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Synthesis and biological evaluation of novel 7-substituted 3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-4-amines as potent Bruton's tyrosine kinase (BTK) inhibitors

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ABSTRACT

A series of novel 7-substituted 3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-4-amines as potent BTK inhibitors were designed, synthesized and evaluated. These thieno[3,2-*c*]pyridin-4-amine derivatives displayed variant inhibitory activities against BTK in vitro. Among these, 7-pyrazol-4-yl substituted 3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-4-amine subseries showed high BTK inhibition and several compounds displayed superior BTK inhibitory activity. Comprehensive SAR was disclosed and compound **13b** showed excellent potency (IC_{50} = 11.8 nM), outstanding hydrophilicity (*A*log*P* = 3.53), and relatively good kinase selectivity, being a promising lead for further evaluation.

1. Introduction

Bruton's tyrosine kinase (BTK) belongs to the Tec family of non-receptor protein tyrosine kinases which is mostly expressed in hematopoietic cells such as B cells, mast cells and macrophages. BTK plays a key role in multiple cellular signaling pathways such as B cell receptor and Fc receptor signaling cascades. Thus, BTK is regarded as a potential therapeutic target for treating hematological malignancies and autoimmune diseases.[1,2] Recently, a small molecular BTK inhibitor ibrutinib (**1**, Imbruvica™) was approved by FDA for treating mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL)[3], which provided the evidence that BTK inhibitors have become effective therapies for hematological malignancies in clinic. Meanwhile, BTK inhibitors are still ongoing in clinical evaluation to identify their use for treating autoimmune diseases such as rheumatoid arthritis (RA). Recently, a BTK agent named HM71224 (**2**) is reported to come through phase I proof-of-mechanism studies, which is developed by Hanmi Pharmaceuticals with the purpose of autoimmune diseases therapy, and now licensed to Lilly.[4] Therefore, BTK inhibitors have now attracted much attention, and many research groups are eager to develop BTK inhibitors.

During recent years, many BTK inhibitors have been reported, and some of them have even advanced to clinical stages, such as ibrutinib (**1**), HM-71224 (**2**), ONO-4059 (**3**), spebrutinib (**4**, CC-292, AVL-292) and RN-486 (**5**).[5] Among these BTK inhibitors, pyrazolo[3,4-*d*]pyrimidines, pyrimidines and 5-phenylpyridinones are the major representative structural scaffolds. Ibrutinib contains the pyrazolo[3,4-*d*]pyrimidine scaffold, whereas ONO-4059 (**3**, phase II) has a nucleus of 7*H*-purin-8(9*H*)-one, that is, structurally derivatized from pyrazolo[3,4-*d*]pyrimidine. Spebrutinib has a pyrimidine central core, while HM71224 contains a thieno[3,2-*d*]pyrimidine skeleton, that is, considered to be a structural mimic of pyrimidine. RN-486 possesses a fundamental core of 5-phenylpyridin-2(1*H*)-one, that was structurally similar to the pharmacophore of CGI-1746 (**6**) and GDC-0834 (**7**)[6–16] (Fig. 1).

Inspired by the pioneering structural scaffolds, our group recently reported our medicinal chemistry work on the discovery of novel BTK inhibitors.[17–19] Among these, one series of thieno[3,2-*c*]pyridine analogues were developed using scaffold hopping strategy.[19,20] In the thieno[3,2-*c*]pyrid-4-amine series, compound **8** was found to exhibit the most BTK inhibitory activity, with IC_{50} value of 12.8 nM. Considering that the

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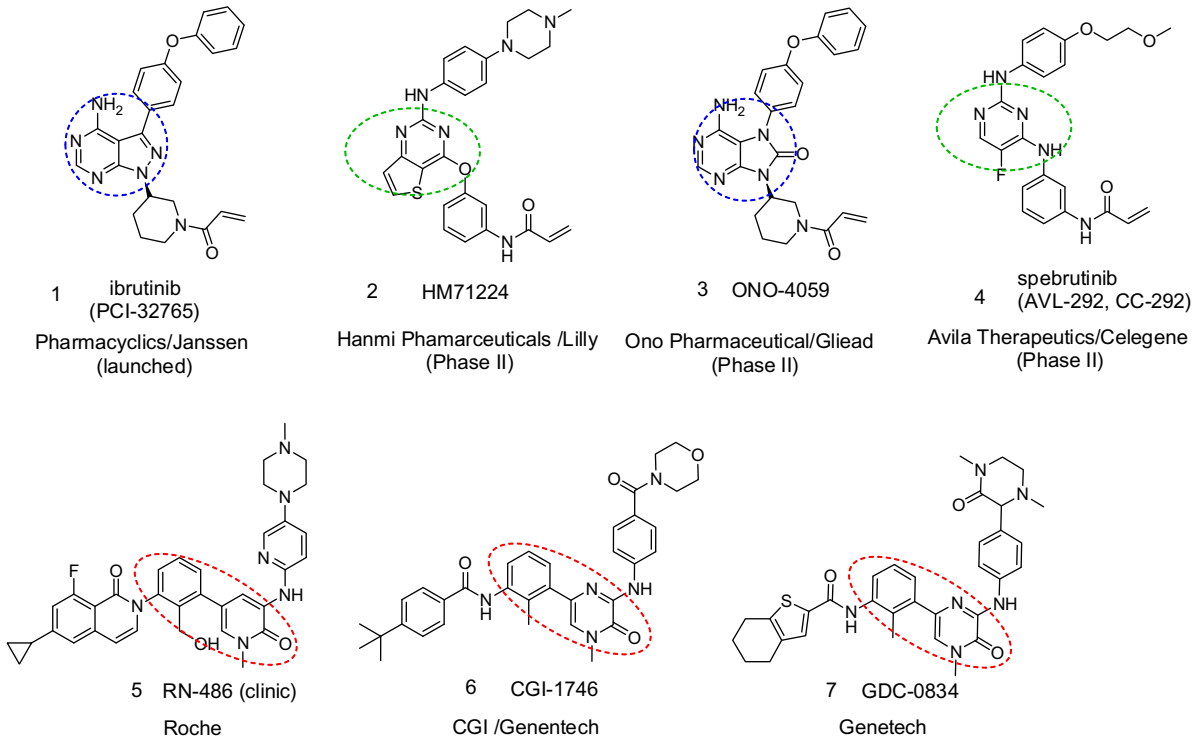


Figure 1. Representative chemical structures of BTK inhibitors.

