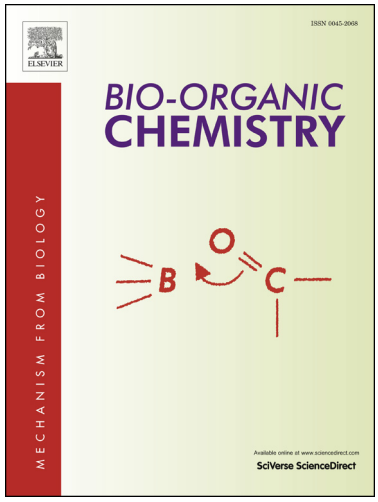

Design, synthesis and biological evaluation of 1H-pyrrolo[2,3-b]pyridine and 1H-pyrazolo[3,4-b]pyridine derivatives as c-Met inhibitors

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Design, synthesis and biological evaluation of 1H-pyrrolo[2,3-b]pyridine and 1H-pyrazolo[3,4-b]pyridine derivatives as c-Met inhibitors

Na Liu [a,†], Yanfen Wang [a,†], Gongchao Huang [b,†], Conghui Ji [b], Wei Fan [a], Haitao Li [b], Ying Cheng [b], Hongqi Tian [c,*]

[a] School of Biological Engineering, Tianjin University of Science and Technology, #29, 13th Avenue, TEDA, Tianjin 300457, China

[b] Tianjin Binjiang Pharma, Inc., #238 Baidi Road, Nankai District, Tianjin 300192, China

[c] Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Sciences & Peking Union Medical College, #238 Baidi Road, Nankai District, Tianjin 300192, China

[†] These authors contributed equally

* Corresponding author. Tel.: +86 186 0261 3300, Fax.: +86 022 65378920, tianhongqi@irm-cams.ac.cn

Abstract

Five novel 1H-pyrrolo[2,3-b]pyridine or 1H-pyrazolo[3,4-b]pyridine derivatives, with a methylene, sulfur, sulfoxide or cyclopropyl group as a linker, were designed, synthesized and biologically evaluated against c-Met and ALK. The development of these methods of compound synthesis may provide an important reference for the construction of novel 7-azaindole and 7-azaindazole derivatives with a single atom linker. The enzyme assay and cell assay *in vitro* showed that compound **9** displayed strong c-Met kinase inhibition with IC_{50} of 22.8 nM, moderate ALK kinase inhibition, and strong cell inhibition with MKN-45 IC_{50} of 329 nM and EBC-1 IC_{50} of 479 nM. In order to find the better candidate compounds, compounds **8**, **9** and **10** have been selected as tool compounds for further optimization.

Keywords: c-Met inhibitor, azaindole, azaindazole, synthesis, biological evaluation

1. Introduction

The tyrosine kinase receptor, c-Met, is also known as hepatocyte growth factor receptor (HGFR), and its ligand, hepatocyte growth factor (HGF), is also known as scatter factor [1]. c-Met is a heterodimer composed of an extracellular α subunit linked by a disulfide bridge to a transmembrane catalytic β subunit. Activation of c-Met occurs through autophosphorylation of Y1234 and Y1235 located in the activation loop [2]. Following activation by its ligand, HGF, c-Met induces a cell program that facilitates malignant tumor behavior, consisting of cell proliferation, cell migration, and invasion, increased cell survival, and cell morphogenesis [3]. Aberrant expression of c-Met and HGF is associated with the development of a wide range of solid tumors, and is regarded as a prognostic marker for malignancy [4]. Because of the known roles of c-Met and its ligand HGF in the pathogenesis and progression of human malignant tumors, c-Met inhibitors would be predicted to have a potential role as targets for therapy in oncology.

At present, some small molecule c-Met inhibitors have been reported to have inhibitory activity against c-Met in human tumors and to inhibit tumor growth [5,6]. Based on the binding modes with c-Met kinase, these inhibitors have been classified to include Type I inhibitors and Type II inhibitors [5,7]. Type I inhibitors comprise the majority of the ATP-competitive inhibitors due to their ability to bind c-Met kinase in the active conformation. Type I inhibitors typically adopt a 'U-shaped' conformation through interactions with both hinge and activation loop residue Y1230. Type II inhibitors recognize a conformation that is sometimes referred to as 'DFG-out', owing to the rearrangement of this motif. The DFG-out conformation then exposes an additional hydrophobic binding site that is adjacent to the ATP binding site (Fig. (9). in reference 5).

