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Design, synthesis and biological evaluation of 4-Aminopyrimidine-5-cabaldehyde oximes as dual inhibitors of c-Met and VEGFR-2

Hao Qiang[a], Weijie Gu[a], DanDan Huang[a,b] ,Wei Shi[a] , Qianqian Qiu[a], Yuxuan Dai[a], Wenlong Huang[a, c]*, Hai Qian[a, c*]

[a] *Center of Drug Discovery, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China.*

[b] *Department of Medicinal Chemistry, Nanjing Sanhome Pharmaceutical Co.,Ltd, Nanjing 210018, PR China*

[c] *Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China*

Abstract: The synergistically collaboration of c-Met/HGF and VEGFR-2/VEGF leads to development of tumor angiogenesis and progression of various human cancers. Therefore, inhibiting both HGF/c-Met and VEGF/VEGFR signaling may provide a novel and effective therapeutic approach for treating patients with abroad spectrum of tumors. Toward this goal, we designed and synthesized a series of derivatives bearing 4-Aminopyrimidine-5-cabaldehyde oxime scaffold as potent dual inhibitors of c-Met and VEGFR2. The cell proliferation assay *in vitro* demonstrated most target compounds have inhibition potency both on c-Met and VEGFR2 with IC_{50} values in nanomolar range, especially compound **14i**, **18a** and **18b**. Based on the further enzyme assay *in vitro*, compound **18a** was considered as the most potent one, the IC50s of which were 210nM and 170nM for c-Met and VEGFR2, respectively. Following that, we docked the compound **10** and **18a** with the proteins c-Met and VEGFR-2, and interpreted the SAR of these analogues. , All the results indicate that **18a** is a dual inhibitors of c-Met and VEGFR2 that holds promising potential.

Key words: Oxime; c-Met; VEGFR-2; synergistically collaborate; cancers

1. Introduction

* Co-corresponding authors: Wenlong Huang and Hai Qian, Center of Drug Discovery, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China. Tel: +86-25-83271302; Fax: +86-25-83271480.
E-mail: ydhuangwenlong@126.com (W.l. Huang), qianhai24@163.com (H. Qian)

Receptor tyrosine kinases (RTKs) play an important role in the regulation of intracellular signal transduction. Aberrant RTKs signaling leads to broad spectrum of tumor.[1] RTKs have become important targets for cancer therapy, and more and more RTKs inhibitors have been designed and synthesized.

The c-Met receptor tyrosine kinase and its natural ligand (HGF) are involved in embryonic development, wound healing, and oncogenesis. c-Met or HGF overexpression, metagenomic amplification, or c-Met mutations leads to aberrant expression of c-Met/HGF signal pathway which induces proliferation, invasion, metastasis, tumor angiogenesis, and drug resistance of cancer cell in broad spectrum of tumor.[2,3] Thus, inhibition of c-Met signaling has been considered as an effective way for cancer therapy. c-Met TKIs for treatment of cancer patients have been developed and evaluated in clinical trials.[3-5] On the basis of action of c-Met and its inhibitor,[6-9] the c-Met TKIs have been classified to type I and type II TKIs. Type II c-Met inhibitors can interrupt c-Met and VEGFR-2 or other kinase signaling pathways[6, 9-12].

In addition, vascular endothelial growth factor receptor 2 (VEGFR2) is a tyrosine kinase receptor expressed in endothelial cells. Once VEGF binding to VEGFR, a conformational change in VEGFR occurred, followed by dimerization and phosphorylation of tyrosine kinase receptor. The VEGF/VEGFR2 signaling pathway is believed to be a major driver of tumor angiogenesis, which supplies oxygen and nutrients to promote tumor growth and plays important roles in tumor malignancy (such as sustaining tumor growth) and in blood-borne metastasis.[7,13-17] Its overexpression has been reported to correlate with the degree of vascularity, aggressive disease, and poor prognosis in the majority of human solid tumors, making the VEGF/VEGFR axis an attractive molecular target for cancer therapy.

Based on the above-mentioned findings, we believe that the synergistically collaboration of c-Met and VEGFR-2 promotes development of angiogenesis and progression of various human cancers[11, 15, 18]. Therefore, molecules that dually inhibit c-Met and VEGFR-2 may have broader advantages over either c-Met- or VEGFR-2 selective inhibitor, since they can inhibit multiple signaling pathways involved in tumor angiogenesis, proliferation, and metastasis. Thus, the dual inhibitors interrupt c-Met and VEGFR-2 signaling pathways represent a promising approach to cancer treatment[19]. Therefore, we initiate synthetic studies of dual inhibitor of c-Met and VEGFR-2 with potent antitumor efficacy.

