 10.89 (s, 1H), 9.26 (s, 1H), 8.01 (s, 3H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.11 (t, *J* = 8.2 Hz, 1H), 7.08 (s, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 1H), 3.17 (d, *J* = 5.2 Hz, 2H), 2.48 (s, 3H), 1.12-1.09 (m, 2H), 1.02-0.99 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.10, 161.42, 150.68, 139.77, 137.20, 136.01, 133.38, 130.38, 128.32, 127.68, 125.11, 122.76, 119.69, 118.10, 117.95, 113.39, 110.66, 46.80, 23.12, 18.06,

13.66. ESI-MS m/z 387.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{23}N_2O_3$ $[M+H]^+$ m/z 387.1703, found 387.1705.

*4.1.8.8. (R)-1-(4-(1-aminoethyl)phenyl)-4-chloro-2-hydroxyphenanthridin-6(5H)-one hydrochloride(**9i**).* Brown solid, yield: 55.7%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (d, *J* = 8.0 Hz, 1H), 7.65-7.62 (m, 2H), 7.52-7.48 (m, 1H), 7.43-7.40 (m, 2H), 7.29 (s, 1H), 7.27-7.20 (m, 2H), 4.62 (q, *J* = 6.8 Hz, 1H), 1.77 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.96, 150.41, 138.51, 138.45, 138.24, 133.96, 131.34, 130.29, 127.82, 127.54, 127.47, 127.41, 127.01, 126.50, 124.53, 118.77, 117.37, 49.77, 20.74. ESI-MS m/z 365.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{21}H_{18}ClN_2O_2$ $[M+H]^+$ m/z 365.1051, found 365.1053.

*4.1.8.9. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-4-chloro-2-hydroxyphenanthridin-6(5H)-one hydrochloride (**9j**).* Brown solid, yield: 72.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 9.72 (s, 1H), 8.32 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.99 (s, 3H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.41 (dd, *J* = 5.2, 2.4 Hz, 1H), 7.33 (s, 1H), 7.26-7.21 (m, 3H), 6.96 (d, *J* = 8.4 Hz, 1H), 3.15-3.07 (m, 3H), 1.36 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.97, 150.43, 142.26, 136.53, 134.06, 131.26, 130.30, 127.84, 127.76, 127.55, 127.46, 127.00, 126.52, 124.80, 118.90, 118.58, 117.37, 44.78, 37.31, 19.51. ESI-MS m/z 379.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{22}H_{20}ClN_2O_2$ m/z $[M+H]^+$ 379.1208, found 379.1208.

*4.1.8.10. (R)-1-(4-(1-aminoethyl)phenyl)-4-chloro-2,7-dihydroxyphenanthridin-6(5H)-one hydrochloride (**9k**).* White solid, yield: 68.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.63 (s, 1H), 11.04 (s, 1H), 10.00 (s, 1H), 8.55 (s, 3H), 7.61-7.59 (m, 2H), 7.39 (s, 1H), 7.30-7.27 (m, 2H), 7.13 (t, *J* = 8.2 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.42 (d, *J* = 8.4 Hz, 1H), 4.52-4.49 (m, 1H), 1.60 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.05, 161.54, 151.20, 138.42, 138.18, 135.11, 133.66, 130.25, 127.40, 127.35, 125.62, 124.70, 119.55, 119.45, 118.10, 117.76, 114.60, 110.84, 49.79, 20.72. ESI-MS m/z 381.1$[M+H]^+$. HRMS (ESI) Calcd for $C_{21}H_{18}ClN_2O_3$ $[M+H]^+$ m/z 381.1000, found 381.1001.

*4.1.8.11. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-4-chloro-2,7-dihydroxyphenanthridin-6(5H)-one hydrochloride (**9l**).* White solid, yield: 87.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.61 (s, 1H), 11.01 (s, 1H), 9.84 (s, 1H), 7.98 (s, 3H), 7.41-7.39 (m, 2H), 7.36 (s, 1H), 7.23-7.18 (m, 2H), 7.12 (t, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.43 (d, *J* = 8.4 Hz, 1H), 3.13-3.06 (m, 3H), 1.35 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (100 MHz,

DMSO-d_6) δ 165.03, 161.45, 151.22, 142.20, 136.44, 135.17, 133.61, 130.24, 127.70, 127.64, 125.58, 124.97, 119.63, 119.24, 118.21, 117.74, 114.50, 110.80, 44.80, 37.27, 19.48. ESI-MS m/z 395.0 $[M+H]^+$. HRMS (ESI) Calcd for $C_{22}H_{20}ClN_2O_3$ $[M+H]^+$ m/z 395.1157, found 395.1157.