The clinically established targeted therapeutic compound, Crizotinib, is a Type I c-Met inhibitor, that displays both anaplastic lymphoma kinase (ALK) and c-Met inhibitory activity [8]. In 2011, Crizotinib has been approved by the US Food and Drug Administration (FDA) for the treatment of ALK-rearranged non-small cell lung carcinoma (NSCLC) as an ALK and c-Met inhibitor. The co-crystalline structure of Crizotinib combined with c-Met (Fig. 1) has been reported by J. Jean Cui and her co-authors [8], in which she revealed that the aminopyridine forms two hydrogen bonds with the backbone CO of P1158 and NH of M1160 of the kinase hinge. The 2,6-dichloro-3-fluorobenzyloxy ring stacks in a coplanar π-interaction against the phenol side chain of Y1230 of the activation loop. In the Crizotinib molecule, both the 2-chloro and 3-fluoro elements on the 3-benzyloxy group point toward the NH of D1222 of the activation loop,

indicating that there may be beneficial electrostatic interactions. The methylene linker allows the two critical binding elements to wrap around M1211 and form hydrogen bonds with the backbone of the hinge and π-stacking interactions with Y1230. The R-methyl group is used to rigidify the benzyl group, and also make favorable hydrophobic interactions with the residues of V1092, L1157, K1110, and A1108 in the pocket. The 5-pyrazol-4-yl group is bound through the narrow lipophilic tunnel surrounded by I1084 and Y1159 and provides the inhibition of c-Met. The terminal piperidine ring that appears to serve as a solubilizing group, attached to the N1 position of the pyrazol-4-yl, extends out into the solvent surrounding the kinase hinge segment. Bringing together all of these groups in Crizotinib compound, results in a strong inhibition of c-Met.

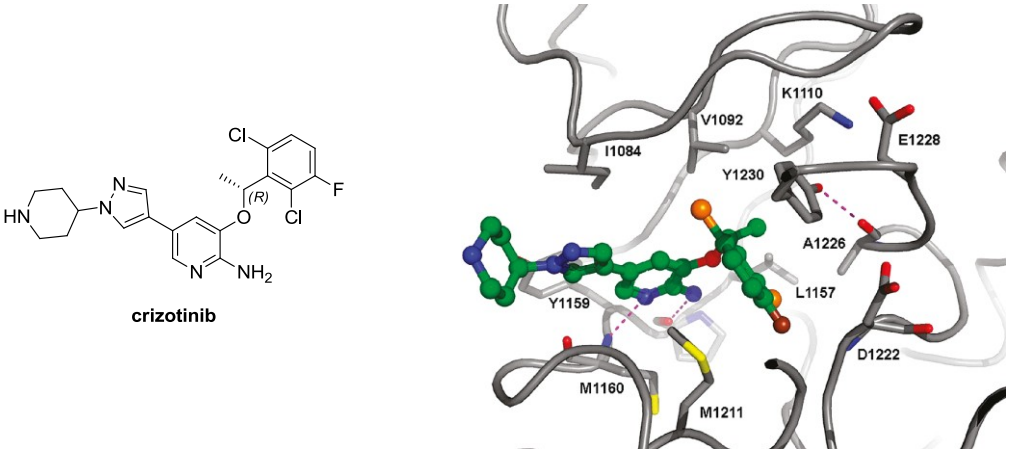


Fig. 1. Cocrystalline structure of Crizotinib combined with c-Met

Replacement of the aminopyridine in Crizotinib with 7-azaindole, resulting in the production of compound **1**, has been previously reported. Compound **1** demonstrated c-Met inhibitory activity [9]. Other 7-azaindole and 7-azaindazole derivatives (compounds **2** and **3**) have also been reported to show c-Met inhibitory activity [10,11]. The structure-activity relationships (SAR) have suggested that 7-azaindole or 7-azaindazole forms two hydrogen bonds to the backbone of P1158 and M1160 of the kinase hinge. A single atom linker, such as methylene, oxygen, sulfur, sulfone or cyclopropyl group (Fig. 2) [9-13], is important, and allow two aromatic appendages to wrap around M1211 and form hydrogen bonds with the backbone P1158 and M1160 of the kinase hinge and π-stacking interactions with Y1230. Furthermore, small substituent group at the benzylic position appears to be tolerated (compounds **1**, **3** and **5**) [9,11,13]. Therefore, exploration of new 7-azaindole or 7-azaindazole derivatives, with a single atom linker, may result in the development

of new c-Met inhibitors.

