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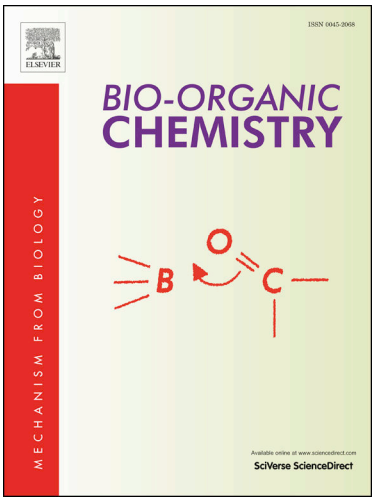

Design, synthesis and biological evaluation of novel 4-phenoxypyridine based 3-oxo-3,4-dihydroquinoxaline-2-carboxamide derivatives as potential c-Met kinase inhibitors

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Design, synthesis and biological evaluation of novel 4-phenoxypyridine based 3-oxo-3,4-dihydroquinoxaline-2-carboxamide derivatives as potential c-Met kinase inhibitors

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Abstract

Blocking c-Met kinase activity by small-molecule inhibitors has been identified as a promising approach for the treatment of cancers. Herein, we described the design, synthesis, and biological evaluation of a series of 4-phenoxypyridine-based 3-oxo-3,4-dihydroquinoxaline derivatives as c-Met kinase inhibitors. Inhibitory activitives against c-Met kinase evaluation indicated that most of compounds showed excellent c-Met kinase activity *in vitro,* and IC_{50} values of ten compounds (**23a, 23e, 23f, 23l, 23r, 23s, 23v, 23w, 23x** and **23y**)were less than 10.00 nM. Notably, three of them (**23v, 23w** and **23y**) showed remarkable potency with IC_{50} values of 2.31 nM, 1.91 nM and 2.44 nM, respectively, and thus they were more potent than positive control drug foretinib (c-Met, IC_{50} = 2.53 nM). Cytotoxic evaluation indicated the most promising compound **23w** showed remarkable cytotoxicity against A549, H460 and HT-29 cell lines with IC_{50} values of 1.57 μM, 0.94 μM and 0.65 μM, respectively. Furthermore, the acridine orange/ethidium bromide (AO/EB) staining, cell apoptosis assays by flow cytometry, wound-healing assays and transwell migration assays on HT-29 and/or A549 cells of **23w** were performed. Especially compound **23w**, which displayed potent antitumor, apoptosis induction and antimetastatic activity, could be used as a promising lead for further development. Meanwhile, their preliminary structure-activity relationships (SARs) were also discussed.

Keywords: 4-Phenoxypyridine derivatives, c-Met inhibitors, Antitumor activity, Apoptosis, Migration.

1. Introduction

The regulation of intracellular signal transduction pathways is a key function of receptor tyrosine kinases (RTKs), and thus, inappropriate activation of RTKs can lead to deleterious consequences with regard to downstream signaling [1]. c-Met is a prototype member of a subfamily of heterodimeric receptor tyrosine kinases (RTKs) and is the receptor for hepatocyte growth factor (HGF) [2]. Binding of HGF to its receptor c-Met, induces several complex signaling pathways that result in cell proliferation, survival, motility, induction of cell polarity, scattering, angiogenesis and invasion [3,4]. The physiological functions of the HGF/c-Met signaling pathway are restricted to embryonic

development, wound healing and tissue regeneration processes [5,6]. However, deregulation of the c-Met /HGF pathway can lead to tumorigenesis and metastasis [7]. Amplification of the c-Met gene, overexpression of c-Met and/or HGF and constitutive activation conferred by sequence mutations are some of the mechanisms of deregulation found in many human cancers [8]. Consequently, pharmacological inhibition of c-Met activity has been considered to be an emerging strategy for cancer therapies. [9].

At present, small molecule c-Met kinase inhibitors were mainly divided into two groups based on the structural features and their different binding mode with the active site: type I inhibitors and type II inhibitors [10–13]. As reported recently, certain mutations near the active site of c-Met may cause resistance of type I inhibitors. Type I inhibitors function as an analog of ATP to competitively bind to the highly conservative hinge region of c-Met kinase [14–17]. In contrast, type II inhibitors not only bind to the same area occupied by the type I inhibitors but also exploit hydrogen bonding and hydrophobic interactions with the allosteric site, which beyond the entrance of c-Met's active site[18-20]. Consequently, type II inhibitors are postulated to be more effective than type I inhibitors against these mutations, and study on type II inhibitors to develop novel chemical entities is much more rational and of great significance. Recently, a series of small molecules with different scaffolds have been reported as type II c-Met inhibitors and some of them are launched or in clinical trials, such as Cabozantinib [21], Foretinib [22], BMS-794833 [23], Altiratinib [24], BMS-777607 [25], NPS-1034[26] and MGCD-516[27] (Fig. 1).

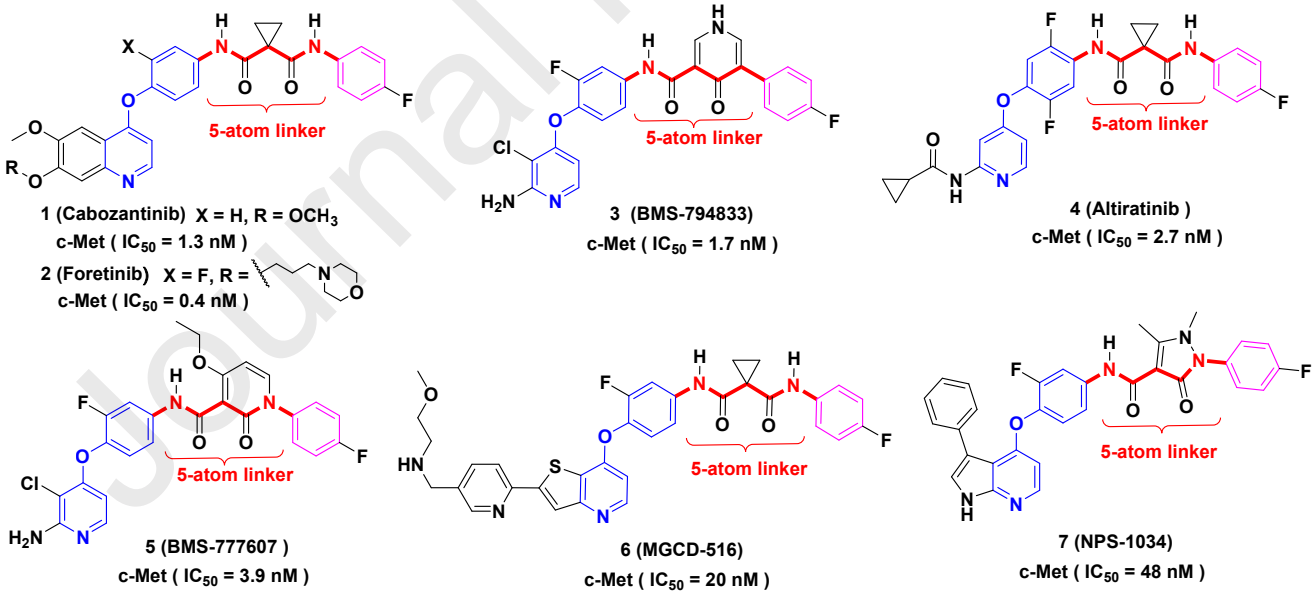


Fig. 1. The representative c-Met kinase inhibitors.

As displayed in Fig 2, most of the type II c-Met inhibitors may be disconnected into three units according to their structures and subunit functions. Reported SARs [28-31] of type II c-Met inhibitors suggested that moiety A (4-phenoxypyridine core: usually substituted 4-phenoxypyridine, 4-phenoxypyrrolopyridine, 4-phenoxythienopyridine and 4-phenoxyquinoline) and moiety B (usually substituted phenyl group) are crucial for kinase activity. The 4-

phenoxypyridine core was essential for furnishing hydrogen bonds with the backbone of c-Met kinase and responsible for π-π stacked interactions with amino acid residue of the DFG motif. In addition, hydrophobic interaction was generated by terminal aryl ring (moiety B) reaching into the hydrophobic pocket formed by amino acid residues. In contrast, various linear chains and heterocyclic rings can be introduced to the main chain of moiety C (linker bridge). Importantly, there should be two structural characteristics on the linker bridge (moiety C) between moiety A and B, one is 5-atoms regulation (i.e., six chemical bonds distance between moiety A and B) and the other is possessing hydrogen-bond donors or acceptors simultaneously [32–34].

To our knowledge, compounds bearing quinoxalinone fragment have been reported to use as a building block in the design of anticancer agents. For example, compounds **8-11** (Fig. 2) displayed a multitude of biological activities [35-38]. Remarkably, the 3-oxo-3,4-dihydroquinoxaline-2-carboxamide framework conforms to "5-atoms regulation", and contains both hydrogen-bond donor and acceptor, which makes it a satisfactory linker. So 3-oxo-3,4-dihydroquinoxaline-2-carboxamide fragment was introduced into the moiety C *via* cyclization strategy based on the '5 atoms regulation'. Meanwhile, 4-phenoxypyridine was used as the moiety A. Substituted phenyl ring was reserved as the moiety B. Small substituent X, R_1 and R_2 were introduced to investigate their effects on activity of the target compounds. Accordingly, a series of 4-phenoxypyridine derivatives were designed and synthesized to study the structure-activity relationships (SARs) and find promising antitumor agents (Fig. 2).

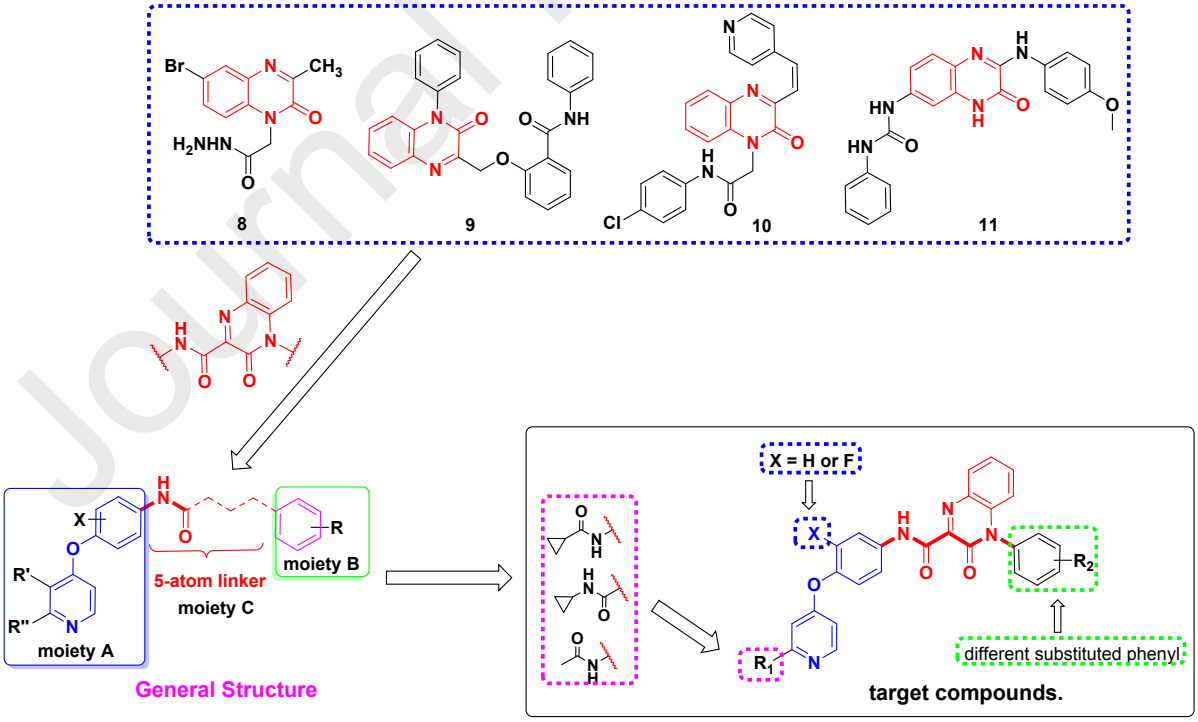


Fig. 2. Design strategy and structures of the target compounds.