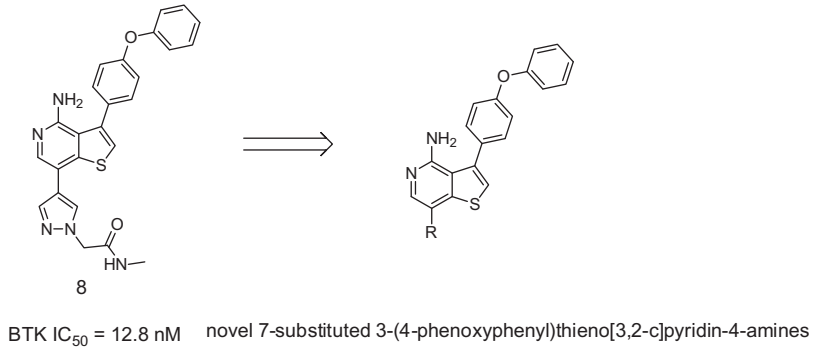


Figure 2. Design of novel 7-substituted 3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-4-amines.

thieno[3,2-*c*]pyrid-4-amines were first reported as potent BTK inhibitors and the derivatives were relatively limited, we are presently interested to further explore extensively structural modification of thieno[3,2-*c*]pyrid-4-amine skeleton and the comprehensively understanding the SAR, thereby designing a series of novel thieno[3,2-*c*]pyrid-4-amine derivatives. Herein, in this paper we report the synthesis and pharmacological evaluation of these novel thieno[3,2-*c*]pyrid-4-amine analogues as potent BTK inhibitors, of which the representative compound **13b** is also evaluated about the kinase selectivity in several other kinase assays (Fig. 2).

2. Chemistry

As summarized in Table 1, the novel 7-substituted thieno[3,2-*c*]pyrid-4-amine derivatives (**13a–j**, **14a–f** and **15a–d**) were designed and synthesized. The synthetic routes for these compounds are depicted in Schemes 1 and 2.

Scheme 1 detailed the synthesis of the desired thieno[3,2-*c*]pyrid-4-amines **13a–j**. As described in the literatures,[19] the key intermediate **12** was synthesized by four steps. Suzuki coupling of commercially available 3-bromothieno[3,2-*c*]pyridin-4-amine and (4-phenoxyphenyl)boronic acid gave compound **9**, which was subsequently iodinated by *N*-iodosuccinimide reagent to afford compound **10**, followed by the Suzuki coupling reaction to provide compound **11**. Then compound **11** was stirred with trifluoroacetic acid to produce the deprotected intermediate **12**. Compound **12** was then reacted with various halides to afford the targeted compounds **13a–j** (Scheme 1).

Scheme 2 depicted the synthesis of compounds **14a–f** and **15a–d**. Compound **14a** was prepared by treating iodide **10** with *N*-Boc-1,4,5,6-tetrahydropyridin-3-ylboronic acid using Suzuki coupling protocols. Deprotection of **14a** with trifluoroacetic acid yielded compound **14b**. Treating **14b** with *N*-methyl chloroacetamide or acryloyl chloride under alkaline condition at −10 °C provided compound **14c** and **14d**, respectively. Hydrogenation reduction of **14b** with Pd/C afforded compound **14e**, which was then treated with acryloyl chloride at −10 °C to provide compound **14f**. Likewise, compounds **15a–d** were synthesized employed the similar synthetic routes. Coupling of **14a** and *N*-Boc-1,4,5,6-tetrahydropyridin-4-ylboronic acid using Suzuki

reaction provided **15a**, which was subsequently reacted with trifluoroacetic acid to generate **15b**. Acylation of **15b** with acryloyl chloride afforded **15c**. Meanwhile, compound **15d** was prepared by hydrogenating **15b** with Pd/C catalyst in ethanol (Scheme 2).

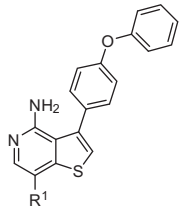
3. Result and discussion

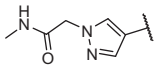
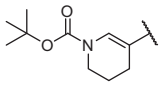
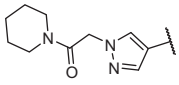
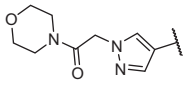
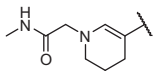
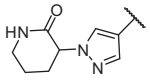
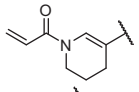
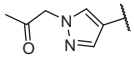
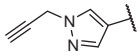
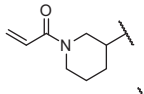
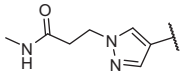
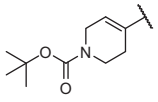
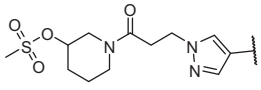
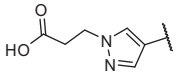
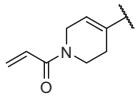
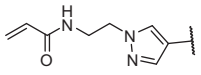
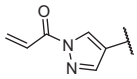
3.1. In vitro BTK kinase inhibition studies for the new compounds

All the newly synthesized 7-substituted thieno[3,2-*c*]pyrid-4-amines (**13a**–**j**, **14a**–**f** and **15a**–**d**) were evaluated in vitro for their capacity to inhibit BTK kinase. The in vitro IC_{50} values are depicted in Table 1.

