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Graphical Abstract

Synthesis and Biological Evaluation of New MET Inhibitors with 1,6-Naphthyridinone Scaffold

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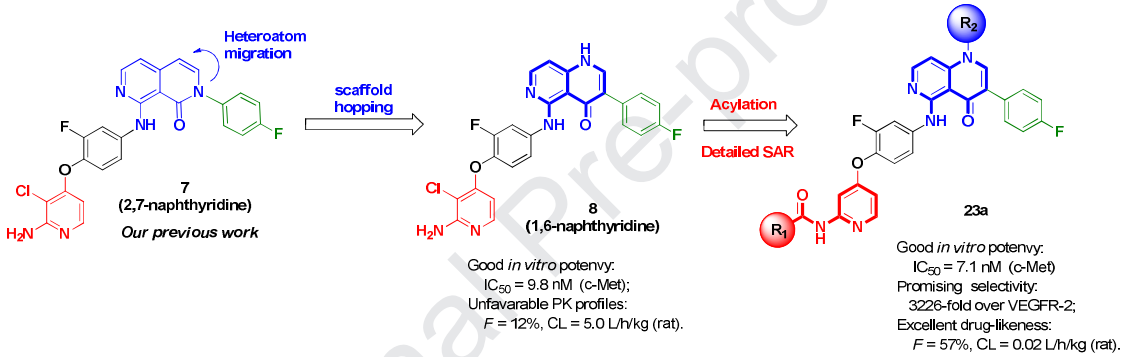

Synthesis and Biological Evaluation of New MET Inhibitors with 1,6-Naphthyridinone Scaffold

Ming-Shu Wang[1,†] Lin-Sheng Zhuo[1,†], Fan-Peng Yang[1,†], Wen-Jie Wang[1], Wei Huang[1]*, and Guang-Fu Yang[1,]*

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ABSTRACT: A potent and novel MET inhibitor, 5-((4-((2-amino-3-chloropyridin-4-yl)oxy)-3-fluorophenyl)amino)-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-ones (**8**), was designed and synthesized via a scaffold-hopping strategy of a 2,7-naphthyridinone MET kinase inhibitor **7**. Lead compound **8** had good potency (IC_{50} of 9.8 nM), but unfavorable pharmacokinetic profiles (***F*** = 12%, CL = 5.0 L/h/kg). Systematic structural optimization of compound **8** resulted in **9g** (MET, IC_{50} = of 9.8 nM) with a comparable MET potency to that of compound **2** and a favorable pharmacokinetic profile (***F*** = 63%, CL = 0.12 L/h/kg). Further study of the derivatization of N(1) amine group of **9g** led to the discovery of **23a** with good MET potency (IC_{50} of 7.1 nM), promising VEGFR-2 selectivity (3226-fold), and a markedly drug-likeness improvement (***F*** = 57.7%, CL = 0.02 L/h/kg). The excellent VEGFR-2 selectivity and favorable drug-likeness of **23g** suggest that the 1,6-naphthyridine moiety could be used as a new scaffold for kinase inhibitor discovery.

KEYWORDS: 1,6-Naphthyridone, MET kinase inhibitor, Pharmacokinetic profiles, VEGFR-2 selectivity

1. Introduction

The heterodimeric transmembrane protein MET is a receptor tyrosine kinase (RTK) known as a hepatocyte growth factor receptor (HGFR). [1-4] Upon activation by hepatocyte growth factor receptor, MET demonstrates pivotal roles in many fundamental cellular processes including survival, proliferation, differentiation, migration, invasion, and motility.[5-7] Dysregulation of MET/HGF signaling has been reported to induce proliferation, invasion, metastasis, tumor angiogenesis, and drug resistance of cancer cells across a broad spectrum of tumors. [8-10] Thus, MET has attracted considerable attention as a potential target for cancer treatment.

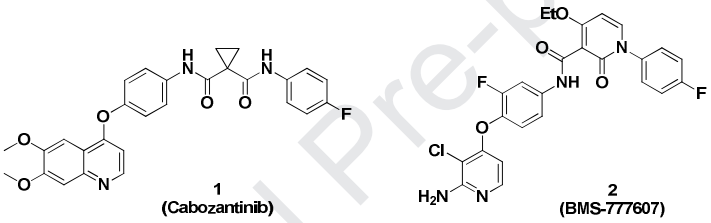


Figure 1. Examples of multi-targeted MET inhibitors.

To date, several MET-targeting agents have been used to disrupt HGF/MET signaling including HGF and MET antibodies and small-molecule kinase inhibitors.[6,11-13] Antibody-based therapies primarily prevent ligand-mediated activation of the receptor, while small-molecule inhibitors can prevent ligand-dependent activation as well as ligand-independent activation; thus, a small-molecule MET inhibitor could benefit a different, and potentially larger, cancer patient population.[14,15] The first small molecule MET inhibitor is cabozantinib (**1**), which was approved by the FDA for the treatment of progressive metastatic medullary thyroid cancer (2012).[16] Since then, many derivatives of cabozantinib have been reported including compound **2** (BMS-777607), which exhibits desirable drug-likeness and modest selectivity to VEGFR-2[17].

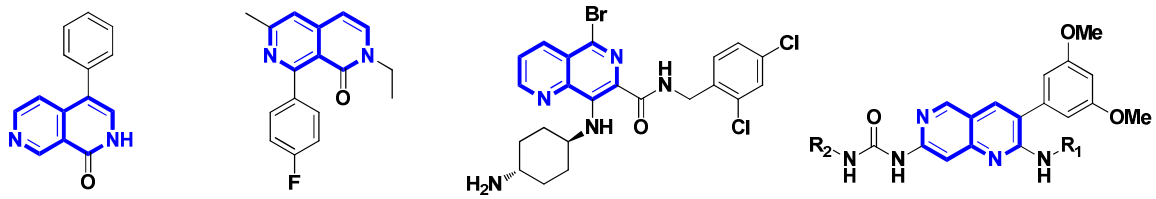


Figure 2. Examples of 2,7-naphthyridine and 1,6-naphthyridine.

Naphthyridines are common in various natural products and are pharmacologically important[18]. Compounds containing a 2,7-naphthyridine scaffold are a novel class of bispyridine structures in many natural alkaloids and synthetic products (Figure 2, compounds **3**, **4**).[19,20] These products usually possess variant biological activities such as antibiotic, cell-protecting, and anticancer activity. This makes them attractive pharmaceutically active fragments for further drug discovery.[21]

The 1,6-naphthyridine is an isomer of 2,7-naphthyridine, and its derivatives can inhibit multiple kinases including Lck, IGF-1R, Abl, FGFR, MET, and ALK (Figure 2, compound **5**).[22] Furthermore, 1,6-naphthyridines were designed as FGFR-1 and VEGFR-2 kinase inhibitors with positions 2- and 7- substituted by an amino and 3- substituted by aryl groups. This suggests that various substitutions on this active scaffold could confer diverse biological activities (Figure 2, compound **6**).[23-25]

In our previous research, knowledge of the binding mode of BMS-777607 in MET kinases led to the introduction of 2,7-naphthyridinone fragments into block C based on the "scaffold hopping strategy." The resulting early candidate **7** showed excellent potency.[26] Here, in order to further explore SARs and optimize the drug-likeness of naphthyridine-based derivatives, compound **7** was selected as a starting point for this research.

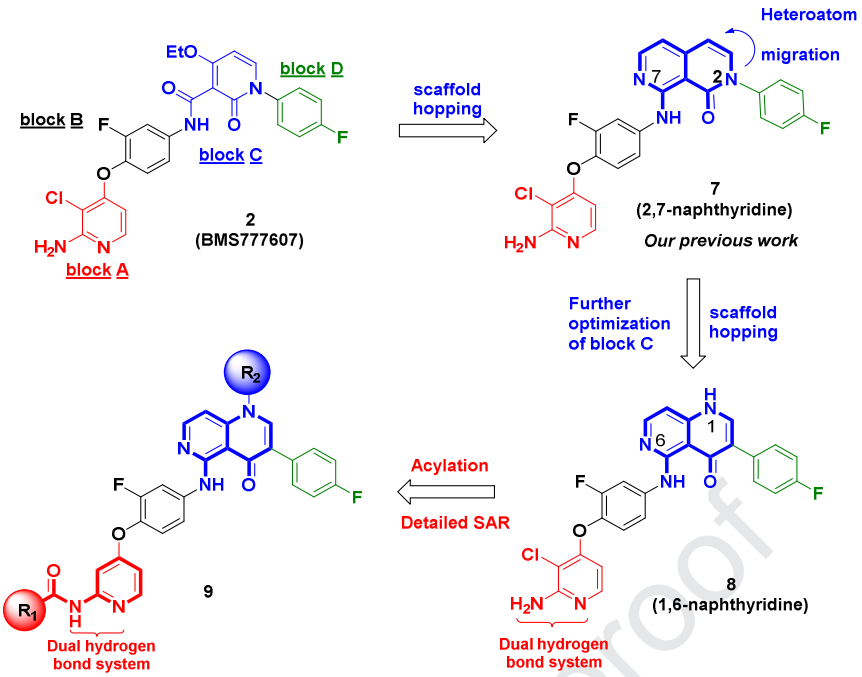


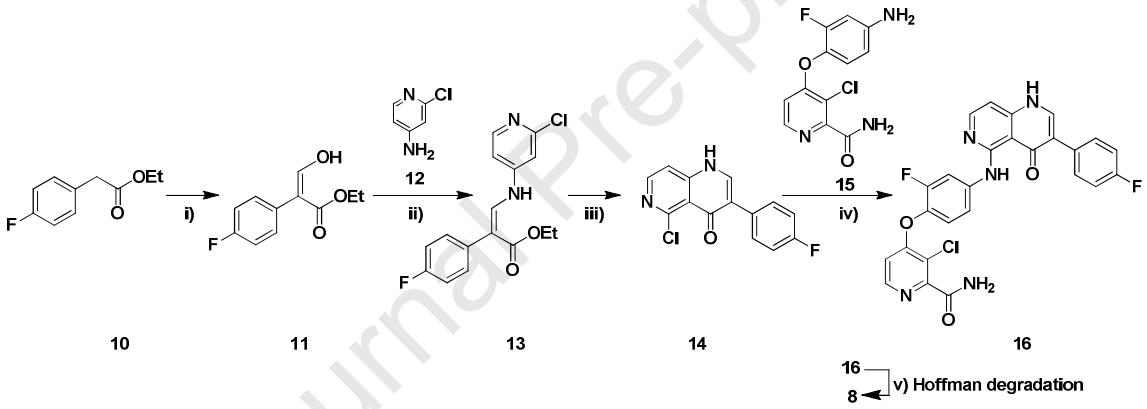
Figure 3. Design of 1,6-naphthyridine derivatives based on a scaffold-hopping strategy.

According to our previous studies, compound **7** displayed comparable MET enzymatic activity as **2** due to the conservation of the key H-bond interactions between the carbonyl group in block C with residues Asp1222 and the amino group in block A with the Met1160.[26] Here, these two key H-bond interactions were reversed, and the 1,6-naphthyridine derivative **8** was designed based on the scaffold-hopping strategy of heteroatom migration to further optimize block C. Furthermore, inspired by the discovery of VEGFR-2/MET inhibitor altiretinib,[27-29] detailed structure activity relationship (SAR) studies of block A and block C were carried out to optimize the drug-likeness.

2. Chemistry

The most attractive route to 5-substituted 1,6-naphthyridines **8** was via an intermediate with a displaceable 5-halo group for direct nucleophilic substitution reactions with the appropriate amines. With this consideration in mind, we designed an efficient synthetic strategy illustrated in Scheme **1.** The aldol reaction of **10** with ethyl formate followed by condensation with 2-chloropyridin-4-amine **12** and cyclization yielded the key intermediate

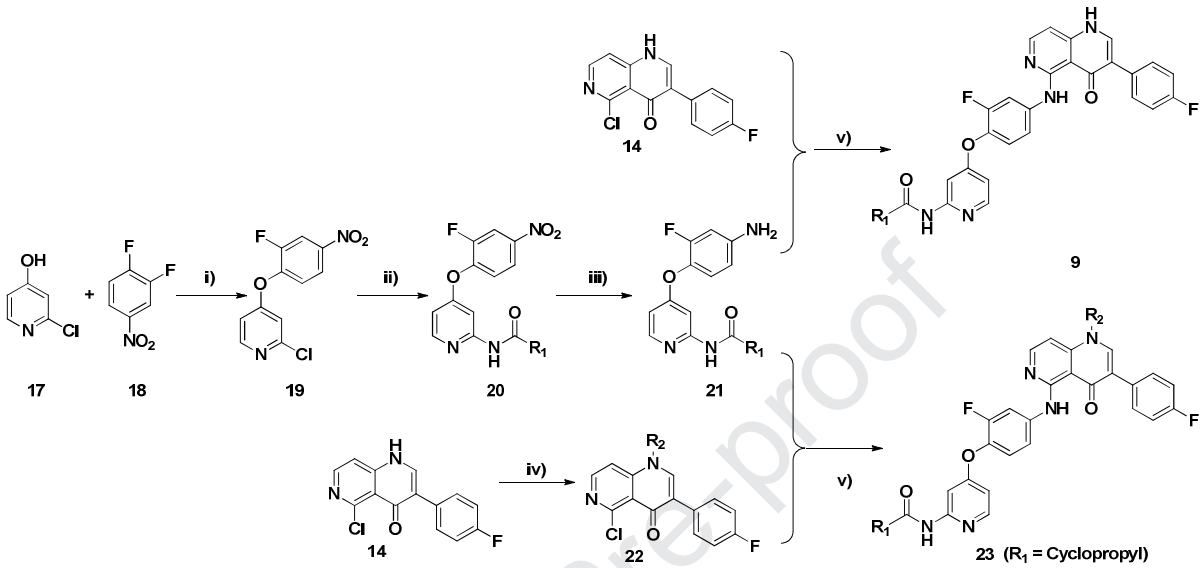
chlorinated 1,6-naphthyridine **14**. The intermediate **15** was smoothly obtained using a method modified from the literature.[17] Due to the presence of the active N(1) amine group in intermediate **14**, the palladium-catalyzed Buchwald coupling reaction failed to afford compound **16**. Thus, we decided to explore more applicable reaction conditions to assemble the chlorinated 1,6-naphthyridine **14** and aromatic amine **15** because protection and deprotection of the amine group would result in a cumbersome synthetic route. Eventually, we determined that the reaction proceeded smoothly in a PTSA acid-isopropanol system. The precipitated compound **16** was easily purified and had excellent yields. Finally, Hoffman degradation delivered **8** in high yield.