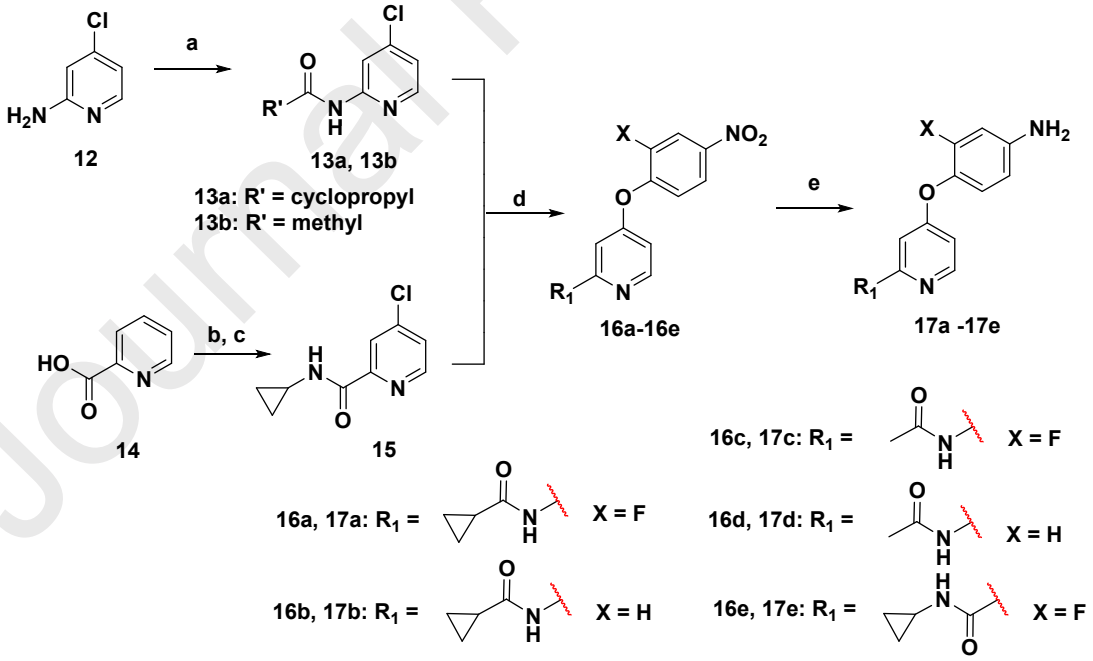
In the current study, all target compounds were synthesized and evaluated for their *in vitro* inhibitory activitives against c-Met kinase and some compounds were further evaluated for their cytotoxic activitives against human lung

adenocarcinoma cell line (A549), human lung cancer cell lines (H460) and human colon cancer cell line (HT-29) cancer cell lines. Their structure-activity relationships (SARs) were further explored. Furthermore, acridine orange/ethidium bromide (AO/EB) staining, apoptosis, wound-healing assays and transwell migration assays of **23w** were performed on HT-29 and/or A549 cell lines. Additionally, a docking analysis was also performed to elucidate the binding mode of the target compound **23w** with c-Met kinase.

2. Results and discussion

2.1. Chemistry

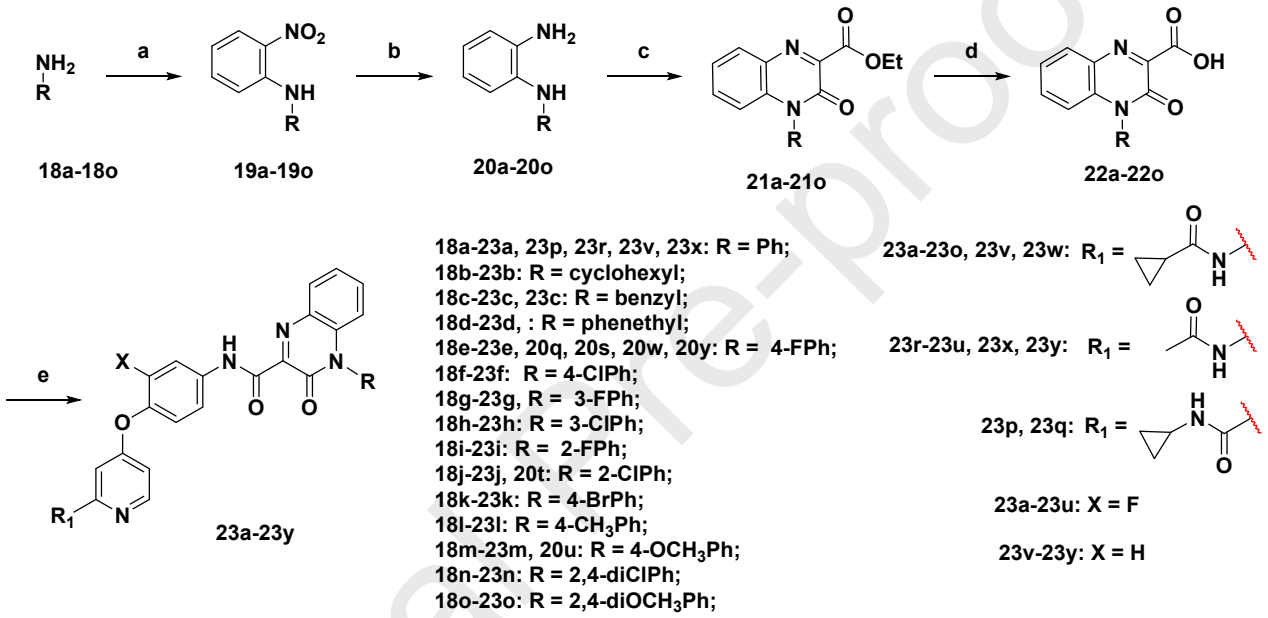
The key intermediate **17a-17e** were synthesized according to the routes outlined in Scheme 1. Commercially available 4-chloropyridin-2-amine was condensed with cyclopropanecarbonyl chloride or acetyl chloride in the presence of Et_3N to provide **13a** and **13b** as a white solid. Meanwhile, the starting material picolinic acid **14** was chlorinated with thionyl chloride, and then treated with cyclopropanamine in an ice-bath to give 4-chloro-N-cyclopropylpicolinamide **15** as a light-yellow solid. Subsequently, a nucleophilic substitution of **13a**, **13b or 15** with 2-fluoro-4-nitrophenol or 4-nitrophenol in refluxing chlorobenzene provided **16a-16e** in a moderate yield. Reduction of the nitro group of **16a-16e** with iron powder and acetic acid in ethyl acetate /water (10:1 v/v) provided aniline compound **17a-17e**.



Scheme 1. Reagents and conditions: (a) cyclopropanecarbonyl chloride or acetyl chloride, Et_3N, CH_2Cl_2, rt, 12 h; (b) $SOCl_2$, NaBr, PhCl, 55 °C, 1 h, reflux, 20 h; (c) cyclopropanamine, Et_3N, THF, 0 °C, 3 h; (d) 2-fluoro-4-nitrophenol or 4-nitrophenol, chlorobenzene, reflux, 40 h; (e) Fe (powder), HOAc, ethyl acetate /water (10:1 v/v), reflux, 2 h.

The synthetic route of the target compounds was shown in **Scheme 2**. The key intermediates 3-oxo-3,4-dihydroquinoxaline-2-carboxylic acid derivatives **22a-22o** were synthesized by a convenient four-step route starting

from 1-fluoro-2-nitrobenzene and different substituted amines **18a-18o**, which was described in detail in our previous study[34, 39]. Commercially available 1-fluoro-2-nitrobenzene was condensed with different substituted amines **18a-18o** in the presence of NaH in DMF at RT for 16 h to obtain intermediates diphenyl amines **19a-19o** as yellow solids, followed by reduction with iron powder and acetic acid in CH_3COOEt/H_2O at reflux condition for 16 h to provide compounds **20a-20o**. Further, intermediate **20a-20o** were treated with diethyl ketomalonate to generate 3-oxo-3,4-dihydroquinoxaline-2-carboxylic acid esters **21a-21o**, which were then converted to acid analogues **22a-22o** by hydrolysis in aqueous lithium hydroxide at RT for 2 h. Finally, acids **22a-22o** were condensed with intermediate **17a-17e** in the presence of HATU and Et_3N in DMF at room temperature to afford the target compounds **23a-23y**.



Scheme 2. Reagents and conditions: (a) amines, NaH, DMF, RT, 16 h; (b) Fe powder, HOAc, CH_3COOEt/H_2O, reflux, 6 h; (c) diethyl ketomalonate, toluene, reflux, 12 h; (d) LiOH, THF/ H_2O, RT, 2 h, then 6 N HCl; (e) **17a-17e**, HATU, Et_3N, DMF, 25 °C,12 h.

2.2 Biological evaluation

2.2.1 In vitro c-Met kinase assays and analysis of the structure-activity relationships

All the newly synthesized 4-phenoxypyridine derivatives (**23a-23y**) were evaluated for their *in vitro* inhibitory activity toward c-Met enzyme using mobility shift assay. Foretinib was used as a positive control, with the results expressed as half-maximal inhibitory concentration (IC_{50}) values presented in Table 1. The IC_{50} values are the average of at least three independent experiments.

As illustrated in Table 1, all the tested compounds (except for **23p** and **23q**) showed excellent c-Met enzymatic activity with IC_{50} values ranging from 1.91 to 202.72 nM, which indicated that introduction of the 3-oxo-3,4-dihydroquinoxaline-2-carboxamide framework to "**5-atom linker**" moiety of pyridine-based compounds maintained the c-Met inhibitory efficacy. Notably, ten of them (**23a, 23e, 23f, 23l, 23r, 23s, 23v, 23w, 23x** and **23y**) exhibited

promising activity against c-Met kinase with IC_{50} values less than 10.00 nM, and compound **23w** demonstrated the best activity with an IC_{50} value of 1.91 nM.

Firstly, a phenyl ring (moiety B) was introduced at the C-1 of quinoxalin-2-one and compound **23a** was obtained. As expected, compound **23a** showed promising inhibition of c-Met kinase with an IC_{50} value of 5.71 nM. Replacement of the phenyl ring by a cyclohexyl ring led to an approximate 10-fold drop in potency (**23a** vs **23b**). In order to know if the "5-atoms regulation" was applicable for the designed compounds, benzyl and phenethyl were also introduced at the C-1 of quinoxalin-2-one. Compared with **23a** (R = Ph, IC_{50} = 5.71 nM), **23c** (R = CH_2Ph, IC_{50} = 23.27 nM) and **23d** (R = CH_2CH_2Ph, IC_{50} = 52.33 nM) decreased potency as the distance between terminal phenyl and quinoxaline increasing, indicated that a phenyl substituent at this position was detrimental to the potency. This characteristic was consistent with our previous reported regulation of five atoms in which moiety A and moiety B are usually connected by six chemical bonds.

Encouraged by the observations described above, further investigations were performed to study the inhibitory potency effect of different substituents on the phenyl ring. The SARs based on IC_{50} values revealed that the inhibition of c-Met kinase of compounds with substituent at 4-position of phenyl ring was higher than those with substituents at 2- or 3-position. For example, compound with 4-fluorophenyl group (**23e**, IC_{50} = 5.02 nM) displayed more potency than those with 3-fluorophenyl (**23g**, IC_{50} = 10.23 nM) or 2-fluorophenyl group (**23i**, IC_{50} = 25.13 nM), and the same trend was observed for the series of chlorophenyl substituted compounds (**23f**, **23h** and **23j**). IC_{50} values indicated that halogen and methyl at the *para* position of the phenyl ring made a good contribution to enzyme activity, with following rank order: -Br < -CH_3 < -Cl < -F. However, the introduction of 4-methoxyphenyl group (**23m**, IC_{50} = 145.96 nM), 2, 4-dichlorophenyl group (**23n**, IC_{50} = 65.68 nM) and 2, 4-dimethoxyphenyl group (**23o**, IC_{50} = 202.72 nM) exhibited an obvious negative effect on the enzyme activity respectively, compared to **23a**, which had no substituent on the phenyl ring.

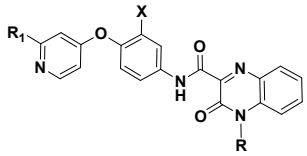
In the following work, our modification focused on the R_1 group. Firstly, the orientation of amide at the C-2 position of pyridine was inverted and two picolinamides were prepared. Unfortunately, the obtained compounds **23p** (IC_{50} = 4110 nM) and **23q** (IC_{50} = 3442 nM) were significantly less potent compared to **23a** (IC_{50} = 5.71 nM) and **23e** (IC_{50} = 5.02 nM). From the results, we could deduced that 2-pyridinylamino amide moiety was the essential group for c-Met kinase inhibitory activity. Meanwhile, replacing the cyclopropyl group with methyl group was very well tolerated (**23r–23u**) and maintained the biological activity, especially IC_{50} values against c-Met kinase of **23r** (IC_{50} = 8.22 nM) and **23s** (IC_{50} = 6.79 nM) were less than 10.00 nM.

Having identified well tolerated group on R and R_1 moiety, our attention was turned to the investigation of

phenoxy part (-X group) and four compounds (**23v-23y**) were prepared. To our delight, the IC_{50} values showed that the fluorine atom was substituted with hydrogen atom resulted a slight boost in c-Met inhibitory activity. Compounds **23v**, **23w** and **23y** showed the most potent activity with IC_{50} values of 2.31 nM, 1.91 nM and 2.44 nM, respectively, which were better than that of positive control foretinib (IC_{50} = 2.53 nM).

Table 1

In vitro c-Met kinase inhibitory activities of target compounds **23a**-**23y**.



Compd.	R	R_1	X	IC_{50} (nM) ± SD [a] c-Met
23a			F	5.71 ± 0.26
23b			F	55.39 ± 3.57
23c			F	23.27 ± 1.31
23d			F	52.33 ± 2.96
23e			F	5.02 ± 0.32
23f			F	7.64 ± 0.98
23g			F	10.23 ± 1.15
23h			F	16.79 ± 1.22
23i			F	25.13 ± 0.89
23j			F	45.28 ± 4.18
23k			F	14.16 ± 1.01
23l			F	8.55 ± 0.45
23m			F	145.96 ± 6.47
23n			F	65.68 ± 5.03
23o			F	202.72 ± 12.65
23p			F	4110 ± 93.27
23q			F	3442 ± 78.53
23r			F	8.22 ± 0.36
23s			F	6.79 ± 0.24
23t			F	62.47 ± 6.17
23u			F	184.36 ± 10.43
23v			H	2.31 ± 0.10
23w			H	1.91 ± 0.12
23x			H	2.96 ± 0.14
23y			H	2.44 ± 0.16
Foretinib[b]	--	--	--	2.53 ± 0.10

[a] Data presented is the mean ± SD value of three independent determinations.
[b] Used as a positive control.