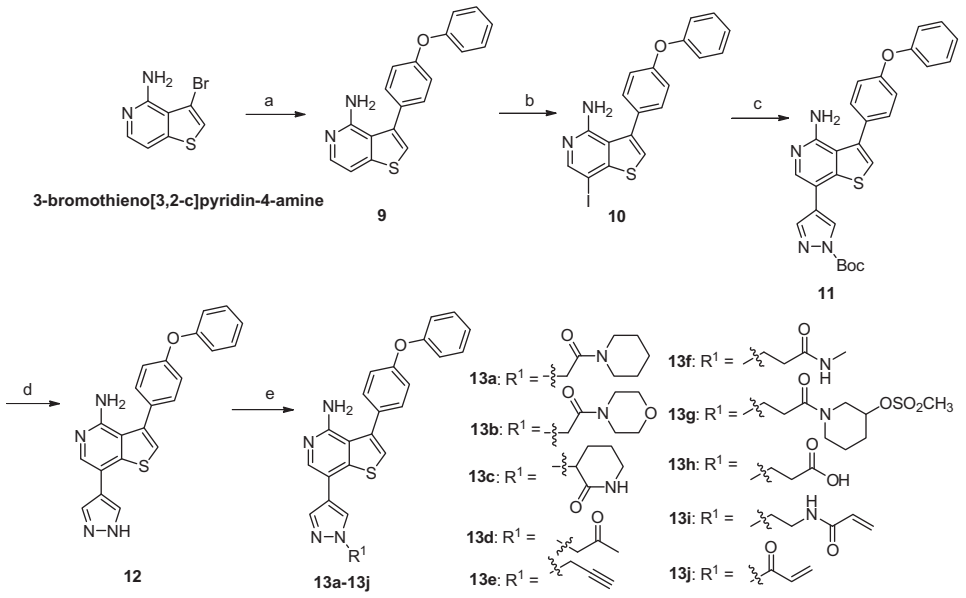
Among the synthesized C7-substituted thieno[3,2-*c*]pyrid-4-amines, the initially designed C7-1*H*-pyrazol-4-yl derivatives displayed moderate to high potency against BTK. In the N1-substituted 1*H*-pyrazol-4-yl analogues, the 2-piperidyl-2-oxoethyl analogue **13a** and the 2-morpholinyl-2-oxoethyl analogue **13b** displayed equivalent potency (**13a**: IC_{50} = 12.2 nM, **13b**: IC_{50} = 11.8 nM), while the 2-oxopiperid-3-yl analogue **13c** showed higher inhibitory activity (**13c**: IC_{50} = 9.5 nM), compared to 2-(methylamino)-2-oxoethyl analogue **8**. Replacing the amide of **8** with a ketone or alkynyl group led to compound **13d** and **13e** showing the highest BTK inhibition, with the IC_{50} values of 7.8 nM and 7.4 nM, respectively. However, the oxoethyl chain of **8** was extended to oxopropyl group, leading to deceased BTK inhibitory activity. The 3-(methylamino)-3-oxopropyl analogue **13g** showed an IC_{50} value of 25.5 nM, while replacing the methylamino group of **13f** with 3-((methylsulfonyl)oxy)piperidin-1-yl group afforded weaker potency (**13g**: IC_{50} = 54.9 nM), whereas the carboxyl group showed tolerant (**13h**: IC_{50} = 21.0 nM). Interestingly, compounds **14i** bearing acrylamide ethyl moiety showed an IC_{50} value of 9.9 nM, which was almost 2.5 fold more potent than

Table 1
In vitro BTK enzymatic inhibition for thieno[3,2-*c*]pyrid-4-amine derivatives

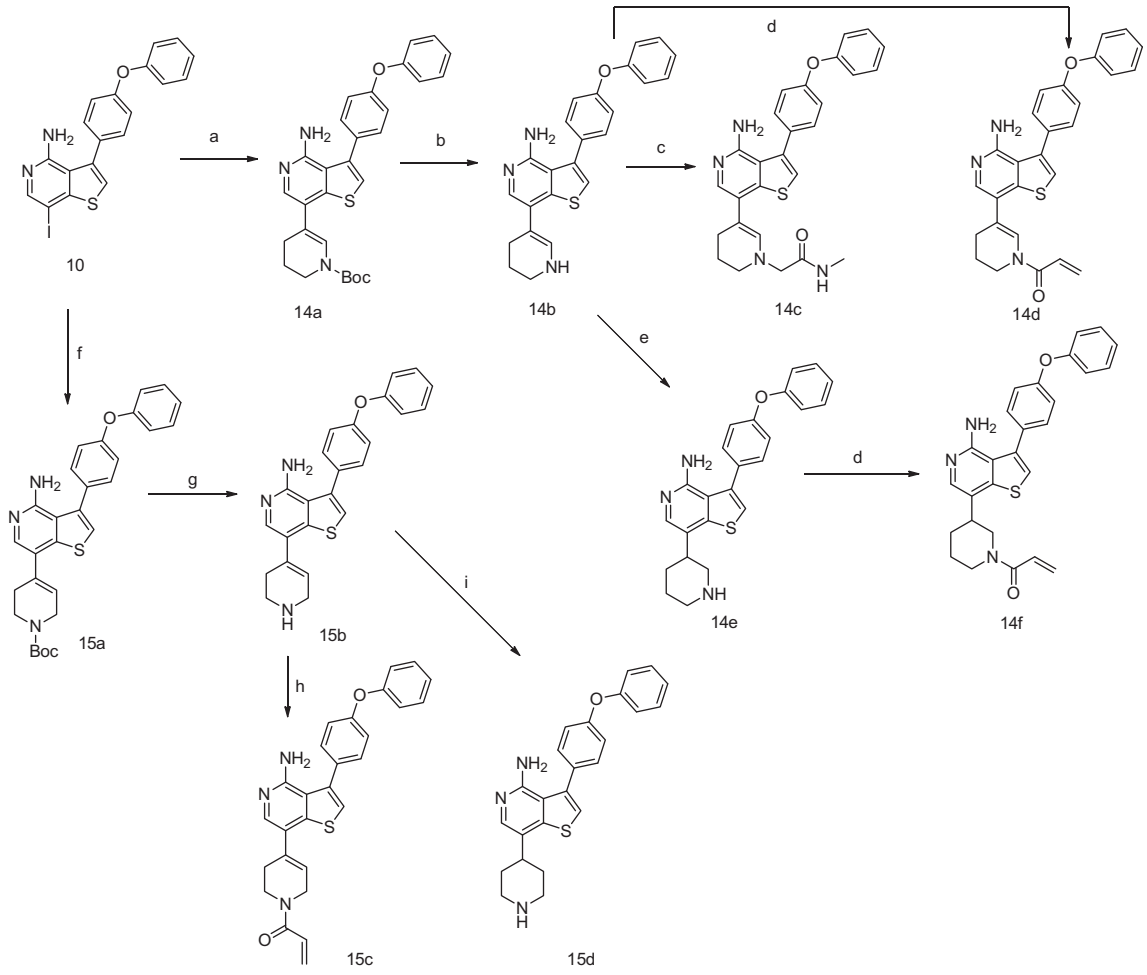


Compds	R^1	Btk IC_{50} [nM][a]	*A*log*P*[b]	Compds	R^1	Btk IC_{50} [nM][a]
8		12.8	3.64	**14a**		2867
13a		12.2	4.76	**14b**		3601
13b		11.8	3.53	**14c**		455.7
13c		9.5	4.23	**14d**		342.6
13d		7.8	4.16	**14e**		>10000
13e		7.4	5.77	**14f**		2295
13f		25.5	3.89	**15a**		1659
13g		54.9	4.03	**15b**		853.9
13h		21.0	4.31	**15c**		183.3
13i		9.9	4.47	**15d**		>10000
13j		1687	—	Ibrutinib	—	4.0

[a] Values are means of three experiments.
[b] *A*log*P* are calculated by Discovery Studio 3.0 using Calculate Molecular Properties Protocol.