Scheme 1. Reagents and conditions: i) NaH, ethyl formate, 0°C; ii) HCl, EtOH, reflux; iii) Ph_2O, 250°C; (iv) PTSA, isopropanol, 80°C; and (v) $PhI(OAc)_2$, $EtOAc/CH_3CN/H_2O$.

The synthesis of target compounds **9** and **23** included a route that started from N-acylated-4-(4-amino-2-fluorophenoxy)pyridin-2-amine (**21**) as the other key intermediate (Scheme **2**). Briefly, commercially available 2-chloropyridin-4-ol (**17**) was reacted with 1,2-difluoro-4-nitrobenzene (**18**) under base conditions to yield 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine (**19**). This was further reacted with different types of acid amides under Pd-catalyzed conditions to produce intermediate **20**. Intermediate **20** was then reduced using iron powder and catalytic amounts of ammonium chloride in ethanol to obtain key intermediate **21**. Another intermediate **22** for the synthesis of

compounds **23** was obtained by the subsequent substitution reaction of **14** with different halides or substituted oxirane in the presence of K_2CO_3. Then, target compounds **9** and **23** were prepared similar to **16**.



Scheme 2. Reaction conditions and reagents: i) K_2CO_3, DMF, r.t.; (ii) NH_2COR, dppf, $Pd_2(dba)_3$, Cs_2CO_3, 1,4-dioxane, 85°C; (iii) Fe, NH_4Cl, EtOH, H_2O, reflux; (iv) K_2CO_3, DMF, r.t.; and (v) PTSA, isopropanol, 80°C.

3. Results and Discussion

We initially studied if the 1,6-naphthyridine moiety can serve as a key pharmacophoric group to demonstrate MET inhibition. Therefore, the 2-aminopyrimidine core and the terminal phenyl ring were reserved based on the chemical structure of compound **7** in the newly designed compound **8**. A generally utilized 2-fluoro-1,4-substituted phenyl linker was also adopted from the newly reported inhibitors. To our delight, **8** (IC_{50} = 9.8 nM) had similar MET potency as **7** (IC_{50} = 9.9 nM) and **2** (IC_{50} = 7.6 nM). This indicated that the scaffold hopping of heteroatom migration was an effective strategy and led to a new scaffold for further optimization.

Next, molecular docking was performed to further verify the design strategy and provide rational direction for optimization. The docking result demonstrated that **8** interacted with protein almost exactly as in the solved cocrystal structure of **2** (Figure 4). The

2-aminopyrimidine core anchors the inhibitor to the hinge region via two hydrogen bonds with Met-1160, whereas the central phenyl ring interacts by π-stacking with Phe-1223; the carbonyl group and the N(1) amine group of 1,6-naphthyridinone fragment interact by H-bonding with Asp1222 and Glu1227, respectively. This implicated that 1,6-naphthyridinone provides a new hydrogen bonding pattern to better fit the subpocket of MET versus 2,7-naphthyridinone; the terminal phenyl ring occupies a allosteric hydrophobic pocket. The docking results revealed that, in addition to the key pharmacophoric group 1,6-naphthyridinone fragment, the dual hydrogen bond system of 2-aminopyrimidine core is essential for maintaining activity. More importantly, the newly introduced functionalizable N(1) amine group of 1,6-naphthyridin-4(1H)-one moiety may provide more optimization options than 2,7-naphthyridin-2(1H)-one.

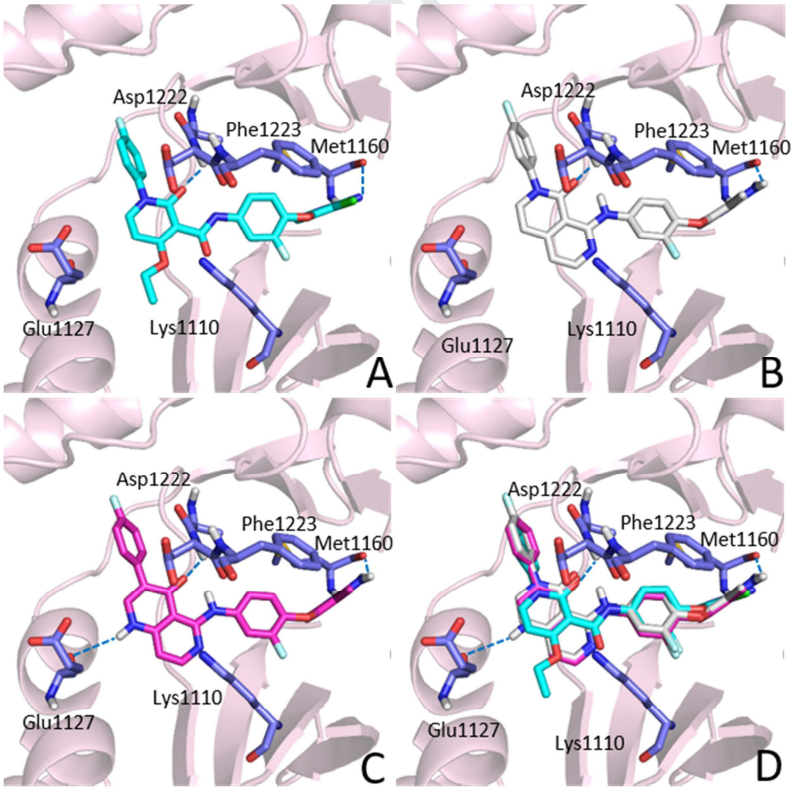
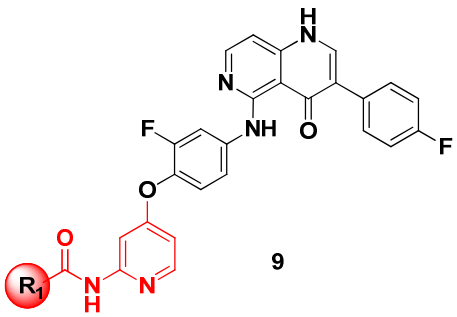


Figure 4. (**A**) Cocrystal structure of **2** bound to the inactive conformation of MET kinase domain; [17] (**B**) Our reported binding mode of **7** with MET;[26] (**C**) The proposed binding mode of **8** with MET; (**D**) The binding mode overlay of **8** (purple), **7** (white), and **2** (light blue) with MET. The navy blue dashed lines represent H-bonds between residues with **8**, **7**, and **2**.

With the binding conformations of **8** in hand and encouraged by the discovery of VEGFR-2/MET inhibitor altiretinib, a SAR study of block A was first performed (Table **1**). A series of acylation modifications of 2-aminopyrimidine core were performed with the dual hydrogen bond system reserved in order to optimize its drug-likeness. Overall, substitution on the 2-aminopyrimidine core was well-tolerated and maintained inhibitory activity. Compound **9a** bearing an acetyl group displayed an IC_{50} value of 21.4 nM, which is approximately 2.1-fold less potent than that of compound **8**. The potency of compound **9b** (MET, IC_{50} = 4.7 nM) with a propionyl group is twice as high perhaps due to the sterics of the propionyl group. When either the butyryl group or pentanoyl group with a long alkyl chain was employed, compound **9c** (MET, IC_{50} = 14.8 nM) and **9d** (MET, IC_{50} = 14.7 nM) only displayed a slight loss (1.5-fold) in enzyme activity, which might benefit from the flexibility of the alkyl chain. Meanwhile, incorporation of an isobutyryl group (**9e)** resulted in a 1.8-fold loss of potency (MET, IC_{50} = 17.7 nM). MET potency was slightly impaired by about 2.9-fold as the sterically bulky pivaloyl group (**9f)** was employed further. The VEGFR-2 selectivity was increased by more than 106.7-fold (MET, IC_{50} = 28.1 nM; VEGFR-2, IC_{50} >3000 nM).

Table 1. Activity of compounds **9a-l** against MET/VEGFR-2 kinase[a] and metabolic stability in human microsomes.



9

Compds.	R_1	IC_{50}, nM[a]		Met Stab ($T_{1/2}$, min)
		MET	VEGFR-2	
8	-	9.8	68	189.8
9a	Me	21.4	38.1	-
9b	Et	4.7	20.2	163.0
9c	n-Pr	14.8	19.4	-
9d	n-Bu	14.7	26.9	-
9e	i-Pr	17.7	230.1	-
9f	t-Bu	28.1	>3000	-
9g		8.1	8.8	792.5
9h		24.0	61.2	-
9i		9.8	>3000	80.4
9j		9.9	58.9	434.0
9k		11.8	288.9	248.9
9l		35.0	>3000	-
2 (BMS-777606)		7.6	138.7	-

[a] *In vitro* kinase assays were performed with the indicated purified recombinant MET or VEGFR-2 kinase domains (nM).

Steric effects might be the main cause of MET potency loss, and steric effects were more crucial for maintaining VEGFR-2 activity. Furthermore, the cyclopropanecarbonyl group (**9g)**, cyclobutanecarbonyl group (**9h)**, and cyclohexanecarbonyl group (**9i)** were also employed. Compound **9g** (MET, IC_{50} = 8.1 nM) and compound **9i** (MET, IC_{50} = 9.8 nM) showed comparable MET activity to **8**, while **9h** (MET, IC_{50} = 24.1 nM) showed a 2.4-fold loss in MET potency. More interestingly, **9g** displayed excellent VEGFR-2 inhibitory activity (IC_{50} = 8.8 nM), and **9i** with a sterically bulky substituent displayed good selectivity against VEGFR-2 (>306-fold). This underscores the importance of steric effects in maintaining VEGFR-2 activity. In addition to the alkyl acyl group, we also explored the 2-hydroxyacetyl group (**9j)**, 2-hydroxypropanoyl group (**9k),** and 2-hydroxy-2-methylpropanoyl group (**9l)**. Similar steric effects were also the main factor

for MET potency and VEGFR-2 selectivity. Compound **9j** (MET, IC_{50} = 9.9 nM) and **9k** (MET, IC_{50} = 11.8 nM) showed comparable MET activity to compound **8**. Compound **9l** (MET, IC_{50} = 35 nM) showed a 3.5-fold loss in MET potency, while compound **9l** also showed good VEGFR-2 selectivity (>306-fold) because of the sterically bulky substituent.