*4.1.8.12. 1-(4-(1-(aminomethyl)cyclopropyl)phenyl)-4-chloro-2,7-dihydroxyphenanthridin-6(5H)-one hydrochloride (**9m**).* White solid, yield: 79.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.61 (s, 1H), 11.00 (s, 1H), 9.93 (s, 1H), 8.02 (s, 3H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.39 (s, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 1H), 3.18 (s, 2H), 1.12-1.10 (m, 2H), 1.03-1.00 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.04, 161.43, 151.30, 140.28, 136.22, 135.14, 133.85, 130.11, 128.44, 125.55, 124.87, 119.62, 119.24, 118.39, 117.73, 114.48, 110.75, 46.74, 23.11, 13.74. ESI-MS m/z 407.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{20}ClN_2O_3$ $[M+H]^+$ m/z 407.1157, found 407.1164.

4.1.8.13.

*(R)-1-(4-(1-aminopropan-2-yl)phenyl)-7-(dimethylamino)-2-hydroxy-4-methylphenanthridin-6(5H)-one dihydrochloride (**9n**).* Light yellow solid, yield: 33.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.83 (br s, 1H), 11.56 (br s, 1H), 9.60 (s, 1H), 8.18 (s, 3H), 8.08 (s, 1H), 7.50-7.38 (m, 3H), 7.22-7.16 (m, 4H), 3.31 (s, 6H), 3.20-3.05 (m, 3H), 2.54 (s, 3H), 1.35 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.42, 151.37, 144.86, 142.17, 136.76, 136.51, 132.31, 130.48, 130.42, 127.95, 127.74, 127.46, 125.45, 122.58, 120.96, 120.36, 117.25, 115.61, 45.94, 44.84, 37.16, 19.49, 18.13. ESI-MS m/z 402.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{25}H_{28}N_3O_2$ $[M+H]^+$ m/z 402.2176, found 402.2180.

*4.1.8.14. (R)-1-(4-(1-aminoethyl)phenyl)-2-hydroxy-4,8-dimethylphenanthridin-6(5H)-one hydrochloride (**9o**).* Off-white solid, yield: 79.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 9.22 (s, 1H), 8.60 (s, 3H), 8.12 (d, *J* = 0.8 Hz, 1H), 7.61-7.58 (m, 2H), 7.28-7.24 (m, 2H), 7.04-7.01 (m, 2H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.51-4.48 (m, 1H), 2.46 (s, 3H), 2.34 (s, 3H), 1.62 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.34, 149.72, 139.39, 137.97, 136.46, 132.31, 132.00, 130.54, 130.51, 128.33, 127.36, 127.28, 127.20, 127.08, 126.76, 124.40, 122.16, 118.90, 117.12, 49.86, 20.82, 20.51, 18.01. ESI-MS m/z 359.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{23}N_2O_2$ $[M+H]^+$ m/z 359.1754, found 359.1761.

4.1.8.15. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-2-hydroxy-4,8-dimethylphenanthridin-6(5H)-one

*hydrochloride (**9p**).* Off-white solid, yield: 72.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 9.04 (s, 1H), 8.11(s, 1H), 8.06 (s, 3H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.19 (t, *J* = 6.0 Hz, 2H), 7.02-7.08 (m, 2H), 6.85 (d, *J* = 8.4 Hz, 1H), 3.16-3.07 (m, 3H), 2.45 (s, 3H), 2.34 (s, 3H), 1.36 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.35, 149.73, 141.74, 137.62, 136.43, 132.40, 131.94, 130.54, 128.33, 127.67, 127.51, 127.37, 127.01, 126.73, 124.21, 122.41, 118.89, 117.22, 44.87, 37.25, 20.50, 19.48, 17.99. ESI-MS m/z 373.3 $[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{25}N_2O_2$ $[M+H]^+$ m/z 373.1911, found 373.1875.

*4.1.8.16. (R)-1-(4-(1-aminoethyl)phenyl)-2-hydroxy-4,7-dimethylphenanthridin-6(5H)-one hydrochloride (**9q**).* Off-white solid, yield: 77.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.14 (s, 1H), 8.45 (s, 3H), 7.52 (t, *J* = 7.2 Hz, 2H), 7.26 (t, *J* = 6.8 Hz, 2H), 7.18-7.16 (m, 1H), 7.02-6.97 (m, 3H), 4.48-4.46 (m, 1H), 2.82 (s, 3H), 2.43 (s, 3H), 1.58 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.63, 149.51, 140.31, 139.42, 137.60, 136.30, 130.78, 130.73, 130.19, 129.54, 129.00, 127.12, 126.91, 126.32, 125.18, 123.71, 122.08, 119.31, 117.45, 49.81, 23.96, 20.73, 17.68. ESI-MS 359.2 m/z $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{23}N_2O_2$ $[M+H]^+$ m/z 359.1754, found 359.1755.

*4.1.8.17. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-2-hydroxy-4,7-dimethylphenanthridin-6(5H)-one hydrochloride (**9r**).* Off-white solid, yield: 79.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 9.04 (s, 1H), 8.02 (s, 3H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.19-7.15 (m, 3H), 7.02-6.98 (m, 3H), 3.11-3.07 (m, 1H), 2.81 (s, 3H), 2.43 (s, 3H), 1.34 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.66, 149.54, 141.44, 140.18, 137.65, 136.41, 130.75, 130.14, 129.51, 128.96, 127.44, 127.26, 126.42, 125.13, 123.46, 122.36, 119.31, 117.56, 44.88, 37.20, 23.95, 19.42, 17.66. ESI-MS m/z 373.1$[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{25}N_2O_2$ $[M+H]^+$ m/z 373.1911, found 373.1913.