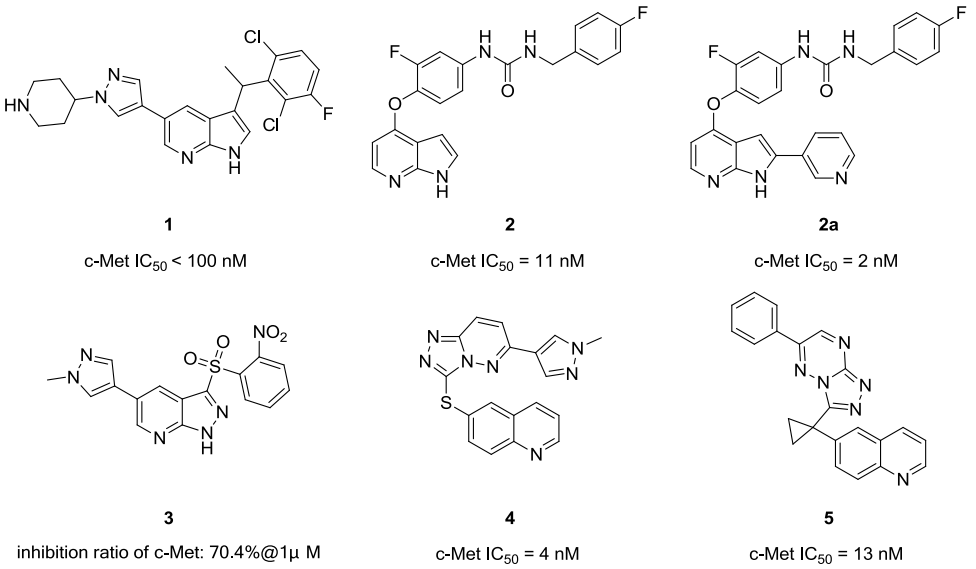


Fig. 2. The structure of compounds **1**, **2**, **3**, **4**, **5** and their c-Met inhibition

2. Results and Discussion

The results of this study included the design, synthesis, and characterization of the five new compounds, following the replacement of the aminopyridine in Crizotinib with the closely related 1H-pyrrolo[2,3-b]pyridine or 1H-pyrazolo[3,4-b]pyridine. The results also included the design, characterization and biological evaluation of the five new compounds directed against c-Met, following the replacement of the linkage between the 7-azaindole or 7-azaindazole and the 2,6-dichloro-3-fluorophenyl with a methylene, sulfoxide or cyclopropyl. The mode of interactions between Crizotinib and c-Met kinase was reported previously by J. Jean Cui and her co-authors in the reference **8** (Fig. 1). The new derivatives, taking Crizotinib and compound 1 as a lead scaffold, were expected to have the similar binding mode. The NH and ring nitrogen of 7-azaindole or 7-azaindazole in new compounds were expected to result in two hydrogen bonds with the backbone CO of P1158 and NH of M1160 of the kinase hinge region, similar to the 2-aminopyridine of Crizotinib. The halogenated phenyl group was maintained owing to its potential co-planar π-interaction with the residue Y1230 of the activation loop. Both the 2-chloro and 3-fluoro elements on the phenyl group were maintained due to the electrostatic interactions with the backbone NH of D1222. The pyrazole group at the C5 position of the 2-aminopyridine was maintained due to its compact size and its ability to modulate physicochemical properties.

The terminal piperidine ring, which attached to the N1 position of the pyrazol-4-yl, was maintained to serve as a solubilizing group.

Compound **2a**, with a 3-pyridyl group at the C2 position of 7-azaindole, demonstrated c-Met inhibition activity [10], indicating that the small lipophilic bulk at the C2 position of 7-azaindole may be tolerated. In order to check this hypothesis, the aminopyridine in Crizotinib was initially replaced with 7-azaindole. By the addition of small lipophilic bulk Me or Cl at C2 position of the 7-azaindole, two novel 7-azaindole derivatives **6** and **7** were synthesized and evaluated for c-Met inhibition. The 7-azaindole was expected to form two hydrogen bonds with the backbone of P1158 and M1160. The Me or Cl substitution at the C2 position of 7-azaindole may be affected the binding of the substrate with the kinase hinge, and make favorable hydrophobic interactions with the amino acid residues in the pocket, to improve inhibitory strength and selectivity. But the enzyme and cell assays of compounds **6** and **7** showed weak kinase and cell inhibition. These findings suggested that the bulkier substituent at the C2 position of 7-azaindole may be not favor the formation of hydrogen bonds of 7-azaindole with the backbone of the hinge, and result in weaker inhibitory effect.

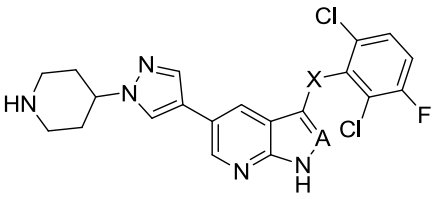
Further optimization resulted from the introduction of 7-azaindazole and replacement of the linkage between the 7-azaindole and 7-azaindazole and the 2,6-dichloro-3-fluorophenyl. Following replacement of the aminopyridine in Crizotinib with 7-azaindole and 7-azaindazole, the methylene linker was replaced with a sulfur and sulfoxide, which was expected to allow the two aromatic appendages to wrap around M1211 and form hydrogen bonds with the backbone P1158 and M1160 and facilitate π-stacking interactions with Y1230. Then two novel 7-azaindole and 7-azaindazole derivatives, compounds **8** and **9**, were synthesized and evaluated against c-Met, both of which displayed strong c-Met kinase and cell inhibitory activity.

In order to further optimize the linkage, a cyclopropyl group was introduced, which was expected not only to stabilize the phenyl group, but also to facilitate the hydrophobic interactions, to allow the two aromatic appendages to wrap around M1211 and form hydrogen bonds to the backbone P1158 and M1160 and to facilitate π-stacking interactions with Y1230. The other 7-azaindazole derivatives, compound **10**, was produced and evaluated against c-Met, which displayed strong c-Met kinase and cell inhibitory activity.

