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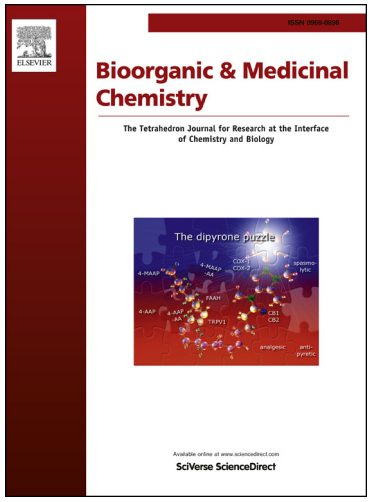

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Design, synthesis, and docking studies of phenylpicolinamide derivatives bearing 1*H*-pyrrolo[2,3-*b*]pyridine moiety as c-Met inhibitors

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Four series of phenylpicolinamide derivatives bearing 1*H*-pyrrolo[2,3-*b*]pyridine moiety (**12a–e, 13a–f, 14a–f** and **15a–i**) were designed, synthesized and evaluated for the IC_{50} values against three cancer cell lines (A549, PC-3 and MCF-7) and c-Met kinase. Five selected compounds (**13b, 15b, 15d, 15e** and **15f**) were further evaluated for the activity against HepG2 and Hela cell lines. Eighteen of the compounds showed excellent cytotoxicity activity and selectivity with the IC_{50} valuables in single-digit μM to nanomole range. Seven of them are equal to more active than positive control Foretinib against one or more cell lines. The most promising compound **15f** showed superior activity to Foretinib, with the IC_{50} values of 1.04 ± 0.11 μM, 0.02 ± 0.21μM and 9.11 ± 0.55 μM against A549, PC-3 and MCF-7 cell lines, which were 0.62 to 19.5 times more active than Foretinib (IC_{50} values: 0.64 ± 0.26 μM, 0.39 ± 0.11 μM, 9.47 ± 0.22 μM) , respectively. Structure–activity relationships (SARs) and docking studies indicated that replacement of quinoline nucleus of the previous active compounds with 1*H*-pyrrolo[2,3-*b*]pyridine moiety maintained even improved the potent cytotoxic activity. The results suggested that the introduction of fluoro atoms to the aminophenoxy part of target compounds or the phenyl group of pyrimidine substituted on C-4 position was benefit for the activity.

Key words: Phenylpicolinamide; 1*H*-pyrrolo[2,3-*b*]pyridine; Synthesis; Docking; c-Met inhibitors; Antitumor activity

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1 Introduction

Cancer is a serious disease that threatens human health and life. There were 14.1 million new cancer cases, 8.2 million deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [1].

c-Met inhibitors are a class of small molecules that inhibit the enzymatic activity of the c-Met tyrosine kinase. These inhibitors may have therapeutic application in the treatment of various types of cancers. Cabozantinib[2], a small molecule that inhibits the activity of multiple tyrosine kinases, including RET, MET, and VEGF receptor 2, was approved by U. S. Food and Drug Administration for the treatment of patients with progressive metastatic medullary thyroid cancer on November 29, 2012[3]. In recent years, many derivatives of Cabozantinib were reported, such as foretinib (GSK1363089) [4], compounds I, II, III[5-6]. The studies revealed that these compounds elicited strong antitumor activity.

In our previous study, we reported a series of quinoline derivatives bearing phenylpicolinamide scaffold as potential c-Met inhibitors [7]. Most of them showed excellent *in vitro* antitumor activity and the structure-activity relationships （SARs） showed that the phenylpicolinamide scaffold is benefit to the activity. In order to investigate the influence of the quinoline nucleus to the activity of this series of compounds, further modifications were carried out in this paper. Firstly, keep the phenylpicolinamide scaffold unchanged, we replaced the quinoline nucleus with 1*H*-pyrrolo[2,3-*b*]pyridine moiety to get compounds **12a**–**e**. However, these compounds showed no activity. Next, we changed the position of the phenyl group on pyrimidine from C-4 to C-5 position to afford **13a**–**f**. These compounds showed excellent cytotoxicity and moderate c-Met kinase activity. What's more, fluoro atom was added to the aminophenoxy part of compounds **12a**–**e** and **13a**–**f** to study the effect to the activity and yielded **14a**–**f** and **15a**–**i**. To our exciting, compounds **15a**–**i** exhibited excellent cytotoxicity and c-Met kinase activity.

Herein we disclosed the synthesis and antitumor activity against A549, PC-3, MCF-7,HepG2 and Hela cancer cell lines, and c-Met kinase of phenylpicolinamide derivatives bearing 1*H*-pyrrolo[2,3-*b*]pyridine moiety. Moreover, docking studies were presented in this paper as well.

