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Design, Synthesis and Evaluation of Highly Selective Pyridone-based Class II MET Inhibitors

Nengfang She[a], Linsheng Zhuo[a], Wen Jiang[a], Xiaolei Zhu[a], Jia Wang[b], Zhihui Ming[b], Xinge Zhao[b], Xin Cong[b] and Wei Huang[a,c,*]

[a]*Key Laboratory of Pesticide and Chemical Biology, Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, P.R.China*

[b]*Jiangsu Key Laboratory of Molecular Targeted Antitumor Drug Research, Jiangsu Simcere Pharmaceutical Co. Ltd, Nanjing 210042, P.R.China*

[c]*State Key Laboratory of Agricultural Microbiology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*

Abstract The high incidence of MET oncogene activation in human malignancies has prompted researchers to develop MET inhibitors. As part of our efforts to developing effective and safe therapeutic agents against MET-dependent tumors, a pyridone-based class II MET inhibitor, namely, 1-(4-((2-amino-3-iodopyridin-4-yl)-oxy)-3-fluorophenyl)-N-(4-fluorobenzyl)-4-methoxy-6-oxo-1,6-dihydropyridine-3-carboxamide (**3s**), was identified. Knowledge of the binding mode of class II MET inhibitors led to the design of new inhibitors that utilize 2-pyridone to conformationally restrain key pharmacophoric groups within the molecule. Integrated molecular docking and SAR studies resulted in the discovery of a novel class of pyridone MET inhibitors with high potency (IC_{50} of 0.005 μM) and efficient selectivity (> 5000 fold) to VEGFR-2, c-Kit and RET kinases.

Keywords: MET; Kinase inhibitor; Selectivity; Pyridone

Footnotes

*Address correspondence to this author at the College of Chemistry, Central China Normal University, No 152, Luoyu Road, Hongshan District, Wuhan 430079, P.R. China; Tel: +86-27-67867958; E-mail: weihuangwuhan@126.com

MET tyrosine kinase is the only known high-affinity receptor of hepatocyte growth factor (HGF), also known as scatter factor.[1] MET is an attractive target of therapeutic agents used in cancer treatment[2] because MET regulates cell proliferation, migration, invasion and survival; these processes are integral to cancer progression and metastasis.

MET inhibitors can be categorized into two classes based on their chemical structures or binding modes.[3] Class I inhibitors bind in a U-shaped conformation to the ATP-binding site at the entrance of a kinase pocket, wrap around Met1211 and bind to a hinge block; as a result, specifically inhibit MET kinases, such as crizotinib. Class II inhibitors bind to a region of MET that extends from the ATP binding site to Ile1145 near the C-c-spiral block. Compoud cabozantinib (**1**), which is an example of a class II MET inhibitor, effectively inhibits the kinase activity of MET, VEGFR-2, c-kit and RET.[4] Studies have suggested that class II inhibitors may be more effective than class I inhibitors against the mutations that disrupt the binding site of class I because their binding interactions extend beyond the entrance of the active site of MET.[5]

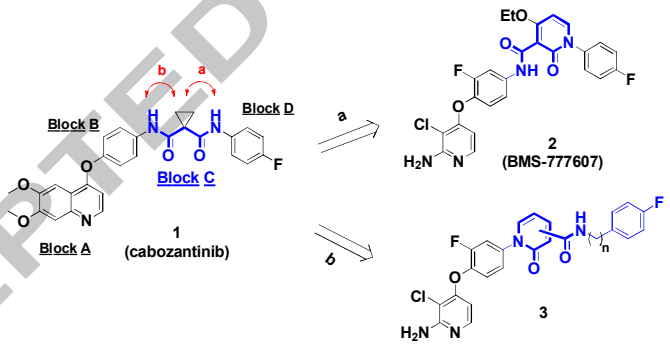
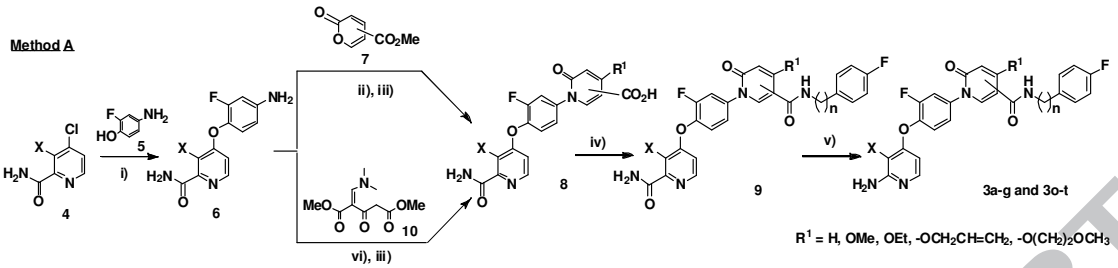


Figure 1. Representative MET inhibitors (**1**, **2**) and designed compounds (**3**)

However, class II MET inhibitors are correlated with many off-target effects of other protein kinases. Although these agents have been generally well characterized, clinical use has shown that these agents elicit unexpected and serious toxic effects in various organs. Moreover, many side effects of class II MET inhibitors occur because these inhibitors exhibit potent but non-specific obstruction of vascular endothelial growth factor function.[3b] Hence, novel MET inhibitors with improved selectivity profiles, particularly to VEGFR-2, and minimal side effects should be developed.