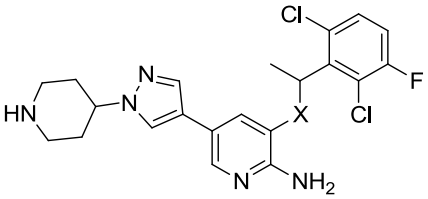
Compound **11b** has been previously synthesized in our laboratory and was submitted for a

patent application (Chinese patent application No. 201210376940.1) on September 29, 2012, but was authorized to another applicant on October 4, 2012 [14]. Compound **11b** has been previously reported to show strong c-Met inhibitory activity [15]. Meanwhile, the racemic compound of Crizotinib, compound **11a**, was also prepared. Crizotinib, **11a**, **11b** and Cisplatin were also evaluated as positive control compounds (Table 1).

Table 1. Structures and biological activities of target compounds **6-10** and positive control compounds:



6 - 10



11a, 11b

Compound				kinase IC_{50} (nM)			cell IC_{50} (nM)	
No	A	X	c-Met	ALK WT	ALK C1156Y	ALK L1196M	MKN-45	EBC-1
6	C-Me	CH-Me	286	504	1517	1664	1979	2383
7	C-Cl	CH-Me	982	2710	4077	3311	6519	9215
8	CH	S=O	63	214	1310	725	1494	1590
9	N	S	22.8	135	651	578	329	479
10	N	$C(CH_2)_2$	47.4	508	2988	735	670	1194
11a		O	14.6	17.6	133	337	97	129
11b		S	51.2	372	2102	2807	1033	1626
Crizotinib			13.7	17.3	7.67	188	ND	ND
Cisplatin			ND	ND	ND	ND	9736	27467

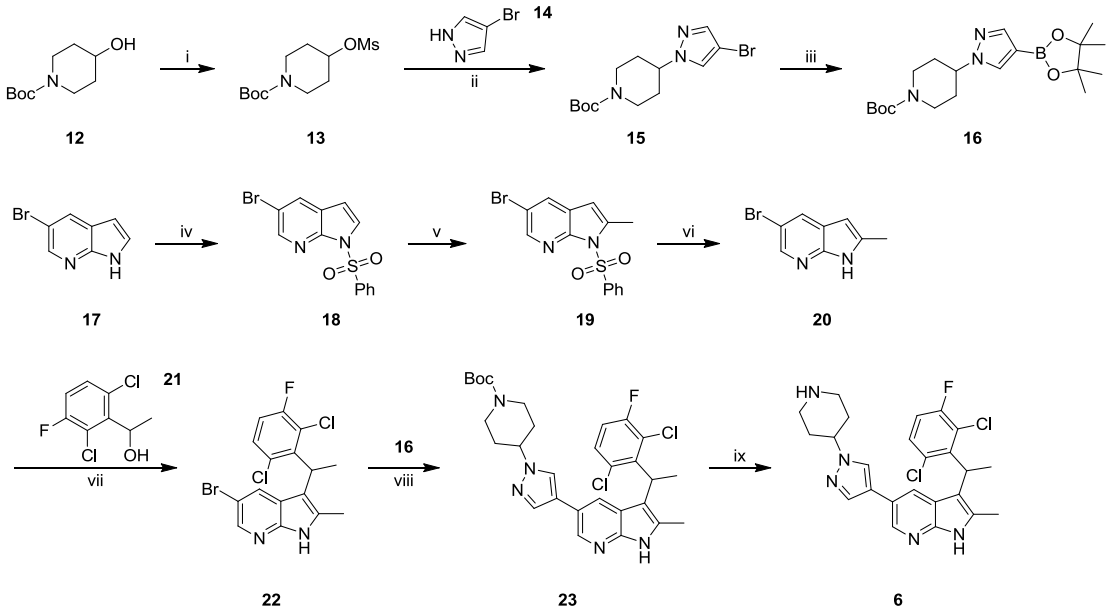
ND: Not determined.

Finally, five novel 7-azaindole and 7-azaindazole derivatives, with a methylene, sulfur, sulfoxide or cyclopropyl group as a linker, were synthesized and biologically evaluated against c-Met and ALK kinase, and MKN-45 and EBC-1 cell line. The results are summarized in Table 1.