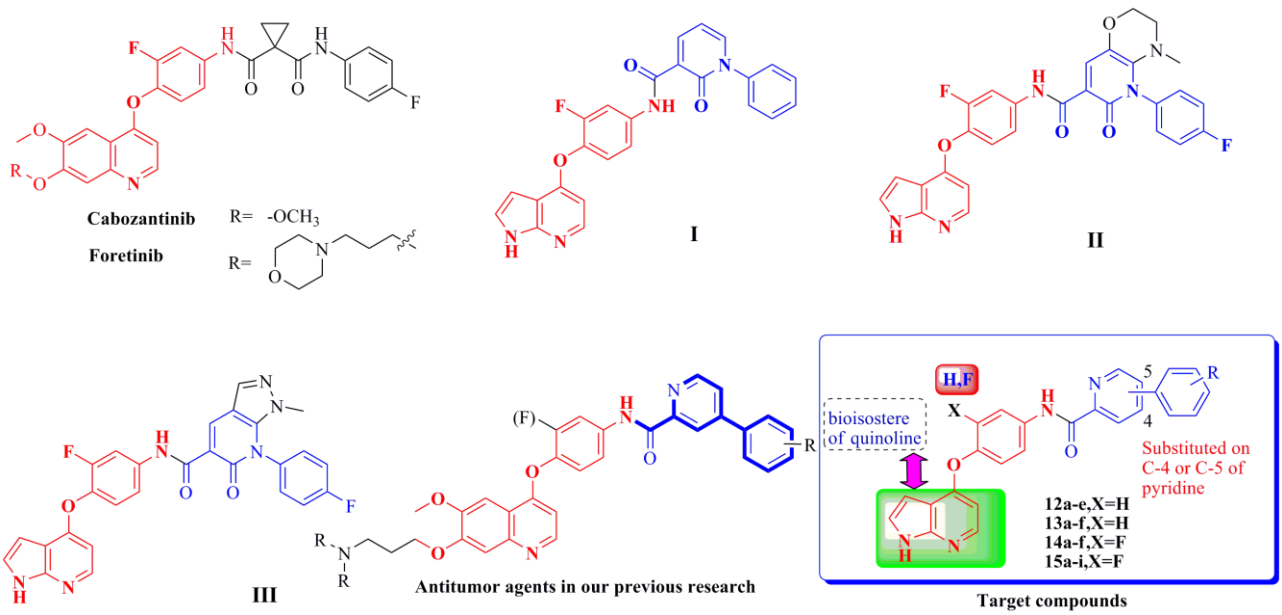


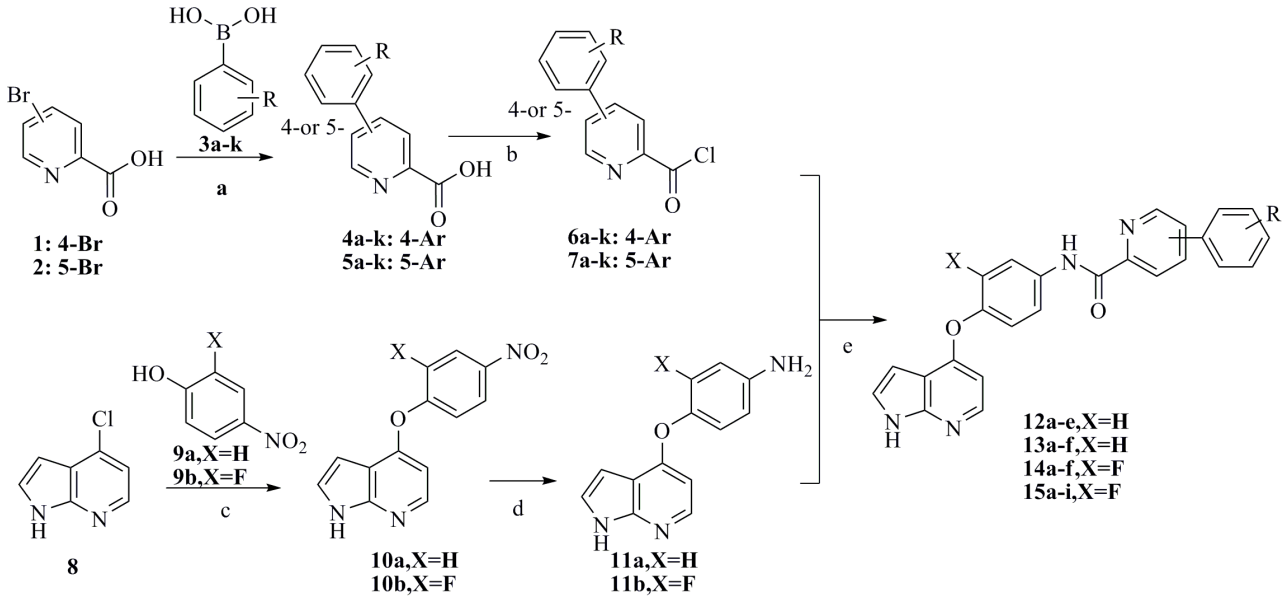
Figure 1 Structures of small-molecule c-Met inhibitors and target compounds.

2. Chemistry

The preparation of target compounds **12a–e, 13a–f, 14a–f** and **15a–i** was described in Scheme 1.

The key intermediates 4-(4-aminophenoxy)-*N*-methylpicolinamide **11a,b** were achieved from 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine **8** *via* substitution reaction with 4-nitrophenol or 2-fluoro-4-nitrophenol **9a,b** and reduction with hydrazine hydrate in sequence as shown in Scheme 1.

4-Bromo-pyridine-carboxylic acid **1** or 5-bromo-pyridine-carboxylic acid **2** was reacted with substituted phenylboronic acids **3a–k** through Suzuki-coupling reaction with bis(triphenylphosphine)palladium(II) dichloride as catalyst to get **4a–k** and **5a–k**, which were then chlorination with oxalyl chloride to obtain **6a–k** and **7a–k**, respectively. Finally, reaction of amides **11a**,**b** with acyl chlorides **6a–k** or **7a–k** promoted by DIPEA in dichloromethane at room temperature yielded target compounds **12a–e, 13a–f, 14a–f** and **15a–i**, respectively.



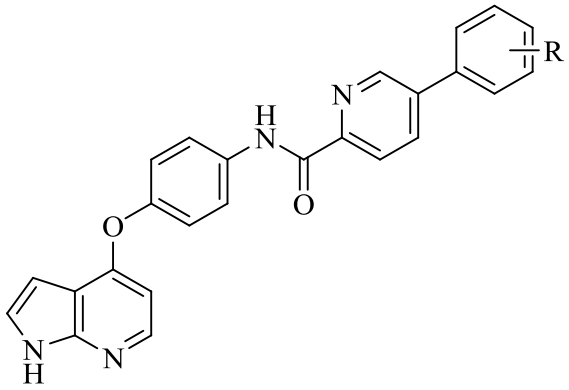
Scheme 1 Synthetic route of target compounds. **Reagents and conditions:** (a) $[(C_6H_5)_3P]_2PdCl_2$, Na_2CO_3, H_2O, 1,4-dioxane, 5-8h; (b) $(COCl)_2$, DMF(cat.), CH_2Cl_2, 0.5h; (c) Phenyl ether, 190℃, 1 h; (d) $N_2H_4{\cdot}H_2O$, $FeCl_3$, activated carbon, EtOH, 10 min; (e) DIPEA, CH_2Cl_2, 0.5h.

3. Results and discussion

3.1 Biological evaluation

Taking c-Met inhibitor Foretinib as reference compound, the target compounds (**12a–e, 13a–f, 14a–f** and **15a–i**) were evaluated for the cytotoxicity against three cancer cell lines A549 (human lung cancer), PC-3 (human prostatic cancer) and MCF-7(human breast cancer) were evaluated by 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) cell proliferation assay. In addition, these compounds were evaluated for the IC_{50} values against c-Met kinase *in vitro* by the Mobility shift assay with ATP concentration at Km, together with reference compound Foretinib. Moreover, five selected compounds (**13b, 15b, 15d, 15e** and **15f**) were further evaluated for the activity against HepG2 and Hela cell lines. The results expressed as inhibition rates or IC_{50} values were summarized in Tables 1–5 and the values are the average of at least two independent experiments.

Table 1 Structures, cytotoxicity and c-Met kinase inhibitory activity of compounds **12a-e**

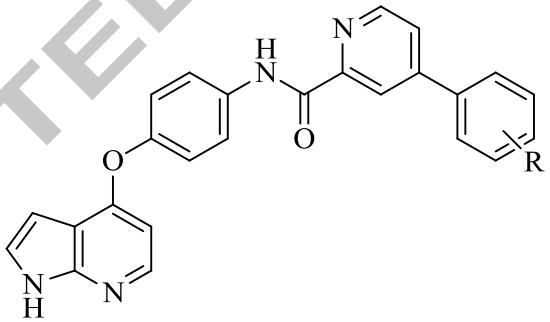


Compd. N.O.	R	IC_{50} (μM)[a]			
		A549	PC-3	MCF-7	c-Met
12a	4-Cl	>10	>10	>10	>10
12b	4-CH_3	>10	>10	>10	7.3
12c	4-OCH_3	>10	>10	>10	8.2
12d	2,4difluo	>10	>10	>10	>10
12e	4-CH_2CH_3	>10	>10	>10	>10
Foretinib[b]	-	0.64 ± 0.26	0.39 ± 0.11	9.47 ± 0.22	0.014

[a] The values are an average of two separate determinations.

[b] Used as a positive control

Table 2 Structures, cytotoxicity and c-Met kinase inhibitory activity of compounds **13a-f**



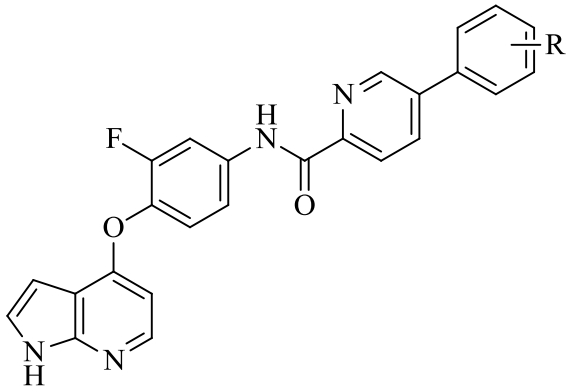
Compd. N.O.	R	IC_{50} (μM)[a]			
		A549	PC-3	MCF-7	c-Met
13a	4-CH_3	>10	>10	>10	2.3
13b	4-OCH_3	6.97 ± 1.01	9.62±1.01	8.21 ± 0.45	1.6
13c	4-CF_3	3.95 ± 1.11	14.22±0.99	>10	>10
13d	4-Cl	4.31 ± 1.03	25.11±0.41	>10	4.6
13e	3-CH_3	7.01 ± 1.10	5.47±0.78	7.47 ± 0.21	ND[c]
13f	H	2.92 ± 1.17	3.27±1.19	3.75 ± 0.45	ND
Foretinib[b]	-	0.64 ± 0.26	0.39 ±0.11	9.47 ± 0.22	0.014

[a] The values are an average of two separate determinations.

