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A series of pyrrolo[2,3-b]pyridine derivatives bearing 1,8-naphthyridin-2-one moiety (23–54) were designed, synthesized and evaluated for their activity against four cancer cell lines and six tyrosine kinases. The most promising compound 32 showed excellent activity in vitro.
Synthesis and antiproliferative activity of pyrrolo[2,3-b]pyridine derivatives bearing the 1,8-naphthyridin-2-one moiety

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Abstract

A series of pyrrolo[2,3-b]pyridine derivatives bearing the 1,8-naphthyridin-2-one moiety were synthesized, and evaluated for their antiproliferative activity against four cancer cell lines (HT-29, A549, H460, and U87MG) and six tyrosine kinases (c-Met, Flt-3, PDGFR-β, VEGFR-2, EGFR, and c-Kit) inhibitory activities in vitro. Most compounds showed moderate to excellent potency, with the most promising analogue 32 showing Flt-3/c-Met IC₅₀ value of 1.16/1.92 nM. Structure-activity relationship studies indicated that the hydrogen atom served as R₁ group was benefited to the potency, and mono-electron-withdrawing groups (mono-EWGs) on the phenyl ring (such as R₃ = 4-F) showed a higher preference for antiproliferative activity.

Keywords: Synthesis; pyrrolo[2,3-b]pyridine derivatives; 1,8-naphthyridin-2-one; antiproliferative activity; c-Met; Flt-3

1. Introduction

Cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015. Globally, nearly 1 in 6 deaths is due to cancer [1]. Despite the efforts to discover and develop small molecule anticancer drugs in the last decade [2–5], development of new antitumor agents with improved safety, efficiency, and tumor selectivity remains desirable.

C-Met inhibitors are a class of small molecules that inhibit the enzymatic activity of the
c-Met tyrosine kinase. Recently, a number of c-Met inhibitors with excellent antitumor activity have been reported. Cabozantinib (1) is a small molecule that inhibits the activity of multiple tyrosine kinases, including RET, MET, and VEGFR-2, was approved on November 2012 by the U.S. FDA for the treatment of patients with progressive metastatic medullary thyroid cancer (MTC) [6]. Recently, many derivatives of Cabozantinib were reported, such as Foretinib (2), BMS777607 (3), MGCD-265 (4), JM800476q-2 (5), BMS-1 (6) listed in Fig. 1 [7-10]. Many structure types of these derivatives were included, such as substitutedquinoline, thieno[2,3-b]pyridine, 2-amino-3-chloropyridine, and pyrrolo[2,3-b]pyridine series [11-17]. However, the main modification of these different series of derivatives was focused on the 5-atom linker, which has two obvious structural characteristics. One is the ‘5 atoms regulation’, which means six chemical bonds distance existing between moiety A and moiety B; the other is the linker containing hydrogen, oxygen, and nitrogen atoms which could form hydrogen-bond donor or acceptor [18-19].

(Figure 1. should listed here)

In our previous study, we introduced 1,4-dihydrocinoline and 1,2,3-triazole fragments into the 5-atom linker based on the “5 atoms regulation”/“hydrogen-bond donor or acceptor”, and the resulting derivatives 7 and 8 (Fig. 2) showed excellent potency [20-21]. 1,8-naphthyridin-2-one fragment was widely used as a building block in the design of anticancer agents. For example, compounds 9 and 10 (Fig. 2) displayed a multitude of biological activities [22-23]. In this work, 1,8-naphthyridin-2-one was introduced to the 5-atom linker, because the carbonyl oxygen and two nitrogen atoms in 1,8-naphthyridin-2-one have high ability to form hydrogen-bonding interactions with c-Met. 4-(2-substitutedphenoxy)-1-substituted-1H-pyrrolo[2,3-b]pyridine was used as the moiety A. Substituted phenyl ring was reserved as the moiety B. Small substituents R1, R2, and R3 were introduced to investigate their effects on activity of the target compounds. Accordingly, we designed a novel series of pyrrolo[2,3-b]pyridine derivatives bearing the 1,8-naphthyridin-2-one moiety (Fig. 3).

(Figure 2. should listed here)

(Figure 3. should listed here)
2. Chemistry

2.1. Synthesis of 3-substituted-4-((1-substituted-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)aniline

The synthesis of the key intermediates of 3-substituted-4-((1-substituted-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)aniline 14a–d was achieved from the commercially available 4-chloro-1H-pyrrolo[2,3-b]pyridine or 4-chloro-1-methyl-1H-pyrrolo[2,3-b]pyridine as shown in Scheme 1, which has been illustrated in detail in our previous study [21].

(Scheme 1. should be listed here)

2.2. Synthesis of the target compounds of 1,8-naphthyridin-2-one-based pyrrolo[2,3-b]pyridine derivatives

The target compounds 23–54 were prepared as illustrated in Scheme 2. Condensation of substituted aniline 16a–h with 2-chloronicotinic acid 15 in AcOH at 100 °C resulted in high yield of intermediates 17a–h as white solids. 17a–h were reduced by LiAlH₄ in THF to afford intermediates 18a–h as white solids, which were oxidized by pyridinium dichromate to get intermediates 19a–h. Acylation of the 19a–h with dimethyl malonate in the presence of piperidine in refluxing ethanol (EtOH) yielded ethyl 2-oxo-1-substituted phenyl-1,2-dihydro-1,8-naphthyridine-3-carboxylates 20a–h. Simple procedures such as hydrolysis and acyl chlorination were used to convert ethyl 20a–h to the corresponding acyl chloride 22a–h; the reactions proceeded with K₂CO₃ and thionyl chloride, respectively. Reaction of anilines 14a–d with acyl chloride 22a–h promoted by DIPEA in dichloromethane at room temperature yielded the target compounds 23–54 [24].

(Scheme 2. should be listed here)

3. Results and Discussion

3.1. In vitro cytotoxic activities and SAR

The cytotoxic activities of the target compounds 23–54 have been evaluated in HT-29 (human colon cancer) and A549 (human lung adenocarcinoma) cell lines using the MTT assay [24-26]. Some potent compounds were further evaluated against the H460 (human lung cancer) and U87MG (human glioblastoma) cell lines. Foretinib is structural optimization of
the marketed drug cabozantinib. Comparing with other small molecule c-Met kinase inhibitors reported, foretinib exhibited excellent antiproliferative activity. In our study, foretinib was used as the positive control, and the results expressed as half-maximal inhibitory concentration (IC$_{50}$) values and are presented in Table 1. The values are the average of three independent experiments.

All the target compounds showed moderate to excellent cytotoxic activity against the different cancer cells with potencies in the single-digit µM range. Ten of these compounds were more potent than foretinib against one or more cell lines, which suggest that 1,8-naphthyridin-2-one is a useful framework in the designing of antitumor agents (Table 1). The IC$_{50}$ values of the most promising compound 32 were 0.036 µM, 0.062 µM, and 0.087 µM against HT29, A549, and H460 cell lines, respectively; these values indicated that this compound was 7.2, 5.2, and 3.2 times more active than foretinib (IC$_{50}$ values: 0.26 µM, 0.32 µM, and 0.28 µM, respectively).

According to the data shown in Table 1, the cell lines data revealed a preference for activity when R$_1$ group was hydrogen atom. For example, the activity of compound 23 (HT29 IC$_{50}$ = 0.29 µM; R$_1$ = H, R$_2$ = H, R$_3$ = H) was more potent than compound 39 (HT29 IC$_{50}$ = 0.62 µM; R$_1$ = CH$_3$, R$_2$ = H, R$_3$ = H), and the same trend was observed in compounds 24/40, 31/47, 32/48, and so on. At the same time, the data showed a preference for activity when the R$_2$ group was fluorine atom. For example, the IC$_{50}$ value of compound 31, 32, 47, and 48 were lower than that of 23, 24, 39, and 40, respectively, against HT29 cells.