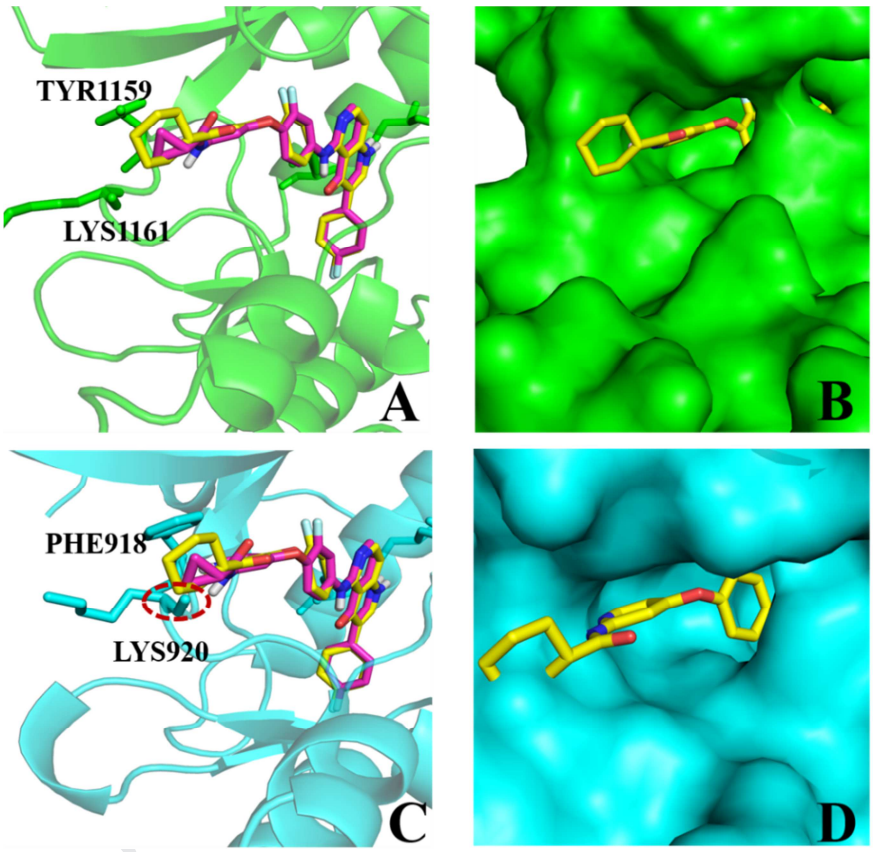


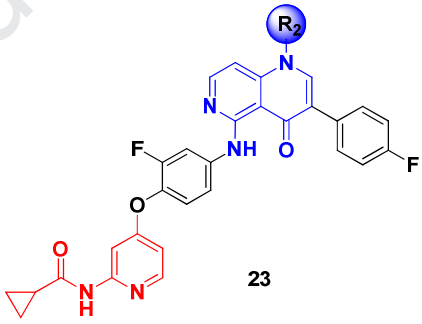
Figure 5. (**A**) The proposed binding mode of **9i** (yellow) and **9g** (pink) with MET; **(B)** Surface representations of **9i** binding to Met; **(C)** The proposed binding mode of **9i** (yellow) and **9g** (pink) with VEGFR-2; **(D)** Surface representations of **9i** binding to VEGFR-2

In order to better understand the SAR of block A and the selectivity between MET and VEGFR-2 of **9i**, molecular docking was further performed. As illustrated in Figure **5,** compound **9g** and **9i** were both docked into the binding site of MET kinase (PDB: 3F82) and VEGFR-2 kinase (PDB: 3U6J). For MET kinase, the cyclopropanecarbonyl group (**9g)** and cyclohexanecarbonyl group (**9i)** all formed well hydrophobic interactions with residues TRY1159 and LSY1161 (Figure **5A**, **5B**). As for VEGFR-2 kinase, sterically smaller

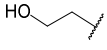
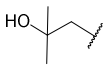
cyclopropanecarbonyl group of **9g** could be well tolerated, however, the replacement of cyclopropanecarbonyl group by sterically bulky cyclohexanecarbonyl group introduced steric clash with residue LYS1161, which should be responsible for the impaired VEGFR-2 potency of **9i** (Figure **5C**, **5D**). Taken together, substitutions on the 2-aminopyrimidine core were more tolerated for MET potency than VEGFR-2. This can lead to the design of selective MET inhibitors against VEGFR-2.

Next, selected potent compounds with good MET inhibitory activity were incubated with human liver microsomes to initially evaluate metabolic stability (Table **1**). Table **1** shows that compound **9b** (bearing propionyl group) had a moderate half-life ($T_{1/2}$ = 163.0 min) comparable to that of **8** ($T_{1/2}$ = 189.8 min). Compound **9g** with a cyclopropanecarbonyl group was very stable in human liver microsomes ($T_{1/2}$ = 792.5 min), and compound **9i** with a cyclohexanecarbonyl group exhibited high clearance rates ($T_{1/2}$ = 80.4 min).

Table 2. Activity of compounds **23a-d** against MET/VEGFR-2 kinase[a] and metabolic stability in human microsomes.



23

Compds.	R_2	IC_{50}, nM[a]		Met Stab ($T_{1/2}$, min)
		MET	VEGFR-2	
23a	Me	7.1	22,910	882.6
23b	Et	15.6	1380	-
23c		33.8	10,450	-
23d		25.9	>30,000	-
2		7.6	138.7	-

[a] *In vitro* kinase assays were performed with the indicated purified recombinant MET or VEGFR-2 kinase domains (nM).

Thus, the potential impact of R_2 substituent on the MET inhibition was investigated with **9g** as the lead compound (Table **2**). The introduction of a methyl group to the N(1)-position (**23a**) led to a slight increase (1.3-fold) of MET potency (MET, IC_{50} = 7.1 nM). More importantly, the methylation of the NH group resulted in a significant increase in VEGFR-2 selectivity by 3226-fold (VEGFR-2, IC_{50} = 22910 nM). The ethyl group was then employed, and **23b** displayed a 1.2-fold MET potency loss and an 88-fold improvement in VEGFR-2 selectivity.

To investigate the optimal substituent on the N(1)-position, compound **23c** (bearing a hydroxyethyl group) and compound **23d** (bearing a propan-2-ol group) were further synthesized. While these compounds had a potency loss against MET (3.4-fold and 2.7-fold), they still maintain high VEGFR-2 selectivity. These results indicate that sterically suitable methyl group might be optimal, and fine-regulation of newly introduced functionalizable N(1) amine group of 1,6-naphthyridin-4(1H)-one moiety compared to 2,7-naphthyridin-2(1H)-one could also lead to selectivity improvement. Even more amazing is that **23a** was slightly more stable ($T_{1/2}$ = 882.6 min, Table **2**) than **9g** in human liver microsomes.

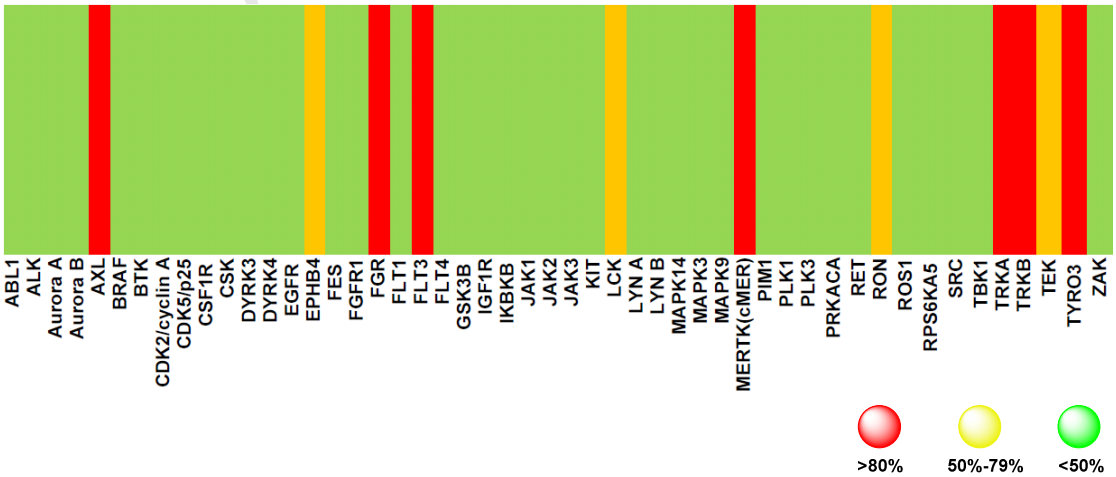


Figure 6. Preliminary results of kinase profile of **23a** (Inhibitory rate in 1 μM)

Then，the broad spectrum kinase profile of **23a** is represented in the form of heat map in Figure **6** using inhibitory rate in 1 μM for fifty other kinases. Like most class II c-Met

inhibitors, **23a** also showed high inhibitory effects against AXL, FGR, FLT3, cMer, TRKA, TRKB and TYRO3 (inhibitory rate > 80% in 1 μM). And the inhibitory activity of **23a** against important kinases was further assayed using the homogeneous time resolved fluorescence (HTRF) method. As shown in Table **3**, In contrast to its high potency against MET (IC_{50} = 7.1 nM), **23a** can also strongly inhibit AXL (IC_{50} = 8.0 nM) and Mer (IC_{50} = 4.2 nM) at low nanomolar level. Moreover, **23a** demonstrated moderate selectivity against FLT4 and RON (83.2 fold and 48.7 fold, respectively), and good selectivity against other forty two kinases (inhibitory rate < 80% in 1 μM).

Table 3. Kinase selectivity profile of **23a**

kinase	IC_{50}, nM	kinase	IC_{50}, nM
AXL	8.2	TRKA	32.9
FLT4	591.0	TRKB	58.8
cMER	4.2	TEK(Tie2)	26.3
RON	346.0	TYRO3(RSE)	41.1

Antiproliferative activities of all the synthesized compounds against NIH-H460 (human lung cancer), HT-29 (human colorectal cancer) and MKN-45 (human gastric cancer) cell lines were also evaluated *in vitro* using Cabozantinib as a positive control**.** As shown in Table **4**, most of the new compounds and Cabozantinib displayed moderate inhibitory activity against the tested cancer cell lines. Compounds **8**, **9b** and **9e** exerted good and broad-spectrum antiproliferative activity against the tested cancer cell lines, especially for compound **9e** (inhibitory rate of 83.4-95.4% in 10 μM).

Table 4. Antiproliferative activity of synthesized compounds against NIH-H460, HT-29 and MKN-45 cell lines.

Compds.	Inhibitory rate (% in 10 μM)[a]		
	NIH-H460	HT-29	MKN-45
8	79.1%	56.2%	85.7%
9a	58.2%	36.8%	77.1%
9b	69.7%	68.7%	92.1%

9c	49.7%	23.5%	64.6%
9d	71.8%	-42.2%	62.7%
9e	84.6%	83.4%	95.4%
9f	56.7%	-45.8%	62.5%
9g	54.6%	25.5%	81.8%
9h	43.6%	46.0%	52.8%
9i	72.1%	8.7%	55.2%
9j	53.6%	18.5%	63.0%
9k	56.0%	47.8%	67.3%
9l	54.3%	-2.9%	57.8%
23a	15.6%	24.8%	30.9%
23b	11.0%	15.8%	31.9%
23c	52.1%	3.5%	41.8%
23d	67.4%	-2.6%	44.3%
Cabozantinib	54.8%	29.0%	49.1%

[a]Data are presented as the means ± SDs of three independent experiments.

Following the *in vitro* experiments, *in vivo* PK properties of three compounds (**8, 9g, 23a**) were determined in rat. As shown in Table **5**, the chemical structure of blocks A and C had a significant influence on the PK properties after oral administration and intravenous injection in rats. The newly designed 1,6-naphthyridine derivatives **8** had a worse oral exposure and oral bioavailability in the rat than the reported 2,7-naphthyridine derivatives. However, **9g,** with the 2-aminopyridine core of block A acylated by the cyclopropanecarbonyl group, afforded a lower total clearance CL (0.12 L/h/kg) after oral dose of 5 mg/kg leading to a 12.8-fold higher plasma $AUC_{0-\infty}$ (42.2 h*μg/mL) and a 1.25-fold longer half-life $T_{1/2}$(6.4 h) compared to **7**. A favorable oral bioavailability (***F*** = 63%) was also observed leading to the favorable overall PK profiles of **9g**. More encouragingly, when the methyl group was introduced into the N(1)-position of **9g**, a particularly lower total clearance CL (0.02 L/h/kg; 7-fold lower to that of **9g**) of **23a** was observed after an oral dose of 5 mg/kg. This led to a markedly higher plasma $AUC_{0-\infty}$ (232.6

h*μg/mL; 69-fold higher to that of **7**) and a much longer half-life $T_{1/2}$ (20.6 h; 4-fold longer to that of **7**). Overall, compound **23a** displayed the best PK profiles. That means that fine-regulation of N(1)-position could lead to improvements in drug-likeness.

Table 5. *In vivo* PK profiles of selected compounds in rat[a,b].

N.O.	7[c]		8		9g		23a	
	2.5 mg/kg[a] (i.v.)	10 mg/kg[a] (p.o.)	2.5 mg/kg[a] (i.v.)	10 mg/kg[a] (i.g.)	1 mg/kg[a] (i.v.)	5 mg/kg[a] (i.g.)	1 mg/kg[a] (i.v.)	5 mg/kg[a] (i.g.)
$t_{1/2}$ (h)	3.2	5.1	0.5	1.0	6.8	6.4	25.4	20.6
t_{max} (h)	-	1.2	0.03	2.7	-	7.3	-	8.7
C_{max} (μg/mL)	-	1.6	16.4	0.6	1.9	2.7	6.5	7.8
$AUC_{0-\infty}$ (h*μg/mL)	3.1	6.7	4.5	2.2	13.5	42.2	80.6	232.6
Vz (L/kg)	3.6	-	0.4	7.5	0.7	1.1	0.4	0.6
CL (L/h/kg)	0.8	-	0.6	5.0	0.08	0.1	0.01	0.02
MRT (h)	-	-	0.3	3.0	8.5	11.4	10.8	32.4
***F* (%)**	-	54	-	12	-	63	-	58

[a] Vehicle: 70% PEG400-30% water. C_{max}, maximum concentration; T_{max}, time of maximum concentration; $T_{1/2}$, half-lif; $AUC_{0-\infty}$, area under the plasma concentration time curve; CL, clearance; V_Z, volume of distribution; and *F*, oral bioavailability. [b] Data reported as the average of three animals. [c]Our previously reported lead compound[26].