[b] Used as a positive control

[c] ND: Not determined

Table 3 Structures, cytotoxicity and c-Met kinase inhibitory activity of compounds **14a-f**

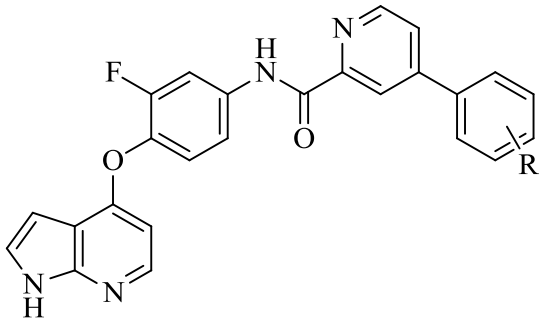


Compd. No	R	IC_{50} (μM) [a]			
		A549	PC-3	MCF-7	c-Met
14a	H	1.25 ± 0.13	0.76 ± 0.12	4.39 ± 1.08	>10
14b	4-CH_3	2.30 ± 0.14	2.64 ± 1.00	8.02 ± 1.05	6.6
14c	4-OCH_3	2.24 ± 0.14	0.65 ± 0.18	10.09 ± 1.02	>10
14d	4-F	6.01 ± 1.14	13.36 ± 0.75	>10	>10
14e	4-Cl	10.00 ± 1.85	3.03 ± 1.27	>10	8.9
14f	2,4-di F	3.10 ± 0.169	4.72 ± 1.51	>10	5.9
Foretinib[b]	-	0.64 ± 0.26	0.39 ± 0.11	9.47 ± 0.22	0.014

[a] The values are an average of two separate determinations.

[b] Used as a positive control

Table 4 Structures, cytotoxicity and c-Met kinase inhibitory activity of compounds **15a-i**



Compd. No.	R	IC_{50} (μM) [a]			
		A549	PC-3	MCF-7	c-Met
15a	H	4.95 ± 1.08	>10	>10	>10
15b	3-CH_3	1.75 ± 0.12	0.86 ± 0.17	9.12 ± 0.56	0.61
15c	4-CH_3	3.12 ± 1.35	3.97 ± 0.94	8.43 ± 0.99	5.23
15d	2,4-di CH_3	4.35 ± 0.15	4.01 ± 1.36	6.22 ± 1.41	0.89
15e	4-CH_2CH_3	3.37 ± 0.15	2.96 ± 1.48	7.47 ± 0.51	1.21
15f	4-OCH_3	1.04 ± 0.11	0.02 ± 0.21	9.11 ± 0.55	0.69

15g	4-F	1.09 ± 0.15	0.71 ± 0.14	9.52 ± 0.21	2.2
15h	4-CF_3	4.92 ± 1.41	4.35 ± 1.16	8.55 ± 0.44	>10
15i	2,4-di F	>10	>10	>10	>10
Foretinib[b]	-	0.64 ± 0.26	0.39 ± 0.11	9.47 ± 0.22	0.014

[a] The values are an average of two separate determinations.
[b] Used as a positive control

Table 5 Cytotoxicity of selected compounds **13b**, **15b**, **15d-f**.

Compd. No	IC_{50} (μM) [a]	
	HepG2	Hela
13b	11.65±1.06	7.53±0.95
15b	11.16 ±0.95	10.32±1.14
15d	13.32 ±1.52	11.89±1.75
15e	1038±0.97	8.63±1.12
15f	13.26±1.32	5.89±1.02

[a] The values are an average of two separate determinations.

As shown in Tables 1–5, eighteen of the compounds exhibited excellent cytotoxicity activity against different cancer cells with potency from the single-digit μM to single-digit nanomole range. In general, the third and the fourth series of compounds (**14a–f** and **15a–i**) which were substituted by fluoro atom on aminophenoxy part are more active than that of the first and the second series (**12a–e** and **13a–f**). What's more, compounds **12a–e**, the phenyl group on pyrimidine of which was C-5 position showed bad activity. However, when fluoro atoms were added to the aminophenoxy part of these compounds or the phenyl group on pyrimidine was changed to C-5 position, the target compounds **14a–f** and **13a–f** were more active than compounds **12a–e**. Similarly, compounds **15a–i** were more potent than compounds **14a–f** and **13a–f**. The results suggested that the introduction of fluoro atoms to the aminophenoxy part of target compounds or the phenyl group of pyrimidine substituted on C-4 position is benefit for the activity. That's why compounds **15a–i** showed the best activity among these four series compounds.

The cytotoxicity activity against A549 and PC-3 cancer cell lines was well while it's moderate or bad against MCF-7, HepG2 and Hela cell lines. It's indicated that the target compounds showed excellent selectivity toward A549 and PC-3 to MCF-7, HepG2 and Hela. Among the eighteen active compounds, seven of them (compounds **14a**, **14c**, **15b**, **15d**, **15f-g** and **15i**) were equal to more potent than Foretinib against one or more cell lines. Especially, compound **15f** showed superior activity to Foretinib, with the IC_{50} values of 1.04 ± 0.11 μM, 0.02 ± μM and 9.11 ± 0.55 μM against A549, PC-3 and MCF-7 cell lines, which were 0.62 to 19.5 times more active than Foretinib (IC_{50} values: 0.64 ± 0.26

μM, 0.39 ± 0.11 μM, 9.47 ± 0.22 μM) , respectively. The results suggested that replacement of quinoline nucleus with 1*H*-pyrrolo[2,3-*b*]pyridine moiety maintained even improved the potent cytotoxic activity.

Furthermore, different substitutions of aryl group affected the cytotoxicity of target compounds. For the second series (**13a-f**), substituted on C-4 position is not beneficial to the activity (**13a-d**) and substituted on C-3 position or none substituents (**13e-f**) is more preferred. For the third series (**14a-f**), it's seem to be that electron withdrawing groups such as halogen atoms (-F,-Cl) in C-2 position or C-4 position is not favor to the activity and electron-donating group is advantageous to the activity (**14a-c** *vs* **14d-f**). For the fourth series (**15a-i**), substituted on C-2 position is not beneficial to the activity (**15d**, **15f**) and substituted on C-3 position or C-4 position is more preferred.

Activity against c-Met kinase of target compounds was further carried out in this paper to investigate the target of these compounds. According to the results of Tables 1–4, we can easily find that the result is similar to the cytotoxicity activity. Compounds **14a**–**f** and **15a**–**i** are more active than that of **12a**–**e** and **13a**–**f** against c-Met kinase, especially compounds **15b**, **15d** and**15f**, with IC_{50} values in nanomole range. The results prompt us that the compounds are series of excellent c-Met kinase inhibitors.

3.2 Molecular docking study

To explore the binding modes of target compounds with the active site of c-Met, molecular docking simulation studies were carried out by using SURFLEX-DOCK module of SYBYL package version. Based on the *in vitro* inhibition results, we selected compound **15f**, our best c-Met inhibitor in this study, as ligand example, and the structure of c-Met was selected as the docking model (PDB ID code : 3LQ8 [5]).