2.2.2 In vitro antiproliferative activity

Based on the *in vitro* c-Met kinase assay results, we selected four compounds which IC_{50} values against c-Met kinase were less than 5.00 nM for their antiproliferative activitives against three c-Met overexpressed human cancer cell lines, namely A549 (human lung adenocarcinoma cell line), H460 (human lung cancer cell line) and HT-29 (human colon cancer cell line) together with foretinib as the positive control by a MTT assay. The results were outlined in Table 2. As shown in Table 2, all the four compounds exhibited excellent antiproliferative activitives against the three tested cell lines. Among them, compound **23v, 23w** and **23x** exhibited remarkable inhibitory activitives against HT-29 cell lines with IC_{50} value of 0.83μM, 0.65 μM and 0.74 μM, respectively, which were more potent than that of the positive control foretinib (IC_{50} = 0.98 μM). Meanwhile, Compound **23y** also showed excellent antiproliferative against H460 cell line with IC_{50} values of 0.79 μM, and it was slightly more potent than foretinib (IC_{50} = 0.83 μM).

Table 2

Antiproliferative activities of selected compounds **23v, 23w, 23x, 23y** and **Foretinib** *in vitro*.

Compd.	IC_{50} (μM) ±SD[a]		
	A549	H460	HT-29
23v	2.17 ± 0.19	0.87 ± 0.06	0.83 ± 0.07
23w	1.57 ± 0.05	0.94 ± 0.03	0.65 ± 0.05
23x	4.84 ± 0.11	1.82 ± 0.16	0.74 ± 0.02
23y	3. 35 ± 0.23	0.79 ± 0.06	1.01 ± 0.09
Foretinib[b]	1.25 ± 0.14	0.83 ± 0.05	0.98 ± 0.06

[a] Data presented is the mean ± SD value of three independent determinations.
[b] Used as a positive control.

*2.2.3. Apoptosis induction ability of **23w***

According to the results of c-Met kinase assay and antiproliferative assay, acridine orange (AO)/ethidium bromide (EB) assay were performed to observe the morphologic changes of apoptosis by fluorescene microscopy after the HT-29 and A549 cells were treatment with compound **23w** for 48 h [40, 41]. As shown in Fig. 3, almost all cells of untreated control group were stained in green and showed normal morphology of regular roundness. Most HT-29 and A549 cells treated with 1.0 μM and 10.0 μM of compound **23w** displayed the morphological changes such as apoptotic body formation, membrane blebbing, cell shrinkage and chromatin condensation, suggesting that compound **23w** induced cell death in HT-29 and A549 cancer cells *via* apoptosis in a dose dependent manner.

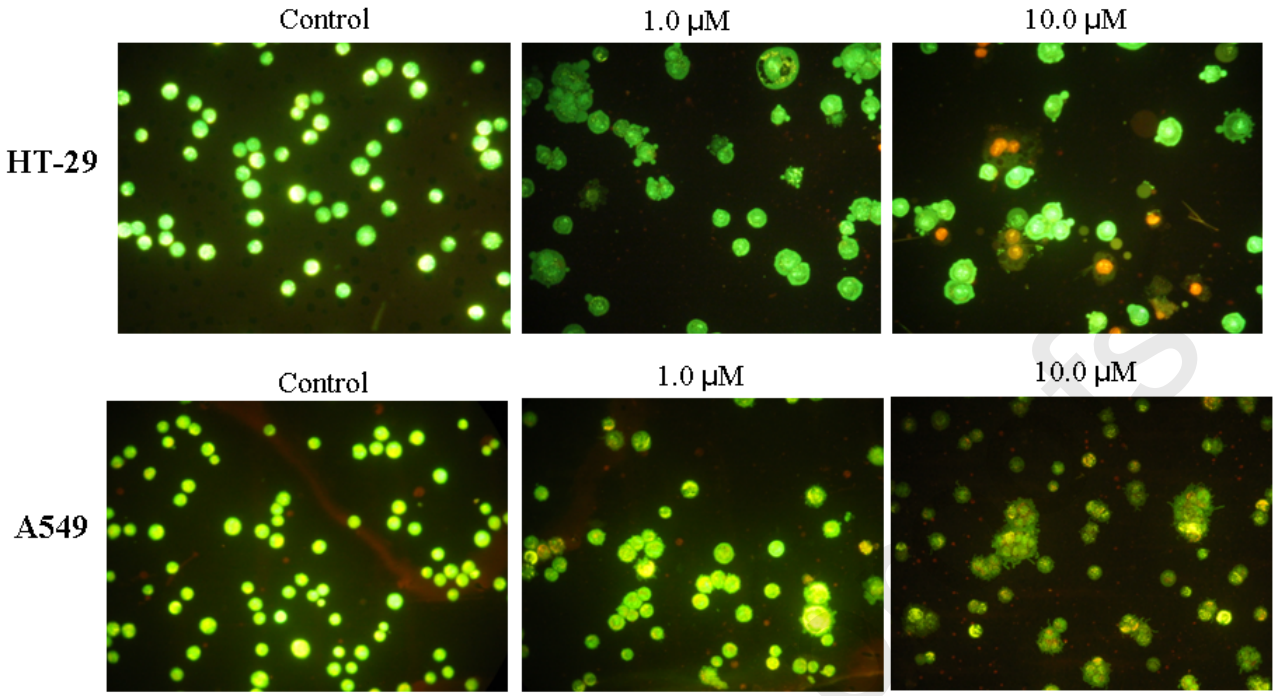


Fig. 3. AO/EB stained apoptosis of HT-29 and A549 cell lines with different concentrations of compound **23w**.

In the next step, flowcytometry analysis with Annexin V/PI double staining was carried out to confirm apoptosis induction by the compound **23w** according to known procedures [42]. HT-29 and A549 cells were seeded per well in six-well plates and were treated with compound **23w** of different concentrations (0, 1.0 and 10.0 μM) for 48 h, respectively. As depicted in Fig. 4, incubation of HT-29 cells with compound **23w** led to a dose dependent apoptosis increase, with the percentage of total apoptotic cells (early and late apoptotic cells) of 17.1 % and 44.2 % at 1.0 μM and 10.0 μM, respectively, compared to the control (13.7%). Meanwhile, we found that treatment of A549 cells with compound **23w** also induced apoptosis in a dose dependent manner as the same trend observed in HT-29 cells. According to these results, we conclude that compound **23w** could effectively induce apoptosis in HT-29 and A549 cells.

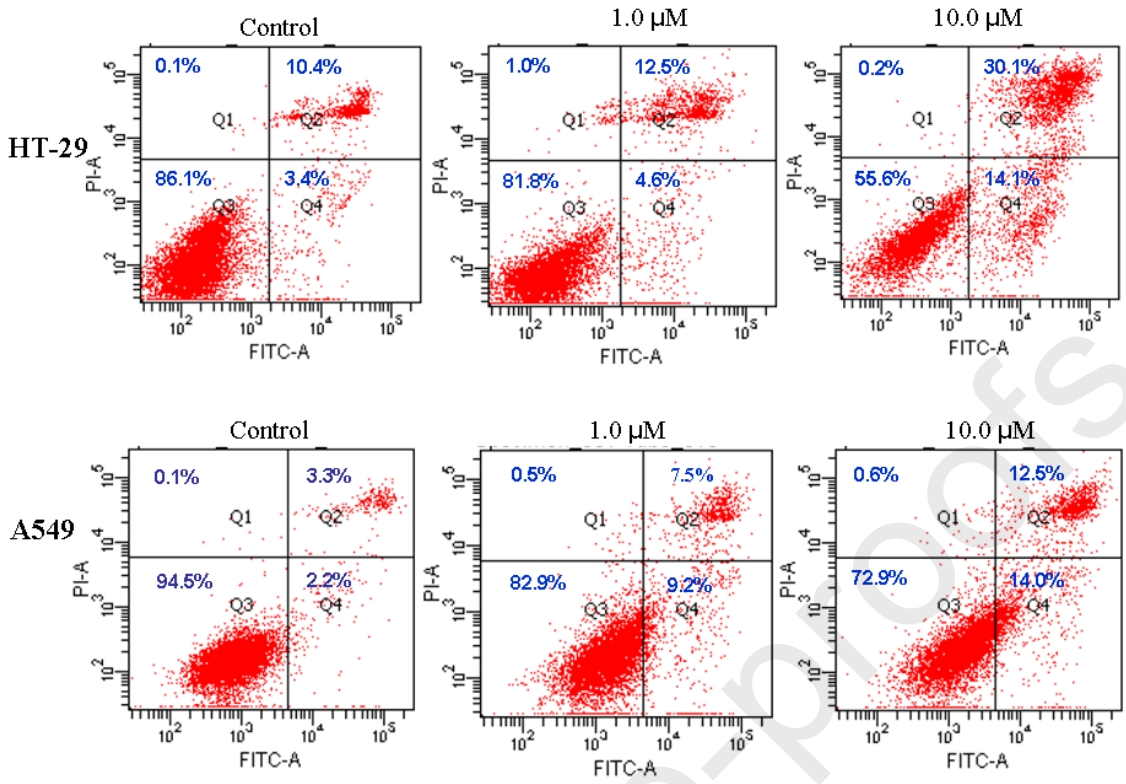


Fig. 4. Compound **23w** induces cell apoptosis of HT-29 and A549 cell line *in vitro*.

*2.2.4. Cell migration inhibition ability of **23w***

As migration is an important characteristic for metastatic cancers [43], we investigated the antimigratory effect of compound **23w** on A549 cells *in vitro* using wound-healing assay. As illustrated in the photomicrographs shown in Fig. 5, untreated A549 cells (control group) almost filled up the wounded area 72 h after scratching the cell monolayer, whereas treatment with 1.0 μM of compound **23w** significantly suppressed wound healing in a time dependent manner. These results indicate that compound **23w** possesses a significant ability to inhibit the metastasis of A549 cells.

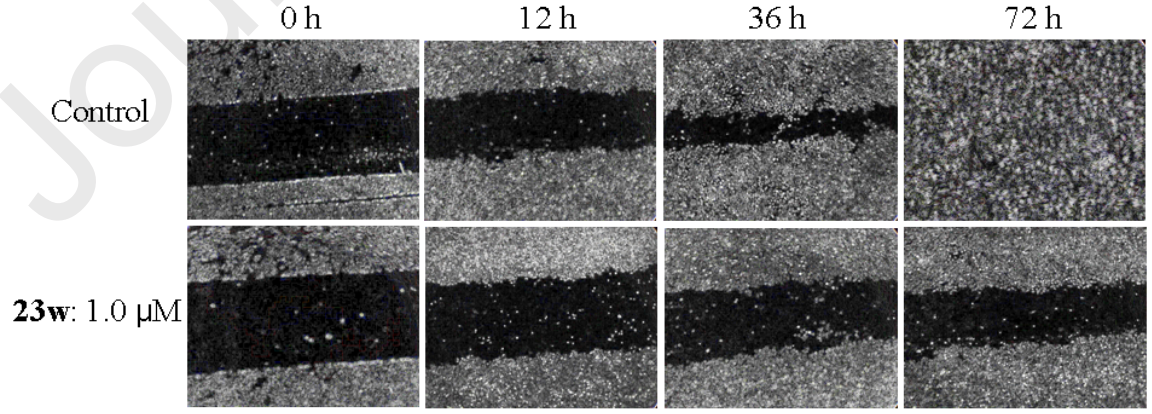


Fig. 5. Anti-migration activity of compound **23w** against A549 cells in wound-healing migration model.

To further determine anti-migration ability of compound **23w**, transwell migration assay was also performed [44].

The results revealed that compound **23w** inhibited the migration of A549 cancer cells obviously at two concentrations (1.0 and 10.0 μM), compared with the control group. Interestingly, the migration of A549 cells was significantly inhibited by compound **23w** and almost completely suppressed by this compound at the concentration of 10.0 μM (Fig. 6).

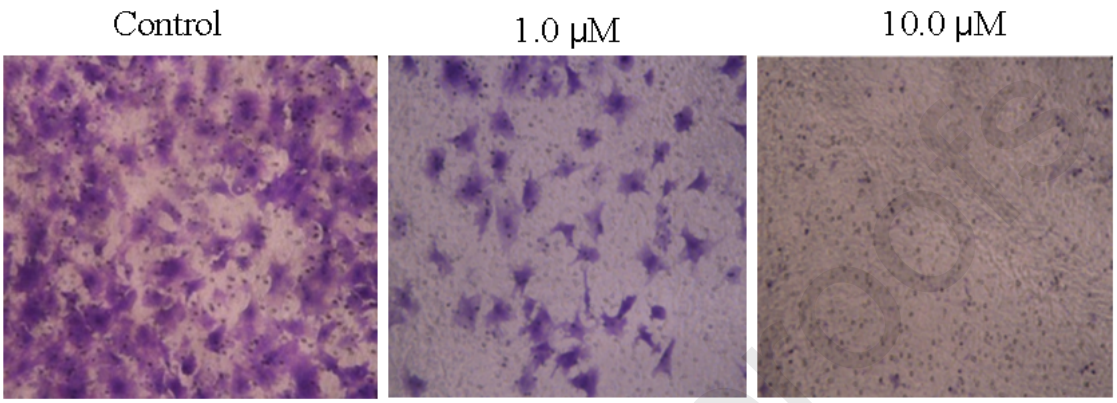


Fig. 6. Anti-migration activity of compound **23w** against A549 cells in transwell migration model.