*4.1.8.18. (R)-1-(4-(1-aminoethyl)phenyl)-2-hydroxy-4,9-dimethylphenanthridin-6(5H)-one (**9s**).* Brown solid, yield: 77.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 9.21 (s, 1H), 8.57 (s, 3H), 8.19 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.04 (s, 1H), 6.68 (s, 1H), 4.52 (d, *J* = 6.0 Hz, 1H), 2.46 (s, 3H), 1.95 (s, 3H), 1.61 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.34, 149.65, 140.36, 139.52, 138.26, 134.69, 130.60, 130.38, 128.85, 127.95, 127.82, 127.31, 126.46, 124.48, 124.44, 122.46, 119.26, 116.95, 49.92, 21.55, 21.33, 18.03. ESI-MS m/z 359.1$[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{23}N_2O_2$ $[M+H]^+$ m/z 359.1754, found 359.1757.

*4.1.8.19. (R)-1-(4-(1-aminopropan-2-yl)phenyl)-2-hydroxy-4,9-dimethylphenanthridin-6(5H)-one hydrochloride (**9t**).* Off-white solid, yield: 84.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 8.19-8.15 (m, 4H), 7.44-7.37 (m, 2H), 7.25-7.18 (m, 3H), 7.05 (s, 1H), 6.62 (s, 1H), 3.22-3.15 (m, 1H), 3.12-3.08 (m, 2H), 2.46 (s, 3H), 1.94 (s, 3H), 1.38 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.33, 149.65, 142.17, 140.14, 137.80, 134.79, 130.57, 130.42, 128.85, 128.06, 127.88, 127.23, 126.98, 124.48, 124.25, 122.73, 119.26, 117.07, 44.89, 37.32, 21.52, 19.68, 18.02. ESI-MS m/z 373.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{25}N_2O_2$ $[M+H]^+$ m/z 373.1911, found 373.1875.

*4.1.9. The representative procedure for the preparation of compound **10***

*(R)-1-(4-(1-(dimethylamino)ethyl)phenyl)-2-hydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**10a**).* To a suspension of **9a** (40 mg), paraformaldehyde (80 mg) in 10 ml MeOH was added 1 drop of HOAc, followed by addition of $NaBH_3CN$ (80 mg). The mixture was stirred at room temperature overnight. It was concentrated and diluted with sat.$NaHCO_3$ aqueous solution, extracted with DCM/MeOH (10:1), concentrated and treated with 3N HCl/MeOH, concentrated again and washed with acetone to give 37 mg desired target as off-white solid, yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 10.46 (s, 1H), 9.26 (s, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 7.72-7.70 (m, 1H), 7.65-7.63 (m, 1H), 7.43 (t, *J* = 7.4 Hz, 1H), 7.34-7.32 (m, 2H), 7.20-7.16 (m, 1H), 7.06 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 4.59-4.56 (m, 1H), 2.80 (d, *J* = 4.4 Hz, 3H), 2.64 (d, *J* = 4.8 Hz, 3H), 2.47 (s, 3H), 1.74 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.38, 149.66, 140.36, 134.68, 133.96, 130.91, 130.81, 130.53, 130.14, 128.81, 128.76, 127.38, 127.17, 126.96, 126.90, 124.72, 122.08, 119.43, 117.00, 64.52, 41.12, 18.03, 16.58. ESI-MS m/z 373.3 $[M+H]^+$. HRMS (ESI) Calcd for $C_{22}H_{18}NO_2$ $[M-N(CH_3)_2]^+$ m/z 328.1332, found 328.1291.

*4.1.9.1. (R)-1-(4-(1-(dimethylamino)propan-2-yl)phenyl)-2-hydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**10b**).* White solid, yield: 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 10.06 (s, 1H), 9.16 (s, 1H), 8.30 (d, *J* = 7.6 Hz, 1H), 7.47-7.40 (m, 3H), 7.22 (t, *J* = 6.4 Hz, 2H), 7.14 (t, *J* = 8.0 Hz, 1H), 7.05 (s, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 3.41 (br s, 3H), 2.80 (s, 3H), 2.77 (s, 3H), 2.47 (s, 3H), 1.38 (d, *J* = 5.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.37, 149.81, 141.91, 137.78, 134.88, 130.70, 130.67, 130.54, 128.71, 127.95, 127.49, 127.31, 126.85, 124.37, 122.57, 119.41, 117.08, 62.39, 43.94, 41.80, 34.94, 20.64,

18.00. ESI-MS m/z 387.3 $[M+H]^+$. HRMS (ESI) Calcd for $C_{25}H_{27}N_2O_2$ $[M+H]^+$ m/z 387.2067, found 387.2071.

*4.1.9.2. 1-(4-(1-((dimethylamino)methyl)cyclopropyl)phenyl)-2-hydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**10c**).* White solid, yield: 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 9.93 (s, 1H), 9.15 (s, 1H), 8.30 (d, *J* = 8.0 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.13-7.10 (m, 1H), 7.05 (s, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 3.55 (s, 2H), 2.72 (s, 6H), 2.46 (s, 3H), 1.18 (s, 2H), 1.12 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.38, 149.80, 140.36, 137.62, 134.84, 130.68, 130.51, 128.80, 128.73, 127.39, 127.31, 126.86, 124.44, 122.40, 119.42, 117.06, 64.26, 42.82, 21.70, 18.00, 13.63. ESI-MS m/z 399.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{26}H_{27}N_2O_2$ $[M+H]^+$ m/z 399.2067, found 399.2067.