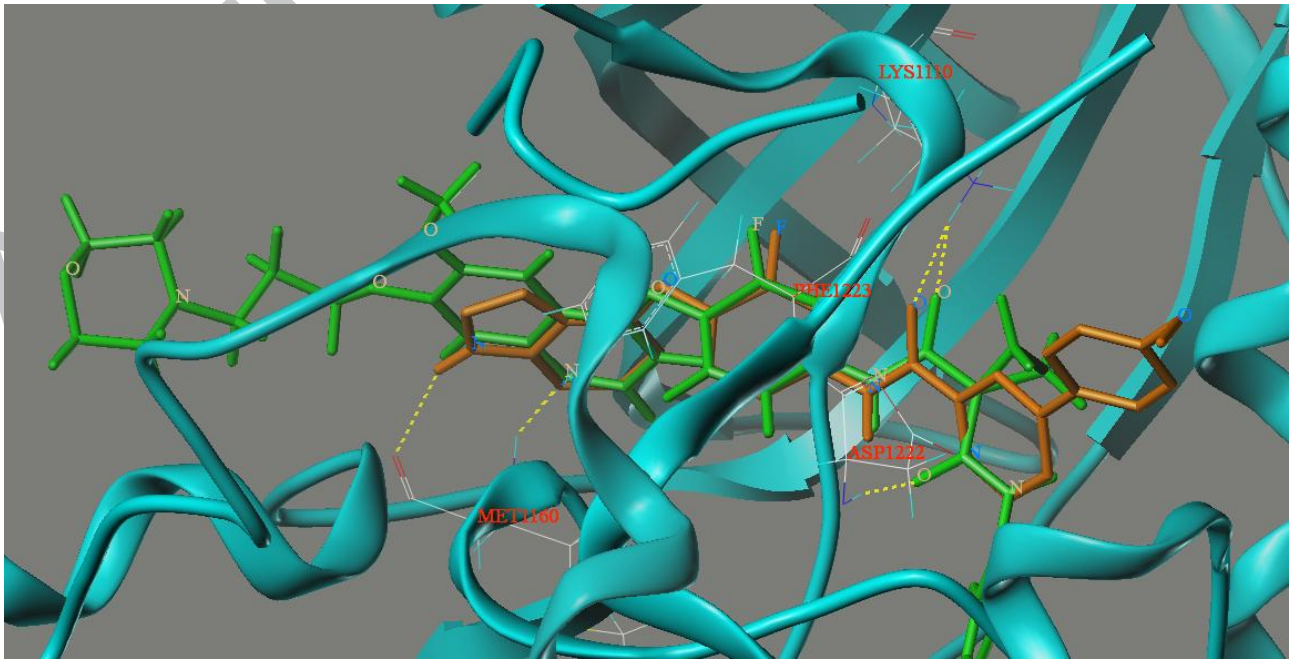


Fig. 2 Binding poses of compound **15f** with c-Met. The proteins were displayed by cyan ribbon. Compound **15f** and

lead compound were displayed by orange and green sticks, respectively. H-bonding interactions between the 15f, lead compound and c-Met were indicated with dashed lines in yellow.

The binding modes of compound **15f** and lead compound Foretinib were shown in Fig. 2. As depicted in Fig. 2, compound **15f** and lead compound can nearly overlap in the binding model and pyrrolo[2,3-*b*]pyridine group and phenylpicolinamide group formed three hydrogen bonds with residues MET1160 and LYS1110, respectively. Analysis of compound **15f**'s binding mode in the active binding site demonstrated that the docking mode of the **15f** is similar to the lead compound with the same H-bond between pyrrolo[2,3-b]pyridine group, phenylpicolinamide group and MET1160, LYS1110. The three hydrogen bonds really play an important role in increasing the inhibitory potency of pyrrolo[2,3-*b*]pyridine derivatives against c-Met kinase according to the docking results. Furthermore, the docking results also give us a new direction to design new c-Met inhibitors. The above-mentioned results of SARs analysis and molecular docking study may allow the rational design of more potent c-Met inhibitors.

4. Conclusions

In summary, we designed and synthesized four series of phenylpicolinamide derivatives bearing 1*H*-pyrrolo[2,3-*b*]pyridine moiety and evaluated for the IC_{50} values against three cancer cell lines and c-Met kinase. Eighteen of the compounds showed excellent cytotoxicity activity with the IC_{50} valuables in single-digit μM to nanomole range. Seven of them are equal to more active than positive control Foretinib against one or more cell lines. The target compounds showed excellent selectivity toward A549 and PC-3 to MCF-7, HepG2 and Hela. The most promising compound **15f** showed superior activity to Foretinib, with the IC_{50} values of 1.04 ± 0.11 μM, 0.02 ± 0.21 μM and 9.11 ± 0.55μM against A549, PC-3 and MCF-7 cell lines, which were 0.62 to 19.5 times more active than Foretinib, respectively. Structure–activity relationships (SARs) and docking studies indicated that replacement of quinoline nucleus with 1*H*-pyrrolo[2,3-b]pyridine moiety maintained even improved the potent cytotoxic activity. The results suggested that the introduction of fluoro atoms to the aminophenoxy part of target compounds or the phenyl group of pyrimidine substituted on C-4 position is benefit for the activity. The fourth series (**15a-i**) exhibited the best activity. Furthermore, different substituted of aryl group affected the cytotoxicity of target compounds. Further study will be carried out to identify the exact action mechanism in near future.

5. Experimental

5.1. Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. NMR spectra were performed using Bruker 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LCMS (Agilent, Palo Alto, CA, USA). The IR spectra were recorded by means of the KBr pellet

technique on WGH-30A drug-beam infrared spectrophotometer. All the materials were obtained from commercial suppliers and used without purification, unless otherwise specified. Yields were not optimized. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). All the materials were obtained from commercial suppliers and used without purification, unless otherwise specied. Yields were not optimized.

5.2 General procedure for the preparation of compounds **4a**–**k** and **5a**–**k**.

To the mixture of an appropriate amount of 4-bromo-pyridine-carboxylic acid **1** (0.90 mmol) or 5-bromo-pyridine-carboxylic acid **2** (0.90 mmol), substituted phenylboronic acid **3a**–**k** (0.18 mmol), anhydrous sodium carbonate (0.29 g, 2.70 mmol), 1,4-dioxane (30 mL), H_2O(10 mL), and bis(triphenylphosphine)palladium(II) dichloride (0.09 g, 0.135 mmol) were added under an atmosphere of nitrogen at room temperature. The reaction mixture then was stirred at 100℃ for 5-8 h and monitored by thin-layer chromatography (TLC). The reaction mixture was cooled to room temperature and the resulting precipitate was isolated by filtration to furnish the acid intermediate as the sodium salt, and then the solution was acidified to pH 5 to furnish the desired target compounds **4a**–**k** and **5a**–**k**.

5.3 Preparation of phenylpyridine chloride **6a**–**k** and **7a**–**k**

Oxalyl chloride (0.5 mmol) was added drop-wise to a stirred mixture of compounds **4a**–**k** or **5a**–**k** (0.2 mmol) and DMF(0.01 mmol) in dichloromethane (10 mL) in room temperature for 10 min, and the mixture was distilled and dissolved in dichloromethane (10 mL) immediately. The solution was used for the next step without further purification.

5.4. General procedure for the key intermediates **11a,b**

The mixture of 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (10 g, 66 mmol) and 4-nitrophenol **9a** (13.62 g, 98 mmol) or 2-fluoro-4-nitrophenol **9b** (15.56 g, 99 mmol) were refluxed in ether at 190℃ for 1 h and monitored by TLC. The mixture was cooled to 70 ℃ and poured into 400 mL ethyl acetate in an ice bath for 30 mins, light yellow solid was precipitated, filtered off and dried to obtain the title compound **10a** or **10b**.

Then **10a** or **10b** (20 mmol) was refluxed with hydrazine hydrate (200 mmol), Ferric chlorid（10 mmol) and an appropriate amount of activated carbon in ethanol (100 mL) for 10min and monitored by TLC. The reaction solution was cooled. Then the insoluble was filtered, the filtrate was evaporated, after that water was added and stirred at room temperature for 30 min. Afterward, the yellow solid was filtered off and dried to obtain the desired target compounds **11a** or **11b**.