4. Conclusions

Our previous research of 2,7-naphthyridinone-based class II MET kinase inhibitors was combined with a scaffold-hopping strategy of heteroatom migration to make compound **8** with a 1,6-naphthyridin-4(1H)-one moiety. This served as the lead compound with good potency but poor pharmacokinetic profiles (***F*** = 12%, CL = 5.0 L/h/kg after an oral dose of 10 mg/kg). The detailed SAR studies of block A resulted in the discovery of the new 1,6-naphthyridone-based MET kinase inhibitor **9g** (MET, IC_{50} of 9.8 nM) with a comparable MET potency to that of compound **2** and favorable pharmacokinetic profiles (***F*** = 63%, CL = 0.1 L/h/kg, $AUC_{0-\infty}$ = 42.2 h*μg/ml, $T_{1/2}$ = 6.4 h after oral dose of 5 mg/kg). Additional work led to the functionalizable N(1) amine group and **23a** (MET, IC_{50} of 7.1 nM). More importantly, by tuning the N(1)-position, **23a** displayed an amazing VEGFR-2 selectivity improvement (3226-fold) as well as an improvement in drug-likeness (***F*** = 58%, CL = 0.02

L/h/kg, $AUC_{0-\infty}$ = 232.6 h*μg/ml, $T_{1/2}$ = 20.6 h after oral dose of 5 mg/kg). Excellent VEGFR-2 selectivity and the favorable drug-likeness of **23a** have shown that the 1,6-naphthyridine moiety could be used as a new scaffold in kinase inhibitor discovery. Further studies are underway.

5. Experimental

5.1. General Methods

Unless otherwise noted, all chemical reagents were commercially available and treated with standard methods. Silica gel column chromatography (CC). silica gel (200-400 Mesh; Qingdao Makall Group Co., Ltd; Qingdao; China). Solvents were dried in a routine way and redistilled. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Melting points of compounds were measured on a Melt-Temp II apparatus and uncorrected. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker BioSpin AG (Ultrashield Plus AV 400) spectrometer as deuterochloroform ($CDCl_3$) or dimethyl sulfoxide-d_6 (DMSO-d_6) solutions using tetramethylsilane (TMS) as an internal standard (δ = 0) unless noted otherwise. MS spectra were obtained on an Agilent technologies 6120 quadrupole LC/MS (ESI). High-resolution mass spectra (HR-MS) were obtained on an Agilent 6224 TOF LC/MS (USA) All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. Yields were of purified compounds and were not optimized.

5.2. General procedures for the synthesis of intermediates 11, 13, 14

5.2.1. (*Z*)-ethyl 2-(4-fluorophenyl)-3-hydroxyacrylate (**11**). A dried flask was charged with sodium hydride (12 mmol), ethyl 2-(4-fluorophenyl)acetate **10** (10 mmol), ethyl format (12 mmol) and anhydrous DMF (50 mL) under nitrogen. The mixture was heated to 100 °C and stirred for 3 h. Ice-cold water was added, and the mixture was extracted with dichloromethane. The organic layer was dried and concentrated. The residue was purified by chromatography (Hexane/EA = 5:1) to yield **11** as a yellow liquid (90%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.03 (s, 1H), 7.87 (s, 1H), 7.32 (t, *J* = 8.0, 5.4 Hz, 2H), 7.12 (t, *J* = 9.0 Hz, 2H), 4.11 (q, *J* = 7.2 Hz, 2H), 1.21 (t, *J* = 7.2 Hz, 3H). MS (ESI): 210.1 $(M)^{+}$.

5.2.2. (*Z*)-ethyl 3-((2-chloropyridin-4-yl)amino)-2-(4-fluorophenyl)acrylate (**13**). A solution of **11** (5 mmol), 2-chloropyridin-4-amine (**12**, 5 mmol) and HCl (20 mmol%) in ethanol (30 mL) was heated to 75 °C under nitrogen for 2 h. The mixture was filtered, and the solid was washed with ice-cold ethanol to yield **13** as a yellow solid (83%). ^{1}H NMR (400 M, DMSO-d_6) δ 8.17 (d, J = 5.6 Hz, 1H), 8.05 (d, J = 13.2 Hz, 1H), 7.41-7.45 (m, 1H), 7.06-7.11 (m, 2H), 7.02 (d, J = 9.6 Hz, 1H), 6.84 (s, 2H), 6.72 (d, J = 5.6 Hz, 1H), 6.54 (d, J = 13.2 Hz, 1H), 4.25 (q, J = 7.2 Hz, 1H), 1.29 (t, J = 7.2 Hz, 1H); MS (ESI): 320.1 $(M)^{+}$.

5.2.3. 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one (**14**). A solution of **13** (3 mmol) in diphenyl oxide (20 mL) was heated to 230 °C under nitrogen for 2 h. The mixture was cooled to room temperature and the resulted solid was washed with petroleum (30-60°C) to afford **14** as yellow solid (11%). ^{1}H-NMR (400 M, DMSO-*d6*) δ 12.40 (bs, 1H), 8.31 (d, J = 5.6 Hz, 1H), 8.16 (s, 1H), 7.66 (d, J = 5.6 Hz, 2H), 7.47 (d, J = 5.6 Hz, 2H), 7.23 (d, J = 6.8 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ173.7, 162.5, 160.9, 150.6, 148.1, 147.6, 138.1, 131.1, 124.4, 117.9, 115.1, 113.0. MS (ESI): 274.0 $(M)^{+}$.

5.3. General procedures for the synthesis of intermediates 15, 19, 20a-l

5.3.1. 4-(4-amino-2-fluorophenoxy)-3-chloropicolinamide **(15)**. The intermediate **15** was smoothly obtained using a modified method from previous published work.[17] ^{1}H NMR (400 MHz, CD3OD) δ 8.29 (d, 1H, J = 5.6 Hz), 7.00 (t, 1H, J = 8.8 Hz), 6.79 (d, 1H, J = 5.6 Hz), 6.63-6.55 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 166.6, 160.8, 154.1, 153.9, 149.0, 148.7, 128.5, 123.7, 115.9, 110.1, 110.0, 101.3; HRMS (ESI): calcd for C12H9N3O2ClF $(M+H^{+})$: 282.0440, Found: 282.0447.

5.3.2. 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **(19)**. A dried flask was charged with 2-chloropyridin-4-ol **17** (38.6 mmol), 1,2-difluoro-4-nitrobenzene **18** (38.6 mmol), K_2CO_3 (46.3 mmol) and DMF (200 ml). The mixture was stirred at room temperature until the completion of the reaction as monitored by TLC analysis, ice-cold water (600 ml) was added, and the mixture was stirred for 5h. Then filtered, the filter cake was washed with water and dried under infrared light to yield the desired product **19** (80%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.44 (d, J = 10.2 Hz, 1H), 8.40 (d, J = 4.2 Hz, 1H), 8.21 (d, J = 9.0 Hz, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.33 (s, 1H), 7.20 (s, 1H). MS (ESI): 268.1 $(M)^{+}$.

5.3.3. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)acetamide **(20a).** A solution of **19** (3.7 mmol), acetamide (4.4 mmol), dppf (0.372 mmol), $Pd_2(dba)_3$ (0.186 mmol) in 1,4-dioxane (25 mL) was heated to 80 °C and stirred under nitrogen until the completion of the reaction as monitored by TLC analysis. The mixture was filtered, and the filtrate was concentrate and purified by chromatography (Hexane/EA = 20:1) to yield **20a** as a pink solid (91%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.42 (d, J = 10.2 Hz, 1H), 8.28 (d, J = 5.4 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.78 (s, 1H), 7.61 (t, J = 8.4 Hz, 1H), 6.83 (s, 1H), 2.06 (s, 3H). MS (ESI): 291.1 $(M)^+$.

5.3.4. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)propionamide **(20b).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and propionamide to yield **20b** as yellow solid (85%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.46 (d, J = 10.2 Hz, 1H), 8.30 (d, J = 5.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.79 (s, 1H), 7.61 (t, J = 8.4 Hz, 1H), 6.83 (s, 1H), 2.44 (q, J = 7.2 Hz, 2H), 1.10 (t, J = 7.2 Hz, 3H). MS (ESI): 305.1 $(M)^+$.

5.3.5. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)butyramide **(20c).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and butyramide to yield **20c** as white solid (92%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.46 (d, J = 10.6 Hz, 1H), 8.31 (d, J = 5.6 Hz, 1H), 8.22 (d, J = 8.8 Hz, 1H), 7.83 (d, J = 2.0 Hz, 1H), 7.64 (t, J = 8.4 Hz, 1H), 6.87 (d, J = 5.6 Hz, 1H), 2.36 (t, J = 7.2 Hz, 2H), 1.57 (m, J = 7.2 Hz, 2H), 0.89 (t, J = 7.6 Hz, 3H). MS (ESI): 319.1 $(M)^+$.

5.3.6. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)pentanamide **(20d).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and pentanamide to yield **20d** as white solid (92%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.43 (dd, J = 10.4, 2.8 Hz, 1H), 8.29 (d, J = 5.6 Hz, 1H), 8.26 – 8.14 (m, 1H), 7.80 (d, J = 2.4 Hz, 1H), 7.62 (t, J = 8.8 Hz, 1H), 6.85 (dd, J = 6.0, 2.4 Hz, 1H), 2.37 (t, J = 7.6 Hz, 2H), 1.52 (q, J = 7.6 Hz, 2H), 1.38 – 1.16 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H). MS (ESI): 333.1 $(M)^+$.

5.3.7. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)isobutyramide **(20e).** Prepared according to the procedure for the preparation of **20a**, from

2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and isobutyramide to yield **20e** as white solid (91%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.43 (d, J = 10.2 Hz, 1H), 8.29 (d, J = 5.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.80 (s, 1H), 7.62 (t, J = 8.4 Hz, 1H), 6.86 (dd, J = 5.4, 1.8 Hz, 1H), 2.73 (dt, J = 13.2, 6.6 Hz, 1H), 1.04 (s, 6H). MS (ESI): 319.1 $(M)^+$.

5.3.8. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)pivalamide **(20f).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and pivalamide to yield **20e** as white solid (87%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.43 (d, J = 10.2 Hz, 1H), 8.30 (d, J = 5.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.80 (s, 1H), 7.62 (t, J = 8.4 Hz, 1H), 6.86 (dd, J = 5.4, 1.8 Hz, 1H), 1.19 (s, 9H). MS (ESI): 333.1 $(M)^+$.

5.3.9. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)cyclopropanecarboxamide **(20g).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and cyclopropanecarboxamide to yield **20g** as white solid (91%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.41 (d, J = 9.0 Hz, 1H), 8.28 (d, J = 5.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.83 (s, 1H), 7.62 (t, J = 8.4 Hz, 1H), 6.84 (d, J = 3.6 Hz, 1H), 1.96 (s, 1H), 0.96 – 0.81 (m, 4H). MS (ESI): 317.1 $(M)^+$.

5.3.10. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)cyclobutanecarboxamide **(20 h).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and cyclobutanecarboxamide to yield **20h** as Light yellow solid (89%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.45 (s, 1H), 8.41 (d, J = 9.0 Hz, 1H), 8.28 (d, J = 5.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.83 (s, 1H), 7.62 (t, J = 8.4 Hz, 1H), 6.84 (d, J = 3.6 Hz, 1H), 3.40 - 3.30 (m, 1H), 2.23 - 2.13 (m, 2H), 2.08 (d, J = 9.0 Hz, 2H), 1.97 -1.85 (m, 1H), 1.78 (s, 1H). MS (ESI): 331.1 $(M)^+$.

5.3.11. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)cyclohexanecarboxamide **(20i).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and cyclohexanecarboxamide to yield **20i** as yellow solid (88%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.45 (d, J = 10.2 Hz, 1H), 8.29 (d, J = 5.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.80 (s, 1H), 7.62 (t, J = 8.4 Hz,

1H), 6.86 (t, *J* = 6.0 Hz, 1H), 2.48 (s, 1H), 1.93 – 1.45 (m, 5H), 1.45 – 0.96 (m, 5H). MS (ESI): 359.1 $(M)^+$.

5.3.12. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)-2-hydroxyacetamide **(20j).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and 2-hydroxyacetamide to yield **20j** as yellow solid (59%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.91 (s, 1H), 8.42 (dd, *J* = 10.4, 2.4 Hz, 1H), 8.28 (d, *J* = 5.6 Hz, 1H), 8.18 (d, *J* = 8.8, 2.4, 1.2 Hz, 1H), 7.76 (d, *J* = 2.4 Hz, 1H), 7.62 (t, *J* = 8.8 Hz 1H), 6.87 (dd, *J* = 5.6, 2.4 Hz, 1H), 5.65 (t, *J* = 6.0 Hz, 1H), 4.01 (d, *J* = 6.0 Hz, 2H). MS (ESI): 307.1 $(M)^+$.

5.3.13. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)-2-hydroxypropanamide **(20k).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and 2-hydroxypropanamide to yield **20j** as yellow solid (70%),^{1}H NMR (600 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.45 (d, *J* = 10.2 Hz, 1H), 8.31 (s, 1H), 8.21 (d, *J* = 9.6 Hz, 1H), 7.78 (s, 1H), 7.65 (t, *J* = 6.0 Hz, 1H), 6.91 (s, 1H), 5.86 (s, 1H), 4.20 (s, 1H), 1.27 (s, 3H). MS (ESI): 321.1 $(M)^+$.

5.3.14. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)-2-hydroxy-2-methylpropanamide **(20l).** Prepared according to the procedure for the preparation of **20a**, from 2-chloro-4-(2-fluoro-4-nitrophenoxy)pyridine **19** and 2-hydroxy-2-methylpropanamide to yield **20l** as yellow solid (83%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.62 (s, 1H), 8.54 (d, *J* = 10.2 Hz, 1H), 8.40 (d, *J* = 5.4 Hz, 1H), 8.30 (d, *J* = 9.6 Hz, 1H), 7.84 (s, 1H), 7.75 (t, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 5.4 Hz, 1H), 6.13 (s, 1H), 1.41 (s, 6H). MS (ESI): 335.1 $(M)^+$.