*4.1.9.3. (R)-1-(4-(1-(dimethylamino)ethyl)phenyl)-2,7-dihydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**10f**).* White solid, yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.84 (s, 1H), 10.95 (s, 1H), 10.63 (s, 1H), 9.37 (s, 1H), 7.69-7.67 (m, 1H), 7.62-7.60 (m, 1H), 7.33-7.30 (m, 2H), 7.09-7.04 (m, 2H), 6.78-6.76 (m, 1H), 6.35-6.33 (m, 1H), 4.58-4.55 (m, 1H), 2.79 (s, 3H), 2.63 (d, *J* = 3.2 Hz, 3H), 2.49 (s, 3H), 1.72 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.11, 161.53, 150.51, 140.23, 135.90, 133.96, 132.89, 130.87, 130.75, 130.01, 128.68, 127.76, 125.47, 122.32, 119.77, 117.83, 117.70, 113.51, 110.82, 64.51, 40.58, 18.10, 16.53. ESI-MS m/z 389.0 $[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{25}N_2O_3$ $[M+H]^+$ m/z 389.1860, found 389.1862.

*4.1.9.4. (R)-1-(4-(1-(dimethylamino)propan-2-yl)phenyl)-2,7-dihydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**10g**).* Brown solid, yield: 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.86 (s, 1H), 10.90 (s, 1H), 10.25 (s, 1H), 9.34 (s, 1H), 7.45-7.42 (m, 2H), 7.21-7.18 (m, 2H), 7.11 (s, 1H), 7.02 (t, *J* = 8.4 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.45 (d, *J* = 8.4 Hz, 1H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.75 (d, *J* = 4.8 Hz, 3H), 2.51 (s, 3H), 1.38 (d, *J* = 5.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.09, 161.48, 150.66, 141.82, 137.65, 136.08, 132.91, 130.66, 130.62, 127.85, 127.71, 127.36, 125.15, 122.81, 119.74, 117.93, 117.82, 113.43, 110.78, 62.37, 43.93, 41.82, 34.91, 20.56, 18.07. ESI-MS m/z 403.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{25}H_{27}N_2O_3$ $[M+H]^+$ m/z 403.2016, found 403.2014.

4.1.9.5.

*1-(4-(1-((dimethylamino)methyl)cyclopropyl)phenyl)-2,7-dihydroxy-4-methylphenanthridin-6(5H)-one hydrochloride (**10h**).* White solid, yield: 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.84 (s, 1H), 10.91 (s, 1H), 9.83 (s, 1H), 9.27 (s, 1H), 7.54 (d, *J* = 6.8 Hz, 2H), 7.19 (d, *J* = 6.8 Hz, 2H), 7.08 (s, 1H), 6.99 (t, *J* = 8.2 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.46 (d, *J* = 8.4 Hz, 1H), 3.56 (s, 2H), 2.72 (s, 6H), 2.48 (s, 3H), 1.17 (s, 2H), 1.11 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.10, 161.49, 150.67, 140.33, 137.52, 136.06, 132.89, 130.75, 130.64, 128.71, 127.72, 125.19, 122.65, 119.78, 117.90, 113.44, 110.78, 64.29, 42.82, 21.69, 18.06, 13.61. ESI-MS m/z 415.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{26}H_{27}N_2O_3$ $[M+H]^+$ m/z 415.2016, found 415.2017.

*4.19.6. (R)-4-chloro-1-(4-(1-(dimethylamino)ethyl)phenyl)-2-hydroxyphenanthridin-6(5H)-one hydrochloride (**10i**).* Off-white solid, yield: 79.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (br s, 1H), 10.46 (s, 1H), 9.85 (s, 1H), 8.33 (dd, *J* = 7.8, 1.4 Hz, 1H),7.75-7.73 (m, 1H), 7.68-7.66 (m, 1H), 7.51-7.47 (m, 1H), 7.38-7.36 (m, 3H), 7.26-7.21 (m, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.62-4.55 (m, 1H), 2.81 (d, *J* = 4.8 Hz, 3H), 2.64 (d, *J* = 4.8 Hz, 1H), 1.74 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.96, 150.27, 139.30, 134.47, 133.88, 131.00, 130.69, 130.59, 130.28, 128.98, 127.89, 127.58, 127.31, 127.16, 126.62, 124.21, 118.91, 118.79, 117.45, 64.49, 40.54, 16.48. ESI-MS m/z 393.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{22}ClN_2O_2$ $[M+H]^+$ m/z 393.1364, found 393.1362.