5.5 General procedure for the preparation of compounds **12a**–**e**, **13a**–**f**, **14a**–**f** and **15a**–**i**

A solution of phenylpyridine chloride **6a**–**k** or **7a**–**k** (0.82 mmol) in dichloromethane (10 mL) was added drop-wise to a solution of aniline **11a,b** (0.41 mmol) and diisopropylethylamine (0.49 mmol) in dichloromethane (10 mL) in an ice

bath. Upon completion of the addition, the reaction mixture was removed from the ice bath and placed in room temperature for 30min and monitored by TLC. The mixture was washed with 10% K_2CO_3 (50 mL ×3) followed by brine (50 mL ×1), and the organic phase was separated, dried, and evaporated to yield **12a–e**, **13a–f**, **14a–f** and **15a–i** which were purified by isopropanol.

5.5.1 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)phenyl)-5-(4-chlorophenyl)picolinamide (**12a**)

This compound was obtained as reddish brown powder in 43.8% yield; ESI-MS m/z: 441.1$[M+H]^+$; m.p. 254.8 ~ 259.6 ℃; ^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H), 10.84 (s, 1H), 9.05 (s, 1H), 8.38 (dd, *J* = 8.2, 2.0 Hz, 1H), 8.25 (d, *J* = 8.2 Hz, 1H), 8.09 (d, *J* = 5.4 Hz, 1H), 8.04 (d, J = 8.9 Hz, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.36 (s, 1H), 7.22 (d, *J* = 8.9 Hz, 2H), 6.43 (d, *J* = 5.4 Hz, 1H), 6.23 (s, 1H).

5.5.2 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)phenyl)-5-*p*-tolylpicolinamide (**12b**)

This compound was obtained as white powder in 56.4% yield; ESI-MS m/z: 421.2$[M+H]^+$; m.p. 269.5 ~ 270.6℃; ^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H), 10.81 (s, 1H), 9.03 (s, 1H), 8.34 (d, *J* = 8.1 Hz, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 8.12 – 8.07 (m, 1H), 8.04 (d, *J* = 8.7 Hz, 2H), 7.75 (d, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 3H), 7.22 (d, *J* = 8.6 Hz, 2H), 6.43 (d, *J* = 5.2 Hz, 1H), 6.23 (s, 1H), 2.39 (s, 3H).

5.5.3 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)phenyl)-5-(4-methoxyphenyl)picolinamide (**12c**)

This compound was obtained as light yellow powder in 55.3% yield; ESI-MS m/z: 437.2$[M+H]^+$; m.p. 238.3 ~ 249.8℃; ^{1}H NMR (400 MHz, DMSO) δ 11.59 (s, 1H), 10.65 (s, 1H), 8.85 (s, 1H), 8.16 (d, *J* = 8.1 Hz, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.92 (t, *J* = 5.8 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.20 (s, 1H), 7.07 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.6 Hz, 2H), 6.27 (d, *J* = 5.4 Hz, 1H), 6.08 (s, 1H), 3.68 (s, 3H).

5.5.4 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)phenyl)-5-(2,4-difluorophenyl)picolinamide (**12d)**

This compound was obtained as light brown powder in 51.4% yield; ESI-MS m/z: 443.1$[M+H]^+$; m.p. 250.1 ~ 250.8℃; ^{1}H NMR (400 MHz, DMSO) δ 11.74 (s, 1H), 10.87 (s, 1H), 8.91 (s, 1H), 8.26 (q, *J* = 8.3 Hz, 2H), 8.09 (d, *J* = 5.4 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.75-7.85 (m, 1H), 7.54 – 7.46 (m, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 2H), 6.43 (d, *J* = 5.4 Hz, 1H), 6.23 (s, 1H).

5.5.5 *N*-(4-((1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)oxy)phenyl)-5-(4-ethylphenyl)picolinamide (**12e)**

This compound was obtained as white powder in 48.4% yield; ESI-MS m/z: 435.2$[M+H]^+$; m.p.261.7 ~ 266.1℃; ^{1}H NMR (400 MHz, DMSO) δ 11.76 (s, 1H), 10.82 (s, 1H), 9.03 (s, 1H), 8.34 (d, *J* = 8.1 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 8.09 (d, *J* = 5.4 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.0 Hz,2H), 7.41 (d, *J* = 7.9 Hz, 2H), 7.35 (t, *J* = 4.9 Hz, 1H), 7.22 (d, *J* = 8.8 Hz,2H), 6.43 (d, *J* = 5.4 Hz, 1H), 6.23 (d, *J* = 3.2 Hz, 1H), 2.69 (q, *J* = 7.4 Hz, 2H), 1.23 (t, *J* = 7.5 Hz, 3H).

5.5.6 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)phenyl)-4-*p*-tolylpicolinamide (**13a**)

This compound was obtained as white powder in 49.9% yield; ESI-MS m/z: 421.2$[M+H]^+$; m.p. 262.1 ~ 266.2 ℃; IR (KBr) cm^{-1}: 3490.0, 3394.0, 3100.0, 3046.0, 3016.0, 3010.0, 1644.0, 1602.0, 1572.0, 1509.0, 1461.0, 1455.0, 1413.0, 1380.0, 1350.0, 1257.0, 858.0, 831.0, 810.0. ^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H), 10.84 (s, 1H), 8.79 (d, *J* = 5.2 Hz, 1H), 8.41 (d, *J* = 1.3 Hz, 1H), 8.09 (d, *J* = 5.4 Hz, 1H), 8.06 – 8.02 (m, 2H), 8.00 (dd, *J* = 5.2, 1.9 Hz, 1H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.37 – 7.34 (m, 1H), 7.25 – 7.20 (m, 2H), 6.43 (d, *J* = 5.4 Hz, 1H), 6.23 (dd, *J* = 3.4, 2.0 Hz, 1H), 2.40 (s, 3H). ESI-HRMS m/z: calcd for $C_{26}H_{20}N_4O_2$ $[M+H]^+$:421.4396; found 421.4389.

5.5.7 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)phenyl)-4-(4-methoxyphenyl)picolinamide (**13b**)

This compound was obtained as pale yellow powder in 61.5% yield; ESI-MS m/z: 437.2$[M+H]^+$; m.p.229.8 ~ 233.4℃; IR (KBr) cm^{-1}: 3478.0, 3370.0, 3100.0, 3058.0, 3022.0, 3010.0, 1674.0, 1584.0, 1539.0, 1497.0, 1461.0, 1455.0, 1413.0, 1380.0, 1347.0, 1266.0, 1050.0, 852.0, 813.0, 804.0.^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H), 10.83 (s, 1H), 8.75 (d, *J* = 5.2 Hz, 1H), 8.39 (d, *J* = 1.4 Hz, 1H), 8.09 (d, *J* = 5.4 Hz, 1H), 8.06 – 8.01 (m, 2H), 7.97 (dd, *J* = 5.2, 1.9 Hz, 1H), 7.92 – 7.86 (m, 2H), 7.38 – 7.33 (m, 1H), 7.25 – 7.20 (m, 2H), 7.14 (dd, *J* = 6.9, 4.9 Hz, 2H), 6.44 (d, *J* = 5.4 Hz, 1H), 6.23 (dd, *J* = 3.4, 2.0 Hz, 1H), 3.84 (d, *J* = 5.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 162.64, 160.75, 157.49, 151.26, 150.71, 149.11, 148.53, 144.33(2C), 135.54, 128.60, 128.43(2C), 124.72, 123.40, 121.97(2C), 120.80(2C), 118.87, 114.85(2C), 110.23, 102.11, 97.26, 55.40. ESI-HRMS m/z: calcd for $C_{26}H_{20}N_4O_3$ $[M+H]^+$:437.4370; found 437.4379.