Initially, compounds **6** and **7** showed weak c-Met and ALK kinase inhibition and cell inhibition, indicating that the bulk substituent at the C2 position of 7-azaindole may interrupt the hydrogen bond formation of 7-azaindole with the backbone of the hinge, resulting in reduced activity. Following replacement of the methylene linker between 7-azaindole or 7-azaindazole and 2,6-dichloro-3-fluorophenyl with a sulfur, sulfoxide or cyclopropyl group, compounds **8**, **9** and **10** were synthesized. The enzyme assay and cell assay *in vitro* showed that compounds **8**, **9** and **10** displayed strong c-Met kinase inhibitory activity, moderate ALK kinase inhibitory activity, and strong cell inhibitory activity. The kinase selectivity of c-Met against ALK is better than Crizotinib. Among the test compounds, compound **9** displayed the best c-Met kinase inhibitory activity with IC_{50} of 22.8 nM, moderate ALK kinase inhibitory activity, and strong cell inhibitory activity with MKN-45 IC_{50} of 329 nM and EBC-1 IC_{50} of 479 nM. In order to find the better candidate compounds, compounds **8**, **9** and **10** have been selected as tool compounds, with 7-azaindole and 7-azaindazole as core and a single atom as linker (such as sulfur, sulfoxide or cyclopropyl group), the further structure optimization is ongoing.

3. Chemistry

*3.1. Synthesis of target compound 3-(1-(2,6-Dichloro-3-fluorophenyl)ethyl)-2-methyl-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (**6**)*

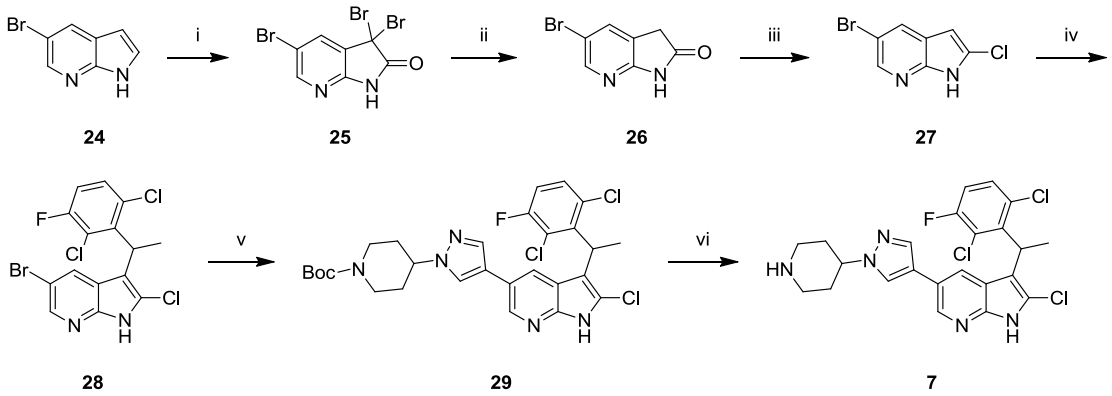


Scheme 1. Reagents and conditions: (i) MsCl, Et_3N, DCM; (ii) NaH, DMF, 100 °C, overnight; (iii)

$Pd(Ph_3P)_2Cl_2$, KOAc, DMSO, 80 °C, 2 h; (iv) $PhSO_2Cl$, NaH, DMF, 0 - 25 °C, 1.5 h; (v) i-Pr_2NH, n-BuLi, MeI, THF, -78 - 25 °C, 3.5 h; (vi) NaOH, MeOH, H_2O, 65 °C, 2 h; (vii) TfOH, DCM, 25 °C, 2 h; (viii) $Pd(dppf)Cl_2$, K_2CO_3, dioxane, H_2O, 100 °C, 12 h; (ix) TFA, DCM, 25 °C, 24 h.

Target compound **6** was produced according to the procedures outlined in Scheme 1. The intermediate **16** was prepared via alkylation of compound **14** with compound **13** followed by palladium-catalyzed coupling with bis(pinacolato)diboron according to published methods [8]. The intermediate **22** was prepared using the synthetic route shown in Scheme 1. Commercially available compound **17** was sulfonylated with Benzenesulfonyl chloride under basic conditions produced compound **18** with a high yield. Methylation of compound **18** was achieved by treatment of compound **18** with Lithium diisopropylamide (LDA) and Iodomethane (MeI), which generated compound **19** with a moderate yield. Treatment of compound **19** with Sodium hydroxide produced compound **20** with a good yield. Fielder-Crafts mutilation of compound **20** with compound **21** generated compound **22** with a good yield. Conventional Suzuki coupling of compound **22** with compound **16** generated compound **2**3 with an excellent yield. Subsequent treatment of compound **23** with acid to remove the butoxy carbonyl (Boc) group produced compound **6** with a good yield.