The lead compound with potent antitumor efficacy was designed by structure-based drug design. At first, we analyzed an X-ray crystal structure of c-Met in complex with the type II c-Met TKIs N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N-(2-phenylacetyl)thiourea(**Kirin Brewery**). The crystal structure indicates that the c-Met protein includes a lipophilic back pocket region and adopts an inactive DFG-out conformation, which is the binding mode of type II c-Met TKIs (PDB code: **3VW8**). The scaffold occupies hinge region which can combine with ATP. These backgrounds lead us to design the type-II dual inhibitors with potent antitumor efficacy by using series scaffolds of VEGFR-2 inhibitors. Compound **I** (in Figure **1**), including quinazoline scaffold, has been reported only as potent VEGFR-2 inhibitor[20] , quinazoline can bound to hinge region by hydrogen bond. 4-aminopyrimidine-5-carbaldehyde oxime is considered as isosteric structure of quinazoline since the intramolecular hydrogen bond forming a pseudo six-membered ring (**II** in Figure **1**). A series of VEGFR-2 inhibitors with 4-aminopyrimidine-5-carbaldehyde oxime have been described, exemplified by compound **II** and **III** (**JNJ-38158471**).[21] Cabozantinib[22](in Figure **1**) and Foretinib[23](in Figure **1**), compounds for treatment of medullary thyroid cancer, were previously reported to inhibit c-Met and VEGFR-2 simultaneously.[24] The difference biological profiles of these compounds indicated that the cyclopropane-1,1-dicarboxamide moiety might be critical for the potent dual inhibition of c-Met and VEGFR-2.[25] These research in this area inspired the design of first dual inhibitor **10** (in **Figure1)**, which was a split structure containing both the cyclopropane-1, 1-dicarboxamide moiety and the 4-aminopyrimidine-5-carbaldehyde oxime for inhibiting c-Met and VEGFR-2. The total compounds contain solvent region, scaffold and side chain region. We preliminary studied the SAR of solvent region and scaffold.

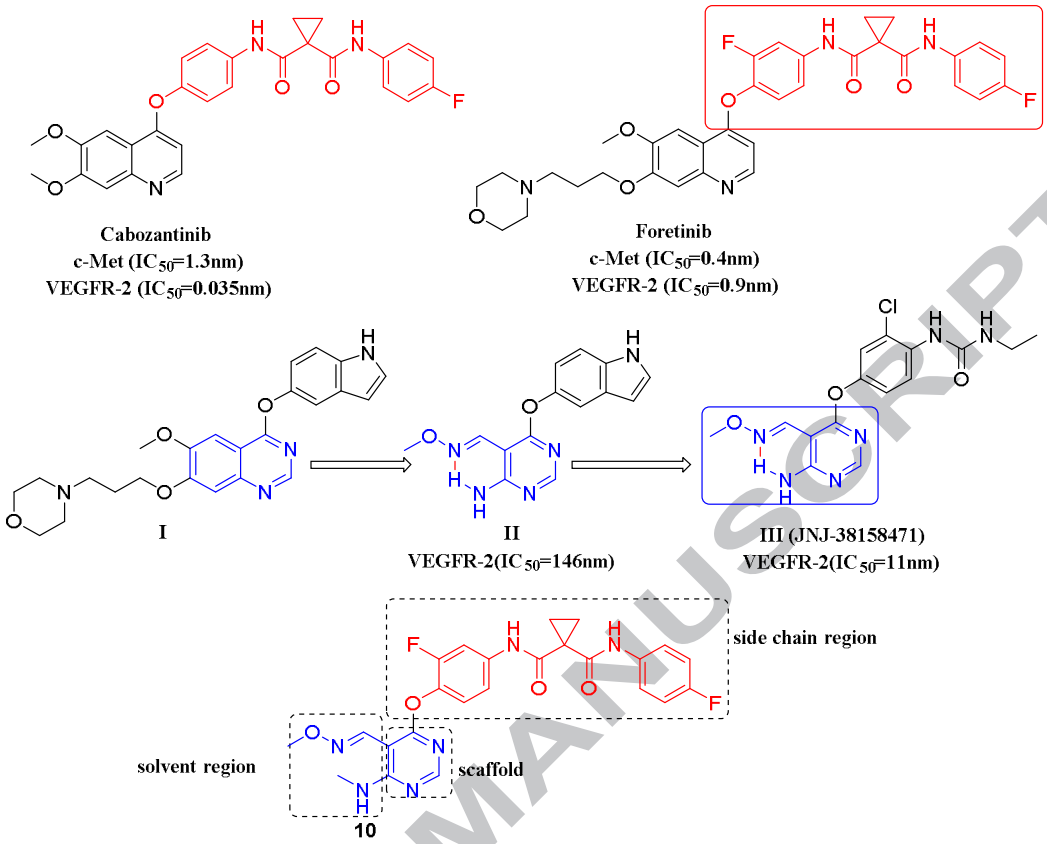
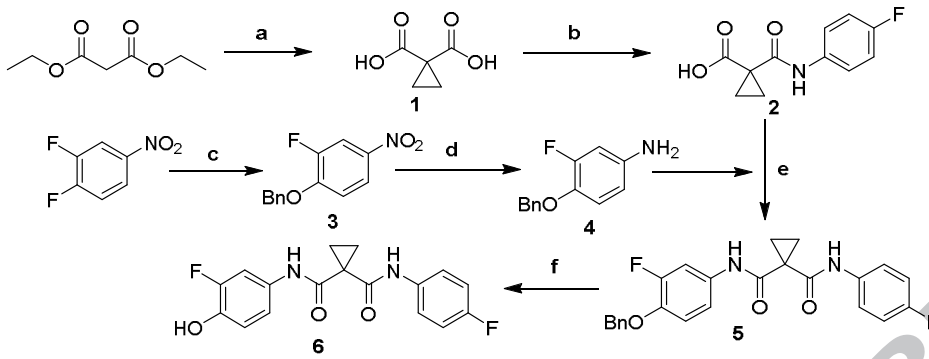