5.5.8 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)phenyl)-4-(4-(trifluoromethyl)phenyl)picolinamide (**13c**)

This compound was obtained as white powder in 55.6% yield; ESI-MS m/z: 475.2$[M+H]^+$; m.p.225.8 ~ 229.7℃; ^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H), 10.88 (s, 1H), 8.88 (d, *J* = 5.1 Hz, 1H), 8.48 (d, *J* = 1.3 Hz, 1H), 8.15 (d, *J* = 8.2 Hz, 2H), 8.11 – 8.07 (m, 2H), 8.07 – 8.02 (m, 2H), 7.94 (d, *J* = 8.3 Hz, 2H), 7.37 – 7.33 (m, 1H), 7.25 – 7.20 (m, 2H), 6.44 (d, *J* = 5.4 Hz, 1H), 6.25 – 6.21 (m, 1H).

5.5.9 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)phenyl)-4-(4-chlorophenyl)picolinamide (**13d**)

This compound was obtained as light brown powder in 48.6% yield; ESI-MS m/z: 441.1$[M+H]^+$; m.p.258.5 ~ 262.4℃; ^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H), 10.86 (s, 1H), 8.83 (d, *J* = 5.3 Hz, 1H), 8.42 (d, *J* = 1.3 Hz, 1H), 8.09 (d, *J* = 5.4 Hz, 1H), 8.05 (d, *J* = 2.1 Hz, 1H), 8.04 – 8.01 (m, 2H), 7.98 – 7.93 (m, 2H), 7.66 – 7.61 (m, 2H), 7.37 – 7.34 (m, 1H), 7.25 – 7.20 (m, 2H), 6.44 (d, *J* = 5.4 Hz, 1H), 6.23 (dd, *J* = 3.4, 2.0 Hz, 1H).

5.5.10 *N*-(4-((1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)oxy)phenyl)-4-(m-tolyl)picolinamide (**13e**)

This compound was obtained as light brown powder in 49.5% yield; ESI-MS m/z: 421.2$[M+H]^+$; m.p 211.5 ~ 212.6℃; ^{1}H NMR (400 MHz, DMSO) δ 11.74 (s, 1H), 10.86 (s, 1H), 8.80 (d, *J* = 5.1 Hz, 1H), 8.41 (s, 1H), 8.11 – 8.07 (m, 1H), 8.04 (t, *J* = 8.3 Hz, 2H), 7.99 (t, *J* = 5.9 Hz, 1H), 7.73 (s, 1H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 7.4 Hz, 2H), 7.23 (d, *J* = 8.8 Hz, 2H), 6.43 (d, *J* = 5.4 Hz, 1H), 6.23 (s, 1H), 2.43 (s, 3H).

5.5.11 *N*-(4-((1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)oxy)phenyl)-4-phenylpicolinamide (**13f**)

This compound was obtained as white powder in 46.9% yield; ESI-MS m/z: 407.1$[M+H]^+$; m.p 205.8 ~ 206.5℃; ^{1}H NMR (400 MHz, DMSO) δ 11.74 (s, 1H), 10.86 (s, 1H), 8.82 (d, *J* = 5.1 Hz, 1H), 8.43 (s, 1H), 8.08 (t, *J* = 4.6 Hz, 1H), 8.06 (s, 1H), 8.05 – 8.01 (m, 2H), 7.91 (d, *J* = 7.3 Hz, 2H), 7.59 (t, *J* = 7.1 Hz, 2H), 7.55 (d, *J* = 6.9 Hz, 1H), 7.36 (s, 1H), 7.23 (d, *J* = 8.8 Hz, 2H), 6.43 (d, *J* = 5.4 Hz, 1H), 6.23 (s, 1H).

5.5.12 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-5-phenylpicolinamide (**14a**)

This compound was obtained as white powder in 44.4% yield; ESI-MS m/z: 425.1$[M+H]^+$; m.p.231.5 ~ 232.9℃; ^{1}H NMR (400 MHz, DMSO) δ 11.78 (s, 1H), 11.05 (s, 1H), 9.06 (s, 1H), 8.38 (d, *J* = 7.9 Hz, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 8.19 - 8.13 (m, 1H), 8.10 (d, *J* = 5.2 Hz, 1H), 7.91 (d, *J* = 9.0 Hz, 1H), 7.86 (d, *J* = 7.4 Hz, 2H), 7.62 – 7.54 (m, 2H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.45 - 7.38 (m, 2H), 6.41 (d, *J* = 5.4 Hz, 1H), 6.28 (s, 1H).

5.5.13 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-5-p-tolylpicolinamide (**14b**)

This compound was obtained as yellow powder in 48.9% yield; ESI-MS m/z: 477.2$[M+K]^+$; m.p.248.5 ~ 250.4℃; IR (KBr) cm^{-1}: 3478.0, 3160.0, 3100.0, 3064.0, 3058.0, 3010.0, 1656.0, 1593.0, 1539.0, 1497.0, 1461.0, 1455.0, 1407.0, 1380.0, 1350.0, 1236.0, 840.0.^{1}H NMR (400 MHz, DMSO) δ 11.78 (s, 1H), 11.02 (s, 1H), 9.03(s, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.27 – 8.20 (m, 1H), 8.18 - 8.13 (m, 1H), 8.09 (d, *J* = 5.4 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.74 (d, *J* = 7.7 Hz, 1H), 7.42 (t, *J* = 6.9 Hz, 1H), 7.38 (d, *J* = 7.3 Hz, 3H), 6.41 (d, *J* = 5.2 Hz, 1H), 6.28 (s, 1H), 2.40 (s, 3H). ^{13}C NMR

(101 MHz, DMSO) δ 162.63, 157.05, 152.11, 150.97, 147.99, 146.07, 144.12, 138.48, 138.30, 135.40(2C), 132.98, 129.77(2C), 126.95(2C), 124.73, 123.50, 122.67, 116.87, 109.24, 108.93, 108.70, 100.54, 96.65, 20.64. ESI-HRMS m/z: calcd for $C_{26}H_{19}FN_4O_2$ $[M+H]^+$:439.4285; found 439.4290.

5.5.14 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-5-(4-methoxyphenyl)picolinamide (**14c**)

This compound was obtained as white powder in 56.9% yield; ESI-MS m/z: 493.1$[M+K]^+$; m.p.241.7 ~ 243.6℃; ^{1}H NMR (400 MHz, DMSO) δ 11.78 (s, 1H), 11.01 (s, 1H), 9.02 (s, 1H), 8.33 (d, *J* = 8.2 Hz, 1H), 8.23 (d, *J* = 8.0 Hz, 1H), 8.18 - 8.13 (m, 1H),, 8.09 (s, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 9.2 Hz, 1H), 7.39 (s, 1H), 7.13 (d, *J* = 8.3 Hz, 2H), 6.41 (d, *J* = 4.8 Hz, 1H), 6.28 (s, 1H), 3.85 (s, 3H).

5.5.15 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-5-(4-fluorophenyl)picolinamide (**14d**)

This compound was obtained as yellow powder in 54.3% yield; ESI-MS m/z: 481.1$[M+K]^+$; m.p.285.7 ~ 287.1℃; ^{1}H NMR (400 MHz, DMSO) δ 11.77 (s, 1H), 11.03 (s, 1H), 9.05 (s, 1H), 8.37 (d, *J* = 8.3 Hz, 1H), 8.26 (d, *J* = 7.9 Hz, 1H), 8.18-8.13 (d, 1H), 8.09 (d, *J* = 5.3 Hz, 1H), 7.92 (t, *J* = 9.7 Hz, 3H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.39 (s, 2H), 6.41 (d, *J* = 5.3 Hz, 1H), 6.28 (s, 1H).

5.5.16 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-5-(4-chlorophenyl)picolinamide (**14e**)

This compound was obtained as yellow powder in 53.3% yield; ESI-MS m/z: 459.1$[M+H]^+$; m.p.242.5 ~ 243.7℃; ^{1}H NMR (400 MHz, DMSO) δ 11.77 (s, 1H), 11.06 (s, 1H), 9.04 (s, 1H), 8.39 (d, *J* = 8.1 Hz, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 8.18 -8.12 (m, 1H), 8.08 (t, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 3H), 7.64 (d, *J* = 8.4 Hz,2H), 7.45 – 7.37 (m, 2H), 6.41 (d, *J* = 5.2 Hz, 1H), 6.28 (s, 1H).