5.4. General procedures for the synthesis of intermediates 21a-l

5.4.1 N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)acetamide **(21a).** To the mixture of N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)acetamide (**20a**, 2.06 mmol), NH_4Cl (6.18 mmol), ethanol (18 mL) and H_2O (2 ml) added iron powder (6.18 mmol) slowly. Upon the completion of addition, the reaction mixture was heated at reflux for 1 h, and then filtered immediately. Subsequently, the filtrate was concentrated and anhydrous ether (5.0 mL) was added to the residue, then stirred for 0.5 h and filtrated to give the compound **21a** as a yellow solid (81%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.13 (s, 1H), 7.60 (s, 1H), 6.95 (s, 1H), 6.79 – 6.15 (m, 3H), 5.44 (s, 2H), 2.02 (s, 3H). ^{13}C NMR (150 MHz,

DMSO-*d6*) δ 170.1, 164.0, 154.5, 154.0, 152.3, 150.5, 145.0, 123.4, 121.9, 114.2, 108.5, 101.3, 24.3. MS (ESI): 261.1 $(M)^+$.

5.4.2. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)propionamide **(21b)**. Prepared according to the procedure for the preparation of **21a**, from **20b** to yield **21a** as a brown solid (85%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.11 (s, 1H), 7.61 (s, 1H), 6.88 (s, 1H), 6.74 – 6.23 (m, 3H), 5.44 (s, 2H), 2.33 (s, 2H), 0.99 (s, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 173.4, 166.7, 155.6, 154.3, 149.7, 148.8, 129.5, 124.3, 110.5, 107.3, 101.9, 99.5, 29.7, 9.7. MS (ESI): 276.1 ($M+H^+$).

5.4.3. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)butyramide **(21c)**. Prepared according to the procedure for the preparation of **21a**, from **20c** to yield **21c** as a white solid (88%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 1H), 8.16 (d, *J* = 5.6 Hz, 1H), 7.66 (d, *J* = 2.0 Hz, 1H), 6.99 (t, *J* = 8.8 Hz, 1H), 6.64 (dd, *J* = 5.6, 2.4 Hz, 1H), 6.55 (dd, *J* = 13.2, 2.4 Hz, 1H), 6.46 (dd, *J* = 8.8, 1.6 Hz, 1H), 5.52 (s, 2H), 2.34 (t, *J* = 7.2 Hz, 2H), 1.57 (h, *J* = 7.6 Hz, 2H), 0.89 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 172.6, 166.7, 155.5, 153.9, 149.7, 148.9, 129.5, 124.3, 110.5, 107.3, 101.9, 99.6, 38.4, 18.6, 14.0. MS (ESI): 289.1 $(M)^+$.

5.4.4. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)pentanamide **(21d)**. Prepared according to the procedure for the preparation of **21a**, from **20d** to yield **21d** as a white solid (80%),^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 8.14 (d, *J* = 5.6 Hz, 1H), 7.64 (s, 1H), 6.97 (t, *J* = 9.2 Hz, 1H), 6.62 (dd, *J* = 5.6, 2.4 Hz, 1H), 6.50 (dd, *J* = 12.4, 2.0 Hz, 1H), 6.43 (d, *J* = 8.4 Hz, 1H), 5.46 (s, 2H), 2.34 (t, *J* = 7.2 Hz, 2H), 1.63 – 1.42 (m, 2H), 1.27 (q, *J* = 7.2 Hz, 2H), 1.01 – 0.73 (m, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 172.8, 166.7, 155.6, 154.3, 149.7, 129.5, 129.4, 124.3, 110.5, 107.3, 101.9, 99.6, 36.2, 27.3, 22.1, 14.10. MS (ESI): 303.1 $(M)^+$.

5.4.5. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)isobutyramide **(21e)**. Prepared according to the procedure for the preparation of **21a**, from **20e** to yield **21e** as a yellow solid (81%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.44 (s, 1H), 8.14 (s, 1H), 7.63 (s, 1H), 6.96 (s, 1H), 6.64 (s, 1H), 6.50 (d, *J* = 12.0 Hz, 1H), 6.41 (s, 1H), 5.46 (s, 2H), 2.70 (s, 1H), 1.03 (s, 6H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 176.7, 166.7, 155.6, 154.4, 149.6, 148.8, 129.5, 124.4, 110.5, 107.4, 101.9, 99.5, 34.9, 19.7. MS (ESI): 289.1 $(M)^+$.

5.4.6. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)pivalamide **(21f)**. Prepared according to the procedure for the preparation of **21a**, from **20f** to yield **21f** as a white solid (89%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.16 (d, *J* = 5.4 Hz, 1H), 7.64 (s, 1H), 6.97 (t, *J* = 9.0 Hz, 1H), 6.67 (s, 1H), 6.52 (d, *J* = 13.2 Hz, 1H), 6.43 (d, *J* = 8.4 Hz, 1H), 5.47 (s, 2H), 1.19 (s, 9H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.7, 166.7, 155.6, 154.5, 149.4, 148.8, 129.5, 124.4, 110.5, 107.6, 101.9, 100.0, 27.2, 27.2. MS (ESI): 303.2 $(M)^{+}$.

5.4.7. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclopropanecarboxamide **(21g)**. Prepared according to the procedure for the preparation of **21a**, from **20g** to yield **21g** as a white solid (89%), ^{1}H NMR (600 MHz, DMSO—d_6) δ 10.79 (s, 1H), 8.15 (d, *J* = 5. 4 Hz, 1H), 7.59 (s, 1H), 6.95 (t, *J* = 9.0 Hz, 1H), 6.67-6.61 (m, 1H), 6.49 (dd, *J* = 13.2, 2.4 Hz, 1H), 6.40 (d, *J* = 8.4 Hz, 1H), 5.44 (s, 2H), 2. 03-1.88 (m, 1H), 0. 76 (br, 4H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 173.2, 166.7, 155.6, 154.3, 149.7, 148.8, 129.5, 124.3, 110.4, 107.4, 101.9, 99.5, 14.6, 8.1. MS (ESI): 287.1 $(M)^{+}$.

5.4.8. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclobutanecarboxamide **(21h)**. Prepared according to the procedure for the preparation of **21a**, from **20h** to yield **21h** as a yellow solid (80%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.15 (s, 1H), 7.67 (s, 1H), 6.99 (s, 1H), 6.80 – 6.06 (m, 3H), 5.51 (s, 2H), 3.38 (s, 1H), 2.17 (s, 2H), 2.06 (s, 2H), 1.90 (s, 1H), 1.77 (s, 1H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 174.3, 166.7, 155.6, 154.4, 149.7, 148.9, 124.3, 110.5, 107.3, 101.9, 99.6, 39.6, 24.8, 18.0. MS (ESI): 301.1 $(M)^{+}$.

5.4.9. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclohexanecarboxamide **(21i)**. Prepared according to the procedure for the preparation of **21a**, from **20i** to yield **21i** as a white solid (80%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.10 (d, *J* = 5.6 Hz, 1H), 7.59 (d, *J* = 2.0 Hz, 1H), 6.94 (t, *J* = 8.8 Hz, 1H), 6.61 (dd, *J* = 5.6, 2.4 Hz, 1H), 6.48 (dd, *J* =13.2, 2.4 Hz 1H), 6.39 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.46 (s, 2H), 2.44 (s, 1H), 1.71 – 1.67 (m, 5H), 1.43 – 1.05 (m, 5H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 175.8, 166.7, 155.6, 154.4, 149.6, 148.9, 129.5, 124.3, 110.5, 107.3, 101.9, 99.5, 44.7, 29.4, 25.8, 25.6. MS (ESI): 329.2 $(M)^{+}$.

5.4.10. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)-2-hydroxyacetamide **(21j)**. Prepared according to the procedure for the preparation of **21a**, from **20j** to yield **21j** as a light brown solid (82%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (s, 1H), 8.13 (d, *J* = 5.6 Hz,

1H), 7.59 (s, 1H), 6.95 (t, *J* = 9.2 Hz, 1H), 6.64 (dd, *J* = 5.6, 2.4 Hz, 1H), 6.49 (dd, *J* = 13.2, 2.4 Hz, 1H), 6.40 (dd, *J* = 8.8, 2.0 Hz, 1H), 5.75 (t, *J* = 6.0 Hz, 1H), 5.47 (s, 2H), 3.97 (d, *J* = 6.0 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 171.9, 166.8, 155.5, 153.2, 150.0, 149.0, 129.4, 124.3, 110.5, 107.8, 101.8, 99.4, 61.9. MS (ESI): 277.1 $(M)^+$.

5.4.11. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)-2-hydroxypropanamide **(21k)**. Prepared according to the procedure for the preparation of **21a**, from **20k** to yield **21k** as a white solid (81%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.65 (s, 1H), 8.17 (d, *J* = 4.8 Hz, 1H), 7.62 (s, 1H), 6.98 (t, *J* = 8.4 Hz, 1H), 6.70 (s, 1H), 6.51 (d, *J* = 12.6 Hz, 1H), 6.42 (d, *J* = 8.4 Hz, 1H), 5.86 (d, *J* = 4.8 Hz, 1H), 5.48 (s, 2H), 4.16 (t, *J* = 6Hz, 1H), 1.26 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 174.3, 166.9, 155.5, 153.2, 150.0, 148.9, 129.4, 110.5, 107.9, 103.9, 101.9, 99.2, 67.7, 21.0. MS (ESI): 291.1 $(M)^+$.

5.4.12. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)-2-hydroxy-2-methylpropanamide **(21l)**. Prepared according to the procedure for the preparation of **21a**, from **20l** to yield **21l** as a white solid (79%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.19 (s, 1H), 7.64 (s, 1H), 6.99 (s, 1H), 6.88 – 6.29 (m, 3H), 5.97 (s, 1H), 5.44 (s, 2H), 1.35 (s, 6H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 176.1, 167.0, 155.5, 153.0, 150.0, 148.9, 129.4, 124.4, 110.5, 108.1, 101.9, 98.8, 72.9, 27.7. MS (ESI): 305.2 $(M)^+$.

5.5. General procedures for the synthesis of intermediates 22a-d

5.5.1. 5-chloro-3-(4-fluorophenyl)-1-methyl-1,6-naphthyridin-4(1H)-one **(22a)**. A dried flask was charged with 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** (1.8 mmol), iodomethane (2.18 mmol), K_2CO_3 (5.3 mmol) and DMF (5 ml). The mixture was stirred at room temperature for 3h. Then, the reaction mixturewas poured intowater (60.0 mL), the precipitate was collected by filtration and washed with water, and dried under infrared light to yield the desired product **22a**. ^{1}H NMR (600 MHz, DMSO-d_6) *δ* 8.43 (d, *J* = 6.0 Hz, 1H), 8.29 (s, 1H), 7.69 (d, *J* = 6.6 Hz, 1H), 7.67 (d, *J* = 6.0 Hz, 1H), 7.61 (d, *J* = 6.0 Hz, 1H), 7.25 (t, *J* = 8.7 Hz, 2H), 3.85 (s, 3H). ^{13}C-NMR (100 M, DMSO-d_6) δ 173.2, 162.6, 161.0, 151.0, 148.5, 148.0, 143.9, 131.0, 124.1, 118.4, 115.0, 111.4, 41.1. MS (ESI): 288.1 $(M)^+$.

5.5.2. 5-chloro-1-ethyl-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **(22b)**. Prepared according to the procedure for the preparation of **22a**, from

5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and iodoethane to yield **22b** as a white solid (86%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.42 (d, J = 6.0 Hz, 1H), 8.31 (s, 1H), 7.75 – 7.68 (m, 3H), 7.25 (t, J = 8.7 Hz, 2H), 4.33 (q, J = 7.2 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H). ^{13}C-NMR (100 M, DMSO-d_6) δ 173.3, 162.623, 161.030, 151.403, 148.57336, 147.1, 142.8, 131.1, 124.6, 118.7, 115.1, 111.1, 48.4, 14.3. MS (ESI): 302.1 $(M)^+$.

5.5.3. 5-chloro-3-(4-fluorophenyl)-1-(2-hydroxyethyl)-1,6-naphthyridin-4(1H)-one **(22c)**. Prepared according to the procedure for the preparation of **22a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and oxirane to yield **22c** as a white solid (46%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.39 (d, J = 6.0 Hz, 1H), 8.19 (s, 1H), 7.74 (d, J = 6.0 Hz, 1H), 7.72 – 7.65 (m, 2H), 7.24 (t, J =8.4 Hz, 2H), 5.01 (s, 1H), 4.38 (s, 2H), 3.76 (s, 2H). ^{13}C-NMR (100 M, DMSO-d_6) δ 173.4, 162.6, 161.0, 151.3, 148.3, 147.8, 144.0, 131.0, 123.8, 118.7, 115.2, 111.4, 59.2, 55.2. MS (ESI): 348.1 $(M)^+$.

