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Discovery of novel c-Met kinase inhibitors bearing a thieno[2,3-*d*]pyrimidine or furo[2,3-*d*]pyrimidine scaffold

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ABSTRACT

A series of thieno[2,3-*d*]pyrimidines and furo[2,3-*d*]pyrimidines were synthesized and evaluated for the c-Met inhibition. Thieno[2,3-*d*]pyrimidine **6b** stood out as the most potent showing an IC_{50} of 35.7 nM. This compound displayed high inhibitory effect on cell proliferation in BaF3-TPR-Met cells and showed high selectivity for c-Met family against other 14 tested kinases. However, compound **6b** was found ineffective in the c-Met-dependent U-87MG human gliobastoma xenograft model that may be relevant to its poor PK profile.

1. Introduction

c-Met is a member of the receptor tyrosine kinase (RTK) family. It is the cell surface receptor for hepatocyte growth factor (HGF), and is normally expressed by epithelial cells of many organs during embryogenesis and in adulthood.[1,2] Aberrant HGF/c-Met signaling has been found affecting cell proliferation, survival and motility, leading to tumor growth, angiogenesis and metastasis.[3–9] Therefore, c-Met has emerged as a promising therapeutic target for cancer therapy. Many small molecules selectively targeting the ATP binding site of c-Met kinase have been identified and exerted significant therapeutic effects in treating human cancers clinically.[10–15] Among these, acylthiourea **1**[15] developed by Kirin (Fig. 1) represented one of the earliest c-Met inhibitors possessing potent inhibition of c-Met phosphorylation in epidermoid carcinoma cells with IC_{50} value of 121 nM, and showing inhibition to tumor growth by 70% at a dosage of 100 mg/kg against human brain tumor cell (U-87MG) transplanted in nude mice.[15] As a structural mimic of the widely used quinoline scaffold in **1**, MethylGene Inc.[16,17] recently reported a series of c-Met inhibitors by replacing the quinoline core with a thieno[3,2-*b*]pyridine framework. The lead compound **2a** showed significantly enhanced in vitro activity (IC_{50}, 27 nM) and favorable pharmacokinetic characteristics. It exhibited remarkable tumor regression at an oral dosage of 40 mg/kg in the MKN-45 human xenograft mouse model.[17] Meanwhile, Bristol-Myers Squibb[18] reported a series of pyrrolopyridines, also as analogues of **1**, among which compound **2b** displayed highly potent c-Met potency (IC_{50}, 1.8 nM) and demonstrated significant in vivo antitumor activity in the GTL-16 human gastric carcinoma xenograft model at doses of 25–100 mg/kg. Interestingly, all compounds **1**, **2a**, and **b** showed significant potency as well for the vascular endothelial growth factor receptor (VEGFR), another key factor implicated in the development and progression of various human cancers. Therefore, to clarify the therapeutic benefit of c-Met-targeted drugs, potent and selective c-Met inhibitors are highly needed.

During drug screening of our internal compound libraries, we found that thieno- or furo-[2,3-*d*]pyrimidine cores (**I** or **II**, Fig. 1) that were widely used in other categories of therapies displayed potent and selective c-Met inhibitory activity upon introducing an appropriate aryloxy substituent at the C4- as that in **2b**. Herein, we report the synthesis and pharmacological evaluation of these two novel series of compounds.

2. Results and discussion

2.1. Chemistry

As described in Scheme 1, the 4,5-disubstituted thieno [2,3-*d*]pyrimidine series was prepared from the key intermediates

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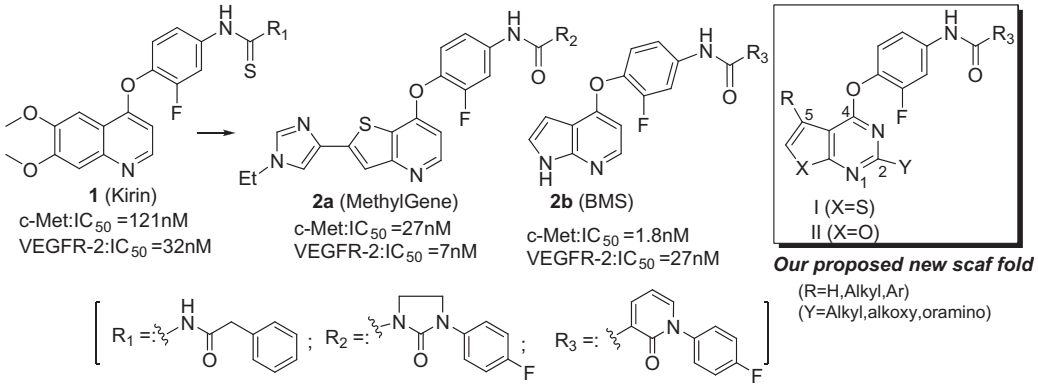
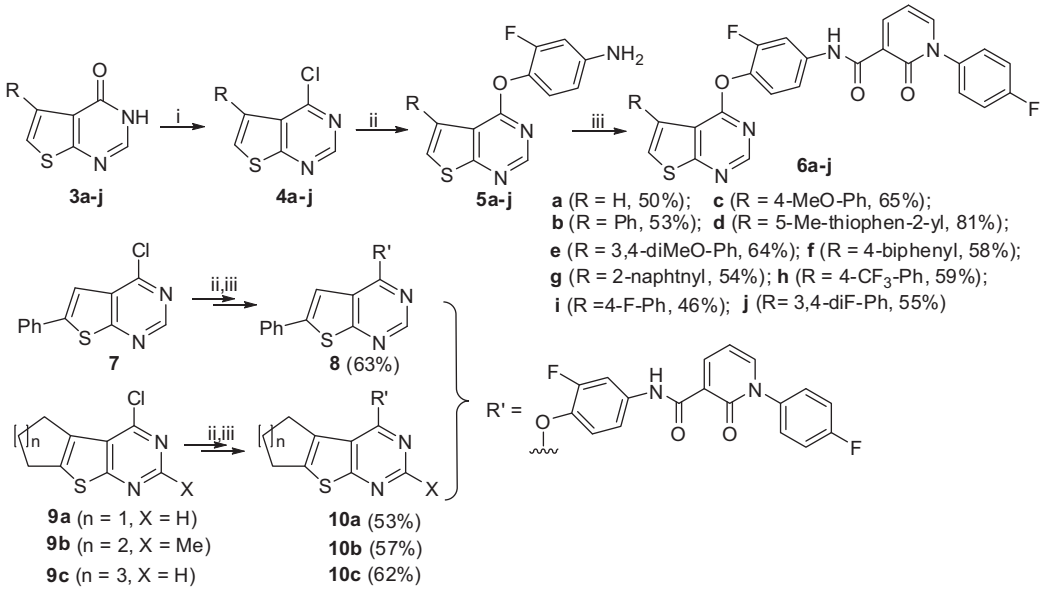
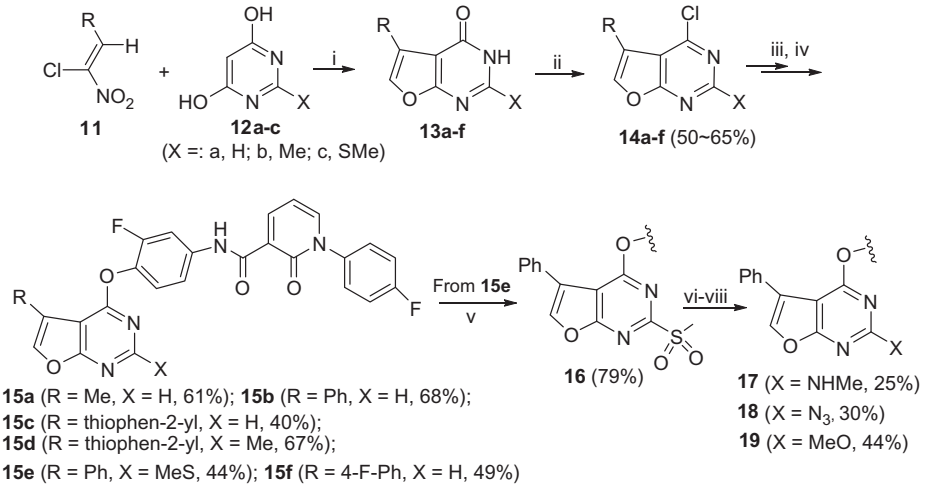


Figure 1. Representative c-Met inhibitors (**1** and **2**) and our proposed thieno- and furo-[2,3-*d*]pyrimidines **I** and **II**.



Scheme 1. Reagents and conditions: (i) $POCl_3$, 100 °C, 1 h, ~50%; (ii) 4-amino-2-fluorophenol, NaH, DMF, 0 °C, 2 h, ~90%; (iii)1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid, TBTU, DIPEA, DMF, 0 °C, 4 h.



Scheme 2. Reagents and conditions: (i) DBU, EtOH, 120 °C, overnight, 20–50%; (ii) $POCl_3$, 100 °C, 0.5 h, ~50%; (iii) 4-amino-2-fluorophenol, NaH, DMF, 0 °C, 0.5 h, 60–90%; (iv) 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid, TBTU, DIPEA, DMF, 0 °C to rt, 4 h, 40–70%; (v) *m*CPBA, DCM, 0 °C, 2 h; (vi) CH_3NH_2·HCl, DIPEA, DMF, 140 °C, microwave, 1 h; (vii) NaN_3, DMF, rt, 12 h; (viii) NH_3·CH_3OH, rt, overnight.