Further studies were performed to examine the effect of different substituents on the phenyl ring (moiety B) on potency. The stronger mono-electron-withdrawing groups (mono-EWGs) introduced to the phenyl ring increased the cytotoxic activity to a higher extent. For example, compound 31, with no substituent on the phenyl ring, showed a HT29 IC$_{50}$ value 0.16 µM. Introduction of stronger mono-EDGs to R$_3$ (32, R$_3$ = 4-F, IC$_{50}$ = 0.036 µM, increased 4.4-fold) increased the inhibitory efficacy greater than that of weaker mono-EDGs (33, R$_3$ = 4-Cl, IC$_{50}$ = 0.082 µM, increased 2.0-fold; 34, R$_3$ = 4-Br, IC$_{50}$ = 0.12 µM, increased 1.3-fold). However, incorporation of mono-electron-donating groups (mono-EDGs) and double electron-withdrawing groups (double-EWGs) could decrease the potency of the compounds. Incorporation of mono-EDGs decreased the cytotoxic activity. The inhibitory
efficacy of 51 \((R_3 = 4\text{-OCH}_3, \text{IC}_{50} = 1.29 \mu\text{M})\) and 52 \((R_3 = 3\text{-Cl-4-F, IC}_{50} = 1.46 \mu\text{M})\) are 2.5 times and 2.9 times lower than 47 \((R_3 = \text{H, IC}_{50} = 0.51 \mu\text{M})\), respectively. Therefore, this could be demonstrated that mono-EWGs (such as \(R_3 = 4\text{-F}\)) had a positive effect on the cytotoxic activity, especially for the stronger mono-EWGs.

(Table 1. should be listed here)

3.2. In vitro enzymatic assays

The c-Met enzymatic assays of eight pyrrolo[2,3-b]pyridine derivatives were evaluated using homogeneous time-resolved fluorescence (HTRF) assay [27-29]. The results suggested that the inhibition of c-Met may be one mechanism of the antitumor effect of these derivatives (Table 2). Compound 32 showed the most potent activity with an \(\text{IC}_{50}\) value of 1.92 nM, which was comparable to that of the positive control foretinib \((\text{IC}_{50} = 1.56 \text{nM})\), and this compound should be studied further.

(Table 2. should be listed here)

3.3. Enzymatic selectivity assays

As shown in Table 3, compound 32 was chosen for further evaluation of the selectivity on c-Met over other tyrosine kinases. Compared with its high potency against c-Met \((\text{IC}_{50} = 1.92 \text{nM})\), 32 also exhibited high inhibitory effects against Flt-3 \((\text{IC}_{50} = 1.16 \text{nM})\) and PDGFR-\(\beta\) \((\text{IC}_{50} = 1.81 \text{nM})\). While, compound 32 showed inhibitory effects against VEGFR-2, EGFR, and c-Kit, although the potency was 79.3-, 329.6-, and 356.5-fold lower than that of c-Met. These data suggested that compound 32 is a promising multitarget kinase inhibitor.

(Table 3. should be listed here)

4. Binding model analysis

To further elucidate the binding mode of these pyrrolo[2,3-b]pyridine derivatives, docking analysis was performed. The docking simulation was conducted using SURFLEX-DOCK module of SYBYL 8.1 package version. The co-crystal structure of foretinib (GSK1363089) with c-Met kinase were obtained from RCSB Protein Data Bank. The binding model was exemplified by the interaction of compound 32 with c-Met. As shown in Fig. 4, the hydrogen atom connected to nitrogen atom of pyrrolidine and the nitrogen atom
of the pyridine ring in 32 formed two hydrogen bonds with MET1160. In the 5-atom linker, the oxygen atom of the amide and 1,8-naphthyridin-2-one formed two hydrogen bonds with LYS1110. Therefore, compound 32 formed four hydrogen-bonding interactions with c-Met.

5. Conclusions

In summary, a series of pyrrolo[2,3-b]pyridine derivatives bearing the 1,8-naphthyridin-2-one moiety were designed and synthesized. Four human cancer cell lines (HT29, A549, H460, and U87MG) and six tyrosine kinases were used to evaluate the potency of the synthesized compounds. Compared with foretinib, ten of the derivatives were more potent against one or more cell lines. With a Flt-3/c-Met IC\textsubscript{50} value of 1.16/1.92 nM, compound 32 (a multitarget tyrosine kinase inhibitor) showed the strongest cytotoxic activities against HT-29, A549, and H460 cell lines, which was 7.2, 5.2, and 3.2 times more active than foretinib against these three cell lines, respectively. Analysis of SARs indicated that the hydrogen atom served as R\textsubscript{1} group was benefited to the potency, and mono-EWGs on the phenyl ring (such as R\textsubscript{3} = 4-F) showed a higher preference for antiproliferative activity.

6. Experimental

6.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. Reactions’ time and purity of the products were monitored by TLC on FLUKA silica gel aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm. Column chromatography was run on silica gel (200–300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). All melting points were obtained on a Bühchi Melting Point B-540 apparatus (Bühchi Labortechnik, Flawil, Switzerland) and were uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra were recorded on Bruker ARX-400, 400MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy) [29].

6.2. General procedure for 3-substituted-4-((1-substituted-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)aniline (14a–d)
The preparation of the key intermediates 14a–d has been illustrated in detail in our previous work [21], and so the synthesis method would not be listed here.

6.2.1. 4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)aniline (14a)

Light yellow solid; Yield: 55%; M.p.: 182–185°C; ¹H NMR (400 MHz, DMSO-d₆) δ 11.61 (s, 1H), 8.01 (d, J = 5.3 Hz, 1H), 7.30 (d, J = 2.3 Hz, 1H), 6.88 (d, J = 8.5 Hz, 2H), 6.63 (d, J = 8.5 Hz, 2H), 6.28 (d, J = 5.4 Hz, 1H), 6.21 (s, 1H), 5.08 (s, 2H); MS (ESI) m/z(%): 226.5 [M+H]+.

6.2.2. 3-fluoro-4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)aniline (14b)

Light yellow solid; Yield: 49%; M.p.: 184–188°C; ¹H NMR (400 MHz, DMSO-d₆) δ 11.67 (s, 1H), 8.03 (d, J = 5.3 Hz, 1H), 7.33 (s, 1H), 7.02 (t, J = 9.0 Hz, 1H), 6.52 (d, J = 13.3 Hz, 1H), 6.44 (d, J = 8.6 Hz, 1H), 6.29 (d, J = 5.5 Hz, 1H), 6.24 (s, 1H), 5.42 (2H); MS (ESI) m/z(%): 244.2 [M+H]+.

6.2.3. 4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)aniline (14c)

Light yellow solid; Yield: 53%; M.p.: 188–191°C; ¹H NMR (400 MHz, DMSO-d₆) δ 8.01 (d, J = 5.3 Hz, 1H), 7.31 (d, J = 2.5 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 6.62 (d, J = 8.6 Hz, 2H), 6.26 (d, J = 5.3 Hz, 1H), 6.23 (m, 1H), 5.10 (s, 2H), 3.96 (s, 3H); MS (ESI) m/z(%): 240.8 [M+H]+.

6.2.4. 3-fluoro-4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)aniline (14d)

Light yellow solid; Yield: 56%; M.p.: 192–195°C; 1H NMR (DMSO-d₆) 8.02 (m, 1H), 7.32 (s, 1H), 7.01 (t, J = 9.0, 1H), 6.51 (dd, J = 13.2, 1H), 6.43 (dd, J = 8.6, 1H), 6.28 (d, J = 5.4 Hz, 1H), 6.24 (m, 1H), 5.42 (2H), 4.09 (s, 3H); MS (ESI) m/z(%): 258.7 [M+H]+.