Figure 1. C-Met and/or VEGFR-2 dual inhibitor and the design of novel inhibitor for both c-Met and VEGFR-2 kinase

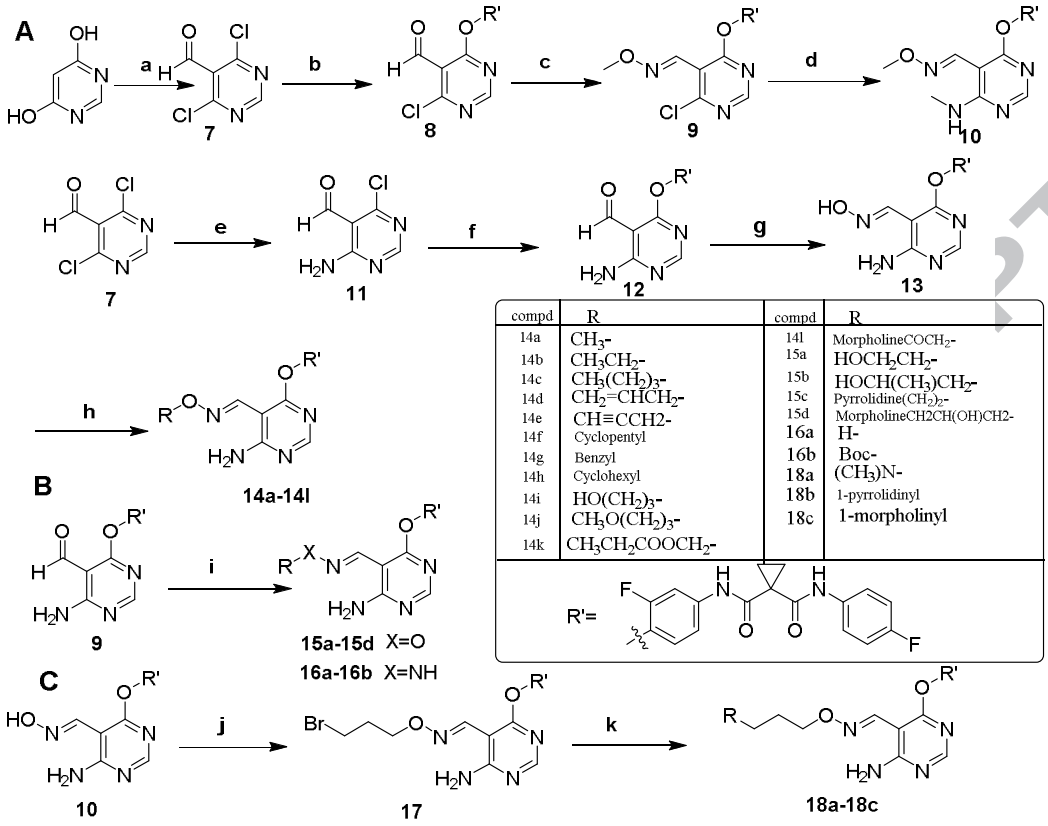
2. Chemistry

The compound **1** was prepared by condensation of diethyl malonate and 1, 2-dibromoethane under 50% sodium hydroxide solution. The compound **1** was coupled with equal equivalence 4-fluoroaniline by utilizing sulfoxide chloride and triethylamine to obtain **2**. On the other way, **3** was synthesized by benzyl alcohol and 3, 4-difluoronitrobenzene under basic condition at reflux temperature. The amine of **4** was prepared by reduction of the nitro of **3**. The compound **5** was prepared by condensation of **2** and **4** under basic condition at room temperature. Under the hydrogen atmosphere, the benzyl of **5** had been removed by hydrogenolysis.



Scheme 1. **Reagents and conditions:** a. Dibromoethane, TEBA, NaOH/H_2O, rt, 3h; b. 4-Fluoroaniline, $SOCl_2$, Et_3N, THF, 0 ^{0}C -rt, 3h; c. Benzyl alcohol, KOH, CH_3CN, rt, overnight; d. iron powder, ammonium formate, Toluene/H_2O, reflux, 8h; e. compd. **2** and **4**, $SOCl_2$, Et_3N, THF, 0 ^{0}C, overnight; f. Pd/C, H_2, THF/MeOH, rt, 7h.