5.5.4. 5-chloro-3-(4-fluorophenyl)-1-(2-hydroxy-2-methylpropyl)-1,6-naphthyridin-4(1H)-one **(22d)**. Prepared according to the procedure for the preparation of **22a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and 2,2-dimethyloxirane to yield **22d** as a white solid (46%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.41 (d, J = 6.0 Hz, 1H), 8.21 (s, 1H), 7.76 (d, J = 6.0 Hz, 1H), 7.72 – 7.64 (m, 2H), 7.26 (t, J = 8.7 Hz, 2H), 5.09 (s, 1H), 4.38 (s, 2H), 1.21 (s, 6H). ^{13}C-NMR (100 M, DMSO-d_6) δ 173.4, 162.6, 161.0, 151.1, 148.9, 147.7, 144.8, 130.9, 123.3, 118.5, 115.1, 112.7, 70.9, 61.1, 27.6. MS (ESI): 346.1 $(M)^+$ ..

5.6. General procedures for the synthesis of targets 8, 9a-o and 23a-d

5.6.1. 5-(4-(2-amino-3-chloropyridin-4-yloxy)-3-fluorophenylamino)-3-(4-fluorophenyl)-1,6-naphthyridin-4(*1H*)-one **(8)**. A solution of 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** (0.5 mmol), 4-(4-amino-2-fluorophenoxy)-3-chloropicolin-amide **15** (0.5 mmol) and PTSA (0.5 mmol) in isopropanol (10 mL) was heated to 90 °C under nitrogen for 2 h. The mixture was filtered, and the solid was washed with ice-cold ethanol to yield 3-chloro-4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)- amino)phenoxy)picolinamide **16** as yellow solid (80%). A solution of above carboxy amide derivative **16** (1 mmol) and (diacetoxyiodo)benzene (1.1 mmol) in acetonitrile (10 mL) was stired at 0 °C for 1 h. The resulted solution was concentrated in

vacuum and purified by chromatography (CH_2Cl_2/MeOH = 20:1) to yield **8** as a yellow solid (33%), mp 295-296 °C. ^{1}H-NMR (400 M, DMSO-d_6) δ 13.14 (s, 1H), 12.41 (bs, 1H), 8.33 (d, J = 10.8 Hz, 1H), 8.16-8.18 (m, 2H), 7.70-7.76 (m, 3H), 7.46 (d, J = 6.8 Hz, 1H), 7.23-7.29 (m, 3H), 6.86 (d, J = 4.8 Hz, 1H), 6.35 (s, 2H), 5.95 (d, J = 4.8 Hz, 1H); ^{13}C-NMR (100 M, DMSO-d_6) δ 178.0, 162.2, 160.1, 157.4, 155.6, 154.4, 152.0, 148.4, 147.2, 146.0, 139.0, 138.3, 134.2, 131.0, 130.8, 123.3, 122.7, 116.0, 114.7, 108.1, 106.9, 103.2, 100.1. MS (ESI). 492.1 $[M+H]^+$. HR-MS (EI) m/z calcd for $C_{25}H_{16}ClF_2N_5O_2$, 491.0961; found 492.1039 $[M+H]^+$.

5.6.2.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)acetamide 4-methylbenzenesulfonate **(9a)**. A solution of 5-chloro-3-(4-fluorophenyl)-1,6- naphthyridin-4(1H)-one **14** (0.5 mmol), N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)acetamide **21a** (0.5 mmol) and PTSA (0.5 mmol) in isopropanol (10 mL) was heated to 90 °C under nitrogen for 2 h. The mixture was filtered, and the solid was washed with ice-cold ethanol to yield N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)acetamide 4-methylbenzenesulfonate **(9a)** as white solid (61%), m.p. 248-249 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.42 (s, 1H), 13.18 (s, 1H), 11.57 (s, 1H), 8.31 (s, 2H), 8.16 – 7.94 (m, 2H), 7.74 (s, 2H), 7.61 – 7.41 (m, 4H), 7.37 (s, 1H), 7.32 – 7.20 (m, 2H), 7.16 – 7.06 (m, 3H), 7.04 (s, 2H), 2.28 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.2, 171.6, 168.5, 162.9, 154.8, 154.4, 153.2, 151.4, 147.5, 145.7, 144.4, 139.2, 138.3, 136.1, 131.8, 131.2, 130.5, 128.5, 125.8, 124.9, 124.6, 121.8, 115.3, 115.2, 109.0, 107.1, 104.4, 100.2, 24.4, 21.2. HRMS (ESI): calcd for $C_{27}H_{19}F_2N_5O_3$ $[M+Na]^+$ 522.1348, found: 522.1349.

5.6.3.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)propionamide 4-methylbenzenesulfonate **(9b)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)propionamide **21b** to yield **9b** as a white

solid (52%), m.p. 257-258 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.42 (s, 1H), 13.16 (s, 1H), 11.53 (s, 1H), 8.36 – 8.25 (m, 2H), 8.12 (s, 1H), 8.03 (d, *J* = 5.4 Hz, 1H), 7.80 – 7.64 (m, 2H), 7.54 (s, 2H), 7.51 – 7.43 (m, 2H), 7.38 (s, 1H), 7.35 – 7.20 (m, 2H), 7.17 – 7.07 (m, 3H), 7.07 – 6.97 (m, 2H), 2.45 (q, *J* = 7.2 Hz, 2H), 2.29 (s, 3H), 1.07 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.2, 175.3, 168.8, 162.9, 161.3, 154.8, 154.4, 153.2, 151.2, 147.6, 145.6, 143.8, 139.2, 138.4, 131.3, 130.5, 128.6, 125.9, 125.0, 124.7, 115.4, 115.3, 109.2, 107.0, 104.4, 100.2, 29.9, 21.2, 9.1. HRMS (ESI): calcd for $C_{28}H_{21}F_2N_5O_3$ $[M+Na]^+$ 536.1505, found: 536.1505.

5.6.4.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)butyramide 4-methylbenzenesulfonate **(9c)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)butyramide **21c** to yield **9c** as a white solid (57%), m.p. 251-252 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.44 (s, 2H), 11.82 (s, 1H), 8.32 (d, *J* = 4.8 Hz, 2H), 8.09 (s, 1H), 8.02 (d, *J* = 6.6 Hz, 1H), 7.80 – 7.72 (m, 2H), 7.60 – 7.51 (m, 2H), 7.51 – 7.44 (m, 3H), 7.29 (t, *J* = 8.4 Hz, 2H), 7.23 (s, 1H), 7.15 – 7.06 (m, 3H), 2.44 (t, *J* = 6.6 Hz, 2H), 2.29 (s, 3H), 1.62 (q, *J* = 7.2 Hz, 2H), 0.91 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.2, 174.5, 168.7, 162.9, 161.3, 154.8, 154.4, 153.2, 151.3, 147.6, 145.7, 143.9, 139.2, 138.3, 131.3, 130.5, 128.6, 125.9, 125.0, 124.7, 115.4, 115.3, 109.1, 107.0, 104.4, 100.4, 38.4, 21.2, 18.2, 13.8. HRMS (ESI): calcd for $C_{29}H_{23}F_2N_5O_3$ $[M+Na]^+$ 550.1661, found: 550.1662.

5.6.5.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)pentanamide 4-methylbenzenesulfonate **(9d)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)pentanamide **21d** to yield **9d** as a white solid (54%), m.p. 250-251 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.44 (s, 1H), 13.26 (s, 1H), 11.68 (s, 1H), 8.38 – 8.26 (m, 2H), 8.10 (s, 1H), 8.02 (d, *J* = 6.0 Hz, 1H), 7.77 – 7.72 (m,

2H), 7.63 – 7.51 (m, 2H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.38 (s, 2H), 7.30 (t, *J* = 9.0 Hz, 2H), 7.19 (d, *J* = 5.4 Hz, 1H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.06 (d, *J* = 6.6 Hz, 1H), 2.43 (t, *J* = 4.8 Hz, 2H), 2.34 (, 3H), 1.57 (q, *J* = 7.2 Hz, 2H), 1.31 (dt, *J* = 7.2 Hz, 2H), 0.88 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.2, 174.7, 168.8, 162.9, 161.3, 154.8, 154.3, 153.2, 151.1, 147.6, 145.5, 143.7, 139.2, 138.4, 136.2, 131.3, 131.2, 130.5, 128.6, 125.9, 125.1, 124.7, 115.4, 115.3, 109.3, 107.0, 104.4, 100.3, 36.3, 26.8, 22.0, 21.2, 14.1. HRMS (ESI): calcd for $C_{30}H_{25}F_2N_5O_3$ $[M+Na]^+$ 564.1817, found: 564.1819.

5.6.6.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)isobutyramide 4-methylbenzenesulfonate **(9e)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)isobutyramide **21e** to yield **9e** as a white solid (54%), m.p. 215-216 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.43 (s, 1H), 13.19 (s, 1H), 11.55 (s, 1H), 8.35 – 8.28 (m, 2H), 8.10 (s, 1H), 8.02 (d, *J* = 6.0 Hz, 1H), 7.74 (s, 2H), 7.54 (s, 2H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.44 (s, 1H), 7.28 (t, *J* = 8.4 Hz, 2H), 7.17 (s, 1H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.04 (d, *J* = 6.6 Hz, 1H), 2.85 – 2.63 (m, 1H), 2.28 (s, 3H), 1.11 (m, 6H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 178.5, 177.2, 168.9, 162.9, 161.3, 154.8, 154.3, 153.2, 151.3, 147.6, 145.5, 143.6, 139.2, 138.4, 131.3, 130.4, 128.6, 125.9, 125.1, 124.8, 122.5, 115.4, 115.3, 109.4, 107.0, 104.4, 100.4, 35.5, 21.2, 19.2. HRMS (ESI): calcd for $C_{29}H_{23}F_2N_5O_3$ $[M+Na]^+$ 550.1661, found: 550.1660.

5.6.7.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)pivalamide 4-methylbenzenesulfonate **(9f)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)pivalamide **21f** to yield **9f** as light yellow solid (51%), m.p. 196-197 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.40 (s, 1H), 13.02 (s, 1H), 10.97 (s, 1H), 8.38 (d, *J* = 6.6 Hz, 1H), 8.30 (d, *J* = 5.4 Hz, 1H), 8.20 (s, 1H), 8.06 (s, 1H), 7.74 (dd, *J* = 7.8, 6.0 Hz, 2H), 7.56 (s, 3H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.32 – 7.25 (m,

3H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.01 (d, *J* = 5.4 Hz, 1H), 2.35 – 2.13 (m, 3H), 1.25 (s, 9H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 180.0, 177.2, 169.5, 162.9, 161.3, 154.8, 154.5, 153.1, 151.2, 147.5, 145.6, 142.4, 141.2, 139.2, 138.3, 136.5, 131.3, 130.5, 128.6, 125.9, 125.1, 124.7, 115.4, 115.3, 109.9, 107.0, 104.4, 100.7, 26.7, 21.2. HRMS (ESI): calcd for $C_{30}H_{25}F_2N_5O_3$ $[M+H]^+$ 542.1998, found: 542.1994.

5.6.8.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)cyclopropanecarboxamide 4-methylbenzenesulfonate **(9g)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclopropanecarboxamide **21g** to yield **9g** as light yellow solid (46%), m.p. 220-222 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.43 (s, 1H), 13.23 (s, 1H), 11.94 (s, 1H), 8.34 – 8.27 (m, 2H), 8.09 (d, *J* = 10.8 Hz, 1H), 8.01 (d, *J* = 5.4 Hz, 1H), 7.78 – 7.71 (m, 2H), 7.57 – 7.51 (m, 2H), 7.48 (d, *J* = 6.6 Hz, 2H), 7.42 (s, 1H), 7.29 (t, *J* = 7.8 Hz, 2H), 7.16 – 7.09 (m, 3H), 7.05 (d, *J* = 5.4 Hz, 1H), 2.28 (s, 3H), 1.96 (s, 1H), 0.96 – 0.81 (m, 4H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.2, 175.1, 168.7, 162.9, 161.3, 154.8, 154.3, 153.2, 151.1, 147.6, 145.5, 143.9, 139.2, 138.4, 135.9, 131.3, 131.2, 130.4, 128.6, 125.9, 125.1, 124.8, 115.4, 115.3, 109.2, 107.0, 104.4, 100.3, 21.2, 15.2, 9.5. HRMS (ESI): calcd for $C_{29}H_{21}F_2N_5O_3$ $[M+H]^+$ 526.1612 , found: 526.1613.

5.6.9.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)cyclobutanecarboxamide 4-methylbenzenesulfonate **(9h)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclobutanecarboxamide **21h** to yield **9h** as light yellow solid (46%), m.p. 235-236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.68 (s, 1H), 13.41 (s, 1H), 11.61 (s, 1H), 8.34 – 8.26 (m, 2H), 8.17 (s, 1H), 8.08 (d, *J* = 6.0 Hz, 1H), 7.77 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.63 (s, 1H), 7.59 – 7.45 (m, 4H), 7.30 (t, *J* = 8.8 Hz, 2H), 7.21 – 7.11 (m, 5H), 3.47 – 3.36 (m, 1H), 2.31 (s, 3H), 2.28 – 2.20 (m, 2H), 2.20 – 2.09 (m, 2H), 2.02 – 1.90 (m, 1H), 1.87 – 1.75 (s, 1H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.3,

175.5, 167.9, 166.6, 163.6, 162.7, 161.1, 154.8, 153.1, 152.3, 148.5, 147.2, 145.9, 139.8, 138.9, 138.2, 131.3, 130.9, 128.5, 128.4, 125.9, 124.7, 115.3, 115.2, 108.6, 107.1, 104.1, 100.3, 24.7, 21.2, 18.0. HRMS (ESI): calcd for $C_{30}H_{23}F_2N_5O_3$ $[M+Na]^+$ 562.1661 , found: 562.1663.