6.3. General procedure for the preparation of compounds 23–54

To the mixture of an appropriately substituted phenylamine 16a–h (0.054 mol) and glacial acetic acid (100 mL), 2-chloronicotinic acid 15 (0.032 mol) was added at room temperature. Upon the completion of addition, the reaction mixture was stirred at 100 °C for 3–6 h and monitored by thin-layer chromatography (TLC). The reaction mixture was cooled to room temperature and basified with potassium hydroxide to pH 12, and filtered. The filtrate was acidified with hydrochloric acid to pH 3, and stirred for 0.5 h. The precipitate was collected by filtration and dried to give the corresponding 2-(substitutedphenylamino)nicotinic acid 17a–h as white solids.
Lithium aluminum hydride (LiAlH₄, 0.119 mol) was added to tetrahydrofuran (THF, 40 mL) under an atmosphere of nitrogen at 0 °C, and stirred for 10 min. 2-(substitutedphenylamino)nicotinic acid 17a–h (0.027 mol) dissolved in THF was added to the reaction mixture, and stirred for 3.5 h at room temperature. Ethyl acetate was added to the mixture, and basified with potassium hydroxide to pH 12, and filtered. The filtrate was concentrated in a vacuum and the residue was stirred in petroleum ether. The precipitate was collected by filtration and dried to give the corresponding (2-(substitutedphenylamino)pyridin-3-yl)methanol 18a–h as white solids.

(2-(substitutedphenylamino)pyridin-3-yl)methanol 18a–h (0.018 mol) and pyridinium dichromate (0.031 mol) were added to CH₂Cl₂ (75 mL) at room temperature. The reaction mixture was stirred at room temperature for 5–7 h and monitored by thin-layer chromatography (TLC). The solvent was concentrated in vacuum and the residue was stirred in water (50 mL) for 0.5 h. The precipitate was collected by filtration and dried to give the corresponding 2-(substitutedphenylamino)nicotinaldehyde (19a–h) as light yellow solids.

To the mixture of an appropriately 2-(substitutedphenylamino)nicotinaldehyde 19a–h (0.011 mol) and EtOH (75 mL), diethyl malonate (0.022 mol) and piperidine (0.009 mol) was added at room temperature. Upon completion of the addition, the reaction mixture was heated at reflux for 30–35 h. The solvent was concentrated in vacuum and the residue was stirred in H₂O (70 mL) for 0.5 h at room temperature. The precipitate was collected by filtration and dried to give the corresponding 2-oxo-1-substitutedphenyl-1,2-dihydro-1,8-naphthyridine-3-carboxylate (20a–h) as light yellow solids.

To the mixture of dioxane (40 mL) and water (40 mL), 2-oxo-1-substitutedphenyl-1,2-dihydro-1,8-naphthyridine-3-carboxylate 20a–h (8 mmol) and potassium carbonate (30 mmol) was added at room temperature. The reaction mixture was heated at 80 °C for 4–5 h. Water (150 mL) was added to the mixture, and acidified with hydrochloric acid to pH 6 at 0 °C. The precipitate was collected by filtration and dried to give the corresponding ethyl 2-oxo-1-substitutedphenyl-1,2-dihydro-1,8-naphthyridine-3-carboxylic acid 21a–h as light yellow solids.
An appropriate ethyl 2-oxo-1-substitutedphenyl-1,2-dihydro-1,8-naphthyridine-3-carboxylic acid 21a–h (2 mmol) was added to thionyl chloride (10 mL) and refluxed for 6 h. The reaction mixture was evaporated to yield the corresponding 2-oxo-1-substitutedphenyl-1,2-dihydro-1,8-naphthyridine-3-carbonyl chlorides 22a–h, which were used for the next step immediately without further purification.

To a solution of an appropriate aniline 14a–d (1 mmol) and N,N-diisopropylethylamine (3 mmol) in dichloromethane (30 mL), an appropriate carbonyl chloride 22a–h in the previous step dissolved in dried dichloromethane (20 mL) was added drop-wise in an ice bath. Upon completion of the addition, the reaction was removed to room temperature for 5–8 h and monitored by TLC. The mixture was washed with 10% K$_2$CO$_3$ (20 mL × 3) followed by brine (20 mL × 1), and the organic phase was separated, dried, and concentrated in vacuum. The crude product was purified by chromatography on silica gel using MeOH/CH$_2$Cl$_2$ to afford the target compounds 23–54 as white solids.

6.3.1. N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1-phenyl-1,2-dihydro-1,8-naphthyridine-3-carboxamide (23)

Yield: 56%; M.p.: 329.9–330.8 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.91 (s, 1H), 11.85 (s, 1H), 9.16 (s, 1H), 8.59 (d, $J = 6.8$ Hz, 2H), 8.09 - 8.00 (m, 2H), 7.56 (d, $J = 7.1$ Hz, 3H), 7.53 - 7.43 (m, 3H), 7.39 (t, $J = 8.6$ Hz, 4H), 6.41 (d, $J = 5.5$ Hz, 1H), 6.25 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 160.33, 156.86, 152.16, 150.33, 143.35, 143.20, 138.87, 136.38, 135.97, 128.68 (2C), 128.47 (2C), 127.82, 124.72, 124.59, 123.30 (2C), 122.00, 119.28 (2C), 116.13, 114.12, 113.63, 109.17, 108.17, 100.46, 96.39; ESI-MS m/z: 473.15; Anal. calcd. for C$_{28}$H$_{19}$N$_5$O$_3$ (%): C, 61.78; H, 4.04; N, 14.79; Found (%): C, 61.79; H, 4.05; N, 14.80.

6.3.2. N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (24)

Yield: 71%; M.p.: 253.3–254.1°C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.89 (s, 1H), 11.78 (s, 1H), 9.16 (s, 1H), 8.61 (s, 2H), 8.04 (d, $J = 15.3$ Hz, 2H), 7.56 (s, 1H), 7.43 (s, 3H), 7.39 (s, 5H), 6.39 (s, 1H), 6.23 (s, 1H); ESI-MS m/z: 492.14; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 163.46, 161.02, 157.74, 153.01, 151.32, 144.68, 144.15, 139.85, 135.47, 133.54, 131.67,
131.57, 131.48, 125.22, 123.19 (2C), 121.97 (2C), 121.44, 120.32 (2C), 116.67, 116.45, 115.21 (2C), 102.92, 102.62, 97.60; Anal. calcd. for C_{28}H_{18}FN_{5}O_{3} (%): C, 68.43; H, 3.69; N, 14.25; Found (%): C, 68.42; H, 3.70; N, 14.35.

6.3.3. \( \text{N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(4-chlorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (25)} \)

Yield: 63%; M.p.: 233.3-234.1 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 12.69 - 12.69 (m, 1H), 11.66 (s, 2H), 9.08 (s, 1H), 8.52 (d, \(J = 8.0\) Hz, 2H), 8.00 (d, \(J = 5.4\) Hz, 1H), 7.77 (s, 1H), 7.74 (s, 1H), 7.57 (d, \(J = 8.4\) Hz, 2H), 7.39 (t, \(J = 9.4\) Hz, 3H), 7.27 (s, 1H), 7.13 (d, \(J = 8.7\) Hz, 2H), 6.35 (d, \(J = 5.4\) Hz, 1H), 6.12 (s, 1H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 160.78, 157.62, 152.95, 151.61, 151.15, 144.65, 144.14, 139.83, 136.31 (2C), 135.39, 131.67, 133.51, 131.48, 129.72 (2C), 125.13, 123.25, 121.92 (2C), 121.37, 120.32 (2C), 116.54, 115.20, 110.62, 102.68, 97.54; ESI-MS m/z: 507.93; Anal. Calcd. for C_{28}H_{18}ClN_{5}O_{3}(%): C, 66.21; H, 3.57; N, 13.79; Found (%): C, 66.22; H, 3.67; N, 13.78.