The synthesis of 4-aminopyrimidine-5-carbaldehyde oxime derivatives **10, 13**, **14a-14i**, **15a-15d, 16a-16b, 18a-18c** were shown in **Scheme 2.** The compound **7** was synthesized by vilsmeier reaction, the material 4, 6-dihydroxypyrimidine was heated with $POCl_3$ and DMF, then react with **6** under basic condition at reflux temperature to obtain compound **8** which can be condensed in polar solvent to obtain **9**. The **6-Cl** of compound **9** was replaced by methylamine to obtain the first inhibitor **10**. On the other synthetic way, the compound **11** could be prepared by mono-substituted reaction with the materials 7 and ammonia (2M solution in methanol), then, the target compound **12** were obtained by a condensation reaction of **11** and **6**. Compound **13** were synthesized by utilizing hydroxylamine hydrochloride with compound 12. The target compounds **14a-14l** were prepared by condensation reaction of the compound **13** and a series of bromides. To obtain **15a-15d, 16a** and **16b,** we utilize compound **9** and series O-instead hydroxylamine or series substituted hydrazine. The reaction of **10** with 1, 3-dibromopropane could produce compound **17** which could react with series primary amine to obtain **18a-18c**.



compd	R	compd	R
14a	CH_3-	14l	$MorpholineCOCH_2$-
14b	CH_3CH_2-	15a	$HOCH_2CH_2$-
14c	$CH_3(CH_2)_3$-	15b	$HOCH(CH_3)CH_2$-
14d	$CH_2=CHCH_2$-	15c	$Pyrrolidine(CH_2)_2$-
14e	CH≡CCH2-	15d	MorpholineCH2CH(OH)CH2-
14f	Cyclopentyl	16a	H-
14g	Benzyl	16b	Boc-
14h	Cyclohexyl	18a	$(CH_3)N$-
14i	$HO(CH_2)_3$-	18b	1-pyrrolidinyl
14j	$CH_3O(CH_2)_3$-	18c	1-morpholinyl
14k	$CH_3CH_2COOCH_2$-		

Scheme 2. Reagents and conditions: a. $POCl_3$, DMF, 0^0C; 130^0C, 5h; b. compd **6**, DIEA, EA, 2h; c. Methoxyamine hydrochloride, DMSO; d. Methylamine hydrochloride, K_2CO_3, e. Toluene, NH_3 in MeOH, rt; f. compd **6**, K_2CO_3, DMF, rt, 3h; g. hydroxylamine hydrochloride, DMSO, 2h; h. RBr, K_2CO_3, DMF; DMF i. R-O-NH_2.HCl, DMSO, overnight or R-NH-NH_2, EtOH, overnight; j. 1,3-Dibromopropane, K_2CO_3, DMF; k. R_1R_2NH, K_2CO_3, DMF.

3. Results and discussion

3.1 Cell proliferation inhibition and SAR study of target compounds

The inhibitory activities of the compounds prepared against c-Met and VEGFR2 kinases were determined by the elisa assay using anti-phosphotyrosine antibodies. The effects of the compounds on cell proliferation were evaluated using BaF_3-TPR-Met cells, in which the c-Met-dependent cell line, and in VEGF-stimulated human umbilical vein endothelial cells (HUVEC).

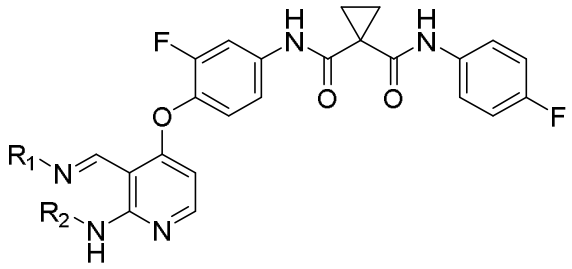
At first, we attempted to dock the first dual inhibitor **10** with the c-Met crystal structure derived from the complex of c-Met and cabozantinib (PDB code: **3LQ8**)[23] and then with the VEGFR-2 crystal structure (PDB code: **3U6J**)[26] by MOE v2008.10. We found that the pyrimidine of 4-aminopyrimidine-5-carbaldehyde oxime could bound to the pocket of ATP in c-Met and

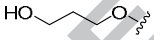
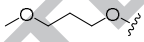
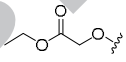
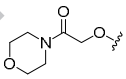
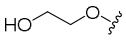
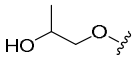
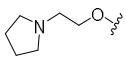
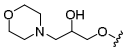
VEGFR-2 receptor proteins by molecular docking. Met_{1160} of c-Met hinge region and Cys_{919} of VEGFR-2 hinge region can form hydrogen bonds with nitrogen-atoms of pyrimidine as shown in **Figure 2A (green lines**). However, the compound **10** could not be stabilized in the binding pocket of c-Met and VEGFR-2 because the loss of hydrogen bonds between 4-amino and residue Met_{1160} of c-Met and Cys_{919} of VEGFR-2 (**Figure 2A**). The free 4-amino of scaffold can bound to Met_{1160} and Cys_{919} by second hydrogen bonds as shown in **Figure 2B(red lines)**. Therefore, the candidate **18a** may have potent inhibitory.