Scheme 1. Reagents and conditions: (a) 4-Phenoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, H_2O, toluene, 90 °C, 3 h, 96%; (b) NIS, DMF, rt, 1 h, 70%; (c) 1-Boc-pyrazole-4-boronic acid pinacol ester, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 100 °C, 1–2 h, 55%; (d) CF_3COOH, DCM, rt, 12 h, 92%; (e) R_1X, K_2CO_3, DMF, 25 °C, 4–12 h, 40–95%.



Scheme 2. Reagents and conditions: (a) $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 100 °C, 1–2 h, *N*-Boc-1,4,5,6-tetrahydropyridin-3-ylboronic acid, 53%; (b) CF_3COOH, DCM, rt, 12 h, 75%; (c) *N*-methyl chloroacetamide, TEA, DCM, −10 °C, 2 h, 53%; (d) TEA, DCM, −10 °C, 2 h, acryloyl chloride, 67% for **14d**; 68% for **14f**; (e) H_2, Pd/C, CH_3OH, rt, 12 h, 77%; (f) $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 100 °C, 1–2 h, *N*-Boc-1,4,5,6-tetrahydropyridin-4-ylboronic acid, 63%; (g) CF_3COOH, DCM, rt, 12 h, 75%; (h) acryloyl chloride, TEA, DCM, −10 °C, 2 h, 77%; (i) H_2, Pd/C, CH_3OH, rt, 12 h, 58%.

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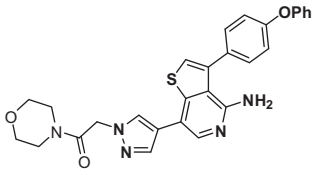
Table 2
Kinase selectivity profiles of compounds **13b**

Compds	Structures	IC_{50} (nM)				
		BTK	JAK2	JAK3	KDR	c-Met
13b		11.8	6864	3068	191	2427

compound **13f**, likely due to that the acrylamide could provide a covalently binding with BTK kinase cysteine residue. However, unfortunately, an attempt of replacing acrylamide ethyl moiety with short acryloyl group (**14h**) led to the loss of BTK inhibitory activity, which appeared that the size of the substituent at N1-position of 1*H*-pyrazol-1-yl was critical for BTK inhibitory activity.

After various substituents at the N1-position of 1*H*-pyrazol-4-yl linked to C7-position of the central thieno[3,2-*c*]pyrid-4-amine skeleton, several piperidyl and tetrahydropyridinyl substituents at the C7-position were attempted to extend the structural diversity. All the tested compounds **14a**–**f** and **15a**–**d** displayed remarkably reduced inhibitory activities. Compounds bearing *N*-Boc-1,4,5,6-tetrahydropyridin-3-yl or 1,4,5,6-tetrahydropyridin-3-yl group were almost inactive against BTK (**14a** and **14b**: IC_{50} > 1000 nM), although the introduction of 2-(methylamino)-2-oxoethyl or acryloyl group led to weak potency (**14c**: IC_{50} = 455.7 nM, **14d**: IC_{50} = 342.6 nM). Moreover, extending the tetrahydropyridin-3-yl to piperid-3-yl group affording the compounds **14e** and **14f**, the BTK inhibitory activities were completely lost (**14e** and **14f**: IC_{50} > 1000 nM). Migration of the nitrogen atom from the 3-position of the tetrahydropyridinyl (or piperidyl) to 4-position afforded several tetrahydropyridin-4-yl (or piperidyl) analogues **15a**–**15d**. It was found that compounds bearing tetrahydropyridin-4-yl moiety showed slightly higher potency than the corresponding tetrahydropyridin-3-yl ones (**15a** vs **14a**, **15b** vs **14b**, **15c** vs **14c**), even though compound **15d** with the piperid-4-yl was inactive for BTK kinase. In this subseries including compounds **14a**–**f** and **15a**–**d**, Compound **15c** bearing an acryloyl tetrahydropyridin-4-yl showed the best potency, with an IC_{50} value of 183.3 nM, however, which was still nearly 25-fold weaker than compound **13e** (Table 1).

Furthermore, in order to assess the drug-like possibility of these compounds with IC_{50} values below 100 nM, their *A*log*P* values were calculated by using Calculate Molecular Properties Protocol of the Discovery Studio 2.5 software package. The results were illustrated in Table 1. Despite compounds **13d** and **13e** displayed higher BTK inhibitory activities in vitro, both showed weaker hydrophilicity properties (**13d**: *A*log*P* = 4.16, **13e**: *A*log*P* = 5.77), compared to compound **8** (*A*log*P* = 3.64). It means both compounds might afford inadequate druggability. Among these compounds, **13b** bearing 2-morpholinyl-2-oxoethyl hydrophilic moiety showed an improved hydrophilicity, with *A*log*P* value of 3.53, moreover, whose BTK inhibition was also favorable. Based on the drug-like prediction study, compound **13b** was considered as the ideal lead candidate, deserving further evaluation (Table 1).

3.2. Kinase selectivity assay

From the BTK inhibition results and the drug-like study above, compound **13b** stood out as the suitable lead candidate. For further evaluating its kinase selectivity, compound **13b** was assessed for the inhibitory activities against 4 kinds of our in-house RTKs and the data were summarized in Table 2. In addition to BTK kinase, compound **13b** only showed moderate inhibitory activities against KDR kinase, with IC_{50} value of 191 nM, which was more than 16-fold weaker than BTK inhibition. This result indicated that **13b** was a relatively selective BTK inhibitor. In future, it should be still further identification against other kinase profiles (Table 2).

4. Conclusion

In summary, we have designed a series of novel 7-substituted 3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-4-amines as potent BTK inhibitors. Twenty thieno[3,2-*c*]pyridin-4-amine derivatives were design, synthesized, and evaluated. These compounds displayed variant inhibitory activities against BTK in vitro. 7-pyrazol-4-yl substituted 3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-4-amine subseries showed high BTK inhibition, and compounds **13d** and **13e** gave the highest potency with the IC_{50} value of 7.8 nM and 7.4 nM, respectively. Compounds **13b** displayed favorable BTK inhibition with an IC_{50} value of 11.8 nM, fine hydrophilicity, and relatively good kinase selectivity, which could be considered as an ideal lead candidate for further evaluation.