2.2.5. Binding model analysis

In order to better understand the binding mode, molecular docking of compound **23w** was performed based on the cocrystal structure of foretinib (GSK1363089) with c-Met (PDB code: 3LQ8). As shown in Fig. 7, binding model of compound **23w** with c-Met (PDB code: 3LQ8) were well consistent with type II c-Met inhibitors binding conformation and bonding model. In the binding model, the 4-phenoxypyridine part (moiety A) was found that the N atom of pyridine and the O atom of cyclopropanecarboxamide interacted with Met1160 *via* two hydrogen bonds. Meanwhile, the pyridinyl ring and the phenyl ring at 4-position of pyridine formed two π–π interactions with Tyr1159 and Phe1223, respectively. In addition, the following interactions were formed in moiety A: (1): five π-Alkyl interaction with TYR1159, Met1160, Ala1108, Ile1084 and Leu1157, respectively; (2): one π-lone pair interaction with 1159; (3): one carbon hydrogen interaction with Pro1158; (4): one π-sigma interaction with Met1121. The 4-fluorophenyl ring (moiety B) fitted into the hydrophobic pocket very well, and formed one π–alkyl interaction with Met1131. The 3-oxo-3,4-dihydroquinoxaline-2-carboxamide moiety (moiety C) formed two hydrogen bonds with Lys1110, one π–anion interaction with Asp1222, one carbonhydrogen bond interaction with Asp1222 and one π–alkyl interaction with Met1131. All these interactions contribute to the tight binding and greatly enhance the inhibitory potency of **23w**.

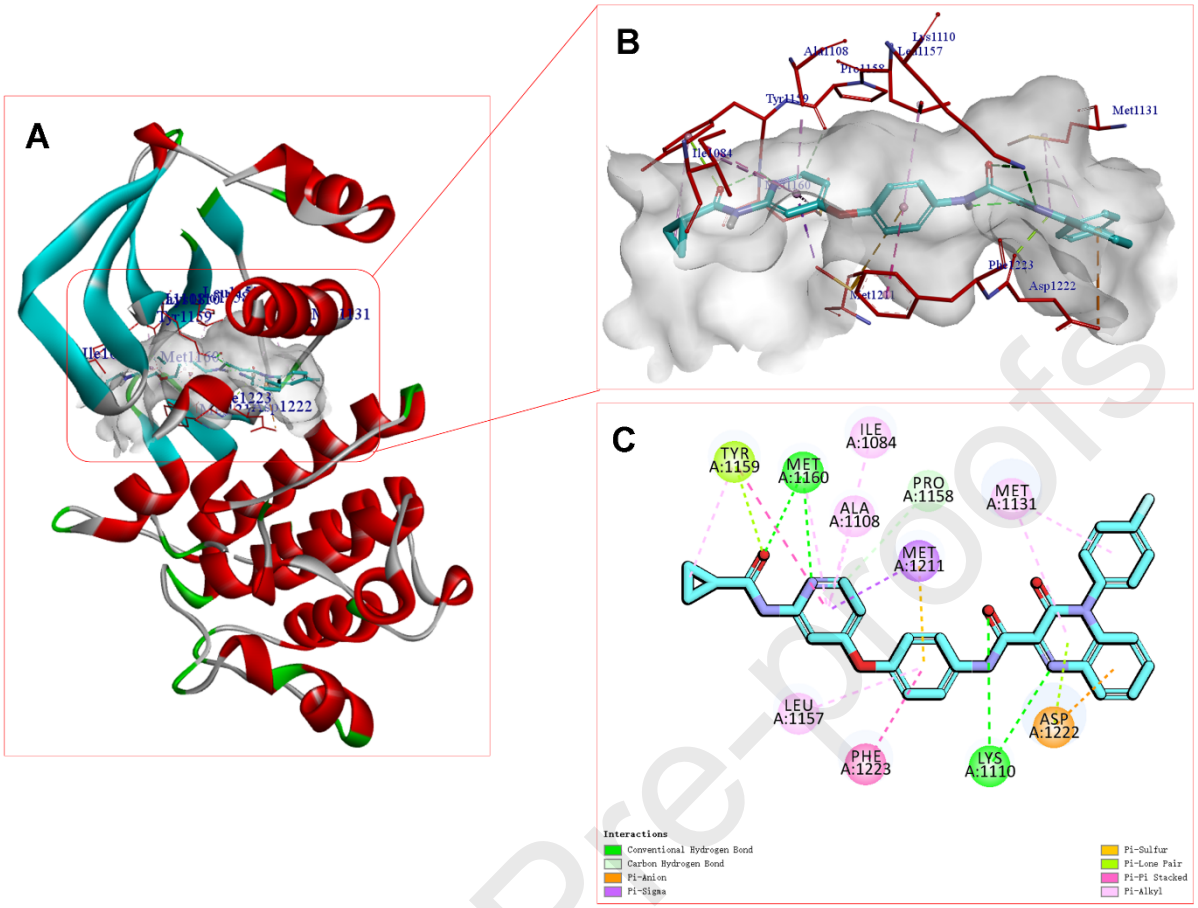


Fig. 7. The mode of compound **23w** bound to c-Met (PDB code: 3LQ8). The amino acid residues were displayed by red sticks and compound was displayed by bright blue sticks. Green dotted lines represent H-bonds. (A) The overview of compound **23w** docked into c-Met. (B) The c-Met active site in complex with compound **23w**. (C) 2D interactions of the docking model of compound **23w** to c-Met.

3. Conclusion

In summary, a series of novel 4-phenoxypyridine derivatives containing 3-oxo-3,4-dihydroquinoxaline moiety as c-Met inhibitors were designed, synthesized and evaluated for their biological activitives. The screening of enzyme activities and cytotoxicity led to the identification of a most promising compound **23w** (c-Met IC_{50} = 1.91 nM) with IC_{50} values of 1.57 μM, 0.94 μM and 0.65 μM against A549, H460, and HT-29 cells, representing a promising lead for further optimization. The The initial structure-activity relationships (SARs) analyses indicated that introduction of the 3-oxo-3,4-dihydroquinoxaline-2-carboxamide framework to "**5-atom linker**" moiety of pyridine-based compounds maintained the c-Met inhibitory efficacy and compounds without substituents or with a fluoro group at 4-position on the terminal phenyl ring (moiety B) were more active than other substituents. Meanwhile, 2-pyridinylamino amide (moiety A) maybe the essential group for c-Met kinase inhibitory activity. Furthermore, AO/EB assays, cell apoptosis assays by flow cytometry, wound-healing assays and transwell migration assay on HT-29 and/or A549 cells results indicated that compound **23w** could induce HT-29 and A549 cells apoptosis, and inhibit A549 cells motility. Further studies on structural optimization and biological activities about these derivatives are still underway in our

laboratory and will be reported in the future.

4. Experimental

4.1. Chemistry

Unless otherwise specified, all melting points were obtained on a Beijing Taike X-4 microscopy melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker Biospin 600 MHz or Bruker Biospin 400 MHz instrument using TMS as the internal standard. All chemical shifts were reported in ppm. IR spectra were recorded as KBr pellets on a Perkin-Elmer Spcetrum one FT-IR spectrometer. MS spectra were obtained on an Agilent 6460 QQQ mass spectrometer (Agilent, USA) analysis system. The elemental analysis of the compounds was performed on a Perkin Elmer 2400 Elemental Analyser (In the mode of measurement C, H, and N, the sample into the combustion tube in pure oxygen atmosphere static combustion and products by a specific reagent after formation of CO_2, H_2O, N_2 and nitrogen oxides, uniform mixing under the atmospheric pressure. The thermal conductivity detector is used for determining the content of C, H and N from mixed gases.). 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). The key intermediates **13a**, **16a**, **17a**, **18a-18o**, **19a-19o**, **20a-20o**, **21a-21o** and **22a-22o** were synthesized according to our previous reported procedures [30, 34, 39].

4.1.1. General procedure for preparation of N-(4-chloropyridin-2-yl)alkylcarboxamide (**13a, 13b**)

Cyclopropanecarbonyl chloride or acetyl chloride (89.00 mmol) was dissolved in dried CH_2Cl_2 (30 mL) and drop-wise added to a mixture of 4-chloropyridin-2-amine (8.80 g, 68.45 mmol), Et_3N (20.78 g, 205.35 mmol) and CH_2Cl_2 (80 mL) in an ice bath, which was then removed to raise the temperature to room temperature and stirred for 12 h. The resulting mixture was sequentially washed with 20% K_2CO_3 (50 mL × 3) and brine (50 mL × 3), and the organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated under reduced pressure. The crude product obtained was purified by silica gel chromatography to give **13a or 13b** as a white solid.

4.1.1.1. N-(4-chloropyridin-2-yl)cyclopropanecarboxamide (**13a**)

Yield: 72.7% %; IR (KBr) cm^{-1}: 3435.3, 3241.8, 1706.5, 1670.1, 1588.4, 1573.5, 1537.4, 1403.5, 1257.0, 1212.6, 1189.5, 1149.6, 959.6, 872.9, 823.8, 710.1; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.31 (s, 1H), 8.16 (d, J = 5.4 Hz, 1H), 7.03 (dd, J = 5.4, 1.6 Hz, 1H), 1.60 – 1.49 (m, 1H), 1.17 – 1.09 (m, 2H), 0.97 – 0.87 (m, 2H); MS (ESI) m/z(%): 197.1 $[M+H]^+$.

4.1.1.2. N-(4-chloropyridin-2-yl)acetamide (**13b**)

Yield: 70.2%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.31 (s, 1H), 8.15 (d, *J* = 5.4 Hz, 1H), 7.05 (dd, *J* = 5.4, 1.8 Hz, 1H), 2.21 (s, 3H); MS (ESI) m/z (%): 171.1 $[M+H]^+$.

4.1.2. 4-Chloro-N-cyclopropylpicolinamide (**15**)

To a solution of picolinic acid (18.0 g, 146.21 mmol) and sodium bromide (3.01 g, 29.24 mol) in chlorobenzene (50 mL), thionyl chloride (60.90 g, 511.80 mmol) was added slowly at room temperature. The reaction mixture was stirred at 50 °C for 30 min, and heated to 85 °C and stirred for another 20 h. The completion of reaction was determined by TLC, at which point the solvent was removed under reduced pressure to give a brown oil, which was immediately dissolved in toluene (50 mL). Then cyclopropanamine (9.2 g, 160.90 mmol) in toluene (30 mL) was added dropwise in an ice-bath. The mixture was stirred at 10 °C for 3.0 h. The solvent was removed under reduced pressure. The residue was dissolved in dried CH_2Cl_2 (100 mL) and the resulting mixture was sequentially washed with saturated aqueous K_2CO_3 (50 mL × 3) and brine (50 mL × 3), and the organic phase was separated, dried, and evaporated. The crude product obtained was purified by silica gel chromatography to afford **15** as a light-yellow solid (16.8 g, yield 58.4%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.83 (d, *J* = 3.9 Hz, 1H), 8.60 (d, *J* = 5.2 Hz, 1H), 8.02 (d, *J* = 2.0 Hz, 1H), 7.75 (dd, *J* = 5.2, 2.1 Hz, 1H), 3.09 – 2.76 (m, 1H), 0.99 – 0.44 (m, 4H); MS (ESI) m/z (%): 197.1 $[M+H]^+$.

4.1.3. General procedure for preparation intermediates of 4-(4-nitrophenoxy)pyridine (**16a-16e**)

A stirring mixture of compound **13a, 13b or 15** (40.68 mmol) and 2-fluoro-4-nitrophenol or 4-nitrophenol (101.71 mmol) in chlorobenzene (100 mL) was heated to 140 °C for about 40 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to yield a pale solid. The solid was dissolved in CH_2Cl_2 (150 mL), and washed with saturated K_2CO_3 aqueous solution (80 mL × 4), then brine (60 mL × 4), and dried over anhydrous Na_2SO_4, concentrated under reduced pressure to afford a brown solid, which was was purified by silica gel chromatography to give **16a-16e** as a light yellow solid.

4.1.3.1. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)cyclopropanecarboxamide (**16a**)

Yield: 55.3%; IR (KBr) cm^{-1}: 3467.0, 3417.7, 1687.3, 1616.7, 1528.7, 1492.5, 1353.9, 1300.4, 1271.9, 1179.8, 1070.0, 875.2, 798.4; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.43 (dd, *J* = 10.3, 2.2 Hz, 1H), 8.30 (d, *J* = 5.7 Hz, 1H), 8.19 (dd, *J* = 9.0, 1.9 Hz, 1H), 7.76 (d, *J* = 2.2 Hz, 1H), 7.61 (t, *J* = 8.5 Hz, 1H), 6.86 (dd, *J* = 5.6, 2.2 Hz, 1H), 2.04 – 1.95 (m, 1H), 0.78 (t, *J* = 6.3 Hz, 4H); MS (ESI) m/z (%): 318.1 $[M+H]^+$.