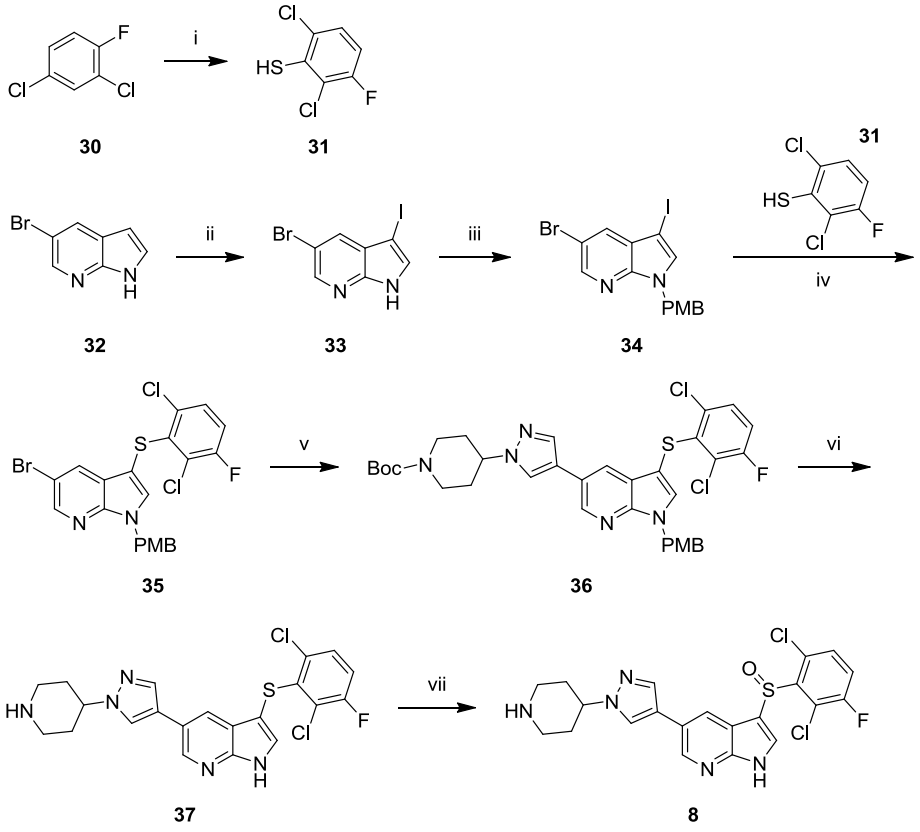
3.2. Synthesis of target compound 2-Chloro-3-(1-(2,6-dichloro-3-fluorophenyl)ethyl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (7)



Scheme 2. Reagents and conditions: (i) Br_2, t-BuOH, H_2O, 25 °C; (ii) Zn, AcOH, 25 °C, 3 h; (iii) $POCl_3$, 110 °C, 4 h; (iv) TfOH, DCM, 25 °C, 16 h; (v) $Pd(dppf)Cl_2$, K_2CO_3, dioxane, H_2O, 100 °C, 12 h; (vi) TFA, DCM, 25 °C, 24 h.

Target compound **7** was synthesized according to the protocol in Scheme 2. Bromination of 5-bromo-7-azaindole (compound **24**) with Bromine produced compound **25** with a high yield. Reduction-debromination of compound **25** with Zinc powder in Acetic acid generated compound **26** with an excellent yield. Treatment of compound **26** with Phosphorus oxychloride ($POCl_3$) generated compound **27** with a high yield. Friedel-Crafts alkylation of compound **27** with compound **21** produced compound **28** with a moderate yield. Suzuki coupling reaction of compound **28** with compound **16** produced compound **29** with an acceptable yield. Subsequent treatment of compound **29** with acid produced compound **7** with a moderate yield.

*3.3. Synthesis of target compound 3-((2,6-Dichloro-3-fluorophenyl)sulfide)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (**8**)*

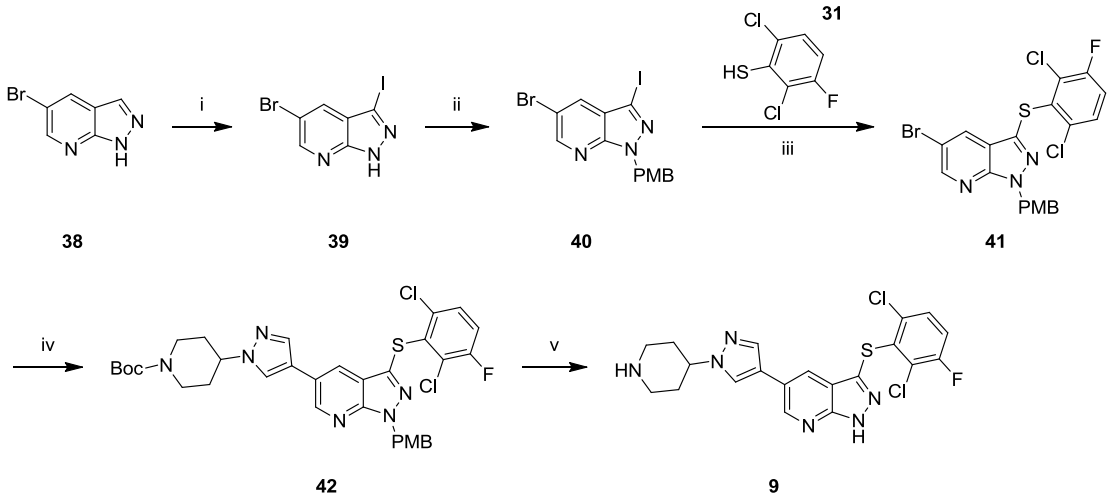


Scheme 3. Reagents and conditions: (i) S, n-BuLi, THF, -78 °C, 3.5 h; (ii) NIS, acetone, 25 °C, 1 h; (iii) PMBCl, K_2CO_3, DMF, 25 °C, 4 h; (iv) CuI, K_2CO_3, ethylene glycol, i-PrOH, 100 °C, 5 h; (v) $Pd(dppf)Cl_2$, K_2CO_3, dioxane, H_2O, 80 °C, 12 h; (vi) TFA, H_2SO_4, DCM, 25 °C, 2 h; (vii)

m-CPBA, 0 °C, 2 h.