*4.1.9.7. (R)-4-chloro-1-(4-(1-(dimethylamino)propan-2-yl)phenyl)-2-hydroxyphenanthridin-6(5H)-one hydrochloride (**10j**).* Light gray solid, yield: 81.5%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.44-8.42 (m, 1H), 7.59 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.56-7.49 (m, 2H), 7.39 (dd, *J* = 8.0 , 1.6 Hz, 1H), 7.34 (dd, *J* = 7.6, 1.6Hz, 1H), 7.31 (s, 1H), 7.26-7.21 (m, 2H), 3.65-3.59 (m, 1H), 3.49-3.41 (m, 2H), 2.96 (s, 6H), 1.49 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 162.74, 152.08, 142.82, 138.96, 135.91, 132.65, 132.51, 132.20, 130.28, 129.07, 129.03, 128.89, 128.53, 127.89, 127.67, 126.37, 121.01, 120.67, 118.51, 64.77, 44.32, 37.15, 21.00. ESI-MS m/z 407.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{24}ClN_2O_2$ $[M+H]^+$ m/z 407.1521, found 407.1490.

*4.1.9.8. (R)-4-chloro-1-(4-(1-(dimethylamino)ethyl)phenyl)-2,7-dihydroxyphenanthridin-6(5H)-one hydrochloride (**10k**).* White solid, yield: 87.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.61 (s, 1H), 11.06 (s, 1H), 10.87 (br s, 1H), 10.01 (s, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 7.72-7.64 (m, 1H), 7.40 (s, 1H), 7.34 (d, *J* = 8.4 Hz,

1H), 7.12 (t, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.34 (d, *J* = 8.4 Hz, 1H). 4.59-4.56 (m, 1H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.62 (d, *J* = 4.4 Hz, 3H), 1.73 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.09, 161.55, 151.07, 139.23, 135.06, 134.41, 130.64, 130.54, 130.18, 128.86, 125.73, 124.41, 119.59, 119.56, 118.00, 114.64, 110.96, 64.47, 40.51, 16.44. ESI-MS m/z 409.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{22}ClN_2O_3$ $[M+H]^+$ m/z 409.1313, found 409.1316.

*4.1.9.9. (R)-4-chloro-1-(4-(1-(dimethylamino)propan-2-yl)phenyl)-2,7-dihydroxyphenanthridin-6(5H)-one hydrochloride (**10l**).* White solid, yield: 79.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.62 (s, 1H), 11.03 (s, 1H), 9.89 (s, 1H), 9.78 (s, 1H), 7.46 (t, *J* = 7.2 Hz, 2H), 7.37 (s, 1H), 7.23 (t, *J* = 7.0 Hz, 2H), 7.07 (t, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.43 (d, *J* = 8.4 Hz, 1H), 3.41 -3.38 (m, 3H), 2.81 (d, *J* = 4.4 Hz, 3H), 2.77 (d, *J* = 4.4 Hz, 3H), 1.36 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.05, 161.50, 151.25, 142.33, 136.65, 135.21, 133.38, 130.40, 130.37, 128.01, 127.57, 125.61, 124.90, 119.61, 119.29, 118.10, 117.78, 114.53, 110.89, 62.31, 43.94, 41.83, 34.90, 20.55. ESI-MS m/z 423.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{24}H_{24}ClN_2O_3$ $[M+H]^+$ m/z 423.1470, found 423.1475.

4.1.9.10.

*4-chloro-1-(4-(1-((dimethylamino)methyl)cyclopropyl)phenyl)-2,7-dihydroxyphenanthridin-6(5H)-one hydrochloride (**10m**).* White solid, yield: 84.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.61 (s, 1H), 11.02 (s, 1H), 9.96 (s, 2H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.41 (s, 1H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.05 (t, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.46 (d, *J* = 8.4 Hz, 1H), 3.55 (s, 2H), 2.70 (s, 6H), 1.18 (s, 2H), 1.11 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.07, 161.49, 151.21, 140.79, 136.48, 135.18, 133.37, 130.38, 128.81, 125.66, 124.75, 119.61, 119.34, 118.20, 117.79, 114.54, 110.91, 64.23, 42.87, 21.71, 13.62. ESI-MS m/z 435.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{25}H_{24}ClN_2O_3$ $[M+H]^+$ m/z 435.1470, found 435.1469.

4.1.9.11.

*(R)-7-(dimethylamino)-1-(4-(1-(dimethylamino)propan-2-yl)phenyl)-2-hydroxy-4-methylphenanthridin-6(5H)-one (**10n**).* Light yellow solid, yield: 44.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.39-7.35 (m, 2H), 7.26-7.21 (m, 2H), 7.02 (s, 1H), 6.95 (t, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 3.03-3.00 (m, 7H), 2.57-2.54 (m, 1H), 2.46-2.41 (m, 4H), 2.24 (s, 6H), 1.33 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.00, 154.06, 148.82, 146.25, 138.67, 134.75, 130.74, 130.63, 130.39, 129.45,

128.66, 128.33, 122.98, 122.44, 119.13, 118.76, 118.09, 115.06, 113.78, 67.50, 45.78, 44.66, 37.94, 20.40, 17.19. ESI-MS m/z 430.4 $[M+H]^+$. HRMS (ESI) Calcd for $C_{25}H_{25}N_2O_2$ $[M-(NCH_3)_2]^+$ m/z 385.1911, found 385.1925.