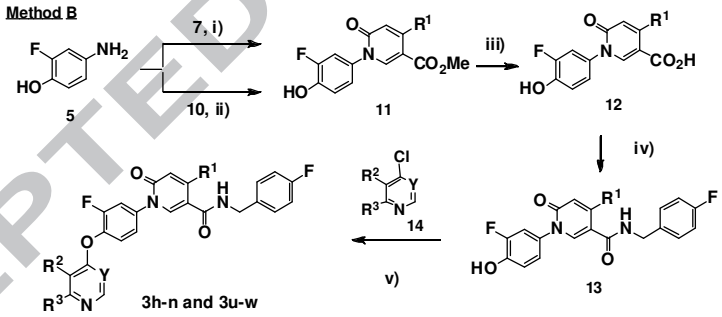
The molecular structure of the class II MET inhibitors can be divided into four blocks known as A, B, C and D (**Figure 1**).[3] Analyzing the crystal structure of foretinib (PDB code, 3LQ8) and MET kinase complex, we found that the chain structure of the block C assumed a pseudocyclic conformation. Therefore, various mono-ring structures, including pyrazole,[6] imidazole[7] and pyridine[8-10] can be successfully used to replace the amide linker of blocks B and C via cyclization strategy **a**, also known as **2** (BMS-777607).[8] In the crystal structure complex of **2** and MET kinase (PDB code, 3F82), block C also adopted a nearly co-planar conformation with maximum deviation from planarity of 0.063 Å in the nitrogen atom of 3-carboxamide group. We proposed that a 2-pyridone ring could be used to modify block C based on the cyclization of the amide linker of blocks B and C (strategy b). The planarity of block C could be conserved to maintain the molecular conformation. The carbonyl groups of 2-pyridone ring and carboxyamide may function as potential H-bond acceptors to retain interactions with key residues in the binding pocket.

Liu *et al.*[5,6] designed a pyrazolone-based MET inhibitor called AMG-458 highly selective (2420 fold) tor VEGFR-2. However, AMG-458 binds covalently to rat and human liver microsomes in the absence of NADPH and forms thioether adducts in vitro and in vivo after aryloxy-quinolines (blocks A and B) are introduced.[3a,11] However, **2** (BMS-777607) exhibits efficient druggability and modest selectivity (46 fold) to VEGFR-2. Therefore, we postulated that block C could be modified and blocks A and B could be reserved in **2** (BMS-777607); such processes may be effective strategies that could be used to discover highly selective class II MET inhibitors with efficient druggability (**Figure 1**). Hence, we disclosed the design and discovery of a novel class of pyridone MET inhibitors **3** with good potency and high selectivity.



Scheme 1. Reagent and conditions: i) *t*-BuOK, DMF, 50°C for 6 h, 76-82%; ii) DMAP, Molecular sieves, MeOH, 60°C for 2 h, 81-85%; iii) NaOH, MeOH, H_2O, 50°C for 2 h, 86-92%; iv) BtOH, EDCI, DMAP, DCM, 25°C for 12 h, 54-85%; v) $PhI(OAc)_2$, EA, DCM, H_2O, 0°C for 2 h, 65-83%; vi) EtOH, 70°C for 2 h, 86-90%.

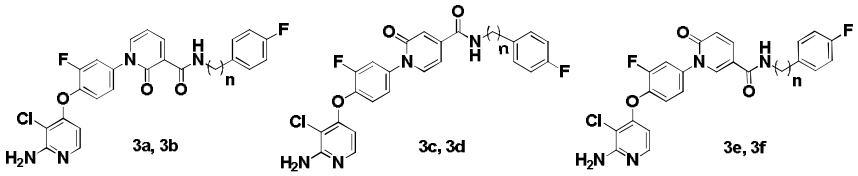
Considering the order at which different blocks were introduced, we selected two kinds of synthetic methods to synthesize the target compound **3**. Method A was utilized when blocks A and B exhibited a benzoxy-pyridine structure. The key carboxylic acid fragment (**8**) was prepared by subjecting chlorinated pyridine (**4**) to nucleophilic substitution with 4-aminophenol (**5**), cyclization with **7**[12] or **10**,[13] and hydrolysis. Compounds **3a–g** and **3o–t** were synthesized on the basis of Hofmann degradation (Scheme **1**) by performing the condensation of carboxylic acid **8** and diverse amine.



Scheme 2. Reagent and conditions: i) DMAP, Molecular sieves, MeOH, 60°C for 2h, 81%; ii) EtOH, 70°C for 2h, 85%; iii) NaOH, MeOH, H_2O, 50°C for 2h, 88-95%; iv) BtOH, EDCI, DMAP, DCM, 25°C for 12h, 62-78%; v) t-BuOK, DMF, 50°C for 1-12h, 65-87%.

Method B was chosen to optimize the structure of block A. The key phenol fragment (**13**) was prepared by subjecting 4-amino-2-fluorophenol (**5**) to cyclization with **7** or **10**, condensation with 4-fluorobenzylamine and hydrolysis. The target compounds **3h–n** and **3u–w** were obtained after **13** was subjected to nucleophilic substitution with chlorinated heterocycle **14** (**Scheme 2**).

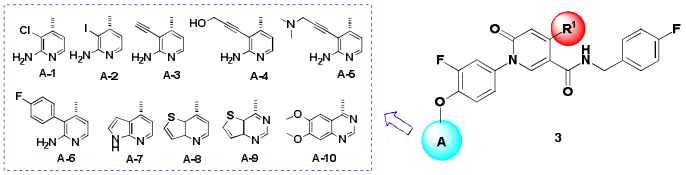
Table 1 MET and VEGFR-2 inhibitory activity of compounds **3a–f**



Compds	n	IC_{50} (μM)	
		MET	VEGFR-2
3a	0	> 25	-
3b	1	> 25	-
3c	0	> 25	-
3d	1	> 25	-
3e	0	> 25	-
3f	1	0.85	>25

The ability of the synthesized compounds prepared in this study was examined to inhibit MET and VEGFR-2 activities by using an enzyme assay with a recombinant kinase domain.[15] In **Table 1**, 1-phenyl-2-pyridone-3-carboxamide derivatives **3a** and **3b** and 1-phenyl-2-pyridone-4-carboxamide derivatives **3c** and **3d** did not inhibit MET even at the highest concentration of 25 μM. The resulting compound **3f** showed a moderate inhibition of MET (IC_{50} = 0.85 μM) when the carboxamide group was moved to 5-position of the 2-pyridone ring and the CH_2 group was introduced between the carboxamide group and block D. This characteristic was inconsistent with the reported regulation of five atoms in which blocks B and D are usually connected by six chemical bonds.[16] **3f** did not inhibit VEGFR-2 even at the highest concentration of 25 μM. As a result, 1-phenyl-2-pyridone-5-carboxamide was used as a potential scaffold to discover highly selective MET inhibitors.