Table 1
c-Met enzymatic activity of selected compounds[a]

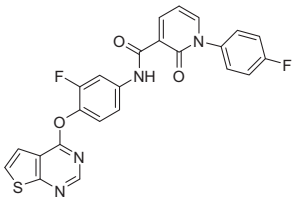
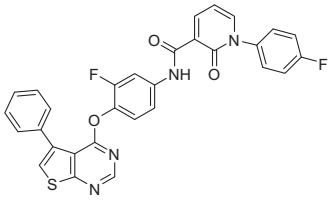
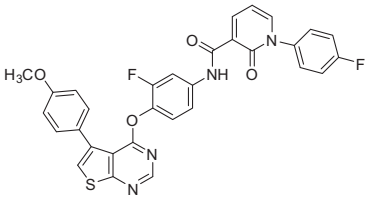
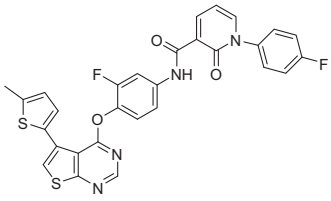
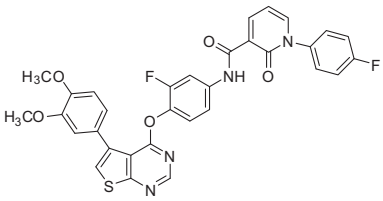
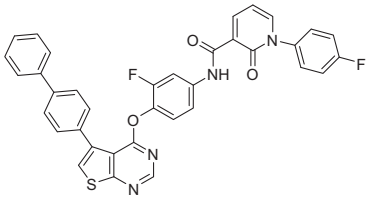
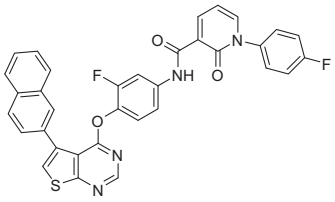
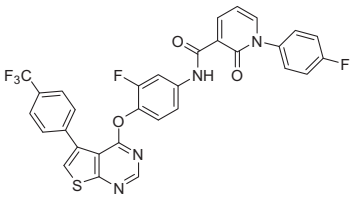
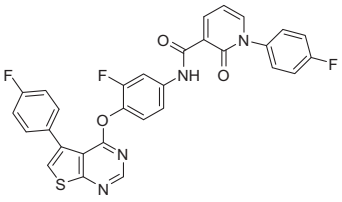
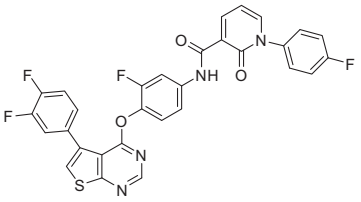
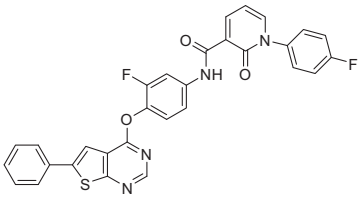
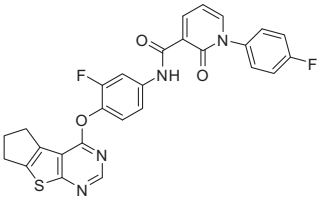
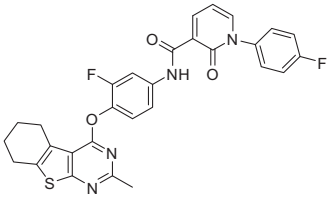
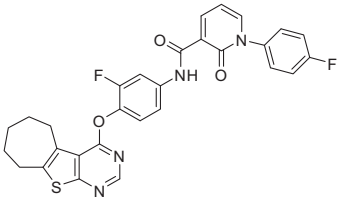
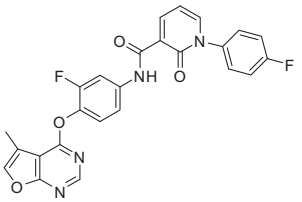
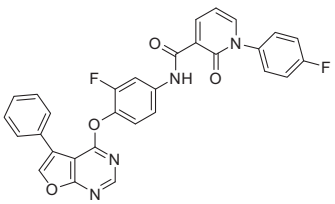
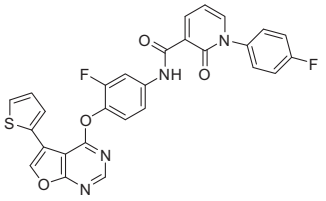
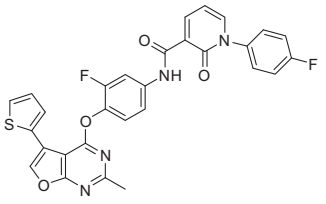
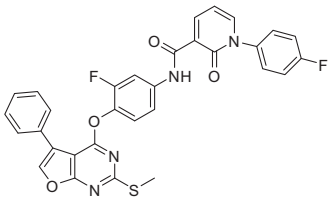
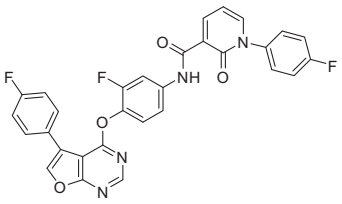
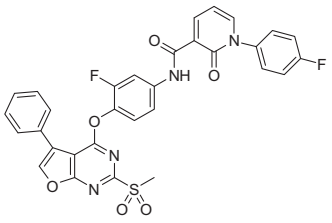
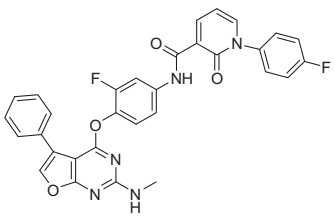
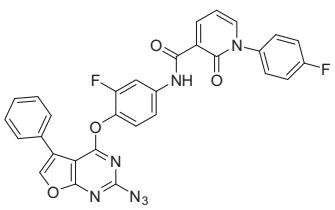
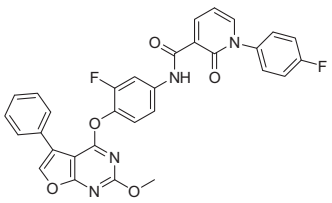
Compound	Structure	c-Met inhibition	
		% at 10 μM	IC_{50} (nM)
6a		14.3	>10 μM
6b		94.3	35.7 ± 3.6
6c		90.0	46.5 ± 6.2
6d		85.5	42.0 ± 10.4
6e		99.9	123 ± 77.1
6f		80.4	>1 μM[b]
6g		92.1	>1 μM[b]
6h		83.8	190.8 ± 40.7

Table 1 (*continued*)

Compound	Structure	c-Met inhibition	
		% at 10 μM	IC_{50} (nM)
6i		71.9	68.0 ± 11.1
6j		88.4	65.7 ± 1.4
8		10.6	>10 μM
10a		12.3	>10 μM
10b		31.2	>10 μM
10c		23.4	>10 μM
15a		16.5	>10 μM
15b		84.9	69.8 ± 11.6

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	c-Met inhibition	
		% at 10 μM	IC_{50} (nM)
15c		46.6	>10 μM
15d		24.3	>10 μM
15e		33.4	>10 μM
15f		80.9	310 ± 138
16		36.7	>10 μM
17		78.2	>1 μM[b]
18		18.5	>10 μM
19		31.7	>10 μM
1	–	–	121[16]
2b	–	–	1.8[20]

[a] IC_{50}s were calculated by Logit method from the results of at least three independent tests with six concentrations each and expressed as means ± SD.
[b] Inhibitory effect less than 50% at 1 μM concentration.

4a–j, which were either obtained from commercial source or prepared by chlorination[19] of thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**3a–j**) in refluxed $POCl_3$ in moderate yields. Condensation[20] of chlorides **4a–j** with 4-amino-2-fluorophenol afforded diaryl ethers 5a–**j** in 90% yield. Treating[18] amines **5a–j** with 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid under TBTU and DIPEA yielded the target compounds **6a–j** in 46–81% yields. 4,6-Disubstituted thieno[2,3-*d*]pyrimidine **8** was prepared from chloride **7** in 63% yield. Similarly, thieno[2,3-*d*]pyrimidines **10a–c** bearing a 5,6-cyclic substituent or a 2-methyl group were prepared from corresponding chlorides **9a–c** in a similar manner in 53–62% yields.

Since chlorides **14a–f** (Scheme 2), the key intermediates to access furo[2,3-*d*]pyrimidines **15a–f** were not commercially available, they were prepared[21] from 2-amino-4-methylfuran-3-carbonitrile **11** and 4,6-dihydroxypyrimidines **12a–c** leading to furo[2,3-*d*]pyrimidinones **13a–f** in moderate yields. Treating[18] **13a–f** with refluxed $POCl_3$ provided the chlorides **14a–f** in 50–65% yields. Similarly, treating[19] chlorides **14a–f** with 4-amino-2-fluorophenol following by condensation[20] with 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid under TBTU and DIPEA yielded target compounds **15a–f** in 33–83% overall yields. Further, oxidation[22] of **15e** with *m*CPBA provided sulfone **16** in 79% yield. Treating sulfone **16** with methylamine under microwave irridation[23] provided **17** in 25% yield. Reaction[24] of sulfone **16** with NaN_3 in DMF was very sluggish and yielded azide **18** in 30% yield. Reacting[25] sulfone **16** with ammonia in MeOH did not yield the corresponding 2-amino product, instead 2-methoxy substituted furo[2,3-*d*]pyrimidine **19** was obtained in 44% isolated yield.