5.5.17 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-5-(2,4-difluorophenyl)picolinamide (**14f**)

This compound was obtained as light yellow powder in 58.7% yield; ESI-MS m/z: 461.1$[M+H]^+$; m.p.266.4 ~ 267.1℃; IR (KBr) cm^{-1}: 3472.0, 3292.0, 3082.0, 3034.0, 3028.0, 3010.0, 1635.0, 1578.0, 1533.0, 1491.0, 1452.0, 1407.0, 1320.0, 1245.0, 798.0, 693.0.^{1}H NMR (400 MHz, DMSO) δ 11.77 (s, 1H), 11.06 (s, 1H), 8.93 (s, 1H), 8.29 (s, 2H), 8.13 -8.18 (m, 1H), 8.09 (d, *J* = 5.4 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.53 – 7.48 (m, 1H), 7.42 (s, 1H), 7.39 (s, 1H), 7.32 (s, 1H), 6.41 (d, *J* = 5.3 Hz, 1H), 6.28 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 162.64, 157.23, 154.74, 152.31, 151.17, 148.84, 148.07, 144.31, 138.17, 137.06, 133.22, 132.39, 124.93, 123.68, 122.64, 117.10, 112.73, 112.52, 109.45, 109.19, 108.96, 105.21, 104.94,100.77, 96.84. ESI-HRMS m/z: calcd for $C_{25}H_{15}F_3N_4O_2$ $[M+H]^+$:461.1225; found 461.1220.

5.5.18 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-phenylpicolinamide (**15a**)

This compound was obtained as light yellow powder in 42.7% yield; ESI-MS m/z: 425.1$[M+H]^+$; m.p.263.6 ~ 271.3℃;ESI-MS m/z: $[M+K]^+$463.1; ^{1}H NMR (400 MHz, DMSO) δ 11.67 - 11.62 (d, 1H), 11.07 (s, 1H), 8.81 (d, *J* = 4.5 Hz, 1H), 8.41 (s, 1H), 8.16-8.12 (d, 1H), 8.07 (d, *J* = 5.1 Hz, 1H), 8.01 (s, 1H), 7.89 (d, *J* = 5.8 Hz, 3H), 7.62 – 7.50 (m, 3H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.36 (s, 1H), 6.38 (d, J = 5.1 Hz, 1H), 6.24 (d, *J* = 14.0 Hz, 1H).

5.5.19 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-m-tolylpicolinamide (**15b**)

This compound was obtained as white powder in 51.3% yield; ESI-MS m/z: 439.2[M+H]+; m.p.224.1 ~ 231.4℃; IR (KBr) cm^{-1}: 3496.0, 3430.0,,3100.0, 3010.0, 1680.0, 1581.0, 1488.0, 1476.0, 1461.0, 1410.0, 1380.0, 1350.0, 1341.0, 1269.0, 1050.0, 771.0, 750.0. ^{1}H NMR (400 MHz, DMSO) δ 11.78 (s, 1H), 11.07 (s, 1H), 8.82 (d, *J* = 5.1 Hz, 1H), 8.42 (d, *J* = 1.3 Hz, 1H), 8.18 – 8.13 (m, 1H), 8.09 (d, *J* = 5.5 Hz, 1H), 8.03 – 8.00 (m, 1H), 7.93 – 7.88 (m, 1H), 7.74 (s, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.49-7.42 (m, 2H), 7.41 – 7.33 (m, 2H), 6.40 (d, *J* = 5.4 Hz, 1H), 6.28 (dd, *J* = 3.5, 2.0 Hz, 1H), 2.44 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 163.15, 157.43(2C), 151.37, 150.68, 149.46, 144.51(3C), 139.03, 136.69, 130.71, 129.56, 127.85, 125.13, 124.58, 124.41, 123.91, 119.95, 117.29, 109.64, 109.36, 109.12, 100.97, 97.04, 21.26. ESI-HRMS m/z: calcd for $C_{26}H_{19}FN_4O_2$ $[M+H]^+$:439.1570; found 439.1578.

5.5.20 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-p-tolylpicolinamide (**15c**)

This compound was obtained as white powder in 53.0% yield; ESI-MS m/z: 477.2$[M+K]^+$; m.p.242.9 ~ 246.7℃; ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 11.22 – 11.00 (m, 1H), 8.89 (d, *J* = 4.7 Hz, 1H), 8.50 (s, 1H), 8.25 (d, *J* = 13.1 Hz, 1H), 8.18 (d, *J* = 5.2 Hz, 1H), 8.10 (d, *J* = 3.2 Hz, 1H), 7.99 (d, *J* = 8.7 Hz, 1H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 6.8 Hz, 1H), 7.48 (d, *J* = 7.2 Hz, 3H), 6.49 (d, *J* = 5.1 Hz, 1H), 6.36 (s, 1H), 6.36 (s, 1H), 2.49 (s, 3H).

5.5.21 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-(2,4-dimethylphenyl)picolinamide (**15d**)

This compound was obtained as white powder in 52.6% yield; ESI-MS m/z: 491.2$[M+K]^+$; ESI-MS m/z: [M+H]+453.2; m.p.227.6 ~ 230.2℃; IR (KBr) cm^{-1}: 3496.0, 3244.0, 3100.0, 3040.0, 3010.0, 1650.0, 1599.0, 1530.0, 1470.0, 1461.0, 1452.0, 1419.0, 1380.0, 1350.0, 1269.0, 810.0, 690.0. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.76 (s, 1H), 11.04 (s, 1H), 8.78 (d, *J* = 4.6 Hz, 1H), 8.15-8.10 (m, 1H), 8.07 (s, 2H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 7.41 (d, *J* = 8.9 Hz, 1H), 7.37 (s, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 7.19 (s, 1H), 7.15 (d, *J* = 7.3 Hz, 1H), 6.38 (d, *J* = 5.1 Hz, 1H), 6.25 (s, 1H), 2.33 (s, 3H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 162.94, 157.22, 154.74, 152.31, 151.18, 150.79, 149.81, 148.56, 144.31, 138.34, 137.05, 135.14, 134.59, 131.51,

129.33, 127.31, 127.11, 124.93, 123.67, 122.66, 117.12, 109.19, 100.77, 96.84,20.74, 19.92, 9.6. ESI-HRMS m/z: calcd for $C_{27}H_{21}FN_4O_2$ $[M+H]^+$:453.1727; found 453.1732.

5.5.22 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-(4-ethylphenyl)picolinamide (**15e**)

This compound was obtained as light yellow powder in 50.2% yield; ESI-MS m/z: 453.2$[M+H]^+$; m.p.225.9 ~ 227.8℃; IR (KBr) cm^{-1}:3496.0, 3190.0, 3100.0, 3070.0, 3040.0, 3010.0, 1680.0, 1590.0, 1572.0, 1530.0, 1500.0, 1464.0, 1461.0, 1410.0, 1380.0, 1350.0, 1269.0, 843.0, 801.0, 696.0. ^{1}H NMR (400 MHz, Acetone) δ 11.78 (s, 1H), 11.05 (s, 1H), 8.81 (d, *J* = 4.8 Hz, 1H), 8.42 (s, 1H), 8.19-8.14 (m, 1H), 8.09 (d, *J* = 5.2 Hz, 1H), 8.02 (d, *J* = 4.2 Hz, 1H), 7.93 – 7.87 (m, 1H), 7.84 (d, *J* = 7.7 Hz, 2H), 7.46 – 7.36 (m, 4H), 6.41 (d, *J* = 5.2 Hz, 1H), 6.28 (s, 1H), 2.74-2.66 (m, 2H), 1.24 (t, *J* = 7.4 Hz, 3H).