Table 2 MET inhibitory activity of compounds **3g–w**



Compds	block A	R^1	IC_{50} (μM)
3g	A-2	H	0.39
3h	A-3	H	0.46
3i	A-4	H	0.28

3j	A-5	H	0.43
3k	A-7	H	1.23
3l	A-8	H	0.21
3m	A-9	H	0.57
3n	A-10	H	0.24
3o	A-1	OMe	0.045
3p	A-1	OEt	0.55
3q	A-1	$-OCH_2CH=CH_2$	0.28
3r	A-1	$-O(CH_2)_2OCH_3$	0.34
3s	A-2	OMe	0.005
3t	A-2	$-O(CH_2)_2OCH_3$	0.15
3u	A-4	OMe	0.099
3v	A-5	OMe	0.21
3w	A-6	OMe	0.12
	1 (cabozantinib)		0.008

The initial SAR study of block A was performed. Chlorine atom (**3f**) was replaced with an iodine atom (**3g**) or an alkynyl group (**3h**, **3i** and **3j**) in the 3-position; as a result, a slight increase in MET inhibitory activity (ranging from 1.8 fold increase to 3.0 fold increase) was observed. The bio-isostere of the pyridine ring with thieno[3,2-b]pyridine (**3l**), thieno[3,2-d]pyrimidine (**3m**) and quinazoline (**3n**) also resulted in a 1.5 fold to 4.0 fold improvement compared with compound **3f**; the substitution of pyrrolo[2,3-b]pyridine (**3k**) resulted in a slight decrease in potency.

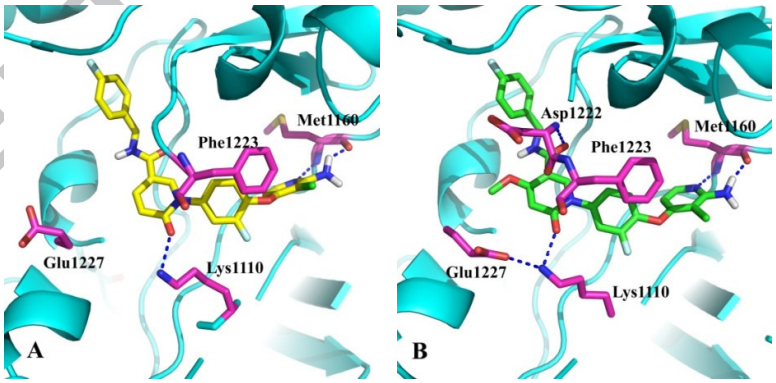


Figure 2. The bindind mode of **3f** (A) and **3o** (B) with MET. The blue line respresents a hydrogen bond. For clarity, only important residues were shown with stick model.

The binding mode of **3f** was investigated using an AutoDock 4.2 program to guide the follow-up structure optimization. Before molecular docking, we systematically analyzed the PDB structures of MET kinase. The result indicated that

the Glu1227 and Lys1110 residues showed different conformations in different PDB structures (data not shown). Furthermore, these residues could be roughly grouped into two conformations. In one of the conformations, a hydrogen bond was formed between Glu1227 and Lys1110 (PDB code, 3LQ8). In the other conformation, Glu1227 acquired an opposite conformation and the H-bond was disrupted (PDB code, 3L82). Accordingly, the IC_{50} values of the ligands in 3LQ8 and 3L82 were 0.5 and 3.9 nM, respectively. In **Figure 2A**, compound **3f** took on a similar binding mode with class II inhibitors and formed H-bonds with Lys1110, Phe1223 and Met1160. However, Glu1227 did not form H-bond with Lys1110. To improve the potency, we proposed that a methoxy group could be introduced to the 4-position of 2-pyridone in block C; this introduction could regulate the conformations of Glu1227 and Lys1110, function as a potential H-bond acceptor, and increase the potency of this inhibitor. The binding mode of **3o** is shown in **Figure 2B**. We observed that Glu1227 indeed formed an H-bond with Lys1110. The binding energy obtained from the AutoDock was -10.23 kcal/mol and -10.98 kcal/mol for **3f** and **3o**, respectively. We were intrigued by this possibility and wondered about the resulting activity of **3o**.

In actuality, **3o** displayed more potent MET inhibitory activity than **3f** (19 fold) when a methoxy group was introduced to the 4-position of 2-pyridone. However, the introduction of a group more bulky than a methoxy group decreased the inhibitory activity at different degrees (**3p**, **3q** and **3r**). Furthermore, the SAR study of block A was performed; **3s** (IC_{50} of 0.005 μM) was substituted with an iodine atom in the 3-position of the pyridine ring and exhibited a 9 fold increase in potency compared with **3o** and comparable potency to **1** (cabozantinib, IC_{50} of 0.008 μM). Bulky groups were introduced to the 3-position of the pyridine ring (**3u**, **3v** and **3w**), resulting in the loss of potency to some extent (2 fold to 42 fold).

Table 3 Kinase selectivity of compounds **3o** and **3s**

Compds	Enzyme, IC_{50}, μM			
	MET	VEGFR-2	c-Kit	RET
3o	0.045	>25	>25	>25
3s	0.005	>25	>25	>25
1 (cabozantinib)	0.008	0.007	0.004	0.016

The inhibitory activity of the most potent compounds **3o** and **3s** against VEGFR-2, c-Kit and RET kinase were also assayed using the homogeneous time resolved fluorescence (HTRF) method.[14] **3o** and **3s** demonstrated extraordinary selectivity against VEGFR-2, c-Kit and RET kinase (> 555 fold and > 5000 fold), while compound **1** (cabozantinib) displayed comparable activity against MET, VEGFR-2, c-Kit and RET kinase without any selectivity.[4]

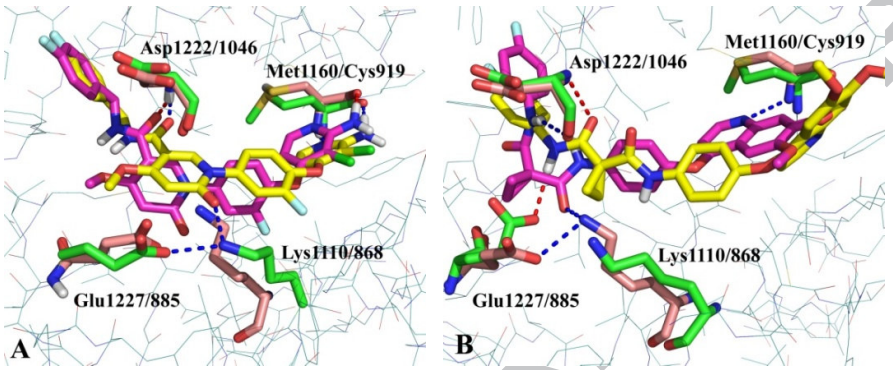


Figure 3. The overlay of the binding model of compounds **3o** (A) and **1** (B) with MET and VEGFR-2 kinases, respectively. The yellow and green stick represent MET complex. The magenta and lightpink stick represent VEGFR-2 complex. The first number of residue represents in MET kinase and the second number refers to in VEGFR-2 kinase.