2.2. c-Met enzymatic activity

All the newly synthesized thieno[2,3-*d*]pyrimidines **6a–j**, **8**, **10a–c**, and furo[2,3-*d*]pyrimidines **15a–f**, **16–19** were evaluated for their ability to inhibit enzymatic activity of the c-Met receptor using a procedure similar to that we reported[26] recently or as that in the literature.[27–30] The results are summarized in Table 1. Among the thieno[2,3-*d*]pyrimidine series, C5-nonsubstituted analogue **6a** is inactive for c-Met, while wide differences in potency were observed for the C5-aryl substituted compounds **6a–j**. 5-Phenyl-, 5-(4-methoxyphenyl)-, and 5-(5-methyl-thien-2-yl)-substituted analogs **6b–d** displayed high c-Met inhibitory potency with similar IC_{50} values of 35.7, 46.5, 42.0 nM, respectively. These results indicated that the C5-aryl substituent in the thieno[2,3-*d*]pyrimidine scaffold may be fitting into a lipophilic binding pocket in c-Met. 3,5-Dimethoxyphenyl substituted congener **6e** displayed slightly (3-fold) decreased potency with an IC_{50} value of 123 nM, suggesting that a bulky C5-substituent is not well tolerated in the proposed lipophilic pocket. This was further supported by the significant reduction in c-Met potency of compounds **6f** and **g** that contain a bulkier substituent. Good potency was retained on 4-fluorophenyl- and 3,4-difluorophenyl substituted thieno[2,3-*d*]pyrimidines **6i** and **j**, which showed IC_{50} values of 68.0 and 65.7 nM, respectively. This result indicated that the electronic property of the substituent in the C5-aryl does not have significant effect on the c-Met potency. It is noteworthy that the C6-aryl substituted thieno[2,3-*d*]pyrimidine **8** is nearly inactive at the c-Met. Similarly, thieno[2,3-*d*]pyrimidines **10a–c** bearing a 5,6-cyclic substituent were also inactive. These results indicated that a substituent at the C6 in the thieno[2,3-*d*]pyrimidine core is not tolerated for c-Met.

Among the furo[2,3-*d*]pyrimidine subseries, C5-methyl substituted analogue **15a** is inactive at the c-Met, whereas C5-phenyl substituted congener **15b** displayed high c-Met inhibitory potency with IC_{50} value of 69.8 nM, only 2-fold less potent than that of thieno[2,3-*d*]pyrimidine **6b**. It is quite disappointing that replacing the C5-phenyl with a thien-2-yl (**15c, d**) or introducing a substituent at the C2 position (**15e**, **16–19)** completely abolished the c-Met activity. 5-(4-Fluorophenyl)-furo[2,3-*d*]pyrimidine **15f** retained moderate c-Met potency with an IC_{50} value of 310 nM. In comparison of the potency between **6b** and **15b**, **6i** and **15f**, thieno[2,3-*d*]pyrimidines **6b** and **i** are generally more potent than their furo[2,3-*d*]pyrimidine congeners **15a** and **f**.

From the results above, thieno[2,3-*d*]pyrimidines **6b–d**, **i**, **j** and furo[2,3-*d*]pyrimidine **15b** stood out as the potent c-Met inhibitors with IC_{50} ranging between 35–70 nM. These compounds were more potent than the Kirin's initial compound **1**, but less potent than Methylgene's compound **2a** and BMS compound **2b** (Table 1).

2.3. Cell proliferation study

Since activation of c-Met ultimately results in cell proliferation, we then evaluated the inhibitory effect of the potent compounds **6a–d**, **i**, **j**, and **15b** on cell proliferation at concentrations of 10, 1, and 0.1 μM in TPR-Met transformed BaF3 cells (BaF3-TPR-Met) that stably express a constitutively active, ligand-independent, oncogenic form of c-Met.[26,31] As shown in Table 2, treatment of BaF3-TPR-Met cells with thieno[2,3-*d*]pyrimidines **6b–d**, **i**, and **j** at 10 μM significantly inhibited cell growth (>80%), while only compounds **6b–d** retained good inhibitory effects at 1 μM concentration. In agreement with the enzymatic results, all the thieno[2,3-*d*]pyrimidines with a C5-aryl substituent had higher inhibition capacity than the C5-nonsubstituted analog **6a** that is inactive at all tested concentrations. Furo[2,3-*d*]pyrimidine **15b** showed good inhibitory effect at 10 μM concentration, however such effect was completely lost at the concentration of 1 μM.

2.4. c-Met phosphorylation study

From the results obtained above, thieno[2,3-*d*]pyrimidines **6b–d** were confirmed active in both c-Met enzymatic and cell growth assays. To determine whether c-Met kinase inhibition of these compounds in cell-free system could be recapitulated in vitro, the inhibition of tyrosine phosphorylation of cellular proteins

Table 2
Effect of the newly synthetic compounds on cell proliferation

Compounds	Inhibition rate (%)		
	10 μM	1 μM	0.1 μM
6a	5.4	−11.8	−4.7
	5.3	−6.5	−12.8
6b	94.7	76.5	−3.4
	94.6	69.5	−10.5
6c	94.3	91.1	−10.1
	94.5	92.6	4.1
6d	95.3	60.6	−2.3
	95.0	64.5	−4.1
6i	92.2	30.8	12.9
	94.5	33.3	13.7
6j	92.3	13.9	−1.1
	89.7	33.7	4.6
15b	93.7	14.6	−4.1
	94.4	14.7	−6.9
15c	−5.4	−9.3	−2.7
	−4.7	−3.0	−12.1
15d	6.2	−2.8	0.6
	−9.7	6.0	29.5
15e	14.9	5.8	10.3
	15.1	2.0	11.7
15f	77.7	6.1	−4.7
	76.3	−5.8	2.8

The inhibition ratio (%) of cell proliferation was calculated using the following equation: $[1 - (A570/A570\ control)] \times 100\%$.[26]

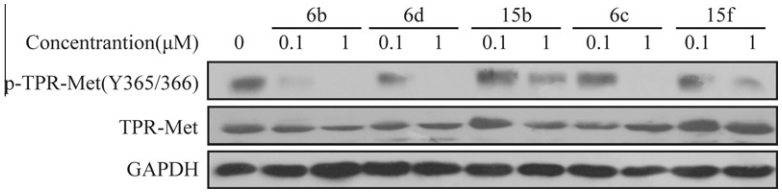


Figure 2. Dose-dependent inhibition of the selected compounds on c-Met phosphorylation in BaF3-TPR-Met cells.

by these compounds in TPR-Met-transformed BaF3 cells were investigated, furo[2,3-*d*]pyrimidines **15b** and **f** were also evaluated as comparison. As shown in Figure 2, compounds **6b–d**, **15b**, and **f** inhibited c-Met autophosphorylation in a dose-dependent manner in BaF3-TPR-Met cells. In general, compound **6b** had higher inhibitory effects than other compounds in the tested cell lines, therefore it was selected for further evaluation.

2.5. Kinase selectivity of lead compound 6b

To examine whether lead compound **6b** is a selective c-Met inhibitor, it was screened against c-Met family member, Ron, along with other 14 tyrosine kinases, including RON, Flt-1, c-Kit, PDGFRα, PDGFRβ, RET, EGFR, ErbB2, ErbB4, c-Src, Abl, EPH-A2, EPH-B2, IGF1R, and FGFR1. Compared to its high potency against c-Met (IC_{50}, 35.7 nM) and RON (IC_{50}, 50.4 nM), compound **6b** produced more than 10,000-fold less potency against other selected kinases, with IC_{50} values greater than 10 μM (Table 3), indicating that compound **6b** is a potent selective c-Met family-targeting inhibitor.

2.6. c-Met downstream signaling study on lead 6b

As shown in Figure 3, exposure to compound **6b** produced significant inhibition of c-Met phosphorylation in BaF3-TPR-Met cells. In addition, the phosphorylation of Erk1/2 and AKT, the key molecules downstream of c-Met that play important roles in cellular proliferation,[2,28] also were significantly inhibited upon treatment with compound **6b**. These data further supported that compound **6b** inhibits c-Met activity as well as subsequent c-Met downstream signaling.

2.7. Antitumor study on lead 6b

To evaluate the inhibition of compound **6b** on c-Met activities in vivo, the antitumor activity of compound **6b** was investigated in the c-Met-dependent U-87MG human gliobastoma xenograft model. We found that compound **6b**, even at the dosage of 100 mg/kg, only showed a marginal effect on tumor growth (Fig. 4). Compound **6b** was well tolerated showing no significant loss of body weight in this xenograft models (data not shown).

2.8. Pharmacokinnetic (PK) profile of lead 6b

The PK parameters of compound **6b** were then evaluated in mice after single iv (10 mg/kg) and po (20 mg/kg) administration.

Table 3
Kinase selectivity of compound **6b**

Kinase	Enzyme IC_{50} (nM)	Kinase	Enzyme IC_{50} (nM)
RON	50.4 ± 1.6	c-Src	>10,000
c-Kit	>10,000	ABL	>10,000
PDGFRα	>10,000	EPH-A2	>10,000
PDGFRβ	>10,000	EPH-B2	>10,000
RET	>10,000	IGF1R	>10,000
EGFR	>10,000	FGFR1	>10,000
ErbB2	>10,000	Flt-1	>10,000
ErbB4	>10,000		

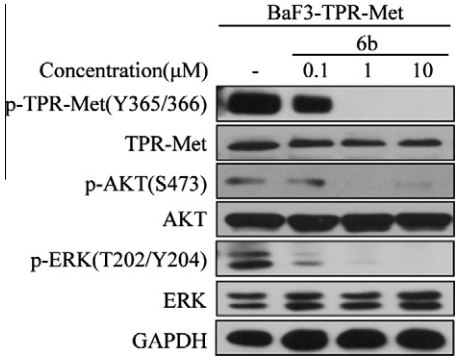


Figure 3. Dose-dependent inhibition of compound **6b** on c-Met phosphorylation and signal transduction pathways in BaF3-TPR-Met cells.