4.1.3.2. N-(4-(4-nitrophenoxy)pyridin-2-yl)cyclopropanecarboxamide (**16b**)

Yield: 58.8%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.31 – 8.26 (m, 2H), 8.23 (d, *J* = 5.7 Hz, 1H), 7.92 (d, *J* = 2.2 Hz, 1H), 7.24 – 7.13 (m, 2H), 6.72 (dd, *J* = 5.7, 2.3 Hz, 1H), 1.64 – 1.45 (m, 1H), 1.11 – 1.05 (m, 2H), 0.93 – 0.86 (m, 2H); MS (ESI) m/z(%): 300.1 M+H]$^+$.

4.1.3.3. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)acetamide (**16c**)

Yield: 51.6%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.21 (d, *J* = 5.7 Hz, 1H), 8.17 – 8.09 (m, 2H), 7.89 (s, 1H), 7.37 – 7.29 (m, 1H), 6.71 (dd, *J* = 5.7, 2.3 Hz, 1H), 2.18 (s, 3H); MS (ESI) m/z(%): 292.1 $[M+H]^+$.

4.1.3.4. N-(4-(4-nitrophenoxy)pyridin-2-yl)acetamide (**16d**)

Yield: 54.3%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 – 8.27 (m, 3H), 8.22 (d, *J* = 5.7 Hz, 1H), 7.93 (s, 1H), 7.20 (d, *J* = 9.1 Hz, 2H), 6.71 (dd, *J* = 5.7, 2.3 Hz, 1H), 2.19 (s, 3H); MS (ESI) m/z(%): 274.1 $[M+H]^+$.

4.1.3.5. N-cyclopropyl-4-(4-nitrophenoxy)picolinamide (**16e**)

Yield: 62.7%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.47 (d, *J* = 5.5 Hz, 1H), 8.21 – 8.09 (m, 2H), 8.02 (s, 1H), 7.71 (d, *J* = 2.5 Hz, 1H), 7.40 – 7.30 (m, 1H), 7.07 (dd, *J* = 5.5, 2.6 Hz, 1H), 3.24 – 2.64 (m, 1H), 0.90 – 0.85 (m, 2H), 0.68 – 0.63 (m, 2H); MS (ESI) m/z(%): 318.1 $[M+H]^+$, 340.0 $[M+Na]^+$.

4.1.4. General procedure for preparation intermediates of 4-(pyridin-4-yloxy)aniline (**17a-17e**)

A mixture of compound **16a-16e** (18.00 mmol), iron powder (90.00 mmol), acetic acid (180.00 mmol), water (10 mL) and ethyl acetate (100 mL) was heated to reflux for 2 h. After completion of the reaction as indicated by TLC, the mixture was filtered immediately. The organic layer of the filtrate was separated, washed with water, dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated under reduced pressure when white solid appeared, which was filtered to obtain **17a-17e** as light yellow solid.

4.1.4.1. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)cyclopropanecarboxamide (**17a**)

Yield: 64.6%; IR (KBr) cm^{-1}: 3411.2, 2025.9, 1736.6, 1617.8, 1510.4, 1426.8, 1207.1, 1163.3, 992.6, 955.9, 866.0, 821.9, 609.7, 468.1; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (s, 1H), 8.15 (d, *J* = 5.7 Hz, 1H), 7.59 (s, 1H), 6.95 (t, *J* = 9.0 Hz, 1H), 6.67 – 6.61 (m, 1H), 6.49 (dd, *J* = 13.1, 2.2 Hz, 1H), 6.40 (d, *J* = 8.7 Hz, 1H), 5.44 (s, 2H), 2.03 – 1.88 (m, 1H), 0.76 (br, 4H); MS (ESI) m/z(%): 288.1 $[M+H]^+$, 310.1 $[M+Na]^+$.

4.1.4.2. N-(4-(4-aminophenoxy)pyridin-2-yl)cyclopropanecarboxamide (**17b**)

Yield: 71.3%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.06 (d, *J* = 5.8 Hz, 1H), 7.75 (s, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 6.69 (d, *J* = 8.6 Hz, 2H), 6.55 (dd, *J* = 5.7, 2.1 Hz, 1H), 3.61 (s, 2H), 1.54 (s, 1H), 1.18 – 0.91 (m, 2H), 0.92 – 0.58 (m, 2H); MS (ESI) m/z (%): 270.1 M+H]$^+$, 292.1 $[M+Na]^+$.

4.1.4.3. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)acetamide (**17c**)

Yield: 67.9%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.49 (s, 1H), 8.14 (d, *J* = 5.7 Hz, 1H), 7.61 (s, 1H), 6.96 (t, *J* = 9.0 Hz, 1H), 6.61 (dd, *J* = 5.6, 2.1 Hz, 1H), 6.56 – 6.46 (m, 1H), 6.42 (d, *J* = 8.6 Hz, 1H), 5.45 (s, 2H), 2.04 (s, 3H); MS (ESI) m/z(%): 262.1 $[M+H]^+$, 284.1 $[M+Na]^+$.

4.1.4.4. N-(4-(4-aminophenoxy)pyridin-2-yl)acetamide (**17d**)

Yield: 70.7%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (s, 1H), 8.05 (d, *J* = 5.8 Hz, 1H), 7.76 (s, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.71 (d, *J* = 8.7 Hz, 2H), 6.55 (dd, *J* = 5.8, 2.3 Hz, 1H), 3.66 (s, 2H), 2.15 (s, 3H); MS (ESI) m/z(%): 244.1M+H]$^+$, 266.1 [M+Na]$^+$.

4.1.4.5. 4-(4-amino-2-fluorophenoxy)-N-cyclopropylpicolinamide (**17e**)

Yield: 76.8%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.33 (d, *J* = 5.6 Hz, 1H), 8.02 (s, 1H), 7.66 (d, *J* = 2.5 Hz, 1H), 7.01 – 6.86 (m, 2H), 6.51 (dd, *J* = 11.9, 2.6 Hz, 1H), 6.45 (dd, *J* = 8.6, 1.7 Hz, 1H), 3.84 (s, 2H), 3.00 – 2.78 (m, 1H), 0.88 – 0.82 (m, 2H), 0.67 – 0.61 (m, 2H); MS (ESI) m/z (%): 288.1 M+H]$^+$, 310.1[M+Na]$^+$.

4.1.5. General procedure for Preparation of the target Compounds **(23a-23y)**

A mixture of the corresponding acids **22a–22o** (1.30mmol), **17a-17e** (1.00 mmol), HATU (1.50 mmol), Et_3N (3.00 mmol), and DMF (8 ml) was stirred at room temperature for 12 hr. The residue was dissolved in CH_2Cl_2 (50 mL) and the resulting mixture was sequentially washed with 20% K_2CO_3 (30 mL × 3) and brine (30 mL × 3), and the organic phase was separated, dried, and evaporated. The crude product obtained was purified by silica gel chromatography to afford **23a-23y** as light yellow solids.

4.1.5.1. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-4-phenyl-3,4-dihydro quinoxaline-2-carboxamide (**23a**).

Yield: 84.1%; M.p.: 254–256 °C; IR (KBr, cm^{-1}): 3395.3, 1693.2, 1646.0, 1596.3, 1578.4, 1535.0, 1512.6, 1420.0, 1306.0, 1201.7, 1171.0, 1092.3, 953.7, 829.4, 767.7, 701.3, 603.0; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.29 (s, 1H), 10.87 (s, 1H), 8.21 (d, *J* = 5.7 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.93 (dd, *J* = 12.6, 2.2 Hz, 1H), 7.70 (t, *J* = 7.6 Hz, 2H), 7.68 – 7.57 (m, 3H), 7.54 (d, *J* = 8.9 Hz, 1H), 7.48 (t, *J* = 8.1 Hz, 3H), 7.41 (t, *J* = 8.9 Hz, 1H), 6.74 (dd, *J* = 5.7, 2.4 Hz, 1H), 6.68 (d, *J* = 8.5 Hz, 1H), 2.04 – 1.90 (m, 1H), 0.89 – 0.65 (m, 4H); MS (ESI) m/z(%): 536.1 [M+H]$^+$, 558.1 [M+Na]$^+$; Anal. calcd. for $C_{30}H_{22}FN_5O_4$ (%): C, 67.28; H, 4.14; N, 13.08. Found (%): C, 67.39; H, 4.19; N, 13.14.

4.1.5.2. 4-cyclohexyl-N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-3,4-dihydro quinoxaline-2-carboxamide (**23b**).

Yield: 79.5%; M.p.: 267–270 °C; IR (KBr, cm^{-1}): 3430.5, 2933.3, 2851.6, 1687.3, 1593.8, 1425.2, 1307.1, 1206.6, 1176.1, 1097.4, 954.8, 864.2, 817.4, 758.8; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.13 (s, 1H), 10.88 (s, 1H), 8.22 (d, *J* = 5.7 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.98 – 7.85 (m, 2H), 7.74 (t, *J* = 7.9 Hz, 1H), 7.66 (d, *J* = 2.2 Hz, 1H), 7.57 – 7.44 (m, 2H), 7.41 (t, *J* = 8.9 Hz, 1H), 6.75 (dd, *J* = 5.7, 2.3 Hz, 1H), 4.73 (s, 1H), 2.55 (s, 2H), 2.06 – 1.92 (m, 1H), 1.87 (d, *J* = 11.6 Hz, 2H), 1.80 – 1.64 (m, 3H), 1.61 – 1.41 (m, 2H), 1.42 – 1.13 (m, 1H), 0.92 – 0.64 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.28, 165.73, 162.90, 154.35, 153.88 (d, *J* = 246.2 Hz), 153.45, 149.96, 137.51 (d, *J*

= 9.7 Hz), 136.22 (d, *J* = 12.3 Hz), 132.45, 130.96, 124.67, 124.44, 116.69, 108.60 (d, *J* = 23.0 Hz), 107.55, 99.62, 28.07, 25.89, 25.23, 14.62, 8.10; MS (ESI) m/z (%): 542.2 $[M+H]^+$, 564.2 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{28}FN_5O_4$ (%): C, 66.53; H, 5.21; N, 12.93. Found (%): C, 66.48; H, 5.26; N, 12.97.

4.1.5.3. 4-benzyl-N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-3,4-dihydro quinoxaline-2-carboxamide (**23c**).

Yield: 84.8%; M.p.: 253–255 °C; IR (KBr, cm^{-1}): 3432.6, 3060.4, 1690.4, 1595.4, 1536.4, 1506.4, 1467.8, 1431.7, 1388.2, 1300.6, 1206.3, 1101.8, 954.8, 874.0, 820.4, 764.0; ^1H NMR (600 MHz, DMSO-d_6) δ 11.25 (s, 1H), 10.88 (s, 1H), 8.22 (s, 1H), 8.07 – 7.96 (m, 1H), 7.93 (dd, *J* = 12.6, 2.2 Hz, 1H), 7.75 – 7.62 (m, 2H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.50 – 7.39 (m, 2H), 7.39 – 7.32 (m, 4H), 7.31 – 7.27 (m, 1H), 6.76 (d, *J* = 3.4 Hz, 1H), 5.59 (s, 2H), 2.10 – 1.80 (m, 1H), 0.96 – 0.44 (m, 4H); MS (ESI) m/z(%): 550.1 $[M+H]^+$, 572.1 $[M+Na]^+$; Anal. calcd. for $C_{31}H_{24}FN_5O_4$ (%): C, 67.75; H, 4.40; N, 12.74. Found (%): C, 67.87; H, 4.44; N, 12.71.

4.1.5.4. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-4-phenethyl-3,4-dihydro quinoxaline-2-carboxamide (**23d**).

Yield: 76.3%; M.p.: 241–243 °C; IR (KBr, cm^{-1}): 3454.5, 1693.5, 1593.2, 1535.3, 1423.5, 1311.6, 1182.4, 761.8; ^1H NMR (600 MHz, DMSO-d_6) δ 11.23 (s, 1H), 10.88 (s, 1H), 8.22 (d, *J* = 5.7 Hz, 1H), 8.01 – 7.96 (m, 1H), 7.93 (dd, *J* = 12.6, 2.3 Hz, 1H), 7.82 – 7.74 (m, 2H), 7.67 (d, *J* = 2.2 Hz, 1H), 7.54 (dd, *J* = 8.8, 1.4 Hz, 1H), 7.51 – 7.47 (m, 1H), 7.42 (t, *J* = 8.9 Hz, 1H), 7.38 (d, *J* = 7.3 Hz, 2H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.26 (t, *J* = 7.3 Hz, 1H), 6.75 (dd, *J* = 5.7, 2.4 Hz, 1H), 4.63 – 4.39 (m, 2H), 3.04 – 2.95 (m, 2H), 2.11 – 1.86 (m, 1H), 0.94 – 0.67 (m, 4H); ^3C NMR (150 MHz, DMSO-d_6) δ 173.29, 165.73, 162.46, 154.35, 153.89 (d, *J* = 246.2 Hz), 152.87, 150.92, 149.98, 138.23, 137.48 (d, *J* = 9.8 Hz), 136.28 (d, *J* = 12.2 Hz), 133.18, 132.88, 131.94, 130.77, 129.27, 128.91, 127.05, 124.68, 124.61, 116.76, 115.45, 108.69 (d, *J* = 23.0 Hz), 107.56, 99.64, 43.71, 33.06, 14.62, 8.11;MS (ESI) m/z(%): 586.1 $[M+Na]^+$; Anal. calcd. for $C_{32}H_{26}FN_5O_4$ (%): C, 68.20; H, 4.65; N, 12.43. Found (%): C, 68.18; H, 4.67; N, 12.48.