5.6.10.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)cyclohexanecarboxamide 4-methylbenzenesulfonate **(9i)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclohexanecarboxamide **21i** to yield **9i** as light white solid (60%), m.p. 238-239 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.39 (s, 1H), 13.14 (s, 1H), 11.50 (s, 1H), 8.30 (s, 2H), 8.15 (s, 1H), 8.03 (s, 1H), 7.73 (s, 2H), 7.52 (s, 2H), 7.49 – 7.42 (m, 2H), 7.37 (s, 1H), 7.31 – 7.24 (s, 2H), 7.17 – 7.07 (m, 3H), 7.02 (s, 1H), 2.45 (s, 1H), 2.27 (s, 3H), 1.85 – 1.55 (m, 5H), 1.42 – 1.08 (m, 5H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.5, 177.2, 169.8, 168.9, 162.9, 161.3, 156.2, 154.8, 154.4, 153.2, 151.3, 147.5, 145.6, 143.5, 139.2, 138.3, 131.3, 130.5, 128.6, 125.9, 125.0, 124.6, 115.4, 115.3, 109.3, 107.0, 104.4, 100.4, 44.9, 28.9, 25.6, 25.3, 21.2. HRMS (ESI): calcd for $C_{32}H_{27}F_2N_5O_3$ $[M+H]^+$ 568.2082 , found: 568.2084.

5.6.11.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)-2-hydroxyacetamide 4-methylbenzenesulfonate **(9j)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)-2-hydroxyacetamide **21j** to yield **9j** as light yellow solid (52%), m.p. 217-218 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.35 (s, 2H), 10.79 (s, 1H), 8.31 (s, 1H), 8.26 (s, 2H), 8.10 (s, 1H), 8.01 (s, 1H), 7.77 – 7.62 (m, 3H), 7.58 – 7.36 (m, 4H), 7.26 (s, 2H), 7.17 – 7.03 (m, 4H), 4.07 (s, 2H), 2.28 (s, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.2, 175.6, 173.8, 168.4, 162.8, 161.2, 154.9, 154.5, 153.2, 151.1, 147.5, 145.7, 145.3, 143.3, 139.0, 138.3, 137.0, 134.4, 131.3, 130.6, 128.6, 125.9, 124.9, 124.5, 115.4, 115.2, 109.1, 107.0, 104.3, 100.8, 62.0, 21.2. HRMS (ESI): calcd for

$C_{27}H_{19}F_2N_5O_4$ $[M+Na]^+$ 538.1297, found: 538.1298.

5.6.12.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)-2-hydroxypropanamide 4-methylbenzenesulfonate **(9k)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)-2-hydroxypropanamide **21k** to yield **9k** as light yellow solid (57%), m.p. 232-233 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.43 (s, 1H), 13.18 (s, 1H), 10.92 (s, 1H), 8.38 (d, *J* = 6.6 Hz, 1H), 8.31 (d, *J* = 5.4 Hz, 1H), 8.17 (s, 1H), 8.04 (d, *J* = 6.0 Hz, 1H), 7.74 (dd, *J* = 8.4, 6.0 Hz, 2H), 7.66 (s, 2H), 7.53 (s, 2H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.29 (t, *J* = 9.0 Hz, 2H), 7.21 (d, *J* = 6.4 Hz, 1H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.04 (d, *J* = 6.6 Hz, 1H), 4.27 (q, *J* = 6.6 Hz, 1H), 2.27 (d, 3H), 1.30 (d, 6.6 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 177.2, 176.6, 169.0, 162.9, 161.3, 154.8, 154.3, 153.2, 150.7, 147.6, 145.6, 144.1, 139.2, 138.3, 136.3, 131.3, 131.2, 130.5, 128.6, 125.9, 125.0, 124.7, 115.4, 115.3, 109.5, 107.0, 104.4, 100.9, 67.9, 21.2, 20.8. HRMS (ESI): calcd for $C_{28}H_{21}F_2N_5O_4$ $[M+Na]^+$ 552.1454, found: 552.1454.

5.6.13.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)-2-hydroxy-2-methylpropanamide 4-methylbenzenesulfonate **(9l)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **14** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)-2-hydroxy-2-methylpropanamide **21l** to yield **9l** as light yellow solid (59%), m.p. 241-242 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.38 (s, 1H), 13.18 (s, 1H), 10.89 (s, 1H), 8.37 (d, *J* = 6.4 Hz, 1H), 8.29 (d, *J* = 5.6 Hz , 1H), 8.12 (s, 1H), 8.00 (d, *J* = 6.4 Hz, 1H), 7.76 – 7.65 (m, 3H), 7.52 (s, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.30 – 7.22 (m, 3H), 7.09 (d, *J* = 7.6 Hz, 2H), 7.03 (d, *J* = 5.6 Hz, 1H), 2.28 (s, 3H), 1.36 (s, 6H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 178.5, 177.2, 169.2, 162.9, 161.3, 154.8, 154.4, 153.2, 150.7, 147.5, 145.7, 143.8, 139.2, 138.3, 137.2, 136.4, 131.3, 130.5, 128.6, 125.9, 125.0, 124.6, 115.4, 115.3, 109.7, 107.0, 104.4, 100.8, 73.0, 27.6, 21.2. HRMS (ESI): calcd for $C_{29}H_{23}F_2N_5O_4$ $[M+Na]^+$ 566.1610, found: 566.1610.

5.6.17.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-1-methyl-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)cyclopropanecarboxamide 4-methylbenzenesulfonate **(23a)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1-methyl-1,6-naphthyridin-4(1H)-one **22a** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclopropanecarboxamide **21g** to yield **23a** as yellow solid (53%), m.p. 216~218°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.56 (s, 1H), 11.87 (s, 1H), 8.39 (s, 1H), 8.34 – 8.12 (m, 3H), 7.75 – 7.66 (m, 2H), 7.54 – 7.40 (m, 4H), 7.33 – 7.22 (m, 3H), 7.12 – 7.05 (m, 3H), 7.03 (d, *J* = 6.4 Hz, 1H), 3.88 (s, 3H), 2.28 (s, 3H), 1.94 (s, 1H), 0.97 – 0.82 (m, 4H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 176.6, 175.2, 169.1, 162.9, 161.1, 155.0, 153.0, 152.0, 150.9, 147.8, 145.6, 145.0, 143.5, 138.4, 131.3, 130.5, 128.6, 125.9, 124.8, 124.2, 115.5, 115.3, 112.5, 109.4, 107.5, 102.7, 100.0, 41.6, 21.2, 15.2, 9.6. HRMS (ESI): calcd for $C_{30}H_{23}F_2N_5O_3$ $[M+Na]^+$ 562.1661 , found: 562.1663.

5.6.18.

N-(4-(4-((1-ethyl-3-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)-2-fluorophenoxy)pyridin-2-yl)cyclopropanecarboxamide 4-methylbenzenesulfonate (**23b**, 0.6eg PTSA). Prepared according to the procedure for the preparation of **9a**, from 5-chloro-1-ethyl-3-(4-fluorophenyl)-1,6-naphthyridin-4(1H)-one **22b** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclopropanecarboxamide **21g** to yield **23b** as light yellow solid (53%), m.p. 195~197°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.50 (s, 1H), 11.10 (s, 1H), 8.50 – 8.20 (m, 4H), 7.77 (s, 3H), 7.60 – 7.45 (m, 3H), 7.45 – 7.21 (m, 3H), 7.20 –7.05 (m, 2H), 6.88 (s, 1H), 4.35 (d, *J* = 6.4 Hz, 2H), 2.31 (s, 1.8H), 1.96 (s, 1H), 1.40 (s, 3H), 0.82 (s, 4H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 176.8, 173.8, 167.0, 162.7, 161.1, 156.4, 154.7, 153.3, 153.0, 148.6, 148.0, 146.2, 143.5, 139.1, 131.3, 131.0, 128.5, 125.9, 124.2, 123.7, 117.6, 115.3, 115.1, 109.5, 108.2, 107.8, 101.8, 99.7, 48.4, 39.5, 21.2, 14.6, 8.6. HRMS (ESI): calcd for $C_{31}H_{25}F_2N_5O_3$ $[M+H]^+$ 554.1925 , found: 554.1922.

5.6.19.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-1-(2-hydroxyethyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)cyclopropanecarboxamide 4-methylbenzenesulfonate

(23c). Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1-(2-hydroxyethyl)-1,6-naphthyridin-4(1H)-one **22c** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclopropanecarboxamide **21g** to yield **23c** as light yellow solid (53%), m.p. 160~161°C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.69 (s, 1H), 11.84 (s, 1H), 8.35 – 8.25 (m, 2H), 8.20 (s, 1H), 8.13 (s, 1H), 7.77 – 7.67 (m, 2H), 7.56 – 7.39 (m, 4H), 7.29 (t, *J* = 8.4 Hz, 2H), 7.23 (s, 1H), 7.19 (d, *J* = 6.0 Hz, 1H), 7.15 – 7.05 (m, 4H), 4.42 (s, 2H), 3.76 (s, 2H), 2.26 (s, 3H), 1.90 (s, 1H), 0.97 – 0.83 (m, 4H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 176.7, 175.0, 168.8, 162.9, 161.3, 155.3, 154.7, 153.2, 153.0, 151.1, 147.5, 145.6, 145.0, 144.0, 138.4, 131.2, 130.5, 128.6, 125.9, 124.7, 123.7, 120.6, 115.5, 115.3, 109.2, 107.8, 102.8, 100.0, 59.3, 55.5, 21.2, 15.2, 9.5. HRMS (ESI): calcd for $C_{31}H_{25}F_2N_5O_4$ $[M+Na]^+$ 592.1772 , found: 592.1773.

5.6.20.

N-(4-(2-fluoro-4-((3-(4-fluorophenyl)-1-(2-hydroxy-2-methylpropyl)-4-oxo-1,4-dihydro-1,6-naphthyridin-5-yl)amino)phenoxy)pyridin-2-yl)cyclopropanecarboxamide bis(4-methylbenzenesulfonate) **(23d)**. Prepared according to the procedure for the preparation of **9a**, from 5-chloro-3-(4-fluorophenyl)-1-(2-hydroxy-2-methylpropyl)-1,6-naphthyridin-4(1H)-one **22d** and N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclopropanecarboxamide **21g** to yield **23d** as light yellow solid (53%), m.p. 2 31~233°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.80 (s, 1H), 11.80 (s, 1H), 8.38 – 8.29 (m, 2H), 8.21 (d, *J* = 11.6 Hz, 1H), 8.12 (d, *J* = 6.8 Hz, 1H), 7.76 (dd, *J* = 8.8, 5.6 Hz, 2H), 7.56 (s, 2H), 7.51 (s, 2H), 7.49 (s, 2H), 7.41 (d, *J* = 6.8 Hz, 1H), 7.35 (t, *J* = 8.8 Hz, 2H), 7.29 (s, 1H), 7.17 – 7.11 (m, 5H), 4.38 (s, 2H), 2.31 (s, 6H), 2.05 – 1.80 (m, 1H), 1.21 (s, 6H), 1.02 – 0.81 (m, 4H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 176.7, 175.1, 168.9, 163.0, 161.3, 160.3, 154.9, 153.1, 151.0, 148.8, 145.8, 145.4, 143.9, 138.5, 136.9, 136.8, 131.2, 130.4, 128.6, 125.9, 124.9, 123.5, 121.5, 115.5, 115.4, 109.3, 107.6, 104.2, 100.0, 70.7, 61.4, 27.5, 21.2, 15.2, 9.6. HRMS (ESI): calcd for $C_{33}H_{29}F_2N_5O_4$ $[M+H]^+$ 598.2188 , found: 598.2187.

5.7. Molecular docking.

The three dimensional (3D) structure of the MET kinase complex (PDB code: 3F82) and VEGFR-2 kinase (PDB code: 3U6J) complex were obtained from PDB database. All water molecules and ligand were removed from the complex structure and hydrogen atoms were added with pH equaling to 7.0 using Sybyl-X. The AutoDock 4.2[30] program was applied to docking compound **8, 9i and 9g** into the binding site of MET kinase and compound **9i and 9g** into the binding site of VEGFR-2 kinase[31-33]. The Gasteiger charges were used for this inhibitors. In the docking process, a conformational search was performed for the ligand using the Solis and Wets local search method, and the Lamarckian genetic algorithm (LGA) [34,35] was applied for the conformational search of the binding complex of ligand with the kinase. Among a series of docking parameters, the grid size was set to be 70x70x80 in 3F82, 46x40x60 in 3U6J, and the used grid space was the default value of 0.375 Å. Among a set of 100 candidates of the docked complex structures, the best one was first selected according to the interaction energy and was then compared with the conformation of ligand (**2**, BMS-777607) extracted from the crystal structure.