As shown in **Table 1**, the 4-substituted amino derivative **10** was inactive against c-Met kinase and VEGFR-2 kinase (IC_{50}>10000nM). On the other hand, the unsubstituted derivative **18a** was found to display both c-Met (IC_{50}=194nM) and VEGFR-2(IC_{50}=260nM) (in **Table 1**) inhibitory activities, as suggested by docking model studies shown potent inhibitory activity, as suggested by the docking model studies.

Next, we focused on the modification of the substituent groups of scaffold to further enhance c-Met/VEGFR2 kinase inhibitory activity. At first, the alkanes (**14a-14c**), olefins/alkynes (**14d**, **14e**), naphthenic groups (**14f**, **14h**) and benzyls (**14g**) were introduced to the solvent region. The increase of carbon atoms of solvent region leads to the increase of Log P and weaks the inhibitory activity for BaF_3-TPR-Met. Comparing the hydroxypropyl substituted compound (**14i**), the hydroxyethyl substituted compound (**15a**) has poor activities. Because the methylation of hydroxyl of compound **14j**, the activity down from 0.11μM to 0.24μM. The compound **14k** was inactive for BaF3-TPR-Met since the introduction of ester group. The introduction of the fragment of formylmorpholine generated general activity (**14l**). For reducing Log P, we introduced the fragments of tertiary in solvent area which lead to the increase of activities (**15c, 18a-18c**). However, the length of carbon chain has little effect on the activity. Dimethyl amino substituted compound **18a**(c-Met IC_{50}=89nm; VEGFR-2 IC_{50}=190nm) is superior to pyrrolidine substituted (**15c, 18c**) and morpholine ring substituted (**18b**) as shown in **Table 1**. When oxime was replaced by pyridine-hydrazone, the activity **was reduced** significantly as shown in **Table 1**(**16a**, **16b**).

Table 1: Structures, Log Ps and IC_{50}s of target compounds against BaF3-TRP-Met *in vitro*



Compd.	R_1	R_2	IC_{50} (μmol/L)[a]	Log P[b]
10	CH_3O	CH_3	>1	3.52
13	HO	H	0.194 ±0.031	2.62
14a	CH_3O	H	0.166 ±0.038	3.04
14b	CH_3CH_2O	H	0.215±0.012	3.41
14c	$CH_3(CH_2)_2CH_2O$	H	0.626±0.024	4.20
14d	CH_2=$CHCH_2O$	H	0.619±0.025	3.65
14e	$CH{\equiv}CCH_2O$	H	0.521±0.041	3.34
14f		H	0.745±0.016	4.27
14g	PhCH2O	H	0.566±0.060	4.45
14h		H	0.879±0.020	4.69
14i		H	0.110±0.018[a]	2.67
14j		H	0.243±0.017	3.18
14k		H	>1	3.15
14l		H	0.397±0.031	2.10
15a		H	0.200±0.018	2.37
15b		H	0.258±0.095	2.75
15c		H	0.162±0.017	3.50
15d		H	0.158±0.036	2.02
16a	NH_2	H	0.765±0.046	2.25
16b	BocNH-	H	0.321±0.028	3.70

18a	H	0.089±0.005[a]	3.23
18b	H	0.119±0.058	3.82
18c	H	0.186±0.074	2.98
Foretinib[c]		0.0092±0.00097	4.28
Cabozantinib[c]		0.0219±0.00282	4.28
Cabozantinib L-Malate[c]		0.01986±0.00251	NT

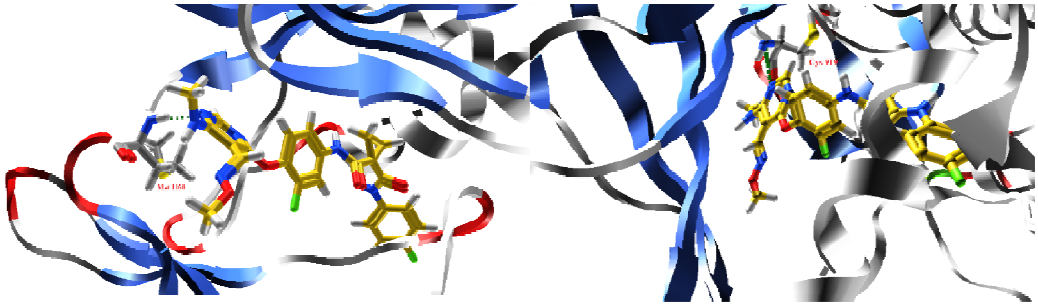
NT, not tested

[a] Data presented is the mean ± SD value of three independent determinations.