*4.1.9.12. (R)-1-(4-(1-(dimethylamino)ethyl)phenyl)-2-hydroxy-4,8-dimethylphenanthridin-6(5H)-one hydrochloride (**10o**).* Off-white solid, yield: 89.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 10.40 (s, 1H), 9.18 (s, 1H), 8.12 (s, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.34-7.28 (m, 2H), 7.02-6.99 (m, 2H), 6.78 (d, *J* = 8.4 Hz, 1H), 4.59-4.56 (m, 1H), 2.81 (d, *J* = 4.4 Hz, 3H), 2.65 (d, *J* = 4.4 Hz, 3H), 2.46 (s, 3H), 2.34 (s, 3H), 1.73 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.60, 140.43, 136.62, 133.94, 132.22, 131.71, 130.91, 130.81, 130.06, 128.84, 128.40, 127.16, 126.84, 124.60, 121.87, 118.96, 117.07, 64.55, 40.67, 20.53, 18.03, 16.63. ESI-MS m/z 387.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{25}H_{27}N_2O_2$ $[M+H]^+$ m/z 387.2067, found 387.2071.

*4.1.9.13. (R)-1-(4-(1-(dimethylamino)propan-2-yl)phenyl)-2-hydroxy-4,8-dimethylphenanthridin-6(5H)-one hydrochloride (**10p**).* Off-white solid, yield: 87.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 9.94 (br s, 1H), 9.09 (s, 1H), 8.11 (s, 1H), 7.44 (t, *J* = 6.0 Hz, 2H), 7.22-7.19 (m, 2H), 7.01 (s, 1H), 6.95 (dd, *J* = 8.6 , 1.8 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 1H), 3.41-3.37 (m, 3H), 2.81 (s, 3H), 2.78 (s, 3H), 2.45 (s, 3H), 2.34 (s, 3H), 1.37 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.34, 149.74, 141.82, 137.86, 136.49, 132.40, 131.70, 130.69, 130.67, 128.34, 127.87, 127.52, 127.28, 127.09, 126.78, 124.27, 122.35, 118.92, 117.17, 62.37, 43.95, 41.80, 34.91, 20.61, 20.51, 17.98. ESI-MS m/z 401.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{26}H_{29}N_2O_2$ $[M+H]^+$ m/z 401.2224, found 401.2236.

*4.1.9.14. (R)-1-(4-(1-(dimethylamino)ethyl)phenyl)-2-hydroxy-4,7-dimethylphenanthridin-6(5H)-one hydrochloride (**10q**).* Off-white solid, yield: 83.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (br s, 1H), 10.10 (s, 1H), 9.16 (s, 1H), 7.64-7.55 (m, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.17 (d, *J* = 7.2 Hz, 1H), 7.01 (s, 1H), 6.98 (d, *J* = 7.2 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 4.56-4.53 (m, 1H), 2.82 (s, 3H), 2.79 (d, *J* = 4.8 Hz, 3H), 2.62 (d, *J* = 4.8 Hz, 3H), 2.43 (s, 3H), 1.71 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.65, 149.44, 140.43, 140.35, 136.22, 133.67, 131.09, 130.97, 130.25, 129.86, 129.24, 129.03, 128.55, 126.28, 125.26, 123.83, 121.82, 119.38, 117.47, 64.52, 40.69, 23.92, 17.69, 16.66. ESI-MS m/z 387.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{25}H_{27}N_2O_2$ $[M+H]^+$ m/z 387.2067, found 387.2072.

*4.1.9.15. (R)-1-(4-(1-(dimethylamino)propan-2-yl)phenyl)-2-hydroxy-4,7-dimethylphenanthridin-6(5H)-one hydrochloride (**10r**).* Off-white solid, yield: 77.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 10.02 (s, 1H), 9.09 (s, 1H), 7.39 (t, *J* = 6.8 Hz, 2H), 7.21-7.15 (m, 3H), 7.01-6.92 (m, 3H), 3.39-3.34 (m, 3H), 2.81 (s, 3H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.74 (d, *J* = 4.8 Hz, 3H), 2.43 (s, 3H), 1.35 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.64, 149.55, 141.55, 140.26, 137.90, 136.43, 130.93, 130.15, 129.26, 129.00, 127.68, 127.23, 126.35, 125.22, 123.51, 122.32, 119.34, 117.53, 62.37, 43.96, 41.80, 34.91, 23.92, 20.61, 17.65. ESI-MS m/z 401.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{26}H_{29}N_2O_2$ $[M+H]^+$ m/z 401.2224, found 401.2266.