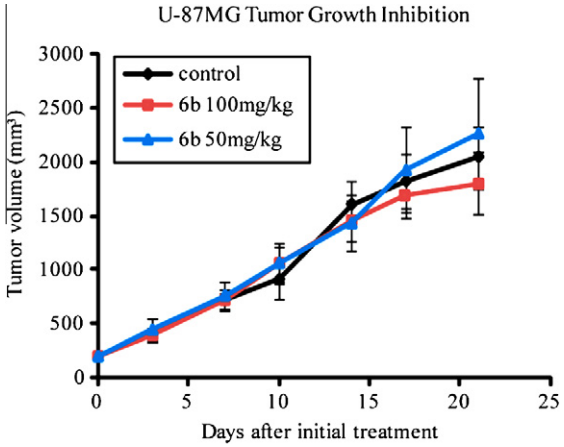


Figure 4. Antitumor efficacy of compound **6b** in the U-87MG xenograft models. Tumor-bearing nude mice were randomly divided in groups when tumor volume reached 100–200 mm^3 and given compound **6b** ip at indicated dose levels or vehicle alone over the designated treatment schedule. Data are presented as means ± SEM. $n = 6$ mice per group.

Compound **6b** displayed a short half-life time (1.67 h), moderate clearance (2.83 L/h/kg, iv), and poor oral bioavailability (5%). This unfavorable PK profile of thieno[2,3-*d*]pyrimidines **6b** along with its poor aqueous solubility may be the major reason for the poor antitumor efficacy. Therefore, more focused structural optimization on the solubility is needed.

3. Conclusion

In summary, a series of thieno[2,3-*d*]pyrimidines and furo[2,3-*d*]pyrimidines were synthesized and evaluated for the c-Met inhibition. Thieno[2,3-*d*]pyrimidine **6b** stood out as the most potent c-Met inhibitor with an IC_{50} of 35.7 nM. This compound displayed high inhibitory effect on cell proliferation in BaF3-TPR-Met cells and showed high selectivity for c-Met family against other 14 tested kinases. However, compound **6b** was found ineffective in the c-Met-dependent U-87MG human gliobastoma xenograft mod-

el. The poor PK profile of **6b** is likely a critical factor to the ineffective antitumor efficacy and will be a major objective for further structural optimization. Meanwhile, further evaluation on other mouse models such as MKN-45 (effective for **2a**) and GTL-16 (effective for **2b**) xenograft models will be conducted as well.

4. Experimental

Reactions were performed under a nitrogen atmosphere in dry glassware with magnetic stirring. The solvents were purified and dried according to the standard methods prior to use. Commercially available reagents were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AC300 spectrometer using tetramethylsilane as an internal reference. Chemical shifts are expressed in ppm and *J* values are given in Hz. Analytical thin-layer chromatography (TLC) was carried out on 0.2 mm Kieselgel 60F 254 silica gel plastic sheets (EM Science, Newark). Column chromatography was used for the routine purification of reaction products. The column output was monitored with TLC. Due to the limited solubility, ^{13}C NMR analysis of compounds **6f**, **g**, **17–19** were not applicable. HPLC analysis of these compounds was conducted on an Agilent 1100 series LC system (Agilent ChemStation Rev.A.10.02; ZORBAX Eclipse XDB-C8, 4.8 mm × 150 mm, 5 μM, 1.0 mL/min, uv 254 nM, rt) with two solvent systems (MeCN/H_2O/TFA, and MeOH/H_2O/TFA). All the assayed compounds displayed a purity of 95–99% in both solvent systems.

4.1. General procedure for preparation of compounds 6a–j

4.1.1. *N*-(3-fluoro-4-(5-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6i)

4.1.1.1. Preparation of 2-(1-(4-fluorophenyl)ethylidene)malononitrile. To a solution of 4-fluoroacetophenone (1.00 g, 7.24 mmol), malononitrile (0.957 g, 14.48 mmol) and ammonium acetate (0.446 g, 5.79 mmol) in 20 mL *m*-xylene, was added 2 mL of glacial acetic acid. The mixture was heated to reflux for 12 h. The solvent was evaporated under reduced pressure. The residue was poured into water and extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4, and evaporated. The crude product was purified by silica gel column chromatograph to afford 2-(1-(4-fluorophenyl)ethylidene)malononitrile (1.07 g, 79.4%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.62 (s, 3H), 7.21 (m, 2H), 7.60 (m, 2H).

4.1.1.2. Preparation of 2-amino-4-(4-fluorophenyl)thiophene-3-carbonitrile. To a solution of 2-(1-(4-fluorophenyl)ethylidene)malononitrile (410 mg, 2.20 mmol) and S_8 (113 mg, 3.53 mmol) in tetrahydrofuran (40 mL), was added dropwise a solution of $NaHCO_3$ (241 mg, 2.87 mmol) in H_2O (20 mL). The mixture was stirred at 35 °C overnight and then evaporated. The residue was poured into water and extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give the crude product, which was purified by silica gel column chromatograph to afford 2-amino-4-(4-fluorophenyl) thiophene-3-carbonitrile (330 mg, 68.8%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.97 (s, 2H), 6.32 (s, 1H), 7.12 (m, 2H), 7.56 (m, 2H).

4.1.1.3. Preparation of 5-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (3i). A mixture of 2-amino-4-(4-fluorophenyl)thiophene-3-carbonitrile (210 mg, 0.96 mmol), formic acid (8 mL) and acetic anhydride (8 mL) was heated at 110 °C for 36 h. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel to afford the title compound **3i** (171 mg, 81.8%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ: 7.02 (t, *J* = 9.0 Hz, 2H), 7.09 (s, 1H), 7.45 (t, *J* = 9.0 Hz, 2H), 7.86 (s, 1H).

4.1.1.4. Preparation of 4-chloro-5-(4-fluorophenyl)thieno[2,3-*d*]pyrimidine (4i). A solution of 5-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **3i** (320 mg, 1.30 mmol) in $POCl_3$ (8 mL) was heated at 100 °C for 1 h. The mixture was evaporated under reduced pressure and the residue was added to ice water, and then extracted with Et_2O. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give the crude product, which was purified by chromatography on silica gel to afford product **4i** (82 mg, 23.9%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.14 (m, 2H), 7.37 (m, 2H), 7.46 (s, 1H), 8.87 (s, 1H).

4.1.1.5. Preparation of 3-fluoro-4-(5-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yloxy)aniline (5i). To the solution of 4-amino-2-fluorophenol (41 mg, 0.32 mmol) in dried DMF (3 mL), was added NaH (13 mg, 0.54 mmol). The mixture was stirred at 0 °C for 10 min, and then a solution of 4-chloro-5-(4-fluorophenyl)thieno[2,3-*d*]pyrimidine **4i** (50 mg, 0.19 mmol) in dried DMF (1 mL) was added. The mixture was stirred at 0 °C for 1.5 h. Ice water (5 mL) was added to quench the reaction and the mixture was extracted by Et_2O (3 × 10 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to obtain the crude product, which was purified by silica gel column chromatograph to give compound **5i** (60 mg, 88.9%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.76 (s, 2H), 6.43 (d, *J* = 9.0 Hz, 1H), 6.49 (m, 1H), 6.89 (t, *J* = 8.4 Hz, 1H), 7.09 (t, *J* = 8.4 Hz, 2H), 7.34 (s, 1H), 7.57 (t, *J* = 8.4 Hz, 2H), 8.63 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 103.2 (d, *J* = 21.4 Hz), 110.4, 114.8 (d, *J* = 21.9 Hz), 116.7, 123.0, 124.0, 130.4 (d, *J* = 13.2 Hz), 131.0 (d, *J* = 8.2 Hz), 131.6, 134.7, 145.8 (d, *J* = 9.5 Hz), 153.1, 154.5 (d, *J* = 245.1 Hz), 162.5 (d, *J* = 245.5 Hz), 163.6, 170.1; MS (EI) *m/z*: 355 (M^+).

4.1.1.6. Preparation of *N* -(3-fluoro-4-(5-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6i). To a solution of aniline **5i** (70 mg, 0.20 mmol), 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid (51.3 mg, 0.22 mmol) and 2-(1*H*-benzotriazole-1-yl)-1,1,3-tetramethyluroniumtetrafluoroborate (TBTU) (87.3 mg, 0.26 mmol) in DMF (4 mL) at 0 °C, was added diisopropylethylamine (93.0 mg, 125 μL, 0.72 mmol). The reaction was allowed to warm to rt and stirred for 2 h. The mixture was quenched with ice water (5 mL) and extracted with Et_2O (3 × 10 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford the crude product, which was purified by silica gel column chromatograph to give the final product **6i** (51 mg, 45.5%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.60 (t, *J* = 7.2 Hz, 1H), 7.07 (m, 3H), 7.27 (m, 3H), 7.35 (s, 1H), 7.40 (m, 2H), 7.58 (m, 3H), 7.93 (d, *J* = 13.8 Hz, 1H), 8.60 (s, 1H), 8.72 (d, *J* = 8.1 Hz, 1H), 11.95 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 107.2, 108.3 (d, *J* = 23.7 Hz), 115.0 (d, *J* = 21.4 Hz), 116.2 (d, *J* = 21.8 Hz), 120.2, 124.5, 125.2, 129.4 (d, *J* = 9.1 Hz), 131.5 (d, *J* = 8.2 Hz), 131.6, 133.6, 134.4 (d, *J* = 12.8 Hz), 136.3, 137.2 (d, *J* = 9.5 Hz), 144.2, 145.1, 152.2, 152.9, 153.0, 153.5 (d, *J* = 243.2 Hz), 161.7, 161.9, 162.0 (d, *J* = 245.1 Hz), 162.6, 169.9; MS-EI *m/z*: 570 (M^+); Anal. Calcd for $C_{30}H_{17}F_3N_4O_3S$: C, 61.69; H, 3.19; N, 9.59. Found: C, 61.90; H, 3.11; N, 9.49.