4.1.5.5. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-4-(4-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**23e**).

Yield: 84.4%; M.p.: 262–264 °C; IR (KBr, cm^{-1}): 3421.4, 1693.6, 1648.8, 1597.0, 1507.8, 1427.3, 1309.3, 1259.3, 1207.2, 1176.4, 954.8, 765.5; ^1H NMR (600 MHz, $CDCl_3$) δ 11.91 (s, 1H), 8.32 (d, *J* = 7.0 Hz, 1H), 8.19 – 8.08 (m, 2H), 7.98 (dd, *J* = 12.1, 2.3 Hz, 1H), 7.79 (s, 1H), 7.63 – 7.56 (m, 1H), 7.55 – 7.49 (m, 1H), 7.45 – 7.33 (m, 5H), 7.15 (t, *J* = 8.6 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 6.60 (dd, *J* = 5.7, 2.2 Hz, 1H), 1.52 – 1.47 (m, 1H), 0.90 – 0.81 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.28, 165.70, 162.68 (d, *J* = 246.6 Hz), 162.02, 154.33, 153.88 (d, *J* = 246.2 Hz), 153.24, 151.19, 149.98, 137.40 (d, *J* = 9.8 Hz), 136.33 (d, *J* = 12.2 Hz), 135.12, 132.74, 131.80, 131.14 (d, *J* = 8.9

Hz), 130.50, 124.82, 124.67, 117.74, 117.59, 116.84, 115.95, 108.78 (d, *J* = 23.0 Hz), 107.56, 99.65, 14.60, 8.10; MS (ESI) m/z(%): 554.1 $[M+H]^+$, 576.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{21}F_2N_5O_4$ (%): C, 65.10; H, 3.82; N, 12.65. Found (%): C, 65.13; H, 3.87; N, 12.61.

4.1.5.6. 4-(4-chlorophenyl)-N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**23f**).

Yield: 86.7%; M.p.: 251–253 °C; IR (KBr, cm^{-1}): 3434.0, 2923.1, 1693.3, 1597.8, 1577.5, 1532.5, 1506.2, 1427.6, 1308.2, 1262.0, 1205.2, 1089.3, 1017.9, 954.8, 828.5, 752.9; ^1H NMR (600 MHz, $CDCl_3$) δ 11.88 (s, 1H), 8.30 (t, *J* = 7.2 Hz, 1H), 8.25 (s, 1H), 8.11 (d, *J* = 5.8 Hz, 1H), 7.97 (dd, *J* = 12.1, 2.3 Hz, 1H), 7.79 (d, *J* = 1.6 Hz, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.61 – 7.56 (m, 1H), 7.55 – 7.49 (m, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.20 – 7.13 (m, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.60 (dd, *J* = 5.8, 2.3 Hz, 1H), 1.54 – 1.47 (m, 1H), 1.09 – 1.03 (m, 2H), 0.89 – 0.83 (m, 2H); MS (ESI) m/z(%): 569.3 $[M]^+$, 592.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{21}ClFN_5O_4$ (%): C, 63.22; H, 3.71; N, 12.29. Found (%): C, 63.31; H, 3.77; N, 12.35.

4.1.5.7. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-4-(3-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**23g**).

Yield: 75.2%; M.p.: 254-257 °C; IR (KBr, cm^{-1}): 3426.4, 3060.4, 1695.1, 1599.2, 1530.9, 1506.7, 1430.8, 1298.4, 1209.8, 1174.2, 1113.9, 872.5, 745.8; ^1H NMR (600 MHz, $CDCl_3$) δ 11.86 (s, 1H), 8.46 (s, 1H), 8.31 (d, *J* = 7.9 Hz, 1H), 8.11 (d, *J* = 5.7 Hz, 1H), 7.97 (dd, *J* = 12.1, 2.1 Hz, 1H), 7.80 (s, 1H), 7.74 – 7.67 (m, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.43 – 7.35 (m, 2H), 7.23 – 7.08 (m, 3H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.59 (dd, *J* = 5.7, 2.1 Hz, 1H), 1.57 – 1.47 (m, 1H), 1.09 – 1.02 (m, 2H), 0.89 – 0.81 (m, 2H); MS(ESI)m/z(%): 554.1 $[M+H]^+$, 576.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{21}F_2N_5O_4$ (%): C, 65.10; H, 3.82; N, 12.65. Found (%): C, 65.23; H, 3.83; N, 12.61.

4.1.5.8. 4-(3-chlorophenyl)-N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**23h**).

Yield: 79.3%; M.p.: 235-238 °C; IR (KBr, cm^{-1}): 3421.9, 3060.4, 2923.1, 1694.0, 1580.4, 1507.1, 1428.3, 1319.6, 1208.5, 1174.5, 1097.4, 955.1, 871.1, 821.4, 751.8; ^1H NMR (600 MHz, DMSO-d_6) δ 11.26 (s, 1H), 10.88 (s, 1H), 8.21 (d, *J* = 5.7 Hz, 1H), 8.08 – 7.99 (m, 1H), 7.94 (dd, *J* = 12.6, 2.3 Hz, 1H), 7.77 – 7.70 (m, 2H), 7.69 (s, 1H), 7.66 (d, *J* = 2.2 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.58 – 7.54 (m, 1H), 7.51 – 7.46 (m, 2H), 7.41 (t, *J* = 8.9 Hz, 1H), 6.97 – 6.49 (m, 2H), 2.09 – 1.79 (m, 1H), 0.98 – 0.52 (m, 4H); MS (ESI) m/z(%): 569.3 $[M]^+$, 592.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{21}ClFN_5O_4$ (%): C, 63.22; H, 3.71; N, 12.29. Found (%): C, 63.20; H, 3.74; N, 12.27.

4.1.5.9. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-4-(2-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide. (**23i**).

Yield: 77.4%; M.p.: 257-259 °C; IR (KBr, cm^{-1}): 3423.6, 3071.4, 2923.1, 1693.7, 1597.6, 1503.3, 1425.6, 1306.4, 1207.0, 1182.5, 1105.7, 954.8, 753.7; ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.84 (s, 1H), 8.40 – 8.26 (m, 2H), 8.11 (d, *J* = 5.8 Hz, 1H), 7.98 (dd, *J* = 12.1, 2.3 Hz, 1H), 7.80 (d, *J* = 1.7 Hz, 1H), 7.72 – 7.64 (m, 1H), 7.63 – 7.57 (m, 1H), 7.56 – 7.51 (m, 1H), 7.50 – 7.38 (m, 4H), 7.15 (t, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.59 (dd, *J* = 5.8, 2.3 Hz, 1H), 1.55 – 1.46 (m, 1H), 1.09 – 1.04 (m, 2H), 0.89 – 0.82 (m, 2H); MS (ESI) m/z (%): 554.1 $[M+H]^+$, 576.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{21}F_2N_5O_4$ (%): C, 65.10; H, 3.82; N, 12.65. Found (%): C, 65.16; H, 3.79; N, 12.69.

4.1.5.10. 4-(2-chlorophenyl)-N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide. (**23j**).

Yield: 80.4%; M.p.: 261-263 °C; IR (KBr, cm^{-1}): 3432.4, 2917.6, 1698.0, 1596.7, 1580.5, 1530.9, 1507.0, 1433.0, 1298.2, 1208.4, 1179.7, 1094.7, 1056.3, 954.8, 864.2, 749.2; ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.87 (s, 1H), 8.63 (s, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 5.8 Hz, 1H), 7.98 (dd, *J* = 12.1, 2.1 Hz, 1H), 7.81 (s, 1H), 7.77 – 7.69 (m, 1H), 7.62 (s, 3H), 7.53 (t, *J* = 7.5 Hz, 1H), 7.42 (dd, *J* = 10.3, 5.0 Hz, 2H), 7.15 (t, *J* = 8.6 Hz, 1H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.59 (dd, *J* = 5.7, 2.1 Hz, 1H), 1.57 – 1.47 (m, 1H), 1.07 (dd, *J* = 7.1, 4.0 Hz, 2H), 0.88 – 0.82 (m, 2H); MS(ESI) m/z(%): 570.1 $[M+H]^+$, 592.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{21}ClFN_5O_4$ (%): C, 63.22; H, 3.71; N, 12.29. Found (%): C, 63.28; H, 3.76; N, 12.31.

4.1.5.11. 4-(4-bromophenyl)-N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**23k**).

Yield: 82.2%; M.p.: 269-271 °C; IR (KBr, cm^{-1}): 3433.9, 2923.1, 1692.9, 1596.7, 1533.1, 1506.2, 1462.3, 1427.9, 1305.9, 1205.9, 1179.7, 1012.4, 828.6; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.26 (s, 1H), 10.88 (s, 1H), 8.21 (d, *J* = 5.7 Hz, 1H), 8.03 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.98 – 7.82 (m, 3H), 7.65 (d, *J* = 2.3 Hz, 1H), 7.63 – 7.57 (m, 1H), 7.55 (dd, *J* = 8.9, 1.4 Hz, 1H), 7.51 – 7.44 (m, 3H), 7.41 (t, *J* = 8.9 Hz, 1H), 6.84 – 6.63 (m, 2H), 2.02 – 1.92 (m, 1H), 0.81 – 0.74 (m, 4H); MS (ESI) m/z(%): 636.0 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{21}BrFN_5O_4$ (%): C, 58.64; H, 3.45; N, 11.40. Found (%): C, 58.60; H, 3.48; N, 11.39.

4.1.10.12. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-4-(p-tolyl)-3,4-dihydro quinoxaline-2-carboxamide (**23l**).

Yield: 85.8%; M.p.: 257-259 °C; IR (KBr, cm^{-1}): 3434.1, 2917.6, 1693.0, 1576.7, 1533.3, 1506,2, 1462.3, 1425.1, 1309.4, 1180.6, 1091.9, 828.6, 769.7; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.28 (s, 1H), 10.88 (s, 1H), 8.21 (d, *J* = 5.7 Hz, 1H), 8.02 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.93 (dd, *J* = 12.6, 2.3 Hz, 1H), 7.66 (d, *J* = 2.3 Hz, 1H), 7.62 – 7.57 (m, 1H), 7.54 (dd, *J* = 8.8, 1.4 Hz, 1H), 7.52 – 7.45 (m, 3H), 7.41 (t, *J* = 8.9 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 6.85 – 6.59 (m, 2H), 2.45 (s, 3H), 2.04 – 1.88 (m, 1H), 0.82 – 0.72 (m, 4H); MS (ESI) m/z (%): 550.2 $[M+H]^+$, 572.1 $[M+Na]^+$; Anal.

calcd. for $C_{31}H_{24}FN_5O_4$ (%): C, 67.75; H, 4.40; N, 12.74. Found (%): C, 67.83; H, 4.49; N, 12.77.

4.1.5.13. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-4-(4-methoxyphenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**23m**).

Yield: 88.9%; M.p.: 210-212 °C; IR (KBr, cm^{-1}): 3423.4, 2913.1, 1693.8, 1643.4, 1597.2, 1577.5, 1508.8, 1462.4, 1424.5, 1303.1, 1252.8, 1182.3, 1031.6, 954.8, 831.8, 767.2; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.29 (s, 1H), 10.87 (s, 1H), 8.21 (d, J = 5.7 Hz, 1H), 8.01 (dd, J = 8.0, 1.1 Hz, 1H), 7.93 (dd, J = 12.6, 2.3 Hz, 1H), 7.66 (d, J = 2.2 Hz, 1H), 7.63 – 7.57 (m, 1H), 7.54 (dd, J = 8.9, 1.4 Hz, 1H), 7.50 – 7.44 (m, 1H), 7.44 – 7.34 (m, 3H), 7.30 – 7.15 (m, 2H), 6.79 – 6.68 (m, 2H), 3.87 (d, J = 9.4 Hz, 3H), 2.14 – 1.71 (m, 1H), 0.98 – 0.47 (m, 4H); MS (ESI) m/z (%): 566.1 $[M+H]^+$, 588.1 $[M+Na]^+$; Anal. calcd. for $C_{31}H_{24}FN_5O_5$ (%): C, 65.84; H, 4.28; N, 12.38. Found (%): C, 65.86; H, 4.31; N, 12.34.

4.1.5.14. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-4-(2,4-dichlorophenyl)-3- oxo-3,4-dihydroquinoxaline-2-carboxamide (**23n**).