[b] LogP values were determined by shake-flask procedure.

[c] Used as positive control.

A



B

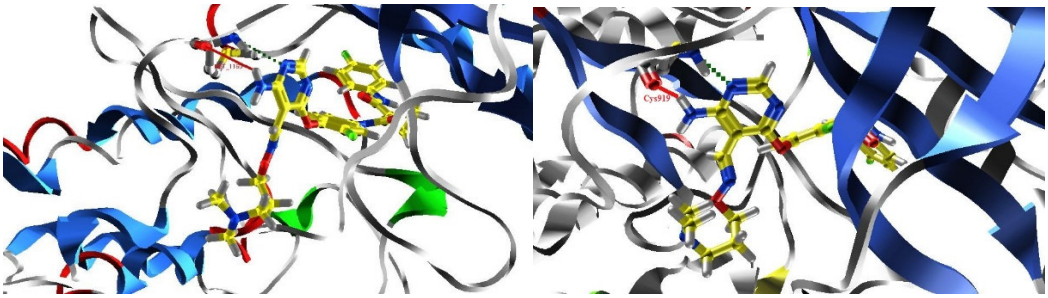


Figure 2.A. the model of **10** bound to c-Met (PDB code: **3LQ8**) and VEGFR-2(PDB code: **3U6J**); B. the model of **18a** with c-Met (PDB code: **3LQ8**) and VEGFR-2(PDB code: **3U6J**)

3.2 Enzymatic activities of optimized compounds

After the determination of the compounds on cell proliferation on BaF_3-TRP-Met and HUVEC, the compounds **13, 14a, 14i, 15c, 15d, 18a, 18b, 18c** were assayed with the enzymatic

activities against c-Met and VEGFR-2 by ELISA, using Cabozantinib[22] and Foretinib[23] as comparisons (**Table 2**). The compound **18a** displays a better enzymatic potency than other compound on c-Met and VEGFR-2 kinase.

Table 2: VEGFR-2 kinase activity of selected compounds and enzymatic activities in vitro

Compd.	HUVEC	c-Met	VEGFR-2
	IC_{50}(μmol/L)[a]	IC_{50}(μmol/L)[a]	IC_{50}(μmol/L)[a]
10	ND	ND	ND
13	0.260±0.072	0.270±0.055	0.140±0.047
14a	0.320±0.21	0.380±0.005	0.120±0.058
14i	0.210±0.18	0.280±0.024	0.560±0.060
15c	0.168±0.116	0.230±0.073	0.250±0.063
15d	0.720±0.20	0.980±0.074	0.890±0.016
18a	0.110±0.005[a]	0.210±0.030	0.170±0.055
18b	0.219±0.014	0.920±0.017	0.340±0.062
18c	0.427±0.026	0.420±0.21	0.220±0.035
Foretinib[b]	0.017±0.016	0.0032±0.29	ND
Cabozantinib[b]	ND	0.0032±0.40	0.003±0.2[a]
Cabozantinib L-Malate[b]	0.045±0.39	ND	ND

ND, not determined

[a] Data presented is the mean ± SD value of three independent determinations.

[b] Used as positive control.

4. Conclusion

In conclusion, novel dual c-Met/VEGFR-2 receptor tyrosine kinase inhibitors based the 4-aminopyrimidine-5-carbaldehyde oxime scaffold were designed and synthesized. The derivatives **13, 14a, 14i, 15c, 15d, 18a, 18b, 18c** contain different substituted oxime ether on 5-position of 4-aminopyrimidine-5-carbaldehyde oxime scaffold have preferable activity. Compound **18a** with the tertiary amino fragment exhibits potent activities in cell proliferation and enzyme assays *in vitro*. Derivative **10** has low inhibition at 1μM concentration, that indicates free 6-amino of pyrimidine ring is essential groups for inhibitory activities. The introduction of

pyrimidine hydrazone on pyrimidine ring weaken c-Met/VEGFR-2 inhibitory activities since the 5-oxime ether is essential for activities. Our findings indicated that compound **18a** could be a lead molecule for the further research of dual inhibitor of c-Met and VEGFR2.

5. Experimental section

5.1 Chemistry

Melting points were determined on RY-1 melting point apparatus, and were not corrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Bruker ACF-300/500 MHz instruments. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd , doublet of doublet; ddd , doublet of doublet of doublets; br, broad singlet; m, multiplet. Coupling constants (J values) are given in hertz (Hz).Mass spectra(MS) were acquired using an Waters UPLC/MS(ACQUITY UPLC/TQD (Waters® UPLC® with the ACQUITY® TQD [Waters Corporation, Milford, MA, USA])) operating in electron spray ionization mode (ESI+) and were used to confirm the purity of each compound. TLCs and preparative TLC were performed on silica gel GF/UV 254. Purifications by column chromatography were carried out over silica gel (200–300 mesh) and visualized under UV light at 254 and 365 nm. Reagents and solvents were obtained from commercial sources and used without further purification.