5. Experimental

5.1. Chemistry

All chemical reagents were purchased from commercial vendors and used without further purification unless noted especially. The melting points (Mp) for the compounds were performed on a Melt-Temp II apparatus and uncorrected. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR (100 MHz) spectra data were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker BioSpin AG (Ultrashield Plus AV 400) spectrometer. MS data were recorded at an Agilent-6120 quadrupole LC/MS (ESI) while HRMS were recorded at a Water Q-Tof micro mass spectrometer. The HPLC study for the compounds was verified using a mixture of solvent (methanol/water or acetonitrile/water) at the flow rate of 2 mL/min and peak detection at 254 nm under UV. Column chromatography was carried out on silica gel (200–300 mesh) purchased from Qindao Ocean Chemical Company of China. Thin-layer chromatography (TLC) analyses were carried out on silica gel GF254. Ibrutinib was synthesized according to the reference literature.[8]

5.1.1. 7-Iodo-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-4-amine (10)

It was prepared as we recently reported,[19] yellow solid (2.4 g, 70.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (s, 1H), 7.57 (s, 1H), 7.48–7.42 (m, 4H), 7.22–7.18 (m, 1H), 7.14–7.10 (m, 4H), 5.61 (s, 2H); MS *m*/*z* 445.0 $[M+H]^+$.

5.1.2. 3-(4-Phenoxyphenyl)-7-(1*H*-pyrazol-4-yl)thieno[3,2-*c*]pyridin-4-amine (12)

It was synthesized according to our recent route,[19] gray solid (59 mg, 75%). Mp: 232.3–234.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6)

δ 13.09 (s, 1H), 8.15 (s, 1H), 8.06 (s, 1H), 7.95 (s, 1H), 7.54 (s, 1H), 7.48–7.43 (m, 4H), 7.20 (t, *J* = 6.80 Hz, 1H), 7.14–7.12 (m, 4H), 5.42 (s, 2H); HPLC 99.7%; MS *m*/*z* 385.1 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{22}H_{16}N_4OS$ $[M+H]^+$ 385.1117, found 385.1128.

5.1.3. 2-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)-1-(piperidin-1-yl)ethanone (13a)

A suspension of **12** (100 mg, 0.26 mmol), 2-chloro-1-(piperidin-1-yl)ethan-1-one (63 mg, 0.39 mmol) and K_2CO_3 (71.7 mg, 0.52 mmol) in DMF (5 mL) was stirred at rt for 12 h. Then the reaction mixture was diluted with the mixed ice and water, the precipitated solid was filtered off and purified by silica gel column using (20–50%) ethyl acetate/petroleum ether) to afford **13a** as a white solid (30 mg, 53.7%). Mp: 155–157 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 8.08 (s, 1H), 7.92 (s, 1H), 7.56 (s, 1H), 7.48–7.43 (m, 4H), 7.20 (t, *J* = 12.80 Hz, 1H), 7.14–7.11 (m, 4H), 5.45 (s, 2H), 5.21 (s, 2H), 3.47 (s, 4H), 1.60–1.55 (m, 4H), 1.29 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 157.5, 156.6, 153.6, 146.7, 139.9, 137.3, 136.8, 131.5, 131.2, 130.7, 129.1, 124.5, 123.8, 119.7, 119.0, 118.7, 118.3, 114.0, 53.5, 45.8, 42.9, 26.4, 25.7, 24.4; HPLC 95.7%; MS *m*/*z* 510.2 $[M+H]^+$; HRMS(ESI) *m*/*z* calcd for $C_{29}H_{27}N_5O_2S$ $[M+H]^+$ 510.1958, found 510.1975.

5.1.4. 2-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)-1-morpholinoethanone (13b)

13b was prepared by the general method as described above for preparation of **13a** (36 mg, 56.4%). Mp: 135–137 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96–7.92 (m, 3H), 7.44–7.39 (m, 4H), 7.20–7.17 (m, 2H), 7.12–7.07 (m, 4H), 5.30 (s, 2H), 5.09 (s, 2H), 3.67 (s, 8H); HPLC 95.1%; MS *m*/*z* 512.2 $[M+H]^+$; HRMS(ESI) *m*/*z* calcd for $C_{28}H_{25}N_5O_3S$ $[M+H]^+$ 512.1953, found 510.1972.

5.1.5. 3-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)piperidin-2-one (13c)

13c was prepared by the general method as described above for preparation of **13a** (21 mg, 38.2%). Mp: 170–172 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 8.09 (s, 1H), 7.93 (s, 1H), 7.91 (s, 1H), 7.52–7.44 (m, 4H), 7.21 (t, *J* = 12.40 Hz, 1H), 7.16–7.12 (m, 4H), 5.46 (s, 2H), 5.07–5.03 (m, 1H), 3.20–3.15 (m, 2H), 2.38–2.35 (m, 1H), 2.32–2.28 (m, 1H), 2.02–1.97 (m, 1H), 1.89–1.85 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.6, 157.5, 156.6, 153.6, 146.7, 140.0, 137.3, 136.7, 131.5, 131.2, 130.7, 128.6, 124.5, 123.7, 119.7, 119.0, 118.6, 117.6, 114.0, 61.1, 41.8, 28.7, 21.4; HPLC 98.2%; MS *m*/*z* 482.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{23}N_5O_2S$ $[M+H]^+$ 482.1645, found 482.1651.

5.1.6. 1-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)propan-2-one (13d)

13d was prepared by the general method as described above for preparation of **13a** (23 mg, 54.7%). Mp: 151–153 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H), 8.09 (s, 1H), 7.97 (s, 1H), 7.57 (s, 1H), 7.51–7.44 (m, 4H), 7.21 (t, *J* = 12.4 Hz, 1H), 7.16–7.12 (m, 4H), 5.48 (s, 2H), 5.23 (s, 2H), 2.15 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.1, 157.5, 156.6, 153.7, 146.7, 140.1, 137.3, 131.5, 131.2, 130.7, 128.9, 124.5, 123.7, 119.7, 118.9, 118.7, 118.6, 113.8, 61.1, 27.4; HPLC 96.1%; MS *m*/*z* 441.2 $[M+H]^+$. HRMS (ESI) *m*/*z* calcd for $C_{25}H_{20}N_4O_2S$ $[M+H]^+$ 441.1379, found 441.1390.