6.3.4. \( \text{N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(4-bromophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (26)} \)

Yield: 57%; M.p.: 264–265 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 12.05 (s, 1H), 11.86 (s, 1H), 9.15 (s, 1H), 8.60 (d, \(J = 6.6\) Hz, 2H), 8.13 (d, \(J = 5.6\) Hz, 1H), 8.05 (d, \(J = 12.8\) Hz, 1H), 7.78 (d, \(J = 8.3\) Hz, 2H), 7.58 (d, \(J = 7.9\) Hz, 1H), 7.50 - 7.35 (m, 6H), 6.48 (d, \(J = 5.4\) Hz, 1H), 6.31 (s, 1H); ESI-MS m/z: 552.39; Anal. Calcd. for C_{28}H_{18}BrN_{5}O_{3}(%): C, 60.88; H, 3.28; N, 12.68; Found (%): C, 60.89; H, 3.38; N, 12.67.

6.3.5. \( \text{N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (27)} \)

Yield: 59%; M.p.: 299.5–301.2°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.95 (s, 1H), 11.81 (s, 1H), 9.14 (s, 1H), 8.59 (t, \(J = 5.8\) Hz, 2H), 8.08 - 8.00 (m, 2H), 7.56 (d, \(J = 8.7\) Hz, 1H), 7.48 - 7.44 (m, 1H), 7.38 (d, \(J = 6.5\) Hz, 2H), 7.28 (d, \(J = 8.7\) Hz, 2H), 7.10 (d, \(J = 8.7\) Hz, 2H), 6.39 (d, \(J = 5.4\) Hz, 1H), 6.24 (s, 1H), 3.84 (s, 1H); ESI-MS m/z: 473.15; Anal. Calcd. for C_{29}H_{21}N_{5}O_{4} (%): C, 69.18; H, 4.20; N, 13.91; Found (%): C, 69.19; H, 4.21; N, 13.92.

6.3.6. \( \text{N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(3-chloro-4-fluorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (28)} \)

Yield: 59%; M.p.: 234.8–235.6 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.81 (s, 2H), 9.17
(s, 1H), 8.61 (d, J = 8.9 Hz, 2H), 8.09 - 8.01 (m, 2H), 7.80 (d, J = 4.6 Hz, 1H), 7.65 (t, J = 8.9 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.52 - 7.46 (m, 2H), 7.43 - 7.32 (m, 2H), 6.40 (d, J = 5.5 Hz, 1H), 6.25 (s, 1H); ESI-MS m/z: 525.92; Anal. calcd. for C_{28}H_{17}FClN_{5}O_{3}(%): C, 63.95; H, 3.26; N, 13.32; Found (%): C, 63.94; H, 3.27; N, 13.31.

6.3.7. **N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(4-bromo-2-fluorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (29)**

Yield: 55%; M.p.: 275–277 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.71 (s, 1H), 11.55 (s, 1H), 9.12 (s, 1H), 8.56 (d, \(J = 5.4\) Hz, 2H), 7.98 (dd, \(J = 20.3, 6.2\) Hz, 2H), 7.82 (d, \(J = 9.6\) Hz, 1H), 7.60 (d, \(J = 8.9\) Hz, 1H), 7.54 - 7.41 (m, 4H), 7.36 - 7.26 (m, 2H), 6.33 (d, \(J = 4.5\) Hz, 1H), 6.17 (s, 1H); ESI-MS m/z: 569.04; Anal. calcd. for C_{28}H_{17}BrFN_{5}O_{3}(%): C, 58.96; H, 3.00; N, 12.28; Found (%): C, 58.95; H, 2.96; N, 12.23.

6.3.8. **N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(2-chloro-4-(trifluoromethyl)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (30)**

Yield: 61%; M.p.: 243.3-244.1 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.73 - 11.45 (m, 2H), 9.10 (s, 1H), 8.54 (d, \(J = 6.7\) Hz, 2H), 8.00 (d, \(J = 10.4\) Hz, 2H), 7.91 (d, \(J = 8.4\) Hz, 1H), 7.79 (dd, \(J = 23.9, 9.8\) Hz, 3H), 7.40 (dd, \(J = 17.5, 9.9\) Hz, 1H), 7.26 (s, 1H), 7.12 (d, \(J = 8.7\) Hz, 2H), 6.35 (d, \(J = 5.1\) Hz, 1H), 6.11 (s, 1H); ESI-MS m/z: 529.95; Anal. calcd. for C_{29}H_{17}F_{3}ClN_{5}O_{3}(%): C, 60.98; H, 2.98; N, 12.16; Found (%): C, 60.97; H, 2.97; N, 12.18.

6.3.9. **N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-2-oxo-1-phenyl-1,2-dihydro-1,8-naphthyridine-3-carboxamide (31)**

Yield: 54%; M.p.: 255.4-256.1 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.91 (s, 1H), 11.80 (s, 1H), 9.16 (s, 1H), 8.62 - 8.56 (m, 2H), 8.09 - 8.00 (m, 2H), 7.57 (t, \(J = 7.5\) Hz, 3H), 7.52 - 7.43 (m, 2H), 7.42 - 7.35 (m, 4H), 6.39 (d, \(J = 5.5\) Hz, 1H), 6.24 (s, 1H); ESI-MS m/z: 491.14; Anal. calcd. for C_{28}H_{18}FN_{5}O_{3}(%): C, 68.43; H, 3.69; N, 14.25; Found (%): C, 68.53; H, 3.70; N, 14.35.

6.3.10. **N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (32)**

Yield: 65%; M.p.: 263.3-264.1 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.88 (br, 2H), 9.15 (s, 1H), 8.60 (s, 3H), 8.05 (dd, \(J = 16.3, 8.7\) Hz, 2H), 7.56 (d, \(J = 8.2\) Hz, 1H), 7.46 (s, 3H), 7.42 (s, 1H), 7.38 (s, 2H), 6.39 (d, \(J = 4.6\) Hz, 1H), 6.25 (s, 1H); \(^{13}\)C NMR (100 MHz,
DMSO-$d_6$ δ 162.99, 162.61, 160.78, 157.00, 152.64, 152.35, 151.10, 150.86, 144.19, 143.89, 139.41, 136.45, 132.98, 131.16, 131.08, 124.89, 123.80, 122.50, 119.83, 116.63, 116.17, 115.94, 114.69, 109.41, 108.66, 100.86, 96.70; ESI-MS m/z: 509.15; Anal. calcd. for C$_{28}$H$_{17}$F$_2$N$_5$O$_3$: C, 66.01; H, 3.36; N, 13.75; Found (%): C, 66.11; H, 3.37; N, 13.76.

6.3.11. N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(4-chlorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (33)

Yield: 59%; M.p.: 243.3-244.1°C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.75 (d, $J$ = 27.1 Hz, 2H), 9.10 (s, 1H), 8.53 (d, $J$ = 5.0 Hz, 2H), 8.02 - 7.93 (m, 2H), 7.58 (d, $J$ = 8.5 Hz, 2H), 7.49 (d, $J$ = 8.8 Hz, 2H), 7.38 (d, $J$ = 8.4 Hz, 2H), 7.31 (d, $J$ = 8.2 Hz, 2H), 6.31 (d, $J$ = 5.3 Hz, 1H), 6.17 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 162.97, 161.20, 157.51, 153.14, 151.57, 151.20, 144.70, 144.48, 139.94, 136.93 (2C), 136.27, 133.58, 131.49, 129.77 (2C), 128.23, 125.38, 124.35, 120.39, 117.10, 115.19, 109.87, 109.35, 109.12, 102.00, 101.28, 97.22; ESI-MS m/z: 525.15; Anal. calcd. for C$_{28}$H$_{17}$FCln$_5$O$_3$: C, 63.95; H, 3.28; N, 13.32; Found (%): C, 63.97; H, 3.27; N, 13.31.