The molecular docking method was also used to investigate the selectivity of compounds **3o** and **1**. In **Figure 3A**, **3o** formed H-bonds with Lys1110, Asp1222 and Met1160 in MET kinase and with Asp1046 and Cys919 in VEGFR-2 kinase. **3o** formed an H-bond with Lys1110 in MET kinase and displayed no interactions with the corresponding Lys868 residue in VEGFR-2 kinase. Compound **1** (cabozantinib) formed H-bonds with Lys1110, Asp1222 and Met1160 in MET kinase and formed H-bonds with Asp1046, Glu885 and Cys919 in VEGFR-2 kinase. Therefore, an important H-bond between **3o** and Lys868 was absent in the **3o**/VEGFR-2 kinase complex and this finding is a key factor affecting the selectivity of **3o**. For **1**, another H-bond with Glu885 in VEGFR-2 kinase (Glu1227 in MET kinase) could compensate for the missing of H-bond with Lys868 (Lys1110 in MET kinase), resulting in low selectivity between MET and VEGFR-2 kinase.

The effects of selected pyridone compounds on cell proliferation were then preliminarily evaluated in both MKN-45 and HUVEC cells. **3o** and **3s** elicited

excellent inhibitory effects on the proliferation of HUVEC cells with an IC_{50} of 2.41μM to11.1 μM; by comparison, lower potency was observed in MKN-45 cells (IC_{50} > 25 μM).

We described the design, synthesis and biological screening of a series of conformationally constrained analogues of compound **1** (cabozantinib). These novel compounds contained a structure that replaces the acylthiourea moiety with a novel 2-pyridone-5-carboxamide. On the basis of molecular docking results, we found that the structural modification of block C formed compound **3o** that inhibited MET activity with an IC_{50} of 0.045 μM. Further structural optimization of block A yielded compound **3s** that potently inhibited MET (IC_{50} of 0.005 μM); this result was comparable to the inhibitory activity of compound **1**. Furthermore, **3o** and **3s** showed excellent selectivities (> 555 fold and > 5000 fold, respectively) to VEGFR-2, c-Kit, and RET kinase; by comparison, **1** inhibited the three kinases and MET. Therefore, **1** did not exhibit selectivity. The results of molecular docking clearly indicated that **3o** could form H-bond with Lys1110 in MET kinase but not with VEGFR-2 kinase. In addition, compounds **3o** and **3s** effectively inhibited the proliferationof HUVEC cells. The druggability of **3o** and **3s** is currently evaluated and these two compounds are also optimized. Research progress will be reported in due course.

Acknowledgments

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17. Purity of all final compounds was assessed by HPLC and determined to be >95%. The structures were confirmed by ^{1}H NMR and LC/MS. Analytic data of represented compounds **3a**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 11.84 (s, 1H), 8.34 (d, J = 7.4 Hz, 1H), 7.89 (d, J = 6.5 Hz, 1H), 7.77 (d, J = 12.0 Hz, 1H), 7.51 (d, J = 6.0 Hz, 1H), 7.17-7.23 (m, 5H) , 7.06 (t, J = 9.0 Hz, 1H), 6.48 (t, J = 7.2 Hz, 1H), 6.17 (s, 2H), 5.69 (d, J = 6.0 Hz, 1H). MS (ESI) m/z: 469.0 $[M+H]^+$. HPLC: 95.3%; **3b**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 9.90 (s, 1H), 8.48 (d, J = 7.2 Hz, 1H), 8.05 (d, J = 6.6 Hz, 1H), 7.83 (d, J = 6.0 Hz, 1H), 7.77 (d, J = 12.0 Hz, 1H),