*4.1.9.16. (R)-1-(4-(1-(dimethylamino)ethyl)phenyl)-2-hydroxy-4,9-dimethylphenanthridin-6(5H)-one hydrochloride (**10s**).* Off-white solid, yield: 80.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 10.33 (s, 1H), 9.19 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.34-7.25 (m, 3H), 7.05 (s, 1H), 6.78 (s, 1H), 4.60-4.57 (m, 1H), 2.82 (d, *J* = 4.4 Hz, 3H), 2.66 (d, *J* = 4.4 Hz, 3H), 2.46 (s, 3H), 1.95 (s, 3H), 1.73 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.32, 149.62, 140.45, 140.26, 134.69, 134.06, 130.87, 129.73, 129.06, 128.97, 128.06, 127.68, 127.41, 124.67, 124.60, 122.08, 119.36, 116.84, 64.56, 40.61, 21.54, 18.02, 16.90. ESI-MS m/z 387.2 $[M+H]^+$. HRMS (ESI) Calcd for $C_{25}H_{27}N_2O_2$ $[M+H]^+$ m/z 387.2067, found 387.2066.

*4.1.9.17. (R)-1-(4-(1-(dimethylamino)propan-2-yl)phenyl)-2-hydroxy-4,9-dimethylphenanthridin-6(5H)-one hydrochloride (**10t**).* Off-white solid, yield: 88.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 10.07 (s, 1H), 9.16 (br s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 7.47-7.43 (m, 2H), 7.26-7.20 (m, 3H), 7.05 (s, 1H), 6.69 (s, 1H), 3.41-3.37 (m, 3H), 2.81-2.78 (m, 6H), 2.46 (s, 3H), 1.95 (s, 3H), 1.40 (d, *J* = 5.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.33, 149.69, 142.00, 140.18, 137.94, 134.81, 130.70, 130.56, 128.85, 128.11, 127.92, 127.85, 127.28, 127.08, 124.51, 124.29, 122.64, 119.30, 117.01, 62.28, 43.83, 41.80, 34.80, 21.53, 20.82, 18.02. ESI-MS m/z 401.3 $[M+H]^+$. HRMS (ESI) Calcd for $C_{26}H_{29}N_2O_2$ $[M+H]^+$ m/z 401.2224, found 401.2231.

*4.1.10. (R)-9-(4-(1-aminoethyl)phenyl)-8-hydroxy-6-methylthieno[2,3-c]quinolin-4(5H)-one hydrochloride (Compound **1**).* Following the synthetic procedure of **9a**, compound **1** was obtained as white solid, yield (7 steps): 8.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (s, 1H), 9.27 (s, 1H), 8.50 (s, 3H), 7.66 (d, *J* = 5.6 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.04 (s, 1H), 5.86 (d, *J* = 5.6 Hz, 1H), 4.55-4.53 (m,

1H), 2.50 (s, 3H), 1.62 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.02, 149.49, 141.96, 138.56, 138.34, 132.10, 132.06, 130.64, 129.39, 127.22, 127.19, 126.28, 124.88, 121.37, 118.98, 116.86, 49.83, 20.81, 18.34. HRMS (ESI) Calcd for $C_{20}H_{19}N_2O_2S$ $[M+H]^+$ m/z 351.1162, found 351.1165.

*4.1.11. (R)-9-(4-(1-aminopropan-2-yl)phenyl)-8-hydroxy-6-methylthieno[2,3-c]quinolin-4(5H)-one hydrochloride (**OTS514**).* Following the reference procedure [24], OTS514 was obtained as white solid, yield (7 steps): 6.5%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (d, *J* = 4.0 Hz, 1H), 7.45 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.24 (d, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 6.99 (s, 1H), 6.07 (s, 1H), 3.19-3.11(m, 3H), 2.47 (s, 3H), 1.40 (d, *J* = 4.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.03, 149.55, 142.33, 142.10, 136.61, 132.00, 131.95, 130.67, 130.64, 129.40, 127.58, 127.43, 126.36, 124.70, 121.60, 119.01, 116.97, 44.89, 37.22, 19.48, 18.31. HRMS (ESI) Calcd for $C_{21}H_{21}N_2O_2S$ $[M+H]^+$ m/z 365.1318, found 365.1253.

*4.1.12. (R)-9-(4-(1-(dimethylamino)propan-2-yl)phenyl)-8-hydroxy-6-methylthieno[2,3-c]quinolin-4(5H)-one hydrochloride (**OTS964**).* Following the reference procedure [24], **OTS964** was obtained as white solid, yield (from **OTS514**): 85.1%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.50-7.43 (m, 3H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 6.99 (s, 1H), 6.03 (d, *J* = 5.2 Hz, 1H), 3.54-3.48 (m, 1H), 3.38-3.32 (m, 2H), 2.87 (s, 3H), 2.84 (s, 3H), 2.47 (s, 3H), 1.38 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 151.34, 144.79, 142.80, 138.93, 133.35, 132.97, 132.61, 130.69, 130.21, 128.20, 127.77, 126.75, 123.32, 120.24, 119.00, 64.92, 44.85, 43.74, 37.11, 20.94, 18.26. HRMS (ESI) Calcd for $C_{23}H_{25}N_2O_2$ $[M+H]^+$ m/z 393.1631, found 393.1639.