6.3.12. N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(4-bromophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (34)

Yield: 59%; M.p.: 288.4–289.1 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.05 (s, 1H), 11.86 (s, 1H), 9.15 (s, 1H), 8.60 (d, $J$ = 6.6 Hz, 2H), 8.13 (d, $J$ = 5.6 Hz, 1H), 8.05 (d, $J$ = 12.8 Hz, 1H), 7.78 (d, $J$ = 8.3 Hz, 2H), 7.58 (d, $J$ = 7.9 Hz, 1H), 7.50 - 7.35 (m, 6H), 6.48 (d, $J$ = 5.4 Hz, 1H), 6.31 (s, 1H); ESI-MS m/z: 570.38; Anal. Calcd. for C$_{28}$H$_{16}$BrF$_2$N$_5$O$_3$: C, 58.96; H, 3.00; N, 12.28; Found (%): C, 58.97; H, 3.01; N, 12.38.

6.3.13. N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (35)

Yield: 59%; M.p.: 320.5-321.8 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.80 (s, 1H), 9.16 (s, 1H), 8.60 (d, $J$ = 8.7 Hz, 2H), 8.09 - 8.00 (m, 2H), 7.79 (d, $J$ = 6.7 Hz, 1H), 7.64 (t, $J$ = 8.9 Hz, 1H), 7.57 (d, $J$ = 8.5 Hz, 1H), 7.49 (d, $J$ = 4.8 Hz, 2H), 7.43 - 7.34 (m, 2H), 6.38 (d, $J$ = 5.2 Hz,1H), 6.24 (s,1H); ESI-MS m/z: 521.15; Anal. Calcd. for C$_{29}$H$_{20}$FN$_5$O$_4$: C, 66.79; H, 3.83; N, 13.43; Found (%): C, 66.78; H, 3.84; N, 13.53.

6.3.14. N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(3-chloro-4-fluorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (36)
Yield: 55%; M.p.: 267.8–269.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.80 (s, 1H), 11.78 (s, 1H), 9.16 (s, 1H), 8.60 (d, $J = 8.7$ Hz, 2H), 8.09 - 8.00 (m, 2H), 7.79 (d, $J = 6.7$ Hz, 1H), 7.64 (t, $J = 8.9$ Hz, 1H), 7.57 (d, $J = 8.5$ Hz, 1H), 7.49 (d, $J = 4.8$ Hz, 2H), 7.43 - 7.34 (m, 2H), 6.38 (d, $J = 5.2$Hz, 1H), 6.24 (s, 1H); ESI-MS m/z: 543.91; Anal. Calcd. for C$_{28}$H$_{16}$FCIN$_5$O$_3$(%): C, 61.83; H, 2.97; N, 12.28; Found (%): C, 61.84; H, 2.98; N, 12.38.

6.3.15. N-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(4-bromo-2-fluorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (37)

Yield: 54%; M.p.: 262.2–263.9 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.78 (s, 1H), 11.61 (s, 1H), 9.18 (s, 1H), 8.63 (s, 2H), 8.06 (d, $J = 5.3$ Hz, 1H), 8.02 (d, $J = 13.1$ Hz, 1H), 7.88 (d, $J = 9.2$ Hz, 1H), 7.67 (d, $J = 8.7$ Hz, 1H), 7.57 (d, $J = 8.0$ Hz, 2H), 7.51 (s, 1H), 7.41 - 7.37 (m, 2H), 6.39 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 162.22, 161.00, 159.27, 157.53, 156.75, 155.27, 153.40, 151.51, 150.38, 144.89, 144.66, 140.21, 133.28, 128.94, 125.40, 124.28, 123.21, 120.91, 120.43, 117.22, 115.14, 109.93, 109.47, 109.24, 101.42, 97.24; ESI-MS m/z: 588.37; Anal. calcd. for C$_{28}$H$_{16}$BrF$_2$N$_5$O$_3$(%): C, 57.16; H, 2.74; N, 11.90; Found (%): C, 57.26; H, 2.75; N, 11.91.

6.3.16. N-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(2-chloro-4-(trifluoromethyl)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (38)

Yield: 62%; M.p.: 288.3–289.1 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.76 (d, $J = 6.1$ Hz, 2H), 9.17 (s, 1H), 8.60 (s, 2H), 8.10 - 8.03 (m, 2H), 8.02 - 7.95 (m, 1H), 7.90 (s, 1H), 7.83 (s, 1H), 7.57 (d, $J = 8.5$ Hz, 1H), 7.48 (s, 1H), 7.36 (s, 2H), 6.38 (d, $J = 5.5$ Hz, 1H), 6.23 (s, 1H); ESI-MS m/z: 593.09; Anal. calcd. for C$_{29}$H$_{16}$F$_4$ClN$_5$O$_3$(%): C, 58.65; H, 2.72; N, 11.78; Found (%): C, 58.64; H, 2.73; N, 11.68.

6.3.17. N-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1-phenyl-1,2-dihydro-1,8-naphthyridine-3-carboxamide (39)

Yield: 59%; M.p.: 258.3–259.1 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.94 (s, 1H), 9.18 (s, 1H), 8.61 (s, 2H), 8.13 (d, $J = 5.4$ Hz, 1H), 8.05 (d, $J = 14.1$ Hz, 1H), 7.58 (d, $J = 7.3$ Hz, 3H), 7.54 - 7.50 (m, 1H), 7.47 (dd, $J = 12.8$, 5.1 Hz, 2H), 7.45 (d, $J = 3.3$ Hz, 1H), 7.40 (d, $J = 7.4$ Hz, 3H), 6.44 (d, $J = 5.2$ Hz, 1H), 6.26 (d, $J = 3.4$ Hz, 1H), 3.82 (s, 3H); ESI-MS m/z: 487.16; Anal. calcd. for C$_{29}$H$_{21}$N$_3$O$_3$(%): C, 71.45; H, 4.34; N, 14.37; Found (%): C, 71.44; H, 4.43; N, 14.38.
6.3.18. 1-(4-fluorophenyl)-N-(4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (40)

Yield: 61%; M.p.: 298.3–299.1 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.88 (s, 1H), 9.19 (s, 1H), 8.63 (d, \(J = 9.1\) Hz, 2H), 8.19 (d, \(J = 5.2\) Hz, 1H), 8.07 (d, \(J = 12.9\) Hz, 1H), 7.60 (d, \(J = 8.6\) Hz, 1H), 7.54 - 7.47 (m, 4H), 7.46 (s, 2H), 7.44 - 7.36 (m, 2H), 6.50 (d, \(J = 5.1\) Hz, 1H), 6.29 (d, \(J = 2.9\) Hz, 1H), 3.87 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 163.24, 161.40, 157.73, 153.26, 151.50, 150.66, 144.72, 144.52, 140.03, 133.60, 131.78, 131.70, 129.49, 124.35, 123.14 (2C), 120.45, 117.28, 116.78, 116.55, 115.31 (2C), 110.26, 109.54, 101.72, 96.40, 31.77; ESI-MS m/z: 505.16; Anal. calcd. for C\(_{29}\)H\(_{20}\)FN\(_5\)O\(_3\) (%): C, 68.90; H, 3.99; N, 13.85; Found (%): C, 68.91; H, 3.40; N, 13.86.

6.3.19. N-(4-((1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(4-chlorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (41)

Yield: 57%; M.p.: 273.3–274.1 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.67 (s, 1H), 9.08 (s, 1H), 8.54 - 8.50 (d, 2H), 8.05 (d, \(J = 5.4\) Hz, 1H), 7.75 (d, \(J = 8.9\) Hz, 2H), 7.57 (d, \(J = 8.6\) Hz, 2H), 7.39 (d, \(J = 9.4\) Hz, 2H), 7.33 (d, \(J = 3.4\) Hz, 2H), 7.12 (d, \(J = 8.8\) Hz, 2H), 6.40 (d, \(J = 5.4\) Hz, 1H), 6.12 (d, \(J = 3.4\) Hz, 1H), 3.73 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 163.05, 160.79, 157.81, 152.99, 151.15, 150.43, 144.50, 144.17, 139.85, 136.33 (2C), 135.49 (2C), 133.54, 131.50, 129.76 (2C), 129.18, 123.21, 121.93 (2C), 121.39, 120.35 (2C), 115.21, 110.86, 102.83, 96.66, 31.65; ESI-MS m/z: 521.13; Anal. Calcd. for C\(_{29}\)H\(_{20}\)ClN\(_5\)O\(_3\) (%): C, 66.73; H, 3.86; N, 13.42; Found (%): C, 66.74; H, 3.87; N, 13.52.