The physical characteristics, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis data for all intermediates and target compounds, were reported in the supporting information.

5.2 Cell Proliferation Assay

BaF3-TRP-Met and HUVECs were seeded in 96-well plates in RPMI1640 medium with 10% fetal bovine serum (FBS) and human endothelial serum free medium (Invitrogen), respectively. The following day, serial dilutions of compounds or 0.1% DMSO wereadded to each well. BaF3-TRP-Met were then incubated for further 72 h.Proliferation of HUVECs was stimulated with 60 ng/mL recombinant human VEGF (R&D Systems, Minneapolis, MN) for 120 h. After incubation, cell proliferation was determined using Cell Counting Kit-8 (DOJINDO Laboratories,

Kumamoto, Japan). IC_{50} values werecalculated by nonlinear regression analysis using GraphPad Prism

(GraphPad Software, Inc.).

5.3 *In vitro* c-Met and VEGFR2 enzyme assay

The effects of the compounds on the activities of two tyrosine kinases were determined using enzyme-linked immunosorbent assays (ELISAs) with purified recombinant proteins. Briefly, 20 μg/mL poly (Glu,Tyr)4:1 (Sigma) was pre-coated in 96-well plates as a substrate. A 50 μL aliquot of 10 μmol/L ATP solution diluted in kinase reaction buffer (50 mmol/L HEPES [pH 7.4], 50 mmol/L $MgCl_2$, 0.5 mmol/L $MnCl_2$, 0.2 mmol/L Na_3VO_4, and 1 mmol/L DTT) was added to each well; 1 μL of various concentrations of indicated compounds diluted in 1% DMSO (v/v) (Sigma) were then added to each reaction well. DMSO (1%, v/v) was used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 49μL of kinase reaction buffer. After incubation for 60 min at 37 °C, the plate was washed three times with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). Anti-phosphotyrosine (PY_{99}) antibody was then added. After a 30-min incubation at 37 °C, the plate was washed three times, and horseradish peroxidase-conjugated goat anti-mouse IgG was added. The plate was then incubated at 37 °C for 30 min and washed 3 times. A 100 μL aliquot of a solution containing 0.03% H_2O_2 and 2 mg/ml o-phenylenediamine in 0.1 mol/L citrate buffer (pH5.5) was added. The reaction was terminated by the addition of 50 μL of 2 mol/L H_2SO_4 as the color changed, and the plate was analyzed using a multi-well spectrophotometer (SpectraMAX 190, Molecular Devices) at 490 nm. The inhibition rate (%) was calculated using the following equation: [1-(A490/A490 control)] × 100%. The IC_{50} values were calculated from the inhibition curves in two separate experiments.

5.4 Molecular modeling

Docking simulations were performed using the Molecular Operating Environment (MOE) (The Chemical Computing Group, Montreal, Canada). The crystal structure of c-Met crystal structure(PDB code: **3LQ8**)[23] and VEGFR-2 crystal struture(PDB code: **3U6J**)[26] was downloaded from the Protein Data Bank (PDB). Prior to ligand docking, hydrogens were added and the

crystallized ligand was removed. Subsequently, the structure was prepared with Protonate 3D, and the active site was isolated using MOE Site Finder. The structures were placed in the site with the Triangle Matcher method and then ranked with the London dG scoring function.For the energy minimization in the pocket, MOE Force Field Refinement was used and ranked with the GBVI/WSA dG scoring function.

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References:

[1] Awazu, Y.; Nakamura, K.; Mizutani, A.; Kakoi, Y.; Iwata, H.; Yamasaki, S.; Miyamoto, N.; Imamura, S.; Miki, H.; Hori, A. *Molecular cancer therapeutics* **2013,** *12*, 913.

[2] Gherardi, E.; Birchmeier, W.; Birchmeier, C.; Woude, G. V. *Nature Reviews Cancer* **2012,** *12*, 89.

[3] Cecchi, F.; Rabe, D. C.; Bottaro, D. P. *European journal of cancer* **2010,** *46*, 1260.

[4] Comoglio, P. M.; Giordano, S.; Trusolino, L. *Nature reviews Drug discovery* **2008,** *7*, 504.

[5] Eder, J. P.; Woude, G. F. V.; Boerner, S. A.; LoRusso, P. M. *Clinical Cancer Research* **2009,** *15*, 2207.

[6] Underiner, T. L.; Herbertz, T.; Miknyoczki, S. J. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)* **2010,** *10*, 7.

[7] Folkman, J. Role of angiogenesis in tumor growth and metastasis; Elsevier, 2002;

[8] Fujiwara, Y.; Senga, T.; Nishitoba, T.; Osawa, T.; Miwa, A.; Nakamura, K.; Google Patents, 2008.