4.1.2. *N*-(3-fluoro-4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6a)

This compound was prepared as a white solid from **5a** following a procedure similar to that of preparation of **6i** in 50% yield. ^{1}H

NMR (300 MHz, C_5D_5N) δ: 6.55 (m, 1H), 7.36 (m, 2H), 7.62 (m, 6H), 7.93 (d, J = 6.3 Hz, 1H), 8.49 (d, J = 12.6 Hz, 1H), 8.81 (m, 2H), 12.53 (s, 1H); ^{13}C NMR (100 MHz, C_5D_5N) δ: 107.1, 109.0 (d, J = 23.6 Hz), 116.4, 116.5 (d, J = 23.2 Hz), 118.7, 119.0, 121.7, 123.0, 124.8, 127.2, 129.4 (d, J = 9.2 Hz), 136.8, 138.5 (d, J = 10.2 Hz), 143.2, 145.2, 153.4, 154.9 (d, J = 244.3 Hz), 162.1, 162.6, 162.8 (d, J = 246.2 Hz), 163.2, 170.2; MS (EI) m/z: 476 (M^+); Anal. Calcd for $C_{24}H_{14}F_2N_4O_3S$: C, 58.94; H, 3.17; N, 11.46. Found: C, 58.82; H, 2.78; N, 11.16.

4.1.3. *N*-(3-fluoro-4-(5-phenylthieno[2,3-*d*]pyrimidin-4-yloxy) phenyl)-1-(4-fluoro phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6b)

This compound was prepared as a white solid from **5b** following a procedure similar to that of preparation of **6i** in 33.1% yield. ^{1}H NMR (300 MHz, CD_3Cl) δ: 6.60 (t, J = 6.6 Hz, 1H), 7.06 (t, J = 8.4 Hz, 1H), 7.27 (m, 2H), 7.39 (m, 6H), 7.61 (d, J = 6.9 Hz, 4H), 7.92 (dd, J = 2.4 Hz, J = 12.6 Hz, 1H), 8.62 (s, 1H), 8.73 (dd, J = 2.4, 7.2 Hz, 1H), 11.95 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 107.3, 109.3 (d, J = 23.3 Hz), 116.0, 116.8 (d, J = 22.7 Hz), 122.0, 123.2, 123.7, 127.9, 128.4 (d, J = 8.7 Hz), 129.4, 135.0 (d, J = 13.2 Hz), 135.5, 135.8, 137.1 (d, J = 9.6 Hz), 141.6, 145.2, 153.0, 154.0 (d, J = 245.9 Hz), 161.3, 162.4, 162.7 (d, J = 248.6 Hz), 163.2, 170.4; MS (EI) m/z: 552 (M^+); Anal. Calcd for $C_{30}H_{18}F_2N_4O_3S$: C, 63.65; H, 3.47; N, 9.90. Found: C, 63.80; H, 3.26; N, 9.88.

4.1.4. *N*-(3-fluoro-4-(5-(4-methoxyphenyl)thieno[2,3-*d*] pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6c)

This compound was prepared as a white solid from **5c** following a procedure similar to that of preparation of **6i** in 65.3% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.82 (s, 3H), 6.59 (t, J = 7.2 Hz, 1H), 6.93 (d, J = 7.2 Hz, 1H), 7.07 (t, J = 8.4 Hz, 1H), 7.28 (m, 4H), 7.52 (d, J = 9.0 Hz, 2H), 7.60 (d, J = 4.8 Hz, 1H), 7.93 (d, J = 12.0 Hz, 1H), 8.59 (s, 1H), 8.71 (d, J = 7.2 Hz, 1H), 11.94 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.2, 107.2, 109.2 (d, J = 23.2 Hz), 113.2, 116.0, 116.8, 116.9, 122.0, 122.3, 123.8, 128.0, 128.4 (d, J = 8.6 Hz), 130.5, 135.1 (d, J = 12.8 Hz), 135.5, 135.8, 137.1 (d, J = 9.8 Hz), 141.6, 145.1, 152.9, 154.0 (d, J = 236.0 Hz), 159.3, 161.3, 162.4, 162.7 (d, J = 249.2 Hz), 163.2, 170.4; MS (EI) m/z: 582 (M^+); Anal. Calcd for $C_{31}H_{20}F_2N_4O_4S$: C, 63.91; H, 3.46; N, 9.62. Found: C, 63.57; H, 3.29; N, 9.43.

4.1.5. *N*-(3-fluoro-4-(5-(5-methylthiophen-2-yl)thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6d)

This compound was prepared as a white solid from **5d** following a procedure similar to that of preparation of **6i** in 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.49 (s, 3H), 6.60 (m, 1H), 6.72 (s, 1H), 7.28 (m, 3H), 7.42 (m, 5H), 7.61 (d, J = 5.1 Hz, 1H), 7.95 (d, J = 12.3 Hz, 1H), 8.59 (s, 1H), 8.72 (d, J = 6.9 Hz, 1H), 11.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.3, 107.2, 109.3 (d, J = 23.2 Hz), 116.0, 116.3, 116.8 (d, J = 23.2 Hz), 121.9, 123.0, 123.8, 127.9, 128.3 (d, J = 9.1 Hz), 128.5, 133.9, 135.0, 135.7, 137.2 (d, J = 9.5 Hz), 140.4, 141.7, 145.2, 153.0, 154.1 (d, J = 246.4 Hz), 161.4, 162.4, 162.7 (d, J = 249.1 Hz), 163.1, 170.2; MS (EI) m/z: 572 (M^+); Anal. Calcd for $C_{29}H_{18}F_2N_4O_3S_2$: C, 60.83; H, 3.17; N, 9.78. Found: C, 60.63; H, 3.10; N, 9.67.

4.1.6. *N*-(4-(5-(3,4-dimethoxyphenyl)thieno[2,3-*d*]pyrimidin-4-yloxy)-3-fluoro phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6e)

This compound was prepared as a white solid from **5e** following a procedure similar to that of preparation of **6i** in 64.5% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.85 (s, 3H), 3.90 (s, 3H), 6.59 (t, J = 7.2 Hz, 1H), 6.90 (d, J = 8.1 Hz, 1H), 7.04 (t, J = 8.7 Hz, 1H), 7.16 (m, 2H), 7.28 (m, 3H), 7.34 (s, 1H), 7.39 (m, 2H), 7.61 (dd, J = 2.4 Hz, J = 6.6 Hz, 1H), 7.95 (dd, J = 2.4, 12.6 Hz, 1H), 8.59 (s, 1H), 8.71 (dd, J = 2.4, 7.2 Hz, 1H), 11.96 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 55.7, 55.8, 107.2, 109.2 (d, J = 23.2 Hz), 110.5, 112.9, 116.0, 116.7, 116.8 (d, J = 23.2 Hz), 121.6, 122.0, 122.5, 123.7, 128.4 (d, J = 8.6 Hz), 135.1 (d, J = 13.2 Hz), 135.7 (d, J = 13.7 Hz), 137.2 (d, J = 9.6 Hz), 141.7, 145.2, 148.1, 148.8, 152.9, 153.9 (d, J = 245.5 Hz), 161.3, 162.4, 162.7 (d, J = 249.1 Hz), 163.3, 163.9, 170.4; MS-EI m/z: 612 (M^+); HR-MS (EI) m/z: 612.1279 (calcd for $C_{32}H_{22}F_2N_4O_5S$: 612.1279).

4.1.7. *N*-(4-(5-(biphenyl-4-yl)thieno[2,3-*d*]pyrimidin-4-yloxy)-3-fluorophenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6f)

This compound was prepared as a white solid from **5f** following a procedure similar to that of preparation of **6i** in 57.8% yield. ^{1}H NMR (300 MHz, DMSO) δ: 6.69 (d, J = 6.9 Hz, 1H), 7.39 (m, 7H), 7.58 (m, 2H), 7.74 (m, 6H), 7.94 (m, 2H), 8.09 (dd, J = 1.8, 6.3 Hz, 1H), 8.55 (dd, J = 1.8, 7.2 Hz, 1H), 8.66 (s, 1H), 12.06 (s, 1H); MS-EI m/z: 628 (M^+); HR-MS (EI) m/z: 628.1374 (calcd for $C_{36}H_{22}F_2N_4O_3S$: 628.1381).

4.1.8. *N*-(3-fluoro-4-(5-(naphthalen-2-yl)thieno[2,3-*d*] pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6g)

This compound was prepared as a white solid from **5g** following a procedure similar to that of preparation of **6i** in 54.4% yield. ^{1}H NMR (300 MHz, DMSO) δ: 6.72 (t, J = 6.9 Hz, 1H), 7.41 (m, 4H), 7.58 (m, 4H), 7.85 (d, J = 8.4 Hz, 1H), 7.94 (m, 4H), 8.05 (s, 1H), 8.10 (dd, J = 1.8, 6.6 Hz, 1H), 8.22 (s, 1H), 8.58 (dd, J = 1.5, 7.5 Hz, 1H), 8.70 (s, 1H), 12.04 (s, 1H); MS-EI m/z: 602 (M^+); HR-MS (EI) m/z: 602.1217 (calcd for $C_{34}H_{20}F_2N_4O_3S$: 602.1224).