Yield: 89.6%; M.p.: 240-243 °C; IR (KBr, cm^{-1}): 3433.3, 3076.9, 2923.1, 1693.4, 1596.7, 1533.6, 1506.9, 1428.3, 1303.2, 1205.1, 1097.4, 872.5, 762.8; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.26 (s, 1H), 10.88 (s, 1H), 8.21 (d, J = 5.7 Hz, 1H), 8.15 – 8.03 (m, 2H), 7.93 (dd, J = 12.6, 2.3 Hz, 1H), 7.79 (dd, J = 8.5, 2.3 Hz, 1H), 7.76 – 7.69 (m, 1H), 7.70 – 7.59 (m, 2H), 7.61 – 7.54 (m, 1H), 7.52 (dd, J = 11.2, 4.1 Hz, 1H), 7.42 (t, J = 8.9 Hz, 1H), 6.79 – 6.68 (m, 2H), 2.01 – 1.93 (m, 1H), 0.82 – 0.72 (m, 4H); MS (ESI) m/z (%): 626.0 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{20}Cl_2FN_5O_4$ (%): C, 59.62; H, 3.34; N, 11.59. Found (%): C, 59.58; H, 3.37; N, 11.64.

4.1.5.15. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)-3-fluorophenyl)-4-(2,4-dimethoxyphenyl)-3- oxo-3,4-dihydroquinoxaline-2-carboxamide (**23o**).

Yield: 86.6%; M.p.: 241-244 °C; IR (KBr, cm^{-1}): 3423.5, 3071.4, 2840.6, 1688.3, 1600.9, 1509.3, 1463.8, 1427.6, 1308.5, 1210.8, 1114.2, 1029.6, 954.8, 832.6, 765.5; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.28 (s, 1H), 10.87 (s, 1H), 8.21 (d, J = 5.7 Hz, 1H), 8.00 (d, J = 7.0 Hz, 1H), 7.92 (dd, J = 12.6, 2.3 Hz, 1H), 7.65 (d, J = 2.1 Hz, 1H), 7.62 – 7.58 (m, 1H), 7.55 (d, J = 10.1 Hz, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.41 (t, J = 8.9 Hz, 1H), 7.29 (d, J = 8.6 Hz, 1H), 6.89 (d, J = 2.5 Hz, 1H), 6.80 – 6.71 (m, 3H), 3.89 (s, 3H), 3.72 (s, 3H), 2.02 – 1.88 (m, 1H), 0.84 – 0.70 (m, 4H); MS (ESI) m/z (%): 596.2 $[M+H]^+$, 618.1 $[M+Na]^+$; Anal. calcd. for $C_{32}H_{26}FN_5O_6$ (%): C, 64.53; H, 4.40; N, 11.76. Found (%): C, 64.48; H, 4.45; N, 11.74.

4.1.5.16. N-(4-((2-(cyclopropylcarbamoyl)pyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-4-phenyl-3,4-dihydro quinoxaline-2-carboxamide(**23p**).

Yield: 83.4%; M.p.: 269-271 °C; IR (KBr, cm^{-1}): 3400.5, 3072.6, 2924.1, 1687.7, 1595.1, 1535.3, 1298.1, 1213.2,

1085.9, 916.9, 846.8, 761.9; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.33 (s, 1H), 8.76 (d, J = 4.4 Hz, 1H), 8.52 (d, J = 5.5 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.98 (d, J = 12.1 Hz, 1H), 7.76 – 7.67 (m, 2H), 7.66 – 7.56 (m, 3H), 7.54 – 7.38 (m, 5H), 7.22 (d, J = 2.9 Hz, 1H), 6.68 (d, J = 8.4 Hz, 1H), 2.97 – 2.79 (m, 1H), 0.79 – 0.56 (m, 4H); MS (ESI) m/z (%): 536.1 $[M+H]^+$, 558.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{22}FN_5O_4$ (%): C, 67.28; H, 4.14; N, 13.08. Found (%): C, 67.34; H, 4.16; N, 13.15.

4.1.5.17. N-(4-((2-(cyclopropylcarbamoyl)pyridin-4-yl)oxy)-3-fluorophenyl)-4-(4-fluorophenyl)-3-oxo-3,4-dihydro quinoxaline-2-carboxamide (**23q**).

Yield: 80.5%; M.p.: 210-212 °C; IR (KBr, cm^{-1}): 3371.6, 3072.6, 1687.7, 1591.3, 1510.3, 1296.2, 1199.7, 952.8, 862.5, 761.9; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.76 (d, J = 5.0 Hz, 1H), 8.52 (d, J = 5.6 Hz, 1H), 8.10 – 7.91 (m, 2H), 7.68 – 7.37 (m, 9H), 7.22 (dd, J = 5.6, 2.6 Hz, 1H), 6.72 (d, J = 8.2 Hz, 1H), 3.00 – 2.77 (m, 1H), 0.75 – 0.61 (m, 4H); MS (ESI) m/z (%): 554.2 $[M+H]^+$, 576.2 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{21}F_2N_5O_4$ (%): C, 65.10; H, 3.82; N, 12.65. Found (%): C, 65.16; H, 3.80; N, 12.70.

4.1.5.18. N-(4-((2-acetamidopyridin-4-yl)oxy)-3-fluorophenyl)-3-oxo-4-phenyl-3,4-dihydroquinoxaline-2-carboxamide (**23r**)

Yield: 79.9%; M.p.: 230-233 °C; IR (KBr, cm^{-1}): 3435.1, 2923.1, 1695.1, 1598.1, 1533.6, 1507.2, 1462.3, 1428.9, 1265.2, 1205.1, 1020.6, 971.2, 869.7; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.29 (s, 1H), 10.57 (s, 1H), 8.20 (d, J = 5.7 Hz, 1H), 8.09 – 7.99 (m, 1H), 7.94 (dd, J = 12.6, 2.3 Hz, 1H), 7.75 – 7.58 (m, 5H), 7.55 (dd, J = 8.8, 1.3 Hz, 1H), 7.52 – 7.45 (m, 3H), 7.42 (t, J = 8.9 Hz, 1H), 6.77 – 6.58 (m, 2H), 2.05 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.92, 165.66, 162.11, 154.35, 153.88 (d, J = 246.1 Hz), 153.12, 151.36, 149.99, 137.43 (d, J = 9.8 Hz), 136.38 (d, J = 12.4 Hz), 135.61, 135.04, 132.68, 131.78, 130.69, 130.48, 130.04, 128.75, 124.77, 124.62, 116.82, 115.94, 108.77 (d, J = 22.9 Hz), 107.39, 99.90, 24.28; MS (ESI) m/z(%):532.1 $[M+Na]^+$; Anal. calcd. for $C_{28}H_{20}FN_5O_4$ (%): C, 66.01; H, 3.96; N, 13.75. Found (%): C, 66.12; H, 4.01; N, 13.80.

4.1.5.19. N-(4-((2-acetamidopyridin-4-yl)oxy)-3-fluorophenyl)-4-(4-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2- carboxamide (**23s**).

Yield: 80.9%; M.p.: 254-256 °C; IR (KBr, cm^{-1}): 3429.0, 2917.6, 2851.6, 1694.3, 1596.7, 1583.0, 1533.6, 1507.8, 1426.6, 1265.6, 1204.4, 1152.3, 965.7, 760.0; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.28 (s, 1H), 10.57 (s, 1H), 8.20 (d, J = 5.7 Hz, 1H), 8.07 – 8.00 (m, 1H), 7.94 (dd, J = 12.6, 2.3 Hz, 1H), 7.67 (s, 1H), 7.64 – 7.58 (m, 1H), 7.55 (dd, J = 8.8, 3.9 Hz, 5H), 7.48 (t, J = 7.6 Hz, 1H), 7.42 (t, J = 8.9 Hz, 1H), 6.77 – 6.67 (m, 2H), 2.04 (s, 3H); MS (ESI) m/z (%): 550.1 $[M+Na]^+$; Anal. calcd. for $C_{28}H_{19}F_2N_5O_4$ (%): C, 63.76; H, 3.63; N, 13.28. Found (%): C, 63.88; H, 3.59; N, 13.23.

4.1.5.20. N-(4-((2-acetamidopyridin-4-yl)oxy)-3-fluorophenyl)-4-(2-chlorophenyl)-3-oxo-3,4-dihydroquino xaline-2-carboxamide (**23t**).

Yield: 85.6%; M.p.: 220-222 °C; IR (KBr, cm^{-1}): 3428.9, 2917.6, 2846.2, 1695.5, 1637.9, 1508.9, 1536.3, 1503.4, 1481.5, 1429.8, 1265.4, 1203.9, 1149.6, 1094.7, 749.8; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.28 (s, 1H), 10.58 (s, 1H), 8.20 (d, J = 5.7 Hz, 1H), 8.11 – 8.03 (m, 1H), 7.94 (dd, J = 12.6, 2.3 Hz, 1H), 7.89 – 7.83 (m, 1H), 7.74 – 7.61 (m, 5H), 7.60 – 7.55 (m, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.43 (t, J = 8.9 Hz, 1H), 6.71 (dd, J = 5.7, 2.4 Hz, 1H), 6.62 (d, J = 8.4 Hz, 1H), 2.05 (s, 3H); MS (ESI) m/z (%): 544.1 $[M+H]^+$, 566.1 $[M+Na]^+$; Anal. calcd. for $C_{28}H_{19}ClFN_5O_4$ (%): C, 61.83; H, 3.52; N, 12.88. Found (%): C, 61.76; H, 3.48; N, 12.90.

4.1.5.21. N-(4-((2-acetamidopyridin-4-yl)oxy)-3-fluorophenyl)-4-(4-methoxyphenyl)-3-oxo-3,4-dihydroquinoxa line-2-carboxamide (**23u**).

Yield: 82.4%; M.p.: 239-241 °C; IR (KBr, cm^{-1}): 3435.5, 3076.9, 2917.6, 1684.9, 1646.8, 1603.3, 1510.5, 1465.0, 1423.1, 1297.7, 1251.8, 1198.1, 1166.0, 1113.9, 1078.2, 758.3; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.29 (s, 1H), 10.57 (s, 1H), 8.20 (d, J = 5.7 Hz, 1H), 8.02 (dd, J = 8.0, 1.1 Hz, 1H), 7.94 (dd, J = 12.7, 2.3 Hz, 1H), 7.68 (s, 1H), 7.63 – 7.58 (m, 1H), 7.57 – 7.52 (m, 1H), 7.50 – 7.45 (m, 1H), 7.44 – 7.35 (m, 3H), 7.22 (d, J = 8.9 Hz, 2H), 6.78 – 6.65 (m, 2H), 3.88 (s, 3H), 2.05 (s, 3H); MS (ESI) m/z (%): 562.1 $[M+Na]^+$; Anal. calcd. for $C_{29}H_{22}FN_5O_5$ (%): C, 64.56; H, 4.11; N, 12.98. Found (%): C, 64.64; H, 4.09; N, 13.06.

4.1.5.22. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)phenyl)-3-oxo-4-phenyl-3,4-dihydroquinoxa line-2-carboxamide (**23v**).

Yield: 82.9%; M.p.: 237-239 °C; IR (KBr, cm^{-1}): 3433.3, 1687.7, 1535.3, 1506.4, 1421.5, 1305.8, 1207.4, 758.0; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.10 (s, 1H), 10.82 (s, 1H), 8.19 (d, J = 5.7 Hz, 1H), 8.02 (dd, J = 8.0, 1.2 Hz, 1H), 7.81 (d, J = 8.9 Hz, 2H), 7.70 (t, J = 7.6 Hz, 2H), 7.68 – 7.61 (m, 2H), 7.62 – 7.56 (m, 1H), 7.51 – 7.42 (m, 3H), 7.21 (d, J = 8.9 Hz, 2H), 6.76 – 6.61 (m, 2H), 2.02 – 1.90 (m, 1H), 0.86 – 0.68 (m, 4H); MS (ESI) m/z (%): 540.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{23}N_5O_4$ (%): C, 69.62; H, 4.48; N, 13.53. Found (%): C, 69.74; H, 4.49; N, 13.46.

4.1.5.23. N-(4-((2-(cyclopropanecarboxamido)pyridin-4-yl)oxy)phenyl)-4-(4-fluorophenyl)-3-oxo-3,4-dihydro quinoxaline-2-carboxamide (**23w**).

Yield: 81.6%; M.p.: 245-247 °C; IR (KBr, cm^{-1}): 3422.3, 3063.0, 2374.4, 1687.7, 1591.3, 1508.3, 1425.4, 1305.8, 1217.1, 1166.9, 952.8, 839.0, 763.8; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.09 (s, 1H), 10.82 (s, 1H), 8.19 (d, J = 5.7 Hz, 1H), 8.02 (dd, J = 8.0, 1.1 Hz, 1H), 7.81 (d, J = 8.9 Hz, 2H), 7.65 (d, J = 2.3 Hz, 1H), 7.64 – 7.57 (m, 1H), 7.58 – 7.50 (m, 4H), 7.48 (t, J = 7.6 Hz, 1H), 7.21 (d, J = 8.9 Hz, 2H), 6.76 – 6.64 (m, 2H), 2.05 – 1.84 (m, 1H), 0.85 – 0.65 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.15, 166.21, 162.67 (d, J = 246.5 Hz), 161.76, 154.26, 153.31, 151.62,

149.93, 149.87, 136.03, 135.06, 132.58, 131.84, 131.15 (d, *J* = 8.9 Hz), 130.45, 124.77, 121.92, 121.67, 117.74, 117.59, 115.91, 108.34, 100.70, 14.58, 8.05; MS (ESI) m/z(%): 558.1 $[M+Na]^+$; Anal. calcd. for $C_{30}H_{22}FN_5O_4$ (%): C, 67.28; H, 4.14; N, 13.08. Found (%): C, 67.36; H, 4.10; N, 13.06.