4.2. *In vitro* TOPK inhibition assays

Inhibition of kinase activity by the compounds was assessed against TOPK kinase [35, 36] by contract with Reaction Biology Corp. Kinase Profiling is 10 dose IC_{50} singlet assay. Activity of TOPK kinase was assessed by the HotSpot assay platform, which contained specific kinase/substrate pairs along with required cofactors. Base reaction buffer: 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na_3VO_4, 2 mM DTT, 1% DMSO. Testing compounds were dissolved in 100% DMSO to specific concentration. The serial dilution was conducted by Integra Viaflo Assist in DMSO. The reaction mixture containing the compound and ^{33}P-ATP was incubated at room temperature for 2 h and radioactivity was detected by filter-binding method. Kinase activity data were expressed as the percent remaining kinase

activity in test samples compared to vehicle (dimethyl sulfoxide) reactions. IC_{50} values and curve fits were obtained using Prism (GraphPad Software).

4.3. Cell-based anti-proliferation assay

Human cancer cell lines used in this investigation were obtained from American Type Culture Collection (ATCC, Rockville, MD). HCT116, HCT-15 and A549 cancer cell lines were cultured at 37 °C in a 5% CO_2 incubator in RPMI 1640 medium containing 10% fetal bovine serum (FBS). To estimate cytotoxicity, cells were seeded (1~5 $\times 10^3$ cells per well) in 96-well plates and cultured for 24 h. Cells were then treated with various concentrations of compounds. After culturing for 72 h, the cytotoxicity of the panel of compounds was measured using an MTT assay.

4.4. Western immunoblot analysis

The cancer cell lines were treated by different concentrations of **9g** or for 48 h. And protein lysates were prepared using RIPA lysis buffer (Thermo Scientific, Rockford, IL, USA) with protease inhibitors (Sigma) and phosphatase inhibitors (Roche, Mannenheim, Germany). Protein concentration was determined using the BCA assay (Thermo Scientific). Bound antibodies were detected by developing film from nitrocellulose membranes exposed to chemiluminescence reagent (Immunoblot Western Chemiluminescent Substrate, EMD Millipore, Merck KGaA, Darmstadt, Germany).

4.5. *In vivo* xenograft study

Animal housing and experimental procedures were in line with ethical and legal guidelines and were authorized by local veterinary authorities. Mice used in this study were obtained from Beijing HFK bioscience CO. LTD (Beijing, China). Mice engrafted subcutaneously with 5×10^6 ~1×10^7 HCT116, SW620 and HCT-15 cells were randomly divided into groups when tumor volume was around 100 mm^3 and were administrated by gavage with **9g** 10mg/kg, 15mg/kg or vehicle dissolved in physiological saline with 1.25% DMSO once daily. The tumor size and body weight were measured every 3 days. The mice were sacrificed at an endpoint defined by the tumor volume (~1,000mm^3). Tumor volume was calculated as follows: Volume = $0.52\times a\times b^2$, where a (mm) was the length and b (mm) was the width of the tumor.

4.6. Pharmacokinetic experiments in rats

Animals were maintained in a 12/12 h light/dark cycle with free access to food and water for at least 7

days to adapt the environment. Animal housing and experimental procedures were in line with ethical and legal guidelines and were authorized by local veterinary authorities. **9g** dissolved in physiological saline with 2.5% DMSO was administrated to rats (n = 3) at a single dose (iv 2mg/kg and po 15 mg/kg). Blood samples of 0.2 mL each were collected from the jugular veins into heparinized tubes at 0 h (before drug administration) and at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after dosing. The blood samples were centrifuged at 8,000 rpm for 5 min in 2-8 °C. The plasma samples were separated and store at -80 °C until analysis. The blood drug concentration was then determined with LC-MS/MS to quantify compound concentrations against a calibration curve using analysis specific transitions. WinNonlin® software version 7.0 was used to estimate pharmacokinetic parameters including the peak concentration (Cmax), peak time (T_{max}), plasma clearance (Cl), half-life ($T_{1/2}$), the area under the plasma concentration time curve (AUC(0-t)), the volume of distribution (Vss), the mean residence time (MRT) and bioavailablity (F%).

Conflicts of Interest

The authors declare no conflict of interest about this article.

Acknowledgments

We are grateful for Dr.Wenge Cui's proposal for structure modification and Professor Lijuan Chen's team of State Key Laboratory of Biotherapy (Sichuan University) for HRMS measurements.

Appendix A. Supplementary data

Supplementary data related to this article can be found at:

These data include LCMS, HNMR and ^{13}CNMR of the compounds described in this article.

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- 1-Phenyl Phenanthridin-6(5H)-ones have been discovered as new TOPK inhibitors.
- **9g** markedly inhibited proliferation of colorectal cancer cell lines *in vitro*.
- **9g** suppressed tumor growth *in vivo* and exhibited good pharmacokinetic properties.