6.3.20. 1-(4-bromophenyl)-N-(4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (42)

Yield: 54%; M.p.: 248.4–249.1 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.73 (s, 1H), 9.14 (s, 1H), 8.59 (d, \(J = 6.0\) Hz, 2H), 8.13 (d, \(J = 5.4\) Hz, 1H), 7.82 (d, \(J = 8.8\) Hz, 2H), 7.78 (d, \(J = 8.4\) Hz, 2H), 7.46 (s, 1H), 7.40 (d, \(J = 3.2\) Hz, 2H), 7.37 (s, 1H), 7.21 (s, 1H), 7.18 (s, 1H), 6.47 (d, \(J = 5.3\) Hz, 1H), 6.19 (d, \(J = 3.3\) Hz, 1H), 3.80 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 163.12, 161.45, 157.81, 155.48, 153.33, 153.04, 151.37, 150.75, 144.81, 144.65, 140.13, 136.94, 132.93 (2C), 132.05, 129.58, 124.46 (2C), 123.25, 122.28, 120.61, 117.34, 115.41, 110.34, 109.61, 109.38, 101.81, 96.49, 31.86; ESI-MS m/z: 566.41; Anal. Calcd. for C\(_{29}\)H\(_{20}\)BrN\(_5\)O\(_3\) (%): C, 61.50; H, 3.53; N, 12.46; Found (%): C, 61.51; H, 3.54; N, 12.47.
6.3.21. 1-(4-methoxyphenyl)-N-(4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (43)

Yield: 61%; M.p.: 320.8–321.5 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.83 (s, 1H), 9.13 (s, 1H), 8.12 (d, \(J = 5.3\) Hz, 1H), 7.82 (d, \(J = 8.7\) Hz, 2H), 7.48 - 7.37 (m, 2H), 7.27 (d, \(J = 8.6\) Hz, 2H), 7.19 (d, \(J = 8.6\)Hz, 2H), 7.10 (d, \(J = 8.7\) Hz, 2H), 6.47 (d, \(J = 5.2\) Hz, 1H), 6.19 (d, \(J = 3.2\) Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 162.37, 159.98, 158.58, 156.79, 152.04, 150.53, 150.33, 149.58, 143.61, 142.97, 138.78 (2C), 134.52, 129.45, 128.83, 128.19, 122.21, 121.00, 120.37, 119.32, 119.21, 114.16 (2C), 113.98, 101.95, 95.68, 54.94, 30.65; ESI-MS m/z: 517.18; Anal. Calcd. for C\(_{28}\)H\(_{17}\)N\(_5\)O\(_3\) (%): C, 58.96; H, 3.00; N, 12.28; Found (%): C, 58.97; H, 3.02; N, 12.38.

6.3.22. 1-(3-chloro-4-fluorophenyl)-N-(4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (44)

Yield: 54%; M.p.: 281.4–283.0 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.70 (s, 1H), 9.16 (s, 1H), 8.61 (d, \(J = 8.5\) Hz, 2H), 8.14 (d, \(J = 5.4\) Hz, 1H), 7.84 (d, \(J = 8.8\) Hz, 2H), 7.82 - 7.79 (m, 1H), 7.64 (d, \(J = 9.0\) Hz, 1H), 7.52 - 7.47 (m, 2H), 7.41 (d, \(J = 3.4\) Hz, 1H), 7.20 (d, \(J = 8.8\) Hz, 2H), 6.48 (d, \(J = 5.4\) Hz, 1H), 6.20 (d, \(J = 3.4\) Hz, 1H), 3.81 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 163.20, 161.34, 159.05, 157.81, 156.59, 155.47, 153.35, 153.02, 151.41, 150.74, 144.79, 140.16, 134.46, 132.11, 130.86, 129.57, 124.42, 123.16, 120.67, 118.21, 117.99, 117.35, 115.41, 110.36, 109.62, 109.40, 101.80, 96.50, 31.85; ESI-MS m/z: 537.95; Anal. Calcd. for C\(_{29}\)H\(_{19}\)ClFN\(_5\)O\(_3\) (%): C, 64.51; H, 3.51; N, 12.97; Found (%): C, 64.52; H, 3.52; N, 12.98.

6.3.23. 1-(4-bromo-2-fluorophenyl)-N-(4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (45)

Yield: 60%; M.p.: 321.8–323.4 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.44 (s, 1H), 9.11 (s, 1H), 8.55 (d, \(J = 5.2\) Hz, 2H), 8.06 (d, \(J = 5.0\) Hz, 1H), 7.81 (d, \(J = 9.6\) Hz, 1H), 7.76 (d, \(J = 7.2\) Hz, 2H), 7.60 (d, \(J = 8.5\)Hz, 1H), 7.53 - 7.42 (m, 2H), 7.33 (s, 1H), 7.13 (d, \(J = 7.4\) Hz, 2H), 6.41 (d, \(J = 5.2\) Hz, 1H), 6.12 (s, 1H), 3.73 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 162.34, 160.55, 159.28, 157.74, 156.77, 153.27, 151.33, 150.55, 150.35, 144.67, 140.14, 135.40, 133.30, 129.13, 124.25, 124.11, 123.38, 122.97, 122.88 (2C), 122.02, 121.34 (2C), 120.86, 115.18, 110.84, 102.87, 96.64, 31.62; ESI-MS m/z: 584.2; Anal. calcd. for
C_{29}H_{19}BrF_{2}N_{5}O_{3}(\%): C, 59.60; H, 3.28; N, 11.98; Found (\%): C, 59.61; H, 3.38; N, 11.99.

6.3.24. 1-(2-chloro-4-(trifluoromethyl)phenyl)-N-(4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (46)

Yield: 66\%; M.p.: 293.4–292.0 °C; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) \delta 11.58 (s, 1H), 9.10 (s, 1H), 8.53 (s, 2H), 8.08 - 8.00 (m, 2H), 7.91 (d, \textit{J} = 8.1 Hz, 1H), 7.76 (d, \textit{J} = 7.4 Hz, 3H), 7.41 (s, 1H), 7.33 (s, 1H), 7.12 (d, \textit{J} = 8.0 Hz, 2H), 6.40 (s, 1H), 6.12 (s, 1H), 3.72 (s, 3H); ESI-MS m/z: 607.1; Anal. calcd. for C_{30}H_{18}F_{4}ClN_{5}O_{3}(\%): C, 59.27; H, 2.98; N, 11.52; Found (\%): C, 59.37; H, 2.99; N, 11.53.

6.3.25. N-(3-fluoro-4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1-phenyl-1,2-dihydro-1,8-naphthyridine-3-carboxamide (47)

Yield: 64\%; M.p.: 299.4–297.2 °C; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) \delta 12.03 (s, 1H), 9.27 (s, 1H), 8.70 (d, \textit{J} = 4.8 Hz, 2H), 8.23 (d, \textit{J} = 5.3 Hz, 1H), 8.13 (s, 1H), 7.60 (d, \textit{J} = 6.7 Hz, 1H), 7.54 (d, \textit{J} = 3.5 Hz, 1H), 7.49 (d, \textit{J} = 6.9 Hz, 2H), 6.53 (d, \textit{J} = 5.4 Hz, 1H), 6.35 (d, \textit{J} = 3.6 Hz, 1H), 5.85 (s, 3H), 3.91 (s, 3H); ESI-MS m/z: 505.51; Anal. Calcd. for C_{29}H_{20}BrF_{2}N_{5}O_{3}(\%): C, 68.90; H, 3.99; N, 13.85; Found(\%): C, 68.89; H, 3.97; N, 13.89.