4.1.9. *N*-(3-fluoro-4-(5-(4-(trifluoromethyl)phenyl)thieno[2,3-*d*] pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6h)

This compound was prepared as a white solid from **5h** following a procedure similar to that of preparation of **6i** in 59.3% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.60 (t, J = 7.2 Hz, 1H), 7.08 (t, J = 8.7 Hz, 1H), 7.27 (m, 3H), 7.41 (m, 2H), 7.45 (s, 1H), 7.62 (d, J = 4.5 Hz, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.95 (d, J = 12.3 Hz, 1H), 8.62 (s, 1H), 8.71 (d, J = 8.1 Hz, 1H), 11.97 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 107.2, 109.2 (d, J = 23.3 Hz), 116.0, 116.4, 116.8 (d, J = 22.8 Hz), 121.9, 122.7, 123.7, 124.8, 124.9, 125.4, 128.3, 129.6, 129.8 (q, J = 32.3 Hz), 134.3, 134.8 (d, J = 12.7 Hz), 135.7, 137.3 (d, J = 10.0 Hz), 139.0, 141.7, 145.1, 153.2, 153.9 (d, J = 254.0 Hz), 161.4, 162.4, 162.7 (d, J = 248.6 Hz), 163.1, 170.5; MS (EI) m/z: 620 (M^+); Anal. Calcd for $C_{31}H_{17}F_5N_4O_3S$: C, 59.57; H, 2.82; N, 8.96. Found: C, 59.59; H, 2.63; N, 8.83.

4.1.10. *N*-(4-(5-(3,4-difluorophenyl)thieno[2,3-*d*]pyrimidin-4-yloxy)-3-fluoro phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (6j)

This compound was prepared as a white solid from **5j** following a procedure similar to that of preparation of **6i** in 55.4% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.60 (t, J = 7.2 Hz, 1H), 7.07 (t, J = 8.4 Hz, 1H), 7.17 (t, J = 9.0 Hz, 1H), 7.31 (m, 3H), 7.40 (m, 5H), 7.62 (d, J = 6.6 Hz, 1H), 7.95 (d, J = 12.6 Hz, 1H), 8.61 (s, 1H), 8.72 (d, J = 7.2 Hz, 1H), 11.97 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 107.2, 109.2 (d, J = 23.7 Hz), 116.0, 116.4, 116.7, 116.8 (d, J = 33.3 Hz), 116.9, 118.5 (d, J = 27.8 Hz), 121.9, 123.7 (d, J = 23.2 Hz), 125.6, 128.4 (d, J = 8.7 Hz), 132.3, 133.6, 134.8 (d, J = 13.2 Hz), 135.7, 137.3, 141.7, 145.2, 149.6 (dd, J = 247.7, 12.3 Hz), 150.1 (dd, J = 247.7, 12.3 Hz), 153.2, 153.9 (d, J = 246.4 Hz), 161.4, 162.4, 162.7 (d, J = 248.7 Hz), 163.1, 170.3;

MS (EI) *m/z*: 588 (M^+); Anal. Calcd for $C_{30}H_{16}F_4N_4O_3S$: C, 61.22; H, 2.74; N, 9.52. Found: C, 61.06; H, 2.73; N, 9.39.

4.2. *N*-(3-fluoro-4-(6-phenylthieno[2,3-*d*]pyrimidin-4-yloxy) phenyl)-1-(4-fluoro phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (8)

3-Fluoro-4-(6-phenylthieno[2,3-*d*]pyrimidin-4-yloxy)aniline was prepared from 4-chloro-6-phenylthieno[2,3-*d*]pyrimidine as a pale yellow solid (87.6%) by following a procedure similar to that of preparation of compound **5i**. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ_H: 6.50 (m, 2H), 7.03 (t, *J* = 8.4 Hz, 1H), 7.40 (m, 3H), 7.70 (m, 3H), 8.53 (s, 1H). ^{13}C NMR (400 MHz, DMSO) δ: 101.3 (d, *J* = 20.8 Hz), 109.8, 113.9, 119.8, 124.2, 126.6, 128.3 (d, *J* = 13.0 Hz), 129.6, 132.5, 143.0, 148.6 (d, *J* = 10.2 Hz), 153.2, 154.4 (d, *J* = 241.6 Hz), 162.8, 168.1; MS-EI *m/z* 337 (M^+).

Carboxamide **8** was obtained from 3-fluoro-4-(6-phenylthieno[2,3-*d*]pyrimidin-4-yloxy)aniline as a white solid (52.7%) by following a procedure similar to that of preparation of compound **6i**. ^{1}H NMR (300 MHz, C_5D_5N) δ: 6.53 (t, *J* = 7.2 Hz, 1H), 7.41 (m, 5H), 7.62 (m, 4H), 7.91 (m, 4H), 8.51 (d, *J* = 12.6 Hz, 1H), 8.78 (m, 2H), 12.53 (s, 1H). ^{13}C NMR (400 MHz, CF_3COOD) δ: 112.7, 113.8 (d, *J* = 22.7 Hz), 114.8, 119.5 (d, *J* = 23.6 Hz), 121.1, 121.6, 125.4, 125.5, 128.2, 128.8, 129.9, 130.3 (d, *J* = 8.8 Hz), 131.5, 131.7, 132.3, 133.4, 136.2, 136.7, 138.5 (d, *J* = 13.8 Hz), 146.1, 149.7, 152.5, 155.8 (d, *J* = 249.0 Hz), 158.7, 165.4, 166.3 (d, *J* = 250.8 Hz), 166.6, 166.9; MS-EI *m/z* 552 (M^+); Anal. Calcd for $C_{30}H_{18}F_2N_4O_3S{\cdot}0.3H_2O$: C, 64.58; H, 3.36; N, 10.04. Found: C, 64.54; H, 3.40; N, 9.96.

4.3. *N*-(3-fluoro-4-(6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (10a)

The precursor 3-fluoro-4-(6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yloxy)aniline was obtained from **9a** as a white solid (93.2%) by following a procedure similar to that of preparation of **5i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.52 (m, 2H), 3.05 (t, *J* = 8.4 Hz, 2H), 3.14 (t, *J* = 8.4 Hz, 2H), 3.78 (s, 2H), 6.50 (t, *J* = 9.6 Hz, 2H), 7.03 (t, *J* = 8.1 Hz, 2H), 8.48 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.7, 29.1, 30.0, 103.4 (d, *J* = 21.3 Hz), 110.6, 115.6, 124.2, 131.1 (d, *J* = 12.9 Hz), 136.2, 141.4, 145.7 (d, *J* = 9.3 Hz), 151.9, 154.8 (d, *J* = 245.7 Hz), 162.5, 173.9; MS (EI) *m/z*: 301 (M^+). This compound was then converted to target compound **10a** as a white solid (52.8%) by following a procedure similar to that of preparation of compound **6i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.78 (m, 4H), 1.92 (m, 2H), 2.95 (t, *J* = 5.4 Hz, 2H), 3.31 (t, *J* = 5.4 Hz, 2H), 6.60 (t, *J* = 7.2 Hz, 1H), 7.23 (m, 3H), 7.40 (m, 3H), 7.61 (dd, *J* = 2.1, 6.6 Hz, 1H), 7.95 (dd, *J* = 2.1, 12.0 Hz, 1H), 8.44 (s, 1H), 8.73 (dd, *J* = 2.1, 7.2 Hz, 1H), 11.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.7, 29.1, 30.0, 107.2, 109.3 (d, *J* = 23.2 Hz), 115.6, 116.0, 116.8 (d, *J* = 23.2 Hz), 122.0, 123.8, 128.4 (d, *J* = 8.8 Hz), 135.5 (d, *J* = 12.9 Hz), 135.8, 136.1, 137.1 (d, *J* = 9.7 Hz), 141.7 (d, *J* = 5.1 Hz), 145.2, 151.7, 154.2 (d, *J* = 246.6 Hz), 161.4, 162.1, 162.4, 162.7 (d, *J* = 249.0 Hz), 174.1; MS (EI) *m/z*: 517 (M^+); Anal. Calcd for $C_{27}H_{18}F_2N_4O_3S$: C, 62.78; H, 3.51; N, 10.85. Found: C, 62.79; H, 3.54; N, 10.85.

4.4. *N*-(3-fluoro-4-(2-methyl-tetrahydro[1]benzothieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (10b)

This compound was prepared as a white solid (56.5%) by following a procedure similar to that of preparation of compound **6i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.89 (m, 4H), 2.52 (s, 3H), 2.83 (s, 2H), 3.03 (s, 2H), 6.60 (t, *J* = 7.2 Hz, 1H), 7.30 (m, 5H), 7.61 (d, *J* = 6.6 Hz, 1H), 7.90 (d, *J* = 12.3 Hz, 1H), 8.72 (d, *J* = 7.5 Hz, 1H), 11.96 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.3, 22.9, 25.4, 25.5, 25.8, 107.2, 109.1 (d, *J* = 23.1 Hz), 115.8, 116.2, 116.8 (d, *J* = 23.1 Hz), 122.0, 123.9, 127.0, 128.4 (d, *J* = 8.8 Hz), 134.4, 135.7, 135.8, 136.6 (d, *J* = 9.7 Hz), 141.6, 145.1, 163.5 (d, *J* = 246.2 Hz), 161.4, 161.9, 162.1, 162.4, 162.7, 169.1; MS-EI *m/z*: 544 (M^+); HR-MS (EI) *m/z*: 544.1386 (calcd for $C_{19}H_{22}F_2N_4O_3S$: 544.1381).