4.1.5.24. N-(4-((2-acetamidopyridin-4-yl)oxy)phenyl)-3-oxo-4-phenyl-3,4-dihydroquinoxaline-2-carboxamide (**23x**).

Yield: 79.5%; M.p.: 243-245 °C; IR (KBr, cm^{-1}): 3267.4, 3061.0, 1693.5, 1591.3, 1537.3, 1502.6, 1425.4, 1301.9, 1211.3, 1159.2, 1091.7, 844.8; ^1H NMR (600 MHz, DMSO-d_6) δ 11.10 (s, 1H), 10.52 (s, 1H), 8.18 (d, *J* = 5.7 Hz, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 2H), 7.74 – 7.56 (m, 5H), 7.51 – 7.42 (m, 3H), 7.21 (d, *J* = 8.9 Hz, 2H), 6.71 – 6.61 (m, 2H), 2.04 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.81, 166.14, 161.85, 154.28, 153.20, 151.76, 150.00, 149.88, 136.04, 135.65, 134.98, 132.52, 131.82, 130.69, 130.43, 130.02, 128.76, 124.73, 121.84, 121.66, 115.91, 108.22, 100.96, 24.29; MS (ESI) m/z (%): 492.2 $[M+H]^+$, 514.2 $[M+Na]^+$; Anal. calcd. for $C_{28}H_{21}N_5O_4$ (%): C, 68.42; H, 4.31; N, 14.25. Found (%): C, 68.38; H, 4.34; N, 14.19.

4.1.5.25. N-(4-((2-acetamidopyridin-4-yl)oxy)phenyl)-4-(4-fluorophenyl)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**23y**).

Yield: 79.4%; M.p.: 253-256 °C; IR (KBr, cm^{-1}): 3064.9, 1695.4, 1587.4, 1367.5, 1220.9, 11161.2, 1020.3, 968.3, 846.8; ^1H NMR (600 MHz, DMSO-d_6) δ 11.09 (s, 1H), 10.52 (s, 1H), 8.18 (d, *J* = 5.7 Hz, 1H), 8.02 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 2H), 7.67 (s, 1H), 7.65 – 7.43 (m, 6H), 7.21 (d, *J* = 8.9 Hz, 2H), 6.71 (d, *J* = 8.5 Hz, 1H), 6.66 (dd, *J* = 5.7, 2.4 Hz, 1H), 2.04 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.81, 166.13, 162.67 (d, *J* = 246.6 Hz), 161.75, 154.28, 153.33, 151.58, 150.01, 149.88, 136.02, 135.06, 132.58, 131.85, 131.15 (d, *J* = 9.1 Hz), 130.46, 124.77, 121.83, 121.68, 117.74, 117.59, 115.91, 108.22, 100.96, 24.28; MS (ESI) m/z (%): 510.2 $[M+H]^+$, 532.2 $[M+Na]^+$; Anal. calcd. for $C_{28}H_{20}FN_5O_4$ (%): C, 66.01; H, 3.96; N, 13.75. Found (%): C, 66.05; H, 4.00; N, 13.74.

4.2. Pharmacology

4.2.1. c-Met kinase assay

The *in vitro* enzymatic assays were performed by mobility shift assay. The solution of peptide substrates, ATP, appropriate kinase (Carna), and various concentrations of tested compounds were mixed with the kinase reaction buffer (50 mM HEPES, pH 7.5, 0.0015% Brij-35, 10 mM $MgCl_2$, 2 mM DTT), with blank DMSO as the negative control. The kinase reaction was initiated by the addition of tyrosine kinase proteins diluted in 39 μL of kinase reaction buffer solution and incubated at 28 °C for 1 h. Then, 25 μL of stop buffer (100 mM HEPES, pH = 7.5, 0.015% Brij-35, 0.2% Coating Reagent #3, 50 mM EDTA) was added to stop reaction. The data were cellected on Caliper at 320 nm and 615 nm and converted to inhibition values. IC_{50} was presented in MS Excel and the curves fitted by XLfit excel add-in

version

4.2.2. MTT assay in vitro

The anti-proliferative activities of tested compounds were evaluated against A549, H460 and HT-29 cell lines using the standard MTT assay *in vitro*, with Foretinib as the positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplement 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 tested compounds 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 DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with an ELISA reader. All compounds were tested three times in each of the cell lines. 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.

4.2.3. AO/ EB assay

A549 or HT-29 cells were added to a final concentration of 1×10^6/mL in a 6-well plate, and the plate was incubated for 24 h. Cells were treated with various concentrations of compound **23w**. After being cultured for 48 h, control cells and treated cells were washed with PBS which stored at 4 °C, and then dual fluorescent staining solution (20 μL) containing 100 μg/mL AO and 100 μg/mL EB was added to each well for 10 min, and then covered with a coverslip. The morphology of apoptotic cells was examined using fluorescent microscope.

4.2.4. Apoptosis assay

Apoptosis was measured by flow cytometry using Annexin V/propidium iodide (PI) double staining. HT-29 or A549 cells were added to a final concentration of 1×10^6/mL in a 6-well plate, and the plate was incubated for 24 h. Cells were treated with various concentrations of compound **23w**. After being cultured for 48 h, control cells and treated cells were harvested and washed with PBS, and then resuspended in 100μL 1×binding buffer incubated in the mixture of 5μL Annexin V-FTIC and 5μL PI for 10 min at room temperature in dark place. The cells were resuspended in 400μL 1×binding buffer just before flow cytometric analysis.

4.2.5. Wound-healing assay

A549 cells were added to a final concentration of 1×10^6 cells /mL in a 6-well plate, and the plate was incubated for 24 h. Twenty-four hours later, when the cells reached confluency, scratches were created with sterile 1.0 mL pipette

tips and images were captured using phase contrast microscopy at 0 h, 12 h, 36 h and 72 h after treatment with 1.0 μM of compound **23w**.

4.2.6. Transwell migration assay

A549 cells were plated at a concentration of $1x10^5$ cells / mL per well in 6-well plates for 8 h and then treated with various concentrations of compound **23w** for 24 h. Then cells were harvested and resuspended as single cells in RPMI1640 with 1.0% FBS at the concentration of $1x10^5$cells/mL. Abave cells solution 100 μL were added to the upper chamber and the lower chamber was filled with 600 μL RPMI1640 supplemented with 10% FBS. Cells were allowed to migrate for 24 h at 37 °C in a 5% CO_2 humidified incubator. The experiment was terminated by discarding the medium and fixing the cells with MeOH for 30 min. Non-invading cells on the upper side of the insert were removed by a cotton-tipped applicator. Staining of the cells on the bottom of the membrane was performed with crystal violet (0.1%) for 10 min at room temperature and washed with PBS. Then lower surface was captures and counted under an inverted microscope (Olympus).

4.2.7. Molecular docking study

The crystal structure of c-Met (PDB code: 3LQ8) in complex with foretinib (GSK1363089) was used for molecular modeling. The docking simulation was conducted using Glide XP (Schrödinger 2014), since Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. The protein coordinates (PDB code: 3LQ8) were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/). For enzyme preparation, the hydrogen atoms were added. The whole c-Met enzyme was defined as a receptor and the site sphere was selected on the basis of the ligand binding location of foretinib. Compound foretinib was removed and compound **23w** was placed.. Accelrys Discovery Studio 6.0 system was used for graphic display.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version,

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Legends

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

Fig. 2. Design strategy and structures of the target compounds.

Scheme 1. Reagents and conditions: (a) cyclopropanecarbonyl chloride or acetyl chloride, Et_3N, CH_2Cl_2, rt, 12 h; (b) $SOCl_2$, NaBr, PhCl, 55 °C, 1 h, reflux, 20 h; (c) cyclopropanamine, Et_3N, THF, 0 °C, 3 h; (d) 2-fluoro-4-nitrophenol or 4-nitrophenol, chlorobenzene, reflux, 40 h; (e) Fe (powder), HOAc, ethyl acetate /water (10:1 v/v), reflux, 2 h.

Scheme 2. Reagents and conditions: (a) amines, NaH, DMF, RT, 16 h; (b) Fe powder, HOAc, CH_3COOEt/H_2O, reflux, 6 h; (c) diethyl ketomalonate, toluene, reflux, 12 h; (d) LiOH, THF/ H_2O, RT, 2 h, then 6 N HCl; (e) **17a-17e**, HATU, Et_3N, DMF, 25 °C,12 h.

Table 1 *In vitro* c-Met kinase inhibitory activities of target compounds **23a**-**23y**.

Table 2 Antiproliferative activities of selected compounds **23v, 23w, 23x, 23y** and **Foretinib** *in vitro*.

Fig. 3. AO/EB stained apoptosis of HT-29 and A549 cell lines with different concentrations of compound **23w**.

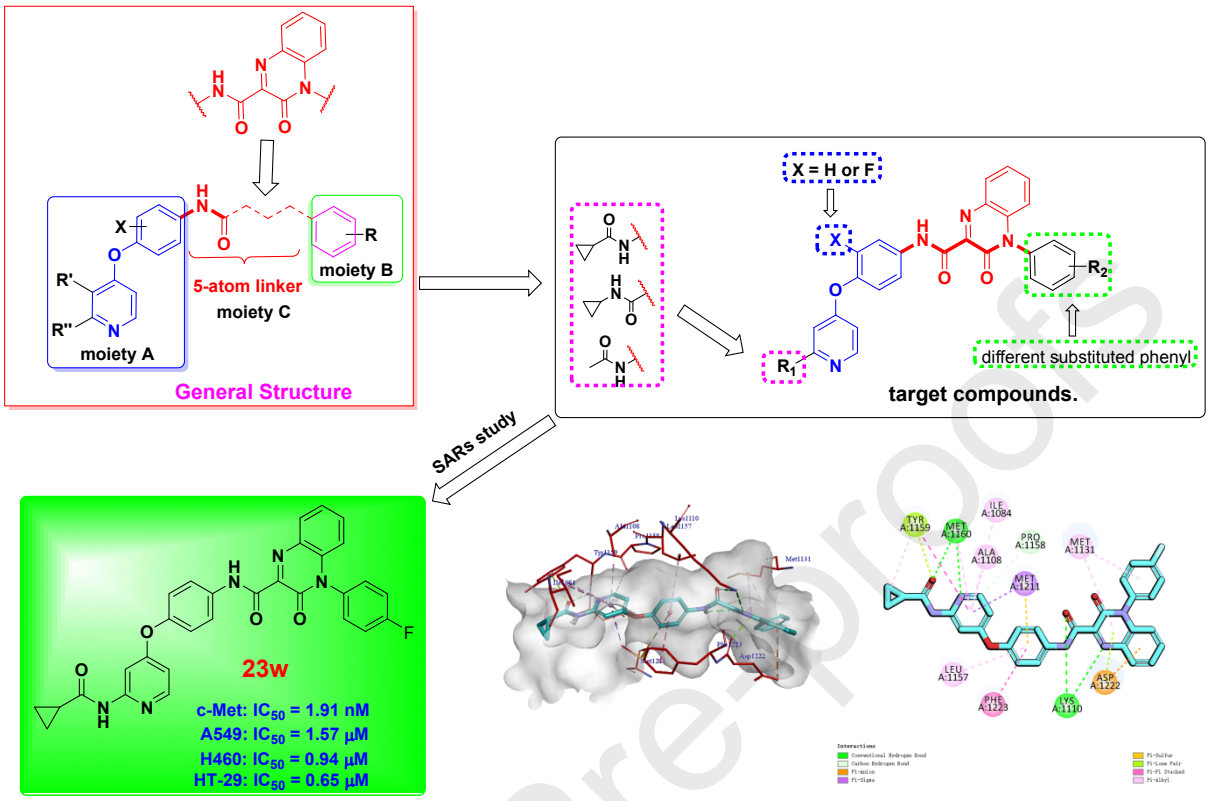
Fig. 4. Compound **23w** induces cell apoptosis of HT-29 and A549 cell line *in vitro*.

Fig. 5. Anti-migration activity of compound **23w** against A549 cells in wound-healing migration model.

Fig. 6. Anti-migration activity of compound **23w** against A549 cells in transwell migration model.

Fig. 7. The mode of compound **23w** bound to c-Met (PDB code: 3LQ8). The amino acid residues were displayed by red sticks and compound was displayed by bright blue sticks. Green dotted lines represent H-bonds. (A) The overview of compound **23w** docked into c-Met. (B) The c-Met active site in complex with compound **23w**. (C) 2D interactions of the docking model of compound **23w** to c-Met.

Graphical abstract



A series of novel 4-phenoxypyridine derivatives containing 3-oxo-3,4-dihydroquinoxaline moiety were synthesized and evaluated for their c-Met kinase activities and cytotoxic activities against A549, H460, HT-29 cancer cell lines. Furthermore, acridine orange/ethidium bromide staining, apoptosis, wound-healing assay and transwell migration assay on HT-29 and/or A549 cells of **23w** were performed.

Highlights

- A series of novel 4-phenoxypyridine derivatives were designed and synthesized.
- The target compounds showed c-Met kinase activities and cytotoxic activities
- Compound **23w** showed an IC_{50} value of 1.91 nM against c-Met kinase, and more potent than foretinib.
- The cytotoxic activities of **23w** were more potent than foretinib against HT-29 cells.
- Compound **23w** could induce cells apoptosis and inhibit cells motility.