6.3.26. N-(3-fluoro-4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (48)

Yield: 61\%; M.p.: 309.4–307.2 °C; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) \delta 11.88 (s, 1H), 9.16 (s, 1H), 8.61 (d, \textit{J} = 2.3 Hz, 1H), 8.59 (d, \textit{J} = 4.0 Hz, 1H), 8.14 (d, \textit{J} = 5.4 Hz, 1H), 8.04 (dd, \textit{J} = 12.8, 2.1 Hz, 1H), 7.57 (d, \textit{J} = 8.7 Hz, 1H), 7.50 - 7.34 (m, 7H), 6.45 (d, \textit{J} = 5.4 Hz, 1H), 6.26 (d, \textit{J} = 3.4 Hz, 1H), 3.82 (s, 3H); \textsuperscript{13}C NMR (101 MHz, DMSO-\textit{d}_6) \delta 163.49, 163.11, 161.31, 161.05, 157.89, 155.24, 153.15, 151.38, 150.01, 144.41, 144.18, 139.92, 133.46, 131.66, 131.57, 129.58, 124.27, 123.01, 120.34, 117.17, 116.66, 116.44, 115.19, 110.31, 109.42, 109.19, 101.62, 96.45, 31.80; ESI-MS m/z: 523.15; Anal. Calcd. for C_{29}H_{20}BrF_{2}N_{5}O_{3}(\%): C, 66.54; H, 3.55; N, 13.38; Found(\%): C, 66.51; H, 3.52; N, 13.39.

6.3.27. 1-(4-chlorophenyl)-N-(4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (49)

Yield: 66\%; M.p.: 299.4–297.0 °C; \textsuperscript{1}H NMR (400 MHz, DMS-\textit{d}_6) \delta 11.79 (s, 1H), 9.14 (s, 1H), 8.58 (d, \textit{J} = 7.4 Hz, 2H), 8.13 (d, \textit{J} = 5.3 Hz, 1H), 8.01 (d, \textit{J} = 12.7 Hz, 1H), 7.78 (d, \textit{J} = 8.1 Hz, 2H), 7.33 (s, 1H), 7.12 (d, \textit{J} = 8.0 Hz, 2H), 6.40 (s, 1H), 6.12 (s, 1H), 3.72 (s, 3H); ESI-MS m/z: 541.15; Anal. Calcd. for C_{29}H_{20}BrF_{2}N_{5}O_{3}(\%): C, 59.60; H, 3.28; N, 11.98; Found(\%): C, 59.61; H, 3.38; N, 11.99.
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= 8.3 Hz, 2H), 7.54 (d, J = 8.6 Hz, 1H), 7.46 (dd, J = 7.2, 5.0 Hz, 1H), 7.43 - 7.33 (m, 4H), 6.45 (d, J = 5.2 Hz, 1H), 6.24 (d, J = 3.1 Hz, 1H), 3.82 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$_d_6$) δ 163.04, 161.36, 157.72, 153.24, 151.29, 150.65, 144.71, 144.57, 140.05, 136.86 (2C), 133.68, 132.84 (2C), 131.96, 129.50 (2C), 124.38, 123.16, 122.19, 120.52, 117.25, 115.33, 110.25, 109.52, 109.29, 101.70, 96.40, 31.78; ESI-MS m/z: 539.15; Anal. calcd. for C$_{29}$H$_{19}$FClN$_5$O$_3$: C, 64.51; H, 3.55; N, 12.97; Found (%): C, 64.39; H, 3.4; N, 12.93.

6.3.29. 1-(4-bromophenyl)-N-(3-fluoro-4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (50)

Yield: 56%; M.p.: 250.4–252.3 °C; $^1$H NMR (400 MHz, DMSO-$_d_6$) δ 11.81 (s, 1H), 9.17 (s, 1H), 8.61 (d, J = 8.5 Hz, 2H), 8.12 (d, J = 5.1 Hz, 1H), 8.04 (d, J = 12.8 Hz, 1H), 7.79 (d, J = 6.9 Hz, 1H), 7.65 (t, J = 9.0 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.49 (d, J = 4.8 Hz, 2H), 7.44 - 7.34 (m, 2H), 6.43 (d, J = 5.1 Hz, 1H), 6.24 (d, J = 3.2 Hz, 1H), 3.80 (s, 3H); ESI-MS m/z: 565.07; Anal. Calcd. for C$_{29}$H$_{20}$BrF$_2$N$_5$O$_3$: C, 61.49; H, 3.51; N, 12.36; Found (%): C, 61.51; H, 3.52; N, 12.34.

6.3.29. N-(3-fluoro-4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-1-(4-methoxylphenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (51)

Yield: 69%; M.p.: 278.4–277.0 °C; $^1$H NMR (400 MHz, DMSO-$_d_6$) δ 11.96 (s, 1H), 9.13 (s, 1H), 8.58 (t, J = 6.0 Hz, 2H), 8.11 (d, J = 5.3 Hz, 1H), 8.03 (d, J = 12.9 Hz, 1H), 7.55 (d, J = 9.1 Hz, 1H), 7.47 - 7.33 (m, 3H), 7.27 (d, J = 8.7 Hz, 2H), 7.10 (d, J = 8.8 Hz, 2H), 6.41 (d, J = 5.3 Hz, 1H), 6.24 (d, J = 3.4 Hz, 1H), 3.81 (d, J = 15.0 Hz, 6H); ESI-MS m/z: 535.17; Anal. Calcd. for C$_{30}$H$_{22}$FN$_5$O$_4$: C, 67.28; H, 4.14; N, 13.08; Found (%): C, 67.38; H, 4.15; N, 13.19.

6.3.30. 1-(3-chloro-4-fluorophenyl)-N-(3-fluoro-4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (52)

Yield: 63%; M.p.: 281.4–283.0 °C; $^1$H NMR(400 MHz, DMSO-$_d_6$) δ 11.81 (s, 1H), 9.17 (s, 1H), 8.61 (d, J = 8.5 Hz, 2H), 8.12 (d, J = 5.1 Hz, 1H), 8.04 (d, J = 12.8 Hz, 1H), 7.79 (d, J = 6.9 Hz, 1H), 7.65 (t, J = 9.0 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.49 (d, J = 4.8 Hz, 2H), 7.44 - 7.34 (m, 2H), 6.43 (d, J = 5.1 Hz, 1H), 6.24 (d, J = 3.2 Hz, 1H), 3.80 (s, 3H); ESI-MS m/z: 557.15; Anal. Calcd. for C$_{29}$H$_{18}$F$_2$ClN$_5$O$_3$: C, 62.43; H, 3.25; N, 12.55; Found (%): C, 62.43; H, 3.26; N, 12.54.
6.3.31. 1-(3-chloro-4-fluorophenyl)-N-(3-fluoro-4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (53)

Yield: 56%; M.p.: 240.4–241.0 °C; \(^{1}\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.55 (s, 1H), 9.11 (s, 1H), 8.56 (d, \(J = 5.7\) Hz, 2H), 8.05 (d, \(J = 5.3\) Hz, 1H), 7.96 (d, \(J = 13.0\) Hz, 1H), 7.81 (d, \(J = 9.1\) Hz, 1H), 7.60 (d, \(J = 8.0\) Hz, 1H), 7.53 - 7.42 (m, 3H), 7.33 (dd, \(J = 18.2, 6.2\) Hz, 1H), 6.36 (d, \(J = 5.3\) Hz, 1H), 6.17 (d, \(J = 3.5\) Hz, 1H), 3.74 (s, 3H); ESI-MS m/z: 601.06; Anal. calcd. for \(C_{29}H_{18}BrF_{2}N_{5}O_{3}\) (%): C, 57.82; H, 3.01; N, 11.63; Found (%): C, 57.83; H, 3.21; N, 11.64.