4.5. *N*-(3-fluoro-4-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (10c)

This compound was prepared as a white solid (62.0%) by following a procedure similar to that of preparation of compound **6i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.78 (m, 4H), 1.92 (m, 2H), 2.95 (t, *J* = 5.4 Hz, 2H), 3.31 (t, *J* = 5.4 Hz, 2H), 3.78 (s, 2H), 6.60 (t, *J* = 5.4 Hz, 1H), 7.36 (m, 6H), 7.61 (dd, *J* = 2.1, 6.6 Hz, 1H), 7.95 (dd, *J* = 2.1, 12.0 Hz, 1H), 8.42 (s, 1H), 8.73 (dd, *J* = 2.1, 7.2 Hz, 1H), 11.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 26.9, 27.3, 28.6, 30.1, 32.1, 107.2, 109.2 (d, *J* = 23.6 Hz), 116.0, 116.8 (d, *J* = 23.2 Hz), 119.8, 122.0, 123.9, 128.4 (d, *J* = 8.8 Hz), 132.5, 135.4 (d, *J* = 13.0 Hz), 135.8, 137.1 (d, *J* = 9.7 Hz), 140.4, 141.6, 145.2, 151.5, 154.2 (d, *J* = 246.6 Hz), 161.3, 162.4, 162.7 (d, *J* = 249.0 Hz), 167.2; MS (EI) *m/z*: 544 (M^+); Anal. Calcd for $C_{29}H_{22}F_2N_4O_3S$: C, 63.75; H, 4.10; N, 10.25. Found: C, 64.03; H, 4.07; N, 10.28.

4.6. General procedure for synthesis of compounds 15a–f

4.6.1. *N*-(3-fluoro-4-(5-methylfuro[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluoro phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (15a)

This compound was obtained from 3-fluoro-4-(5-methylfuro[2,3-*d*]pyrimidin-4-yloxy)aniline as a white solid (61.0%) by following a procedure similar to that of preparation of **6i**. ^{1}H NMR (300 MHz, DMSO) δ: 2.34 (s, 3H), 6.71 (t, *J* = 6.9 Hz, 1H), 7.41 (m, 4H), 7.58 (m, 2H), 7.95 (m, 2H), 8.08 (m, 1H), 8.44 (s, 1H), 8.57 (m, 1H), 12.07 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ: 9.4, 105.3, 107.2, 108.3 (d, *J* = 23.6 Hz), 113.9, 116.2 (d, *J* = 23.1 Hz), 120.2, 124.6, 129.4 (d, *J* = 8.8 Hz), 134.7, 134.9, 136.3, 137.3 (d, *J* = 9.2 Hz), 141.4, 144.3, 145.2, 152.7, 153.6 (d, *J* = 244.3 Hz), 161.8, 161.9, 162.5 (d, *J* = 235.5 Hz), 168.5; MS (EI) *m/z*: 474 (M^+); Anal. Calcd for $C_{25}H_{16}F_2N_4O_4$: C, 62.70; H, 3.47; N, 11.70. Found: C, 62.61; H, 3.16; N, 11.56.

4.6.2. *N*-(3-fluoro-4-(5-phenylfuro[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluoro phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (15b)

This compound was obtained as a white solid (68.5%) by following a procedure similar to that of preparation of **6i**. ^{1}H NMR (300 MHz, CD_3Cl) δ: 6.61 (t, *J* = 6.6 Hz, 1H), 7.26 (m, 3H), 7.40 (m, 6H), 7.63 (d, *J* = 6.0 Hz, 1H), 7.75 (m, 3H), 7.97 (d, *J* = 12.3 Hz, 1H), 8.52 (s, 1H), 8.73 (d, *J* = 6.0 Hz, 1H), 11.99 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 103.6, 107.3, 109.3 (d, *J* = 23.1 Hz), 116.0, 116.8 (d, *J* = 23.0 Hz), 121.5, 122.0, 123.7, 128.2, 128.4 (d, *J* = 8.5 Hz), 128.5, 128.6, 130.0, 135.3 (d, *J* = 13.0 Hz), 135.7, 137.3 (d, *J* = 9.7 Hz), 139.9, 141.7, 145.2, 153.1, 154.1 (d, *J* = 246.7 Hz), 161.4, 162.4, 162.7 (d, *J* = 258.1 Hz), 163.3, 169.1; MS (EI) *m/z*: 536 (M^+); Anal. Calcd for $C_{30}H_{18}F_2N_4O_4$: C, 66.16; H, 3.50; N, 10.29. Found: C, 66.42; H, 3.48; N, 10.21.

4.6.3. *N*-(3-fluoro-4-(5-(thiophen-2-yl)furo[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (15c)

This compound was obtained as a white solid (40.5%) by following a procedure similar to that of preparation of **6i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.61 (t, *J* = 6.6 Hz, 1H), 7.10 (t, *J* = 4.2 Hz, 1H),

7.25 (m, 4H), 7.40 (m, 3H), 7.62 (m,2H), 7.86 (s, 1H), 7.97 (dd, J = 2.4, 12.3 Hz, 1H), 8.51 (s, 1H), 8.73 (dd, J = 1.8, 7.2 Hz, 1H), 11.99 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 103.1, 107.2, 109.2 (d, J = 23.2 Hz), 115.1, 116.0, 116.8 (d, J = 23.2 Hz),121.8, 123.7, 125.4, 127.7, 127.9, 128.3 (d, J = 9.1 Hz), 130.9, 135.0 (d, J = 13.3 Hz), 135.7, 137.4 (d, J = 10.0 Hz), 139.3, 141.7, 145.2, 153.2, 154.0 (d, J = 246.4 Hz), 161.4, 162.3, 162.6 (d, J = 254.7 Hz), 163.1, 168.8; MS (EI) m/z: 542 (M^+) HR-MS (EI) m/z: 542.5128 (calcd for $C_{28}H_{16}F_2N_4O_4S$: 542.5120).

4.6.4. *N*-(3-fluoro-4-(2-methyl-5-(thiophen-2-yl)furo[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (15d)

This compound was obtained as a white solid (67.4%) by following a procedure similar to that of preparation of **6i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.57 (s, 3H), 6.61 (t, J = 6.9 Hz, 1H), 7.08 (m, 1H), 7.26 (m, 4H), 7.37 (m, 3H), 7.64 (m, 2H), 7.77 (s, 1H), 7.94 (dd, J = 2.4, 12.3 Hz, 1H), 8.73 (dd, J = 1.8, 7.2 Hz, 1H), 11.97 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.5, 100.2, 107.3, 109.1 (d, J = 22.8 Hz), 115.0, 115.8, 116.8 (d, J = 22.8 Hz), 121.9, 123.8, 125.1, 127.5, 127.9, 128.3 (d, J = 8.7 Hz), 131.4, 135.3 (d, J = 13.2 Hz), 135.7, 136.9 (d, J = 10.0 Hz), 138.5, 141.7, 145.2, 154.2 (d, J = 246.4 Hz), 161.4, 162.4, 162.5, 162.7 (d, J = 253.7 Hz), 163.8, 169.5; MS (EI) m/z: 556 (M^+). HR-MS (EI) m/z: 556.5394 (calcd for $C_{29}H_{18}F_2N_4O_4S$: 556.5396.

4.6.5. *N*-(3-fluoro-4-(2-(methylthio)-5-phenylfuro[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (15e)

This compound was obtained as a white solid (43.7%) by following a procedure similar to that of preparation of **6i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.35 (s, 3H), 6.59 (t, J = 5.4 Hz, 1H), 7.22 (m, 4H), 7.38 (m, 5H), 7.60 (m, 2H), 7.73 (d, J = 10.5 Hz, 2H), 7.91 (d, J = 10.5 Hz, 1H), 8.72 (d, J = 7.2 Hz, 1H), 11.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 99.4, 107.2, 108.8 (d, J = 23.7 Hz), 115.6, 116.7 (d, J = 23.2 Hz), 121.4, 121.8, 123.8, 128.0, 128.3, 128.6, 130.1, 135.2 (d, J = 12.8 Hz), 135.7, 137.0 (d, J = 9.2 Hz), 138.2, 141.7, 145.1, 154.1 (d, J = 246.0 Hz), 161.4, 162.1, 162.4, 162.6 (d, J = 252.8 Hz), 167.2, 169.8; MS (EI) m/z: 582 (M^+). HR-MS (EI) m/z: 582.5767 (calcd for $C_{31}H_{20}F_2N_4O_4S$: 582.5780).

4.6.6. *N*-(3-fluoro-4-(5-(4-fluorophenyl)furo[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (15f)

This compound was obtained as a white solid (49.2%) by following a procedure similar to that of preparation of **6i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.61 (t, J = 7.2 Hz, 1H), 7.14 (m, 3H), 7.23 (m, 2H), 7.41 (m, 3H), 7.62 (m, 1H), 7.71 (m, 3H), 7.97 (dd, J = 2.4, 12.0 Hz, 1H), 8.52 (s, 1H), 8.73 (dd, J = 2.1, 7.5 Hz, 1H), 11.99 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 103.6, 107.3, 109.3 (d, J = 23.1 Hz), 115.7 (d, J = 21.3 Hz), 116.0, 116.8 (d, J = 23.2 Hz), 120.6, 122.0, 123.7, 126.0, 128.4 (d, J = 8.8 Hz), 130.3 (d, J = 7.8 Hz), 135.2 (d, J = 13.0 Hz), 135.7, 137.3 (d, J = 9.9 Hz), 139.7, 141.7, 145.2, 153.2, 154.0 (d, J = 246.2 Hz), 161.4, 162.4, 162.7 (d, J = 250.4 Hz), 162.8 (d, J = 246.2 Hz), 163.2, 169.1; MS (EI) m/z: 554 (M^+); HR-MS (EI) m/z: 554.1203 (calcd for $C_{30}H_{17}F_3N_4O_4$: 554.1202).