Target compound **8** was prepared according to the procedure in Scheme 3. Compound **31** was generated via treatment of 2,4-Dichloro-1-fluorobenzene with n-Butyllithium (n-BuLi) and Sulfur with a high yield. Iodination of 5-Bromo-7-azaindole (compound **32**) with N-Iodosuccinimide (NIS) produced compound **33** with an excellent yield. Treatment of compound **33** with 1-(chloromethyl)-4-methoxybenzene (PMBCl) under basic conditions produced compound **34** with a high yield. Treatment of compound **34** with compound **31** via a copper-catalyzed reaction produced compound **35** with a good yield. Suzuki coupling of compound **35** with compound **16** generated compound **36** with a moderate yield. Subsequent treatment of compound **36** with acid to remove the butoxy carbonyl (Boc) group and the 4-methoxybenzyl (PMB) group produced compound **37** with a good yield. Oxidation of compound **37** with 3-Chloroperoxybenzoic acid (m-CPBA) produced compound **8** with a good yield.

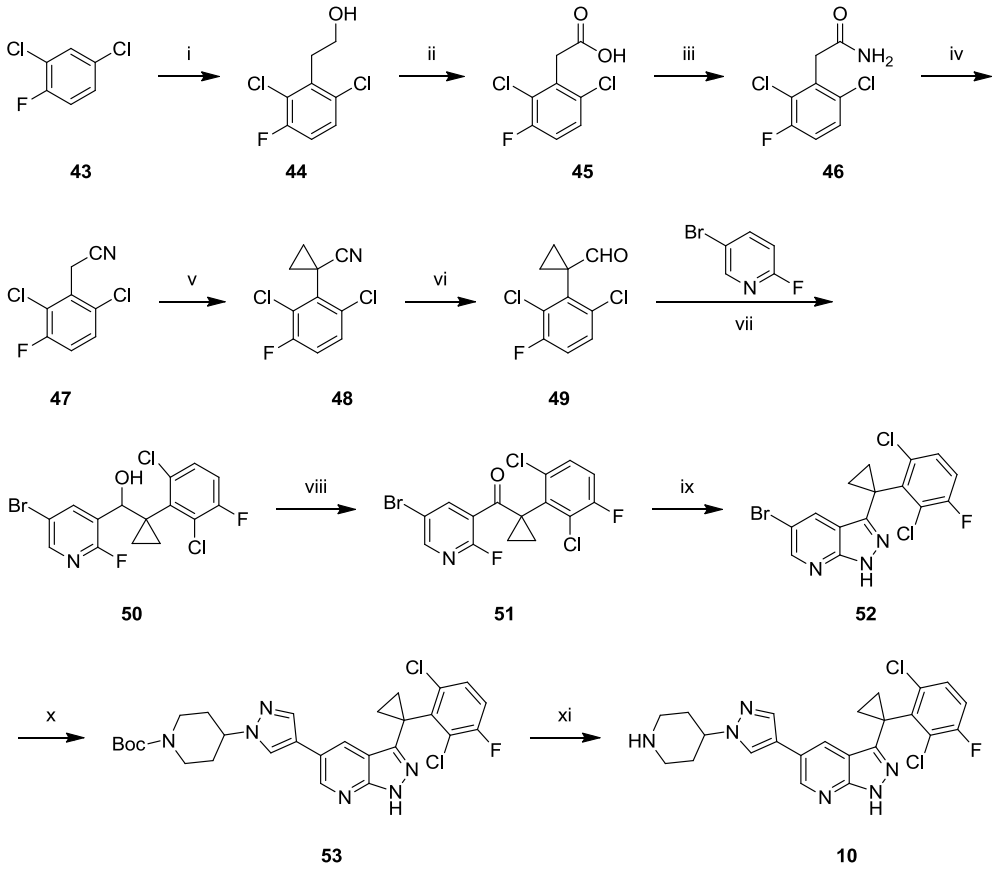
*3.4. Synthesis of target compound 3-((2,6-Dichloro-3-fluorophenyl)thio)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrazolo[3,4-b]pyridine (**9**)*



Scheme 4. Reagents and conditions: (i) I_2, KOH, DMF, 25 °C, 4 h; (ii) PMBCl, K_2CO_3, DMF, 25 °C, 4 h; (iii) CuI, K_2CO_3, ethylene glycol, i-PrOH, 100 °C, 5 h; (iv) $Pd(Ph_3P)_4$, Cs_2CO_3, DME, H_2O, 80 °C, 12 h; (v) TFA, H_2SO_4, DCM, 25 °C, 2 h.