5.1.7. 3-(4-Phenoxyphenyl)-7-(1-(prop-2-yn-1-yl)-1*H*-pyrazol-4-yl)thieno[3,2-*c*]pyridin-4-amine (13e)

13e was prepared by the general method as described above for preparation of **13a** (31 mg, 78.5%). Mp: 150.3–152.5 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.99 (s, 1H), 7.87 (s, 1H), 7.44–7.38 (m, 4H), 7.20–7.18 (m, 2H), 7.11–7.08 (m, 4H), 5.18 (s, 2H), 4.83 (s, 2H), 2.83 (s,1H). HPLC 96.6%; MS *m*/*z* 424.1 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{18}N_4OS$ $[M+H]^+$ 424.1226, found 424.1243.

5.1.8. 3-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)-N-methylpropanamide (13f)

13f was prepared by the general method as described above for preparation of **13a** (15 mg, 46.0%). Mp: 140–142 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.89 (s, 1H), 7.84 (s, 1H), 7.44–7.38 (m, 4H), 7.19–7.17 (m, 2H), 7.11–7.08 (m, 4H), 5.71 (s, 1H), 4.88 (s, 2H), 4.53–4.48 (m, 2H), 2.80–2.75 (m, 2H), 2.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.3, 157.5, 156.6, 153.7, 146.7, 140.0, 137.3, 136.8, 131.5, 131.2, 130.7, 127.6, 124.5, 123.7, 119.7, 118.9, 118.7, 118.0, 114.0, 48.3, 36.3, 26.0; HPLC 96.2%; MS *m*/*z* 470.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{26}H_{23}N_5O_2S$ $[M+H]^+$ 492.1464, found 492.1468.

5.1.9. 1-(3-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)propanoyl)piperidin-3-yl methanesulfonate (13g)

13g was prepared by the general method as described above for preparation of **13a** (34 mg, 42.5%). Mp: 155–157 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 8.00 (s, 2H), 7.75 (s, 1H), 7.53–7.48 (m, 4H), 7.23–7.17 (m, 5H), 6.10 (s, 2H), 4.80–4.68 (m, 1H), 4.48–4.42 (m, 2H), 3.72 (s, 3H), 3.23–3.18 (m, 4H), 1.95–1.90 (m, 2H), 1.50–1.25 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3, 157.8, 156.5, 137.9, 137.1, 131.5, 130.7, 130.0, 128.7, 126.2, 124.5, 119.7, 119.4, 118.9, 116.5, 114.5, 76.2, 49.6, 48.3, 44.9, 38.3, 29.8, 29.2, 22.3; HPLC 95.0%; MS *m*/*z* 618.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{31}H_{31}N_5O_5S_2$ $[M+H]^+$ 618.1839, found 618.1844.

5.1.10. 3-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)propanoic acid (13h)

13 h was prepared by the general method as described above for preparation of **13a** (30 mg, 41.7%). Mp: 245–247 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H), 8.18 (s, 1H), 8.04 (s, 1H), 7.89 (s, 1H), 7.56 (s, 1H), 7.49–7.43 (m, 4H), 7.20 (t, *J* = 12.40 Hz, 1H), 7.14–7.11 (m, 3H), 5.44 (s, 2H), 4.42–4.38 (m, 2H), 2.75–2.70 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 157.5, 156.5, 153.6, 146.8, 140.0, 137.3, 136.6, 131.5, 131.2, 130.7, 127.7, 124.5, 123.8, 119.7, 118.9, 118.7, 117.9, 114.0, 48.8, 29.4; HPLC 99.1%; MS *m*/*z* 457.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{20}N_4O_3S$ $[M+H]^+$ 457.1328, found 457.1332.

5.1.11. N-(2-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)ethyl)acrylamide (13i)

13i was prepared by the general method as described above for preparation of **13a** (36 mg, 75%). Mp: 158–160 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.34–8.31 (m, 1H), 8.14 (s, 1H), 8.05 (s, 1H), 7.94(s, 1H), 7.55(s, 1H), 7.50–7.43 (m, 4H), 7.20 (t, *J* = 14.76 Hz, 1H), 7.14–7.11 (m, 4H), 6.25–6.18 (m, 1H), 6.09 (dd, *J* = 2.24 Hz, *J* = 2.24 Hz, 1H), 5.59 (dd, *J* = 2.24 Hz, *J* = 2.20 Hz, 1H), 5.43 (s, 2H), 4.34–4.29 (m, 2H), 3.64–3.58 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 157.5, 156.6, 153.7, 146.7, 140.1, 137.3, 132.0, 131.2, 130.7, 127.9, 125.9, 124.5, 123.7, 119.7, 118.9, 118.7, 118.2, 114.0, 51.2, 29.5; HPLC 95.7%; MS *m*/*z* 482.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{23}N_5O_2S$ $[M+H]^+$ 482.1645, found 482.1652.

5.1.12. 1-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-1*H*-pyrazol-1-yl)prop-2-en-1-one (13j)

13j was prepared by the general method as described above for preparation of **13a** (33 mg, 68.1%). Mp: 163–165 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 8.10 (s, 2H), 7.95 (s, 1H), 7.70 (s, 1H), 7.46–7.43 (m, 4H), 7.19–7.10 (m, 4H), 6.94 (d, *J* = 8.40 Hz, 2H), 6.50 (d, *J* = 4.0 Hz, 1H), 6.35–6.29 (m, 1H), 5.79 (d, *J* = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 156.5, 153.7, 148.8, 147.0, 140.2, 138.9, 137.3, 131.6, 131.5, 130.8, 128.0, 124.6, 124.5, 123.7, 121.5, 119.8, 118.9, 118.7, 117.6,

114.9, 114.3; HPLC 95.1%; MS *m*/*z* 439.1 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{18}N_4O_2S$ $[M+H]^+$ 439.1223, found 439.1233.

5.1.13. Tert-butyl-5-(4-amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-3,4-dihydropyridine-1(2*H*)-carboxylate (14a)

A solution of **10** (150 mg, 0.337 mmol), N-Boc-1,4,5,6-tetrahydropyridin-3-ylboronic acid (77 mg, 0.337 mmol) and Pd(dppf)Cl_2·CH_2Cl_2 (27 mg, 0.0337 mmol) in 1,4-dioxane (10 mL) was degassed with nitrogen for 5 min followed by addition Na_2CO_3 (107 mg, 2 M in water) under continuous flow of nitrogen. The reaction mixture was stirred at 100 °C for 2 h, AND the catalyst was removed by filtration through Celite. The filtrate was concentrated and purified by column chromatography to afford compound **14a** (89 mg, 53.1%). Mp: 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.45–7.40 (m, 4H), 7.20–7.17 (m, 2H), 7.13–7.06 (m, 4H), 6.29 (s, 1H), 5.58 (s, 2H), 4.25–4.21 (m, 2H), 1.98–1.92 (m, 2H),1.61–1.57 (m, 2H), 1.48 (s, 9H); HPLC 96.3%; MS *m*/*z* 500.2 $[M+H]^+$; HRMS(ESI) *m*/*z* calcd for $C_{29}H_{29}N_3O_3S$ $[M+H]^+$ 500.2002, found 500.2017.