7.35-7.42 (m, 2H) ,7.11-7.25 (m, 4H), 6.66 (t, *J* = 7.0 Hz, 1H), 6.47 (s, 2H), 6.02 (d, *J* = 5.4 Hz, 1H), 4.50 (d, *J* = 6.0 Hz, 2H). MS (ESI) m/z: 483.0 $[M+H]^+$. HPLC: 95.9%; **3c**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 10.52 (s, 1H), 7.80-7.87 (m, 5H), 7.40-7.47 (m, 2H) , 7.20 (t, *J* = 6.0 Hz, 2H), 7.08 (d, *J* = 6.0 Hz, 1H), 6.71 (d, *J* = 7.5 Hz, 1H), 6.46 (s, 2H), 6.06 (d, *J* = 5.5 Hz, 1H). MS (ESI) m/z: 469.0 $[M+H]^+$. HPLC: 96.8%; **3d**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 10.52 (s, 1H), 9.29 (d, *J* = 6.0 Hz, 1H), 7.67-7.81 (m, 3H), 7.33-7.42 (m, 3H), 7.17 (t, *J* = 9.0 Hz, 2H), 6.96 (s, 1H), 6.67 (d, *J* = 6.0 Hz, 2H), 6.49 (s, 1H), 6.03 (d, *J* = 6.0 Hz, 1H), 4.44 (d, *J* = 5.7 Hz, 2H). MS (ESI) m/z: 483.0 $[M+H]^+$. HPLC: 98.0%; **3e**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 10.13 (s, 1H), 8.53 (d, *J* = 7.2 Hz, 1H), 8.04 (d, *J* = 9.6 Hz, 1H), 7.70-7.82 (m, 2H), 7.52-7.68 (m, 2H), 7.48-7.52 (m, 2H), 7.16 (t, *J* = 9.0 Hz, 2H), 6.63 (d, *J* = 10.2 Hz, 1H), 6.51 (s, 2H), 6.06 (d, *J* = 6.0 Hz, 1H). MS (ESI) m/z: 469.1 $[M+H]^+$. HPLC: 95.0%; **3f**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.83 (t, *J* = 6.0 Hz, 1H), 8.35 (s, 1H), 7.95 (d, *J* = 10.0 Hz, 1H), 7.73-7.83 (m, 2H), 7.42 (d, *J* = 8.5Hz, 2H), 7.36 (d, *J* = 8.5Hz, 2H), 7.14-7.19 (m, 2H), 6.55 (d, *J* = 10.0 Hz, 1H), 6.25 (s, 2H), 6.04 (d, *J* = 5.5 Hz, 1H), 4.42 (d, *J* = 6.0 Hz, 2H). MS (ESI) m/z: 482.7 $[M+H]^+$. HPLC: 96.2%; **3g**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.85 (t, *J* = 5.7 Hz, 1H), 8.35 (s, 1H), 7.95 (d, *J* = 9.9 Hz, 1H), 7.80 (d, *J* = 6.0 Hz, 1H), 7.75 (d, *J* = 10.8 Hz, 1H), 7.32 -7.42 (m, 4H), 7.15 (t, *J* = 5.5 Hz, *J* = 6.0 Hz, 2H) , 6.55 (d, *J* = 9.6 Hz, 1H), 6.25 (s, 2H), 5.93 (d, *J* = 5.5 Hz, 1H), 4.42 (d, *J* = 5.7 Hz, 2H). MS (ESI) m/z: 575.0 $[M+H]^+$. HPLC: 95.6%; **3h**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.83 (t, *J* = 6.0 Hz, 1H), 8.34 (s, 1H), 7.96 (d, *J* = 9.5 Hz, 1H), 7.86 (d, *J* = 6.0 Hz, 1H), 7.73 (d, *J* = 11.3 Hz, 1H), 7.42-7.47 (m, 2H), 7.33-7.36 (m, 2H), 7.16 (t, *J* = 9.5 Hz, 2H), 6.56 (d, *J* = 9.5 Hz, 1H), 6.25 (s, 2H), 5.93 (d, *J* = 5.5 Hz, 1H), 4.59 (s, 1H), 4.42 (d, *J* = 6.0 Hz, 2H). MS (ESI) m/z: 473.1 $[M+H]^+$. HPLC: 96.4%; **3i**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.83 (t, *J* = 5.6 Hz, 1H), 8.34 (s, 1H), 7.96 (d, *J* = 9.5 Hz, 1H), 7.83 (d, *J* = 6.0 Hz, 1H), 7.72 (d, *J* = 11.3 Hz, 1H), 7.41-7.48 (m, 2H), 7.33-7.36 (m, 2H), 7.14 (t, *J* = 8.7 Hz, 2H), 6.56 (d, *J* = 9.6 Hz, 1H), 6.37 (s, 2H), 5.91 (d, *J* = 5.8 Hz, 1H), 5.24 (t, *J* = 6.0 Hz, 1H), 4.42 (d, *J* = 5.8 Hz, 2H), 4.35 (d, *J* = 5.9 Hz, 2H). MS (ESI) m/z: 503.1 $[M+H]^+$. HPLC: 97.5%; **3j**: ^{1}H NMR 500 MHz, DMSO-*d6*) δ 8.83 (t, *J* = 5.9 Hz, 1H), 8.33 (s, 1H), 7.96 (d, *J* = 9.5 Hz, 1H), 7.85 (d, *J* = 5.8 Hz, 1H), 7.71 (d, *J* = 11.5 Hz, 1H), 7.38-7.43 (m, 2H), 7.33-7.36 (m, 2H), 7.14 (t, *J* = 6.7 Hz, 2H), 6.56 (d, *J* = 9.6 Hz, 1H), 6.28 (s, 2H), 6.01 (d, *J* = 5.8 Hz, 1H), 4.42 (d, *J* = 5.8 Hz, 2H), 4.35 (d, *J* = 5.9 Hz, 2H), 2.18 (s, 6H). MS (ESI) m/z: 530.2 $[M+H]^+$. HPLC: 95.5%; **3k**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.84 (t, *J* = 5.9 Hz, 1H), 8.38 (s, 1H), 8.13 (d, *J* = 5.4 Hz, 1H), 7.97 (d, *J* = 9.6 Hz, 1H), 7.76 (d, *J* = 11.2 Hz, 1H), 7.51 (t, *J* = 8.7 Hz, 1H), 7.41-7.45 (m, 2H), 7.33-7.37 (m, 2H), 7.15 (t, *J* = 6.7 Hz, 2H), 6.57 (d, *J* = 9.6 Hz, 1H), 6.48 (d, *J* = 5.3 Hz, 1H), 6.30-6.31 (m, 1H), 6.01 (d, *J* = 5.8 Hz, 1H), 4.42 (d, *J* = 5.8 Hz, 2H). MS (ESI) m/z: 473.1 $[M+H]^+$. HPLC: 95.4%; **3l**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.87 (t, *J* = 6.0 Hz, 1H), 8.58 (d, *J* = 5.3 Hz, 1H), 8.38 (s, 1H), 8.21 (d, *J* = 5.4 Hz, 1H), 7.97 (d, *J* = 9.6 Hz, 1H), 7.82 (d, *J* = 11.3 Hz, 1H), 7.63-7.71 (m, 2H), 7.33-7.52 (m, 3H), 7.15 (t, *J* = 9.0 Hz, 2H), 6.72 (d, *J* = 5.3 Hz, 1H), 6.58 (d, *J* = 9.6 Hz, 1H), 4.43 (d, *J* = 5.7 Hz, 2H). MS (ESI) m/z: 491.1 $[M+H]^+$. HPLC: 95.1%; **3m**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.76-8.85 (m, 2H), 8.58 (d, *J* = 5.1 Hz, 1H), 8.42 (s, 1H), 7.99 (d, *J* = 9.6 Hz, 1H), 7.71-7.78 (m, 2H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.31-7.39 (m, 3H), 7.02-7.18 (m, 2H), 6.59 (d, *J* = 9.6 Hz, 1H), 4.41 (d, *J* = 5.7 Hz, 2H). MS (ESI) m/z: 490.1 $[M+H]^+$. HPLC: 98.3%; **3n**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.86 (t, *J* = 6.0 Hz, 1H), 8.60 (s, 1H), 8.41 (s, 1H), 7.97 (d, *J* = 9.6 Hz, 1H), 7.76 (d, *J* = 11.3 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.60 (s, 1H), 7.41-7.59 (m, 2H), 7.33-7.38 (m, 2H), 7.17 (t, *J* = 9.0 Hz, 2H), 6.57 (d, *J* = 9.6 Hz, 1H), 4.42 (d, *J* = 6.0 Hz, 2H), 4.00 (s, 6H). MS (ESI) m/z: 545.2 $[M+H]^+$. HPLC: 99.4%; **3o**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.51 (t, *J* = 6.2 Hz, 1H), 8.11 (s, 1H), 7.80 (d, *J* = 5.7 Hz, 1H), 7.65 (d, *J* = 5.6 Hz, 1H), 7.33-7.44 (m, 4H), 7.15 (t, *J* = 6.6 Hz, 2H), 6.44 (s, 2H), 6.07 (d, *J* = 5.5 Hz, 1H), 6.02 (s, 1H), 4.44 (d, *J* = 6.1 Hz, 2H), 3.28 (s, 3H). MS (ESI) m/z: 513.1 $[M+H]^+$. HPLC: 95.6%; **3p**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.31 (t, *J* = 6.02 Hz, 1H), 8.09 (s, 1H), 7.80 (d, *J* = 5.5 Hz, 1H), 7.65 (d, *J* = 5.6 Hz, 1H), 7.33-7.44 (m, 4H), 7.16 (t, *J* = 6.6 Hz, 2H), 6.45 (s, 2H), 6.06 (d, *J* = 5.5 Hz, 1H), 6.00 (s, 1H),