5.5.23 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-(4-methoxyphenyl)picolinamide (**15f**)

This compound was obtained as light yellow powder in 55.7% yield; m.p.213.5 ~ 215.9℃; ESI-MS m/z: $[M+H]^+$455.2; IR (KBr) cm^{-1}: 3484.0, 3196.0, 3100.0, 3076.0, 3010.0, 1680.0, 1590.0, 1569.0, 1470.0, 1461.0, 1452.0, 1407.0, 1380.0, 1260.0, 1050.0, 1341.0, 837.0, 807.0, 717.0. ^{1}H NMR (400 MHz, DMSO) δ 11.76 (s, 1H), 11.03-10.97 (m, 1H), 9.00 (s, 1H), 8.75 (d, *J* = 5.0 Hz, 1H), 8.37 (s, 1H), 8.29 (s, 1H), 8.22 – 8.16 (m, 1H), 8.12 (s, 1H), 8.07 (d, *J* = 5.3 Hz, 1H), 7.99 – 7.93 (m, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 7.79 (d, *J* = 8.5 Hz, 1H), 7.45 – 7.32 (m, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 6.38 (d, *J* = 5.2 Hz, 1H), 6.26 (s, 1H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 163.01, 160.78, 160.13, 157.23，152.31, 151.17, 150.40, 149.17, 148.58, 147.73, 145.97, 144.31, 138.24, 137.17, 135.13, 128.45,124.92, 123.67, 122.81, 118.99, 117.04, 114.86, 109.12, 100.76, 96.84, 55.41.. ESI-HRMS m/z: calcd for $C_{26}H_{19}FN_4O_3$ $[M+H]^+$: 455.1519; found 455.1514.

5.5.24 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-(4-fluorophenyl)picolinamide (**15g**)

This compound was obtained as light yellow powder in 52.2% yield; ESI-MS m/z: 443.1$[M+H]^+$; m.p. 269.8 ~ 276.5℃; IR (KBr) cm-1: 3478.0, 3226.0, 3100.0, 3082.0, 3052.0, 3010.0, 1671.0, 1593.0, 1542.0, 1485.0, 1413.0, 1320.0, 1254.0, 789.0, 699.0, 846.0. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.75 (s, 1H), 11.03 (s, 1H), 8.80 (d, J = 4.7 Hz, 1H), 8.40 (s, 1H), 8.17-8.10 (m, 1H), 8.07 (d, J = 5.2 Hz, 1H), 8.00 (d, J = 5.0 Hz, 1H), 7.96 (d, J = 6.9 Hz, 2H), 7.88 (d, J = 9.0 Hz, 1H), 7.38 (d, J = 9.4 Hz, 4H), 6.38 (d, J = 5.2 Hz, 1H), 6.26 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ162.88, 157.20, 154.73, 151.16, 150.53, 149.30, 148.02, 144.29(2C), 137.02, 133.01, 129.54, 129.45, 124.91, 124.30, 123.68, 119.69, 117.07, 116.46, 116.24, 109.43, 109.15, 108.92, 100.77, 96.81. ESI-HRMS m/z: calcd for $C_{25}H_{16}F_2N_4O_2$ $[M+H]^+$: 443.1320; found 443.1328.

5.5.25 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-(4-(trifluoromethyl)phenyl)picolinamide (**15h**)

This compound was obtained as white powder in 51.0% yield; ESI-MS m/z: 493.1[M+H]$^+$; m.p. 243.1 ~ 244.3℃; ^{1}H NMR (400 MHz, DMSO) δ 11.86 (s, 1H), 11.17 (s, 1H), 8.98 (d, *J* = 4.4 Hz, 1H), 8.58 (s, 1H), 8.28 – 8.21 (m, 3H), 8.19 (s, 2H), 8.05 – 7.96 (m, 3H), 7.52 (t, *J* = 8.8 Hz, 1H), 7.48 (s, 1H), 6.50 (d, *J* = 4.9 Hz, 1H), 6.37 (s, 1H).

5.5.26 *N*-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yloxy)-3-fluorophenyl)-4-(2,4-difluorophenyl)picolinamide (**15i**)

This compound was obtained as light yellow powder in 51.8% yield; ESI-MS m/z: 461.1[M+H]$^+$; m.p. 279.0 ~ 280.1℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.76 (s, 1H), 11.06 (s, 1H), 8.85 (d, *J* = 4.8 Hz, 1H), 8.30 (s, 1H), 8.14-8.10 (d, 1H), 8.07 (d, *J* = 5.3 Hz, 1H), 7.87 (d, *J* = 9.4 Hz, 2H), 7.85 – 7.79 (m, 1H), 7.48 (t, *J* = 10.0 Hz, 1H), 7.42 (d, *J* = 9.0 Hz, 1H), 7.37 (s, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 6.38 (d, *J* = 5.2 Hz, 1H), 6.25 (s, 1H).

5. 6 Cytotoxicity assay in vitro

The cytotoxic activities of target compounds (**12a–e**, **13a–f**, **14a–f** and **15a–i**) were evaluated with A549, PC-3 and MCF-7 cell lines and the five selected compounds (**13b, 15b, 15d, 15e** and **15f**) were further evaluated for the activity against HepG2 and Hela cell lines by the standard MTT assay *in vitro*, with compounds c-Met inhibitors Foretinib as positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5μg/mL and incubated with cells at 37°C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as inhibition rates or IC_{50} (half-maximal inhibitory concentration) were the averages of two determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

5. 7 c-Met kinase assay in vitro

The target compounds (**12a–e**, **13a–f**, **14a–f** and **15a–i**) are tested for their activity against c-Met kinase through the mobility shift assay [8-9]. All kinase assays were performed in 96-well plates in a 50 μL reaction volume. The kinase buffer contains 50 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 0.0015% Brij-35 and 2 mM DTT. The stop buffer contains 100 mM HEPES, pH 7.5, 0.015% Brij-35, 0.2% Coating Reagent #3 and 50 mM EDTA. Dilute the compounds to 500 μM

by 100% DMSO, then transfer 10 μL of compound to a new 96-well plate as the intermediate plate, add 90 μL kinase buffer to each well. Transfer 5 μL of each well of the intermediate plate to 384-well plates. The following amounts of enzyme and substrate were used per well: kinase base buffer, FAM-labeled peptide, ATP and enzyme solution. Wells containing the substrate, enzyme, DMSO without compound were used as DMSO control. Wells containing just the substrate without enzyme were used as low control. Incubate at room temperature for 10 min. Add 10 μL peptide solution to each well. Incubate at 28 °C for specified period of time and stop reaction by 25 μL stop buffer. At last collect data on Caliper program and convert conversion values to inhibition values. Percent inhibition = (max − conversion)/(max − min) × 100. “max” stands for DMSO control; “min” stands for low control.

5. 8 Docking studies

For docking purposes, the three-dimensional structure of the c-Met (PDB code: 3LQ8) were obtained from RCSB Protein Data Bank [5]. Hydrogen atoms were added to the structure allowing for appropriate ionization at physiological pH. The protonated state of several important residues, such as CYS919, and ASP1046 were adjusted by using SYBYL6.9.1 (Tripos, St. Louis, USA) in favor of forming reasonable hydrogen bond with the ligand. Molecular docking analysis was carried out by the SURFLEX-DOCK module of SYBYL 6.9.1 package to explore the binding model for the active site of c-Met with its ligand. All atoms located within the range of 5.0 Å from any atom of the cofactor were selected into the active site, and the corresponding amino acid residue was, therefore, involved into the active site if only one of its atoms was selected. Other default parameters were adopted in the SURFLEX-DOCK calculations. All calculations were performed on Silicon Graphics workstation.

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Graphical Abstract