5.1.14. 3-(4-Phenoxyphenyl)-7-(1,4,5,6-tetrahydropyridin-3-yl)-thieno[3,2-*c*]pyridin-4-amineine (14b)

A mixture of the **14a** (100 mg, 0.21 mmol) in CF_3COOH (3 mL) was stirred at rt for 2 h. Then the reaction mixture was diluted with the mixed ice and water, and then 1 M NaOH solution was added to alkalify to pH 7–8, the precipitated solid was filtered, and concentrated under reduced pressure. The crude product was purified by columu chromatography to afford **14b** as a gray solid (76 mg, 75.1%). Mp: 210–212 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.42–7.38 (m, 4H), 7.20–7.17 (m, 2H), 7.11–7.06 (m, 4H), 6.36 (s, 1H), 5.53 (s, 2H), 3.78–3.75 (m, 2H), 2.43–2.40 (m, 2H), 2.08–2.03 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.5, 156.6, 154.0, 147.2, 139.6, 137.1, 134.6, 131.4, 131.3, 130.7, 124.5, 124.4, 124.1, 121.6, 119.7, 119.1, 118.6, 42.3, 25.6, 21.6; HPLC 95.3%; MS *m*/*z* 500.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{24}H_{21}N_3OS$ $[M+H]^+$ 400.1478, found 400.1486.

5.1.15. 2-(5-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-3,4-dihydropyridin-1(2*H*)-yl)-*N*-methylacetamide (14c)

A suspension of **14b** (104 mg, 0.26 mmol), N-methyl chloroacetamide (42 mg, 0.39 mmol) and K_2CO_3 (71.7 mg, 0.52 mmol) in DMF (5 mL) was stirred at rt for 12 h. After completion of reaction, the reaction mixture was diluted with the mixed ice and water, and the precipitated solid was filtered off and purified on a silica gel column using (20–50%) ethyl acetate/petroleum ether) as eluent to afford **14c** as a white solid (51 mg, 42.0%). Mp: 143–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.42–7.38 (m, 4H), 7.30 (s, 1H), 7.19–7.14 (m, 2H), 7.11–7.07 (m, 4H), 6.21 (s, 1H), 4.72 (s, 2H), 3.46–3.42 (m, 2H), 3.20 (s, 2H), 2.87 (s, 3H), 2.78–2.75 (m, 2H), 2.46–2.40 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 157.5, 156.6, 154.1, 147.2, 139.6, 137.1, 133.2, 131.4, 131.2, 130.7, 124.5, 124.1, 123.8, 121.2, 119.7, 119.1, 118.6, 61.3, 55.1, 49.8, 26.0, 25.9; HPLC 98.4%; MS *m*/*z* 471.1 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{26}N_4O_2S$ $[M+H]^+$ 471.1849, found 471.1853.

5.1.16. 1-(5-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-3,4-dihydropyridin-1(2*H*)-yl)prop-2-en-1-one (14d)

14d was prepared by the general method as described above for preparation of **14c** (32 mg, 67.8%). Mp: 131–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–7.75 (m, 1H), 7.42–7.38 (m, 4H), 7.20–7.16 (m, 2H), 7.11–7.07 (m, 4H), 6.66–6.61 (m, 1H), 6.37–6.28 (m, 2H), 5.76–5.74 (m, 1H), 4.99 (s, 2H), 4.54 (s, 1H), 4.40 (s, 1H), 3.88–3.76 (m, 2H), 2.46–2.43 (m, 2H); HPLC 98.6%; MS *m*/*z* 454.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{23}N_3O_2S$ $[M+H]^+$ 454.1583, found 454.1584.

5.1.17. 3-(4-Phenoxyphenyl)-7-(piperidin-3-yl)thieno[3,2-*c*]pyridin-4-amine (14e)

A suspension of **14b** (400 mg, 1.0 mmol) in MeOH (20 mL) with the 40 mg 10% palladium carbon was stirred for 5 h at room temperature under an atmosphere of hydrogen gas. The reaction mixture was filtered and concentrated in vacuo to afford **11a** (230 mg, 57.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.42–7.37 (m, 4H), 7.17 (t, *J* = 14.76 Hz, 1H), 7.13 (s, 1H), 7.11–7.07 (m, 4H), 4.61 (s, 2H), 3.26–3.23 (m, 2H), 2.85–2.79 (m, 3H), 2.02–1.99 (m, 2H), 1.88–1.81 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.5, 156.6, 153.7, 148.8, 139.1, 137.5, 131.4, 131.3, 130.7, 124.5, 123.7, 123.4, 119.0, 118.6, 51.8, 46.1, 40.2, 30.7, 26.5; HPLC 97.3%; MS *m*/*z* 402.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{24}H_{23}N_3OS$ $[M+H]^+$ 402.1634, found 402.1639.

5.1.18. 1-(3-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)piperidin-1-yl)prop-2-en-1-one (14f)

14f was prepared by the general method as described above for preparation of **14c** (35 mg, 67.0%). Mp: 148–150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (t, *J* = 16.40 Hz, 1H), 7.41–7.38 (m, 4H), 7.20–7.16 (m, 2H), 7.11–7.07 (m, 4H), 6.68–6.61 (m, 1H), 6.38–6.30 (m, 1H), 5.74–5.70 (m, 1H), 5.43–5.41 (m, 2H), 4.90 (dd, *J* = 12.80 Hz, *J* = 12.40 Hz, 1H), 4.19 (dd, *J* = 12.80 Hz, *J* = 12.4 Hz, 1H), 3.15–3.11 (m, 1H), 2.91–2.88 (m, 1H), 2.24–2.20 (m, 1H), 2.05–1.93 (m, 3H), 1.69–1.60 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 157.5, 156.6, 153.9, 148.7, 139.2, 137.6, 131.4, 131.3, 130.7, 129.0, 127.7, 124.5, 123.6, 123.5, 122.5, 122.0, 119.7, 119.0, 118.6, 51.2, 47.3, 46.1, 29.5, 25.5; HPLC 98.6%; MS *m*/*z* 456.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{25}N_3O_2S$ $[M+H]^+$ 456.1740, found 456.1743.