4.45 (d, *J* = 6.1 Hz, 2H), 4.20 (d, *J* =7.0 Hz, 2H), 1.35 (t, *J* =7.0 Hz, 3H). MS (ESI) m/z: 527.0 $[M+H]^+$. HPLC: 95.2%; **3q**: ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.45 (t, *J* = 6.0 Hz, 1H), 8.10 (s, 1H), 7.80 (d, *J* = 6.0 Hz, 1H), 7.65 (d, *J* = 6.0 Hz, 1H), 7.33-7.43 (m, 4H), 7.11-7.17 (m, 2H), 6.50 (s, 2H), 6.00-6.09 (m, 3H), 5.29-5.44 (m, 2H), 4.74 (d, *J* = 5.4 Hz, 2H), 4.44 (d, *J* =7.0 Hz, 2H). MS (ESI) m/z: 539.0 $[M+H]^+$. HPLC: 96.1%; **3r**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.40 (s, 1H), 7.99 (t, *J* = 5.0 Hz, 1H), 7.82 (d, *J* = 6.0 Hz, 1H), 7.20-7.46 (m, 5H), 7.03 (t, *J* = 6.5 Hz, 2H), 6.18 (d, *J* = 6.0 Hz, 1H), 5.96 (s, 1H), 5.51 (s, 2H), 4.58 (d, *J* = 5.5 Hz, 2H), 4.19 (t, *J* = 4.5 Hz, 2H), 3.66 (t, *J* = 4.5 Hz, 2H), 3.18 (s, 3H). MS (ESI) m/z: 557.0 $[M+H]^+$. HPLC: 95.1%; **3s**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.51 (t, *J* = 6.0 Hz, 1H), 8.10 (s, 1H), 7.79 (d, *J* = 5.5 Hz, 1H), 7.65 (d, *J* = 11.5 Hz, 1H), 7.33-7.41 (m, 4H), 7.15 (t, *J* = 7.0 Hz, 2H), 6.25 (s, 2H), 6.02 (s, 1H), 5.94 (d, *J* = 5.5 Hz, 1H), 4.44 (d, *J* = 6.5 Hz, 2H), 3.92 (s, 3H). MS (ESI) m/z: 605.0 $[M+H]^+$. HPLC: 96.5%; **3t**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.39 (s, 1H), 7.95 (t, *J* = 5.0 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 1H), 7.20-7.35 (m, 5H), 7.04 (t, *J* = 6.0 Hz, 2H), 6.02 (d, *J* = 6.0 Hz, 1H), 5.96 (s, 1H), 5.40 (s, 2H), 4.58 (d, *J* = 5.5 Hz, 2H), 4.19 (t, *J* = 4.5 Hz, 2H), 3.67 (t, *J* = 4.5 Hz, 2H), 3.18 (s, 3H). MS (ESI) m/z: 649.0 $[M+H]^+$. HPLC: 95.8%; **3u**: H NMR (500 MHz, DMSO-*d6*) δ 8.51 (t, *J* = 6.0 Hz, 1H), 8.11 (s, 1H), 7.82 (d, *J* = 10.0 Hz, 1H), 7.64 (d, *J* = 11.0 Hz, 1H), 7.42 (d, *J* = 9.5 Hz, 1H), 7.33-7.36 (m, 3H), 7.15 (t, *J* = 9.5 Hz, 2H), 6.36 (s, 2H), 6.02 (s, 1H), 5.94 (d, *J* = 6.0 Hz, 1H), 5.25 (t, *J* = 6.0 Hz, 1H), 4.44 (d, *J* = 6.0 Hz, 2H), 4.35 (d, *J* = 6.0 Hz, 2H), 3.93 (s, 3H). MS (ESI) m/z: 533.0 $[M+H]^+$. HPLC: 99.0%; **3v**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.51 (t, *J* = 6.0 Hz, 1H), 8.09 (s, 1H), 7.84 (d, *J* = 5.5 Hz, 1H), 7.63 (d, *J* = 11.0 Hz, 1H), 7.33-7.39 (m, 4H), 7.14 (t, *J* = 6.0 Hz, 2H), 6.25 (s, 2H), 6.01-6.06 (m, 2H), 4.44 (d, *J* = 5.8 Hz, 2H), 4.07 (d, *J* = 5.5 Hz, 2H), 3.92 (s, 3H), 2.18 (s, 6H). MS (ESI) m/z: 560.0 $[M+H]^+$. HPLC: 97.1%; **3w**: ^{1}H NMR (500 MHz, DMSO-*d6*) δ 12.2 (s, 1H), 8.53 (s, 1H), 8.29 (d, *J* = 5.5 Hz, 1H), 8.11-8.16 (m, 2H), 7.75 (d, *J* = 11.0 Hz, 1H), 7.62 (t, *J* = 8.5 Hz, 1H), 7.30-7.43 (m, 5H), 7.09-7.16 (m, 4H), 6.56 (d, *J* = 5.0 Hz, 1H), 6.04 (s, 1H), 4.46 (d, *J* = 5.0 Hz, 2H), 3.94 (s, 3H). MS (ESI) m/z: 572.2 $[M+H]^+$. HPLC: 95.2%.