[9] Saavedra, O.; Claridge, S.; Zhan, L.; Raeppel, F.; Granger, M.-C.; Raeppel, S.; Mannion, M.; Gaudette, F.; Zhou, N.; Isakovic, L. *Bioorganic & medicinal chemistry letters* **2009,** *19*, 6836.

[10] Schiering, N.; Knapp, S.; Marconi, M.; Flocco, M. M.; Cui, J.; Perego, R.; Rusconi, L.; Cristiani, C. *Proceedings of the National Academy of Sciences* **2003,** *100*, 12654.

[11] Claridge, S.; Raeppel, F.; Granger, M.-C.; Bernstein, N.; Saavedra, O.; Zhan, L.; Llewellyn, D.; Wahhab, A.; Deziel, R.; Rahil, J. *Bioorganic & medicinal chemistry letters* **2008,** *18*, 2793.

[12] Raeppel, S.; Claridge, S.; Saavedra, O.; Gaudette, F.; Zhan, L.; Mannion, M.; Zhou, N.; Raeppel, F.; Granger, M.-C.; Isakovic, L. *Bioorganic & medicinal chemistry letters* **2009,** *19*, 1323.

[13] Des Guetz, G.; Uzzan, B.; Nicolas, P.; Cucherat, M.; Morere, J.; Benamouzig, R.; Breau, J.; Perret, G. *British journal of cancer* **2006,** *94*, 1823.

[14] Ferrara, N. *Nature Reviews Cancer* **2002,** *2*, 795.

[15] Ferrara, N.; Gerber, H.-P.; LeCouter, J. *Nature medicine* **2003,** *9*, 669.

[16] Olsson, A.-K.; Dimberg, A.; Kreuger, J.; Claesson-Welsh, L. *Nature reviews Molecular cell biology* **2006,** *7*, 359.

[17] Roy, H.; Bhardwaj, S.; Ylä-Herttuala, S. *FEBS letters* **2006,** *580*, 2879.

[18] Mannion, M.; Raeppel, S.; Claridge, S.; Zhou, N.; Saavedra, O.; Isakovic, L.; Zhan, L.; Gaudette, F.; Raeppel, F.; Déziel, R. *Bioorganic & medicinal chemistry letters* **2009,** *19*, 6552.

[19] Kim, K. J.; Li, B.; Winer, J.; Armanini, M.; Gillett, N.; Phillips, H. S.; Ferrara, N. **1993**.

[20] Hennequin, L. F. A.; Google Patents, 2011.

[21] Huang, S.; Li, R.; LaMontagne, K. R.; Greenberger, L. M.; Connolly, P. J. *Bioorganic & medicinal chemistry letters* **2011,** *21*, 1815.

[22] Yakes, F. M.; Chen, J.; Tan, J.; Yamaguchi, K.; Shi, Y.; Yu, P.; Qian, F.; Chu, F.; Bentzien, F.; Cancilla, B. *Molecular cancer therapeutics* **2011,** *10*, 2298.

[23] Qian, F.; Engst, S.; Yamaguchi, K.; Yu, P.; Won, K.-A.; Mock, L.; Lou, T.; Tan, J.; Li, C.; Tam, D. *Cancer research* **2009,** *69*, 8009.

[24] Kurzrock, R.; Sherman, S. I.; Ball, D. W.; Forastiere, A. A.; Cohen, R. B.; Mehra, R.; Pfister, D. G.; Cohen, E. E.; Janisch, L.; Nauling, F. *Journal of Clinical Oncology* **2011,** *29*, 2660.

[25] Zhan, Z.; Ai, J.; Liu, Q.; Ji, Y.; Chen, T.; Xu, Y.; Geng, M.; Duan, W. *ACS medicinal chemistry letters* **2014,** *5*, 673.

[26] Norman, M. H.; Liu, L.; Lee, M.; Xi, N.; Fellows, I.; D'Angelo, N. D.; Dominguez, C.; Rex, K.; Bellon, S. F.; Kim, T.-S. *Journal of medicinal chemistry* **2012,** *55*, 1858.

Design, synthesis and biological activity of 4-Aminopyrimidine-5-cabaldehyde oxime as dual c-Met and VEGFR-2 inhibitor

Hao Qiang[1], DanDan Huang[1,2], Weijie Gu[1], Wei Shi[1], Qianqian Qiu[1], Yuxuan Dai[1], Wenlong Huang[1,3*], Hai Qian[1, 3*]

[1] *Center of Drug Discovery, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China.*

[2] *Department of Medicinal Chemistry, Nanjing Sanhome Pharmaceutical Co.,Ltd, Nanjing 210018, PR China*

[3] *Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China*

The cyclopropane-1, 1-dicarboxamide moiety might be critical for the potent dual inhibition of c-Met and VEGFR-2. 4-Aminopyrimidine-5-cabaldehyde oxime as the scaffold of potent VEGFR-2 inhibitor was combined with the cyclopropane-1,1-dicarboxamide moiety to obtain potent c-Met and VEGFR-2 inhibitor.