6.3.32. 1-(2-chloro-4-(trifluoromethyl)phenyl)-N-(3-fluoro-4-((1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)oxy)phenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (54)

Yield: 63%; M.p.: 293.4–292.0 °C; \(^{1}\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.58 (s, 1H), 9.10 (s, 1H), 8.53 (s, 2H), 8.08 - 8.00 (m, 2H), 7.91 (d, \(J = 8.1\) Hz, 1H), 7.76 (d, \(J = 7.4\) Hz, 3H), 7.41 (s, 1H), 7.33 (s, 1H), 7.12 (d, \(J = 8.0\) Hz, 2H), 6.40 (s, 1H), 6.12 (s, 1H), 3.72 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 161.96, 160.17, 156.63, 154.25, 152.16, 150.12, 149.52, 143.62, 143.69, 139.05, 136.75, 135.92, 134.79, 132.23, 130.39, 130.02, 128.60, 128.41, 123.29, 122.06, 119.58, 116.19, 114.33, 109.14, 109.08, 108.45, 108.21, 100.59, 95.29, 30.68; ESI-MS m/z: 589.11; Anal. calcd. for \(C_{30}H_{19}F_{3}ClN_{5}O_{3}\) (%): C, 61.08; H, 3.25; N, 11.87; Found (%): C, 61.09; H, 3.26; N, 11.88.

6.4. Pharmacology

6.4.1. MTT assay in vitro

The anti-proliferative activities of compounds 23–54 were evaluated against HT-29, H460, A549, and U87MG cell lines using the standard MTT assay in vitro, with foretinib as the positive control, as previously reported protocol. The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximate 4 × 10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO\(_2\) at 37 °C for 24 h. The compounds tested at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 µg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 mL of
DMSO each well, and the absorbency at 492 and 630 nm (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each cell line. The results expressed as IC$_{50}$ (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software [25-26].

6.4.2. Tyrosine kinases assay

The tyrosine kinases activities were evaluated using homogeneous time-resolved fluorescence (HTRF) assays, as previously reported protocol. Briefly, 20 µg/mL poly (Glu, Tyr) 4:1 (Sigma) was preloaded as a substrate in 384-well plates. Then 50 µL of 10 mM ATP (Invitrogen) solution diluted in kinase reaction buffer (50 mM HEPES, pH 7.0, 1 M DTT, 1 M MgCl$_2$, 1 M MnCl$_2$, and 0.1% NaN$_3$) was added to each well. Various concentrations of compounds diluted in 10 µL of 1% DMSO (v/v) were used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 39 µL of kinase reaction buffer solution. The incubation time for the reactions was 30 min at 25 °C, and the reactions were stopped by the addition of 5 µL of Streptavidin-XL665 and 5 µL Tk Antibody Cryptate working solution to all of wells. The plates were read using Envision (PerkinElmer) at 320 nm and 615 nm. The inhibition rate (%) was calculated using the following equation: % inhibition = 100 - [(Activity of enzyme with tested compounds - Min)/(Max - Min)] × 100 (Max: the observed enzyme activity measured in the presence of enzyme, substrates, and cofactors; Min: the observed enzyme activity in the presence of substrates, cofactors and in the absence of enzyme). IC$_{50}$ values were calculated from the inhibition curves [27-29].

7. Acknowledgements

We gratefully acknowledge the generous support provided by National Natural Science Foundation of China (NSFC No. 81660572; NSFC No. 81660692), Natural Science Foundation of Jiangxi Province (20171BAB215071), Science and Technology Project Founded by the Education Department of Jiangxi Province (GJJ150797), Top-notch talent project of Jiangxi Science & Technology Normal University (2016QNBJRC002), Research Fund for the Doctoral Program of Jiangxi Science & Technology Normal University (No.
3000990351), and Jiangxi Provincial Key Laboratory of Drug Design and Evaluation (20171BCD40015).

References


**Legends**

Fig. 1. The representative small-molecule c-Met kinase inhibitors.

Fig. 2. Our previous work on antiproliferative agents bearing pyrrolo[2,3-b]pyridine scaffolds (7 and 8) and potent drugs bearing 1,8-naphthyridin-2-one (9 and 10).

Fig. 3. Design strategy for the pyrrolo[2,3-b]pyridine derivatives bearing the 1,8-naphthyridin-2-one moiety.

Fig. 4. Binding poses of compound 32 with c-Met. The proteins were displayed by silver ribbon. Compound 32 were displayed by multicolor sticks. H-bonding interactions between the 32 and c-Met were indicated with dashed lines in black.

Scheme 1. Reagents and conditions: (i) Diphenyl Ether, 190 °C, 51–55%; (ii) FeCl₃,
N$_2$H$_4$·H$_2$O, Activated Carbon, 80 °C, 78–81%.

Scheme 2. Reagents and conditions: (i) AcOH, 100 °C, 3–6 h; (ii) LiAlH$_4$, N$_2$, THF, r.t. 3.5 h; (iii) Pyridinium dichromate, CH$_2$Cl$_2$, r.t. 5–7 h; (iv) Ethyl acetylacetate, piperidine, EtOH, reflux, 30–35 h; (v) K$_2$CO$_3$, 1,4-dioxane/H$_2$O, 80 °C, 4–5 h (vi) SOCl$_2$, reflux, 6 h; (vii) Appropriate aniline, carbonyl chloride, DIPEA, CH$_2$Cl$_2$, 0 °C, 1 h, r.t., 5–8 h.
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<th>Compd.</th>
<th>$R_1$</th>
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<th>$R_3$</th>
<th>IC$_{50}$ (µM) ± SD</th>
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<td>0.28 ± 0.02</td>
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$a$ Bold values show the IC$_{50}$ values of the target compounds lower than the values of the positive control.

$b$ ND: Not determined.

$c$ Used as the positive control.
Table 2

c-Met kinase activity of selected compounds 24, 25, 31, 32, 33, 40, 48, 49, and foretinib in vitro.

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<th>Compd.</th>
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<td>25</td>
<td>9.25</td>
</tr>
<tr>
<td>31</td>
<td>3.59</td>
</tr>
<tr>
<td>32</td>
<td>1.92</td>
</tr>
<tr>
<td>33</td>
<td>2.23</td>
</tr>
<tr>
<td>40</td>
<td>21.76</td>
</tr>
<tr>
<td>48</td>
<td>32.51</td>
</tr>
<tr>
<td>49</td>
<td>16.83</td>
</tr>
<tr>
<td>Foretinib</td>
<td>1.56</td>
</tr>
</tbody>
</table>
Table 3
Inhibition of tyrosine kinases by compound 32.

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Enzyme IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flt-3</td>
<td>1.16</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>1.81</td>
</tr>
<tr>
<td>VEGFR-2</td>
<td>152.3</td>
</tr>
<tr>
<td>EGFR</td>
<td>632.8</td>
</tr>
<tr>
<td>c-Kit</td>
<td>684.5</td>
</tr>
</tbody>
</table>
$R_1 = \text{H, CH}_3$

$R_2 = \text{H, F}$

Substituted phenyl

"hydrogen-bond donor/acceptor"

$\%$ atoms regulation

Target compounds

$R_1$: \text{H, CH}_3

$R_2$: \text{H, F}

$R_3$: at least one group choosing from \text{H, F, Cl, Br, CH}_3, \text{OCH}_3, \text{and CF}_3$

5 (JM800476q-2, Preclinical)
\[
\text{11a: } X = H \\
\text{11b: } X = \text{CH}_3 \\
\text{12a-b: } R_2 = H \\
\text{12a-b: } R_2 = F \\
\text{13a-d} \\
\text{14a-d}
\]
A series of pyrrolo[2,3-b]pyridine derivatives were designed and synthesized.

The target compounds showed potent antitumor activity.

Compound 32 showed an IC$_{50}$ value of 1.16/1.92 nM against Flt-3/c-Met kinase.