4.7. *N*-(3-fluoro-4-(2-(methylsulfonyl)-5-phenylfuro[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (16)

To a solution of carboxamide **15e** (65 mg, 0.112 mmol) in CH_2Cl_2 (5 mL), was added *m*CPBA (57.6 mg, 0.335 mmol) at 0 °C. The mixture was stirred for 2.5 h, and then quenched with $NaHCO_3$ saturated solution. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatograph to afford the title compound **16** (54 mg, 78.7%) as a white solid. ^{1}H NMR (300 MHz, DMSO) δ: 3.24 (s, 3H), 6.71 (t, J = 7.2 Hz, 1H), 7.42 (m, 3H), 7.51 (m, 4H), 7.59 (m, 2H), 7.81 (d, J = 7.2 Hz, 2H), 8.02 (dd, J = 2.4, 12.6 Hz, 1H), 8.10 (dd, J = 2.4, 6.9 Hz, 1H), 8.56 (dd, J = 1.8, 7.5 Hz, 1H), 8.72 (s, 1H), 12.12 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.1, 105.6, 107.1, 108.0, 116.1 (d, J = 25.5 Hz), 120.1, 120.9, 124.3, 128.6, 128.8, 129.0, 129.3 (d, J = 8.7 Hz), 134.0 (d, J = 12.8 Hz), 136.3, 137.6 (d, J = 9.6 Hz), 144.2, 144.7, 144.9, 153.1 (d, J = 243.7 Hz), 159.0, 161.7, 161.8, 161.9 (d, J = 244.6 Hz), 162.9, 168.0; MS (EI) m/z: 614 (M^+); HR-MS (EI) m/z: 614.1068 (calcd for $C_{31}H_{20}F_2N_4O_6S$: 614.1072).

4.8. N-(3-fluoro-4-(2-(methylamino)-5-phenylfuro[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (17)

To a solution of carboxamide **16** (44 mg, 0.072 mmol) and methylamine hydrochloride (9.7 mg, 0.143 mmol) in 1,4-dioxane (2 mL), was added DIPEA (27.9 mg, 0.216 mmol). The solution was irradiated for 1 h with microwave at 140 °C. The solvent was removed under reduced pressure and the residue was diluted with water and EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatograph to afford compound **17** (10 mg, 24.7%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.89 (s, 3H), 4.99 (q, J = 5.1 Hz, 1H), 6.60 (t, J = 6.3 Hz, 1H), 7.14 (t, J = 8.4 Hz, 1H), 7.24 (m, 1H), 7.33 (m, 2H), 7.40 (m, 6H), 7.61 (m, 1H), 7.73 (m, 2H), 7.88 (dd, J = 2.4, 12.0 Hz, 1H), 8.74 (dd, J = 1.2, 12.0 Hz, 1H), 11.92 (s, 1H); MS (EI) m/z: 565 (M^+); HR-MS (EI) m/z: 565.1567 (calcd for $C_{31}H_{21}F_2N_5O_4$: 565.1562).

4.9. *N*-(4-(2-azido-5-phenylfuro[2,3-*d*]pyrimidin-4-yloxy)-3-fluorophenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (18)

To a solution of methylsulfone **17** (25 mg, 0.041 mmol) in DMF (5 mL) was added NaN_3 (27.9 mg, 0.216 mmol) at 0 °C. The mixture was allowed to warm to rt and was stirred for 1.5 h. The reaction was quenched with water (10 mL) and the mixture was extracted with EtOAc (3 × 10 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatograph to give azide **18** (7 mg, 30.4%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.60 (t, J = 6.9 Hz, 1H), 7.19 (m, 1H), 7.26 (m, 2H), 7.41 (m, 5H), 7.61 (m, 2H), 7.71 (m, 2H), 7.92 (dd, J = 2.7, 12.3 Hz, 1H), 8.73 (dd, J = 2.1, 6.9 Hz, 1H), 11.97 (s, 1H); MS (EI) m/z: 577 (M^+); HR-MS (EI) m/z: 577.1291 (calcd for $C_{30}H_{17}F_2N_7O_4$: 577.1310).

4.10. *N*-(3-fluoro-4-(2-methoxy-5-phenylfuro[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (19)

To a solution of NH_3 in CH_3OH was added methylsulfone **16** (32 mg, 0.052 mmol). The mixture was stirred overnight at rt. The solvent was evaporated under reduced pressure and the residue was poured into water and extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatograph to afford 2-methoxypyrimidine **19** (13 mg, 44.0%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.89 (s, 3H), 6.60 (t, J = 6.3 Hz, 1H), 7.19 (m, 1H), 7.25 (m, 2H), 7.40 (m, 6H), 7.62 (m, 2H), 7.73 (d, J = 7.8 Hz, 2H), 7.92

(d, J = 12.0 Hz, 1H), 8.73 (d, J = 6.9 Hz, 1H), 11.95 (s, 1H); MS (EI) m/z: 566 (M^+); HR-MS (EI) m/z: 566.1399 (calcd for $C_{31}H_{20}F_2N_4O_5$: 566.1402).

4.11. Pharmacological procedure

4.11.1. ELISA kinase assay

The tyrosine kinase activities were evaluated according to the reported protocol. Briefly, in enzyme-linked-immunosorbent assay (ELISA), 20 μg/mL Poly(Glu,Tyr) 4:1 (Sigma) was pre-coated as a substrate in 96-well plates. Fifty microliters of 10 μM ATP solution diluted in kinase reaction buffer (50 mM HEPES pH 7.4, 50 mM $MgCl_2$, 0.5 mM $MnCl_2$, 0.2 mM Na_3VO_4, 1 mM DTT) was added to each well. Various concentrations of compounds diluted in 10 μL of 1% DMSO (v/v) were added to each reaction well, with 1% DMSO (v/v) used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 40 μL of kinase reaction buffer solution. 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). Next, 100 μL anti-phosphotyrosine (PY99) antibody (1:500 diluted in 5 mg/mL BSA T-PBS) was added. After 30 min incubation at 37 °C, the plate was washed three times. Hundred microliters horseradish peroxidase-conjugated goat anti-mouse IgG (1:2000 diluted in 5 mg/mL BSA T-PBS) was added. The plate was reincubated at 37 °C for 30 min, and washed as before. Finally, 100 μL of a solution containing 0.03% H_2O_2 and 2 mg/mL o-phenylenediamine in 0.1 M citrate buffer, pH 5.5, was added and samples were incubated at room temperature until color emerged. The reaction was terminated by the addition of 50 μL of 2 M H_2SO_4, and the plate was read using a multiwell spectrophotometer (VERSAmax™, Molecular Devices, Sunnyvale, CA, USA) at 490 nm. The inhibition rate (%) was calculated using the following equation: $[1 - (A490/A490\ control)] \times 100\%$. IC_{50} values were calculated from the inhibition curves.

4.11.2. Western blot analysis

Cells were cultured under regular growth conditions to exponential growth phase. Then the cells were treatment with indicated concentration of compounds for 4 h at 37 °C and lysed in 1× SDS sample buffer. Those cell lysates were subsequently resolved on 10% SDS–PAGE, and transferred to nitrocellulose membranes. Membranes were probed with, phospho c-Met and c-Met, phospho-ERK1/2 and ERK1/2, phospho-AKT and AKT (All from Cell Signaling Technology, Beverly, MA) and GAPDH (KangChen Biotech) antibody then subsequently with anti-rabbit or anti-mouse IgG horseradish peroxidase. Immunoreactive proteins were detected using an enhanced chemiluminescence detection reagent.

4.11.3. Cell proliferation assay

Cells were seeded in 96-well tissue culture plates. On the next day, cells were exposed to various concentrations of compounds and further cultured for 72 h. Finally, cell proliferation was determined using sulforhodamine B (SRB; Sigma) or Thiazolyl Blue Tetrazolium Bromide (MTT; Sigma) assay.

4.11.4. In vivo antitumor activity assay

Animal experiments were performed according to institutional ethical guidelines of animal care. The cells at density of 5–10×10^6 in 200 μL firstly implanted sc into the right flank of each nude mice and then allowing to grow to 700–800 mm^3, defined as a well-developed tumor. After that, the well-developed tumors were cut into 1 mm^3 fragments and transplanted sc into the right flank of nude mice using a trocar. When the tumor volume reached 100–150 mm^3, the mice were randomly assigned into control and treatment groups. Control groups were given vehicle alone, and treatment groups received compound **6b** as indicated doses via ip administration 7 days per week for 3 weeks. The sizes of the tumors were measured twice per week using microcaliper. The tumor volume (TV) was calculated as: TV = (length × width^2)/2. Tumor volume was shown on indicated days as the median tumor volume ± SE indicated for groups of mice. Percent (%) inhibition values were measured on the final day of study for drug-treated compared with vehicle-treated mice and are calculated as $100 \times \{1-[(\text{treated final day-treated day 1})/(\text{control final day-control day 1})]\}$. Significant differences between the treated versus the control groups ($P \leqslant 0.001$) were determined using Student's t-test.

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Supplementary data

Supplementary data (copies of ^1H and ^{13}C NMR data for all the new compounds as well as the key intermediates) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.038.

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