5.8. Biochemical Kinase Assays.

The ability of compounds to inhibit the activity of a wide variety of kinases was tested *in vitro*. Enzyme assays were run in homogeneous time-resolved fluorescence (HTRF) format in 384-well microtiter plates using purified kinases purchased from Invitrogen. The HTRF KinEASE TK kit (contains substrate-biotin, antibody-cryptate, streptavidin-XL665, 5×enzymatic buffer and detection buffer) was purchased from Cisbio, and the kinase assays were performed according to manufacture's instructions. After the kinases and the compounds incubated at 25~30 °C for 5 min, the reactions were initiated by the addition of 2 μl of mixed substrate solution [mixed solution of ATP (Sigma) and substrate-biotin]. The final concentrations of kinases were at EC_{80} and the total reaction volume was 8 μl. Plates were incubated at 30 °C for 30~60 min, then the reactions were quenched by the addition 8 μl mixed detection solution (mixed solution of antibody-cryptate and streptavidin-XL665 in detection buffer). The fluorescence at 665 nm and 620 nm was measured with PHERAstar FS plate reader (BMG) using a time delay of 50 μs. All kinases assays were conducted

using ATP concentrations below the enzyme K_{mapp} and kinase-specific biotinylated substrate peptides.

The data for dose responses were plotted as percent inhibition calculated with the data reduction formula $100 \times [1 - (U_1 - C_2) / (C_1 - C_2)]$ versus concentration of compound, where U is the emission ratio of 665 nm and 620 nm of test sample, C_1 is the average value obtained for solvent control (2% DMSO), and C_2 is the average value obtained for no reaction control (no kinase sample). Inhibition curves were generated by plotting percentage control activity versus $\log_{10}$ of the concentration of each kinase. The IC_{50} values were calculated by nonlinear regression with Graphpad Prism 5.

5.9. Cell Proliferation Assay.

Cells were seeded in 96-well tissue culture plates. On the next day, cells were exposed to various concentrations of compounds and further cultured for 72 h. Finally, cell proliferation was determined using thiazolyl blue tetrazolium bromide (MTT, Sigma) assay.

5.10. Pharmacokinetic profiles in SD rats

Compound **8**, **9g** or **23a** were dissolved in 70% PEG-400 solution and administered to 3 male SD rats (weight ranging from 180 g to 220 g) for i.v. and p.o. administration. The dosing volume was 2 mL/kg (i.v.) or 10 mL/kg (p.o.). After administration, blood samples were collected at the point including 5 min, 15 min, 30 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 24 h, 48h and 72h (i.v.) or 15 min, 30 min, 45 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 24 h, 48h and 72h (p.o.) for analyses, the collected blood samples were centrifuged at 8000 rpm for 5 min at 4 ^{o}C, and then analyzed after protein precipitation. LC/MS/MS analysis of compound **8**, **9g** or **23a** was performed under optimized conditions to obtain the best sensitivity and selectivity of the analyte in selected reaction monitoring mode (SRM) containing an internal standard. Plasma concentration-time data were measured by a noncompartmental approach using the software WinNonlin Enterprise, version 5.2 (Pharsight Co., Mountain View, CA).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at.

References

[1] D. P. Bottaro, J. S. Rubin, D. L. Faletto, A. M. Chan, T. E. Kmiecik, G. F. Vande Woude, S. A. Aaronson, Identification of the hepatocyte growth factor receptor as the c-Met proto-oncogene product, Science 251 (1991) 802–804.

[2] C. Birchmeier, E. Gherardi, Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase, Trends Cell Boil. 8 (1998) 404-410.

[3] E. Gherardi, W. Birchmeier, C. Birchmeier, G. Vande Woude, Targeting MET in cancer: rationale and progress, Nat. Rev. Cancer 12 (2012) 89-103.

[4] L. Goyal, M. D. Muzumdar, A. X. Zhu, Targeting the HGF/c-MET pathway in hepatocellular carcinoma, Clin. Cancer Res. 19 (2013) 2310-2318.

[5] L. Trusolino, A. Bertotti, P. M. Comoglio, MET signalling: principles and functions in development, organ regeneration and cancer, Nat. Rev. Mol. Cell Biol. 11 (2010) 834-848.

[6] P. M. Comoglio, S. Giordano, L. Trusolino, Drug development of MET inhibitors: targeting oncogene addiction and expedience, Nat. Rev. Drug. Disc. 7 (2008) 504.

[7] E. Gherardi, W. Birchmeier, C. Birchmeier, G. Vande Woude, Targeting MET in cancer: rationale and progress, Nat. Rev. Cancer 12 (2012) 89–103.

[8] C. Boccaccio, P. M. Comoglio, MET, a driver of invasive growth and cancer clonal evolution under therapeutic pressure, Curr. Opin. Cell. Boil. 31 (2014) 98-105.

[9] J. G. Christensen, J. Burrows, R. Salkia, c-Met as a target for human cancer and characterization of inhibitora for therapeutic intervention, Canc. Lett. 225 (2005) 1-26.

[10] P. M. Comoglio, L. Trusolino, C. Boccaccio, Known and novel roles of the MET oncogene in cancer: a coherent approach to targeted therapy, Nat. Rev. Cancer. 18 (2018) 341–358.

[11] B. Peruzzi, D. P. Bottaro, Targeting the c-Met signaling pathway in cancer, Clin. Cancer Res. 12 (2006) 3657-3660.

[12] J. J. Cui, Targeting receptor tyrosine kinase MET in cancer: small molecule inhibitors and clinical progress, J. Med. Chem. 57 (2014) 4427–4453.

[13] M. M. Mohyeldin, B. A. Busnena, M. R. Akl, A.M. Dragoi, K. A. E. Sayed, Novel c-Met inhibitory olive secoiridoid semisynthetic analogs for the control of invasive breast cancer, Eur. J. Med. Chem. 118 (2016) 299-315.

[14] C. Birchmeier, W. Birchmeier, E. Gherardi, G. F. Vande Woude, Met, metastasis, motility and more, Nat. Rev. Mol. Cell. Biol. 4(2003) 915-925.

[15] N. D. D'Angelo, S. F. Bellon, S. K. Booker, Y. Cheng, A. Coxon, C. Dominguez, I. Fellows, D. Hoffman, R. Hungate, P. Kaplan-Lefko, Design, Synthesis, and Biological Evaluation of Potent c-Met Inhibitors, J. Med. Chem. 51 (2008) 5766-5779.

[16] U.S. Food and Drug Administration (Page Last Updated: 01/26/2017) https:// www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ ucm336115.htm, (last accessed: 3 August, 2017).

[17] G. M. Schroeder, Y. An, Z. W. Cai, X. T. Chen, C. Clark, L. A. Cornelius, J. Dai, J. Gullo-Brown, A. Gupta, B. Henley, J. T. Hunt, R. Jeyaseelan, A. Kamath, K. Kim, J. Lippy, L. J. Lombardo, V. Manne, S. Oppenheimer, J. S. Sack, R. J. Schmidt, G. Shen, K. Stefanski, J. S. Tokarski, G. L. Trainor, B. S. Wautlet, D. Wei, D. K. Williams, Y. Zhang, Y. Zhang, J. Fargnoli, R. M. Borzilleri, Discovery of N-(4-(2-amino-3-chloropyridin-4-yloxy)-3-fluorophenyl)-4-ethoxy-1-4-fluorophenyl)-2-oxo-1,2-di hydro-pyridine-3-carboxamide (BMS-777607), a selective and orally efficacious inhibitor of the Met kinase superfamily, J. Med. Chem. 52 (2009) 1251–1254.

[18] M. Maji, K. Chakrabarti, D. Panja, S. Kundu, Sustainable synthesis of N-heterocycles in water using alcohols following the double dehydrogenation strategy, J. Catal. 373 (2019) 93-102.

[19] A. Zhang, C. Ding, C. Cheng, Q. Yao, Convenient synthesis of 2, 7-naphthyridine lophocladines A and B and their analogues, J. Comb. Chem. 9 (2007) 916-919.

[20] Y. B. Cho, J. Lee, J. B. Yi, N. K. Lee, B. Y. Lee, K. C. Hwang, S. Lim, W. Chang, J. H. Chung, B. H. Lee, H. W. Seo, A pharmaceutical compositions containing lactam type pyridine derivatives as an effective ingredient for the prevention and treatment of ischemia, WO 2008123755A1,2008-10-16

[21] D. S. Ziegler, R. Greiner, H. Lumpe, L. Kqiku, K. Karaghiosoff, P. Knochel, Directed Zincation or Magnesiation of the 2-Pyridone and 2, 7-Naphthyridone Scaffold Using TMP Bases, Org. Lett. 19 (2017) 5760-5763.

[22] L. F. Zeng, Y. Wang, R. Kazemi, S. Xu, Z. L. Xu, T.W. Sanchez, L. M. Yang, B. Debnath, S. Odde, H. Xie, Repositioning HIV-1 integrase inhibitors for cancer therapeutics: 1, 6-naphthyridine-7-carboxamide as a promising scaffold with drug-like properties, J. Med. Chem. 55 (2012) 9492-9509.

[23] Y. Wang, Z. L. Xu, J. Ai, X. Peng, J. P. Lin, Y. C. Ji, M. Y. Geng, Y. Q. Long, Investigation on the 1, 6-naphthyridine motif: discovery and SAR study of 1 H-imidazo [4, 5-h][1, 6] naphthyridin-2 (3 H)-one-based c-Met kinase inhibitors, Org. Biomol. Chem. 11 (2013) 1545-1562.

[24] A. M. Thompson, C. J. Connolly, J. M. Hamby, S. Boushelle, B. G. Hartl, A. M. Amar, A. J. Kraker, D. L. Driscoll, R. W. Steinkampf, S. J. Patmore, 3-(3, 5-Dimethoxyphenyl)-1,

6-naphthyridine-2, 7-diamines and related 2-urea derivatives are potent and selective inhibitors of the FGF receptor-1 tyrosine kinase, J. Med. Chem. 43 (2000) 4200-4211.

[25] A. M. Thompson, A. M. Delaney, J. M. Hamby, M. C. Schroeder, T. A. Spoon, S. M. Crean, H. H. Showalter, W. A. Denny, Synthesis and structure−activity relationships of soluble 7-substituted 3-(3, 5-dimethoxyphenyl)-1, 6-naphthyridin-2-amines and related ureas as dual inhibitors of the fibroblast growth factor receptor-1 and vascular endothelial growth factor receptor-2 Tyrosine kinases, J. Med. Chem. 48 (2005) 4628-4653.

[26] L. S. Zhuo, H. C. Xu, M. S. Wang, X. E. Zhao, Z. H. Ming, X. L. Zhu, W. Huang, G. F. Yang, 2, 7-naphthyridinone-based MET kinase inhibitors: A promising novel scaffold for antitumor drug development, Eur. J. Med. Chem. 178 (2019) 705-714.

[27] D. L. Flynn, M. D. Kaufman, Preparation of cyclopropyl dicarboxamides and analogs exhibiting anti-cancer and anti-proliferative activities, WO 2011137342A1, 2011-11-3.

[28] B. D. Smith, M. D. Kaufman, C. B. Leary, B. A. Turner, S. C. Wise, Y. M. Ahn, R. J. Booth, T. M. Caldwell, C. L. Ensinger, M. M. Hood, Altiratinib inhibits tumor growth, invasion, angiogenesis, and microenvironment-mediated drug resistance via balanced inhibition of MET, TIE2, and VEGFR2, Mol. Cancer Ther. 14 (2015) 2023-2034.

[29] Y. Piao, S. Y. Park, V. Henry, B. D. Smith, N. Tiao, D. L. Flynn, J. F. de Groot, Novel MET/TIE2/VEGFR2 inhibitor altiratinib inhibits tumor growth and invasiveness in bevacizumab-resistant glioblastoma mouse models, Neuro-oncology 18 (2016) 1230-1241.

[30] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput Chem. 30 (2009) 2785–2791.

[31] J. F. Yang, F. Wang, W. Jiang, G. Y. Zhou, C. Z. Li, X. L. Zhu, G. F. Hao, G. F. Yang, PADFrag: a database built for the exploration of bioactive fragment space for drug discovery, J. Chem. Inf. Model. 58(2018) 1725–1730.

[32] G. F. Hao, W. Jiang, Y. N. Ye, F. X. Wu, X. L. Zhu, F. B. Guo, G. F. Yang, ACFIS: a web server for fragment-based drug discovery, Nucl. Acids. Res. 44(2016), W550–556.

[33] G. F. Hao, F. Wang, H. Li, X. L. Zhu, W. C. Yang, L. S. Huang, J. W. Wu, E. A. Berry, G. F. Yang, Computational Discovery of picomolar Qo site inhibitors of cytochrome bc1 complex, J. Am. Chem. Soc. 1345(2012) 11168–11176.

[34] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, Automated docking using a lamarckian genetic algorithm and an empirical binding free energy function, J. Comput. Chem. 19 (1998) 1639–1662.

[35] D. Sitkoff, K. A. Sharp, B. Honig, Accurate calculation of hydration free energies using macroscopic solvent models, J. Phys Chem. 98 (1994) 1978–1988.

Highlights:

Synthesis and Biological Evaluation of New MET Inhibitors with 1,6-Naphthyridinone Scaffold

- 1,6-Naphthyridinone was developed as a novel scaffold for the discovery of MET inhibitor.
- The 1,6-naphthyridone fragment was designed via a scaffold-hopping strategy of 2,7-naphthyridinone MET kinase inhibitor.
- Compound **23a** displayed good MET potency, promising VEGFR-2 selectivity and favorable PK profiles.