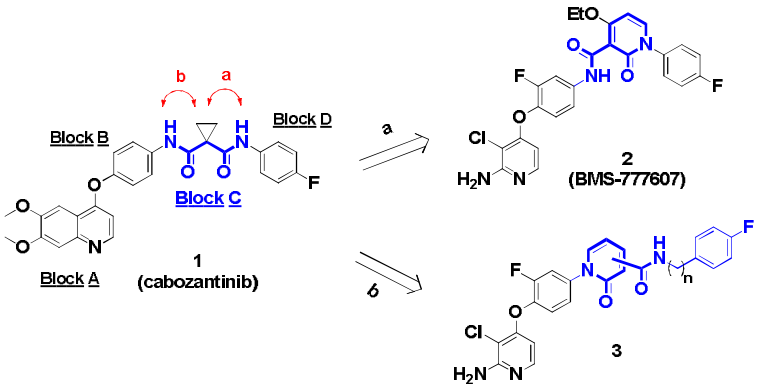


Figure 1. Representative MET inhibitors (**1**, **2**) and designed compounds (**3**)

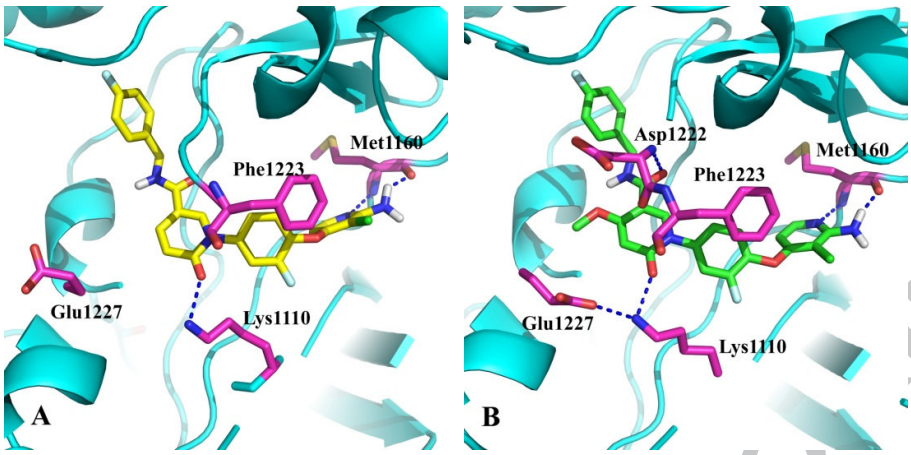


Figure 2. The bindind mode of **3f** (A) and **3o** (B) with MET. The blue line respresents a hydrogen bond. For clarity, only important residues were shown with stick model.

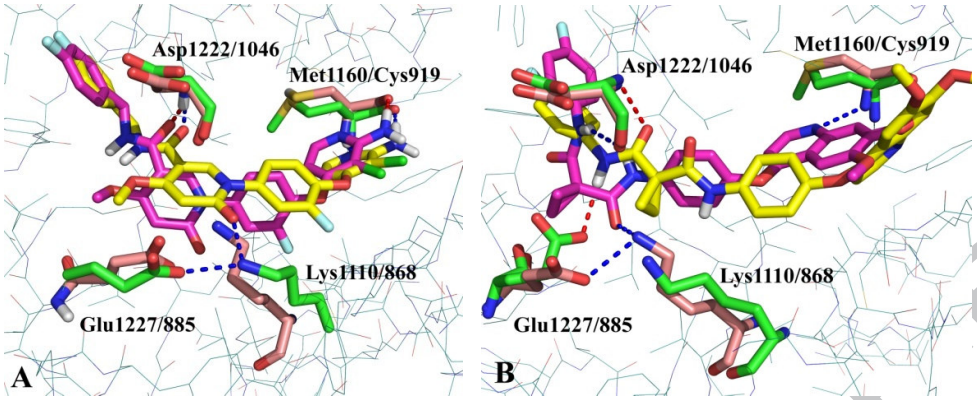
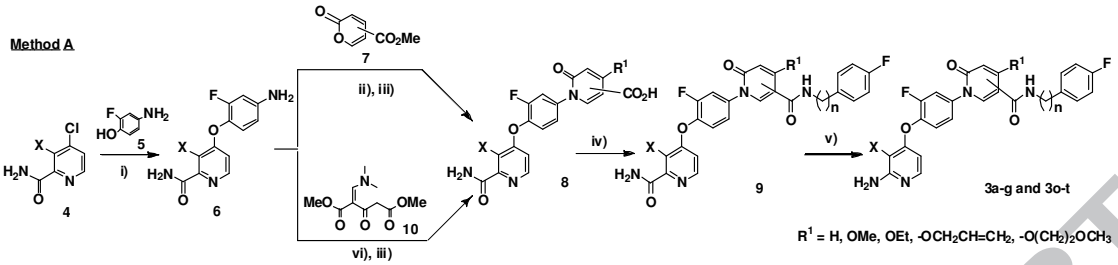
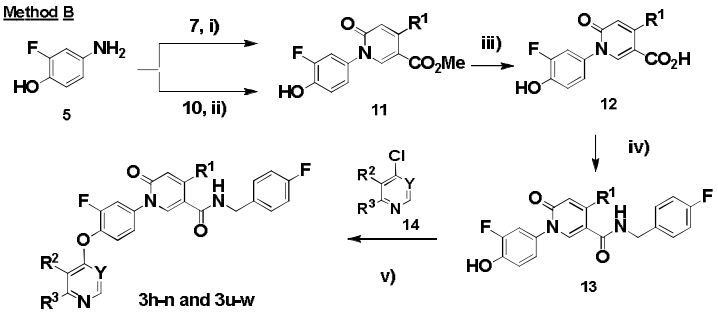


Figure 3. The overlay of the binding model of compounds **3o** (A) and **1** (B) with MET and VEGFR-2 kinases, respectively. The yellow and green stick represent MET complex. The magenta and lightpink stick represent VEGFR-2 complex. The first number of residue represents in MET kinase and the second number refers to in VEGFR-2 kinase.