5.1.19. Tert-butyl-4-(4-amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (15a)

15a was prepared by the general method as described above for preparation of **14a** (235 mg, 63.1%). Mp: 149–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.46–7.38 (m, 4H), 7.19–7.15 (m, 2H), 7.11–7.06 (m, 4H), 6.12 (s, 1H), 4.66 (s, 2H), 4.13 (s, 2H), 3.70 (s, 2H), 2.61 (s, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.5, 156.6, 154.2, 146.7, 140.0, 137.1, 133.2, 132.0, 131.9, 131.5, 131.2, 130.7, 129.3, 129.2, 124.5, 124.1, 122.2, 122.0, 119.7, 119.0, 118.6, 79.4, 43.5, 41.9, 28.6; HPLC 97.7%; MS *m*/*z* 500.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{29}H_{29}N_3O_3S$ $[M+H]^+$ 500.2002, found 500.2020.

5.1.20. 3-(4-Phenoxyphenyl)-7-(1,2,3,6-tetrahydropyridin-4-yl)-thieno[3,2-*c*]pyridin-4-amine (15b)

15b was prepared by the general method as described above for preparation of **14b** (76 mg, 75.1%). Mp: 226–228 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (s, 1H), 7.50 (s, 1H), 7.47–7.43 (m, 4H), 7.20 (t, *J* = 14.76 Hz, 1H), 7.14–7.10 (m, 4H), 6.15 (s, 1H), 5.43 (s, 2H), 3.50 (s, 2H), 3.07–3.04 (m, 2H), 2.53–2.48 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.3, 154.3, 150.8, 147.1, 146.6, 145.3, 140.0, 137.1, 133.2, 131.5, 131.4, 131.2, 130.9, 130.7, 124.5, 124.0, 122.3, 122.0, 121.7, 119.7, 119.0, 118.7, 118.6, 43.5, 41.9, 26.9; HPLC 96.3%; MS *m*/*z* 500.2 $[M+H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{24}H_{21}N_3OS$ $[M+H]^+$ 400.1478, found 400.1476.

5.1.21. 1-(4-(4-Amino-3-(4-phenoxyphenyl)thieno[3,2-*c*]pyridin-7-yl)-3,4-dihydropyridin-1(2*H*)-yl)prop-2-en-1-one (15c)

15c was prepared by the general method as described above for preparation of **14c** (85 mg, 77.4%). Mp: 160.9–161.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.42–7.37 (m, 4H), 7.17 (t, *J* = 14.76 Hz, 1H), 7.13 (s, 1H), 7.11–7.07 (m, 4H), 4.61 (s, 2H), 3.52–3.47 (m, 1H), 3.37–3.34 (m, 1H), 2.87–2.72 (m, 3H), 2.17–2.14 (m, 1H), 1.88–1.80 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.5, 156.5, 154.2, 146.7, 140.0, 137.1, 131.5, 131.2, 130.7,

124.5, 124.1, 122.0, 119.7, 119.0, 118.6, 43.5, 41.9, 28.7; HPLC 98.3%; MS *m/z* 402.2 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{23}N_3OS$ $[M+H]^+$ 402.1634, found 402.1637.

5.1.22. 3-(4-Phenoxyphenyl)-7-(piperidin-4-yl)thieno[3,2-*c*]pyridin-4-amine (15d)

15d was prepared by the general method as described above for preparation of **14e** (230 mg, 57.5%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.42–7.37 (m, 4H), 7.17 (t, J = 14.76 Hz, 1H), 7.13 (s, 1H), 7.11–7.07 (m, 4H), 4.61 (s, 2H), 3.26–3.23 (m, 2H), 2.85–2.79 (m, 3H), 2.02–1.99 (m, 2H), 1.88–1.81 (m, 2H); HPLC 97.3%; MS *m/z* 402.2 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{23}N_3OS$ $[M+H]^+$ 402.1634, found 402.1639.

5.2. BTK enzymatic assay

The HTRF kinase assay (components supplied as kit by Cisbio) was chosen for BTK enzyme assays. It uses time resolved fluorescence resonance energy transfer (TR-FRET) to detect production of a phosphorylated substrate. A peptide substrate is labeled with a biotin that can bind to XL665 labeled streptavidin, and the anti-phosphoresidue antibody is labeled with Eu^+. Upon phosphorylation of the substrate, the antibody binds to phosphorylated substrate that enables TR-FRET detection in homogenous assay format. All the reagents used for the BTK kinase assays including their resources are BTK kinase (Invitrogen), HTRF kinEASE-TK kit (Cisbio Bioassays), ATP(Sigma), DTT(Sunshine), $MgCl_2$ and $MnCl_2$ (Sigma). The assay buffer was composed of 50 mM HEPES (pH 7.0), 5 mM $MgCl_2$, 5 mM DTT, 0.1% NaN_3, 0.1% BSA and 0.1 mM orthovanadate. The HTRF assays were preformed according to the manual in the kit. All reagents were dispensed into each well plate according to the orders as follow: (1) BTK enzyme: 0.5 ng/μl, 4 μl; (2) each compound as well as control: 0.008–50 mM; (3) reagent: 22.4 μM ATP and 0.15 μM substrate, 2 μl; (4) inculation: Ambient, at 25 °C, 5 min; (5) reagent: antibody and XL-665, 8 μl. Then following 1 h incubation at room temperature fluorescence was measured on the PHERAStar FS microplate reader (BMG Lab Technologies). Signal was expressed in terms of HTRF ratio (fluorescence intensity at 665 nm/fluorescence intensity at 620 nm).

5.3. ELISA-based kinase selectivity assay

In vitro kinase inhibition assays were carried out as described elsewhere. Briefly, 96-well plates were pre-coated with 0.2 mg/mL poly (Glu-Tyr, 4:1) (Sigma) overnight at 37 °C. 0.05 mL aliquot of 0.01 mmol/L ATP diluted in kinases and reaction medium were added. Test compounds at various concentrations diluted in 0.01 mL of 1% DMSO (V/V) were added. The reaction mixtures were incubated for 60 min at 37 °C. The wells were washed with PBS containing 0.1% T-PBS for three times. The 0.1 mL Phosphorylated tyrosine substrate was added. The kinase reaction was incubated for 60 min at 37 °C, and then the wells were washed three times and then anti-mouse IgG (ZSGB-BIO; ZB-2305; 0.1 mL/well) coupled with horseradish peroxidase (HRP) was added and incubated for another 30 min. The TMB reaction was quenched by addition of 0.05 mL of 2 M H_2SO_4. The optical density was measured at 450 nm by an ELISA reader. The IC_{50} values were calculated for test compounds by using a regression analysis of the concentration/inhibition data.

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