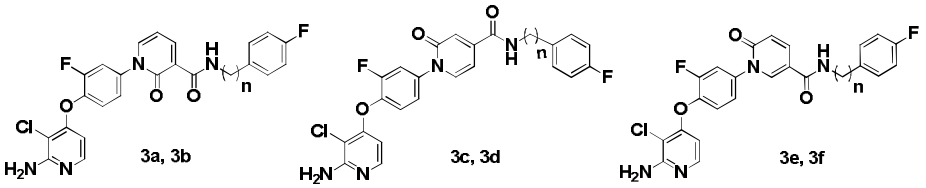


Scheme 1. Reagent and conditions: i) *t*-BuOK, DMF, 50°C for 6h, 76-82%; ii) DMAP, Molecular sieves, MeOH, 60°C for 2h, 81-85%; iii) NaOH, MeOH, H_2O, 50°C for 2h, 86-92%; iv) BtOH, EDCI, DMAP, DCM, 25°C for 12h, 54-85%; v) $PhI(OAc)_2$, EA, DCM, H_2O, 0°C for 2h, 65-83%; vi) EtOH, 70°C for 2h, 86-90%.



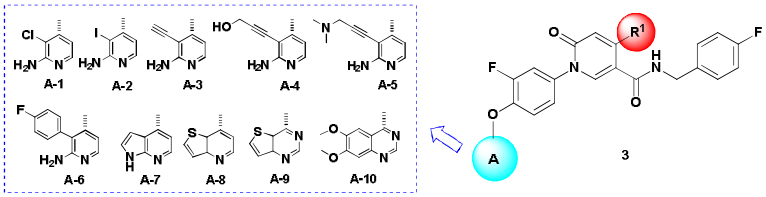
Scheme 2. Reagent and conditions: i) DMAP, Molecular sieves, MeOH, 60°C for 2h, 81% ii) EtOH, 70°C for 2h, 85%; iii) NaOH, MeOH, H_2O, 50°C for 2h, 88-95%; iv) BtOH, EDCI, DMAP, DCM, 25°C for 12h, 62-78%; v) t-BuOK, DMF, 50°C for 1-12h, 65-87%.

Table 1 MET and VEGFR-2 inhibitory activity of compounds **3a–f**



Compds	n	IC_{50} (μM)	
		MET	VEGFR-2
3a	0	> 25	-
3b	1	> 25	-
3c	0	> 25	-
3d	1	> 25	-
3e	0	> 25	-
3f	1	0.85	>25

Table 2 MET inhibitory activity of compounds **3g–w**



Compds	block A	R^1	IC_{50} (μM)
3g	A-2	H	0.39
3h	A-3	H	0.46
3i	A-4	H	0.28
3j	A-5	H	0.43
3k	A-7	H	1.23
3l	A-8	H	0.21
3m	A-9	H	0.57
3n	A-10	H	0.24
3o	A-1	OMe	0.045
3p	A-1	OEt	0.55
3q	A-1	$-OCH_2CH=CH_2$	0.28
3r	A-1	$-O(CH_2)_2OCH_3$	0.34
3s	A-2	OMe	0.005
3t	A-2	$-O(CH_2)_2OCH_3$	0.15
3u	A-4	OMe	0.099
3v	A-5	OMe	0.21
3w	A-6	OMe	0.12
	1 (cabozantinib)		0.008

Table 3 Kinase selectivity of compounds **3o** and **3s**

N.O.	Enzyme, IC_{50}, μM			
	MET	VEGFR-2	c-Kit	RET
3o	0.045	>25	>25	>25
3s	0.005	>25	>25	>25
1 (cabozantinib)	0.008	0.007	0.004	0.016

Graphical Abstract

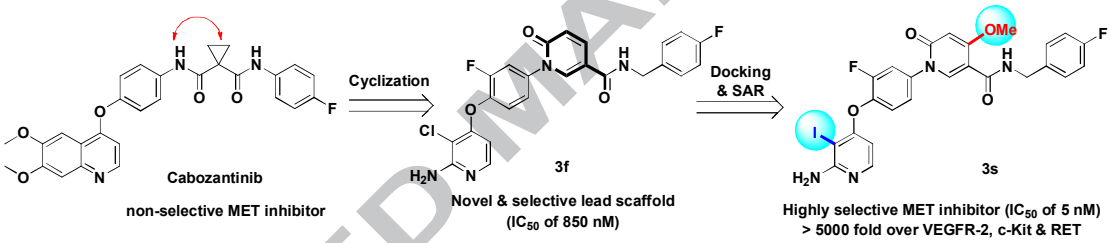
Design, Synthesis and Evaluation of Highly Selective Pyridone-based Class II MET Inhibitors

Nengfang She[a], Linsheng Zhuo[a], Wen Jiang[a], Xiaolei Zhu[a], Jia Wang[b], Zhihui Ming[b], Xinge Zhao[b], Xin Cong[b] and Wei Huang[a,c,*]

[a]*Key Laboratory of Pesticide and Chemical Biology, Ministry of Education, College of Chemistry, Central China Normal University, Wuhan, 430079, P.R.China*

[b]*Jiangsu Key Laboratory of Molecular Targeted Antitumor Drug Research, Jiangsu Simcere Pharmaceutical Co. Ltd, Nanjing 210042, P.R.China*

[c]*State Key Laboratory of Agricultural Microbiology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan* 430070, *China*



A novel series of class II MET inhibitors has been designed that utilize 2-pyridone to conformationally restrain key pharmacophoric groups within the molecule via a scaffold-hopping strategy. Integrated molecular docking and SAR studies resulted in the discovery of a novel class of pyridone MET inhibitor with high potency (IC_{50} of 0.005 μM) and efficient selectivity (> 5000 fold) to VEGFR-2, c-Kit and RET kinase

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