Target compound **9** was prepared according to the method in Scheme 4. Iodination of 5-Bromo-7-azaindazole (compound **38**) with Iodine produced compound **39** with an excellent yield. Treatment of compound **39** with 1-(Chloromethyl)-4-methoxybenzene produced compound **40** with a high yield. Copper-catalyzed coupling of compound **40** with compound **31** produced compound **41** with a good yield. Suzuki coupling of compound **41** with compound **16** produced compound **42** with a moderate yield. Subsequent treatment of compound **42** with acid produced compound **9** with an excellent yield.

*3.5. Synthesis of target compound 3-(1-(2,6-Dichloro-3-fluorophenyl)cyclopropyl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrazolo[3,4-b]pyridine (**10**)*



Scheme 5. Reagents and conditions: (i) n-BuLi, oxirane, THF, -78 °C, 3 h; (ii) $NaClO_2$, TEMPO, 0 - 25 °C, 3 h; (iii) $SOCl_2$, $NH_3.H_2O$, DCM, 0 - 25 °C, 4 h; (iv) $SOCl_2$, pyridine, 0 - 25 °C, 2 h; (v) NaH, $BrCH_2CH_2Br$, 0 - 25 °C, 4 h; (vi) DIBAL-H, PhMe, -78 °C, 1 h; (vii) LiHMDS, THF, -78 °C, 1.5 h; (viii) PCC, DCM, 25 °C, 4 h; (ix) NH_2NH_2, EtOH, 80 °C, 10 h; (x) $Pd(Ph_3P)_4$, Cs_2CO_3, dioxane, H_2O, 80 °C, 12 h; (xi) TFA, DCM, 25 °C, 2 h.

Target compound **10** was synthesized according to the method in Scheme 5. Treatment of compound **43** with n-BuLi and Oxirane generated compound **44** with a moderate yield. Oxidation of compound **44** with 2,2,6,6-Tetramethylpiperidine N-oxide (TEMPO) and sodium chlorite ($NaClO_2$) produced compound **45** with a high yield. Treatment of **45** with Thionyl chloride ($SOCl_2$) and ammonia water produced compound **46** with a high yield. Dehydration of compound **46** with Thionyl chloride produced compound **47** with a high yield. Alkylation of compound **47** with 1,2-Dibromoethane produced compound **48** with an excellent yield. Reduction of compound **48** with Di-isobutylaluminum hydride (DIBAL-H) generated compound **49** with a good yield. Treatment of 5-Bromo-2-fluoropyridine with Lithium bis(trimethylsilyl)amide (LiHMDS) and compound **49** preduced compound **50** with an excellent yield. Oxidation of compound **50** with Pyridinium chlorochromate (PCC) produced compound **51** with a high yield. Treatment of **51** with Hydrazine hydrate produced compound **52** with a moderate yield. Suzuki coupling of compound **52** with compound **16** produced compound **53** with a moderate yield. Subsequent treatment of compound **53** with acid produced compound **10** with an acceptable yield.

4. Conclusion

In conclusion, five novel 1H-pyrrolo[2,3-b]pyridine or 1H-pyrazolo[3,4-b]pyridine derivatives with a methylene, sulfur, sulfoxide or cyclopropyl group as a linker, were designed, synthesized and biologically evaluated as potential c-Met inhibitors. The development of these methods of compound synthesis may provide an important reference for the construction of novel 7-azaindole and 7-azaindazole derivatives with a single atom linker. The enzyme assay and cell assay *in vitro* showed that compound **9** displayed strong c-Met kinase inhibition with IC_{50} of 22.8 nM, moderate ALK kinase inhibition, and strong cell inhibition with MKN-45 IC_{50} of 329 nM and EBC-1 IC_{50} of 479 nM. In order to find the better candidate compounds, compounds **8**, **9** and **10** have been selected as tool compounds, with 7-azaindole and 7-azaindazole as core and with a single atom as linker (such as sulfur, sulfoxide or cyclopropyl group), the further optimization is ongoing in our laboratory and the result will be reported in the future.

5. Experimental Methods

5.1. General Methods for Chemistry

All reagents and solvents were used as purchased from commercial sources. Reactions were carried out under nitrogen atmosphere unless otherwise indicated. 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). ^{1}H NMR spectra were recorded on Bruker ARX-400, 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard and Chloroform-D ($CDCl_3$), Dimethylsulfoxid-d6 (DMSO-d6) or Methanol-d4 (MeOD) as solvents (reported in ppm) with a reference standard of $CDCl_3$ (7.26 ppm) or DMSO-d6 (2.50 ppm). Multiplicities were given as s (singlet), bs (broad singlet), d (doublet), t (triplet), dt (double of triplets), and m (multiplet). Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, U.S.A.).

*5.1.1. Synthesis of target compound 3-(1-(2,6-Dichloro-3-fluorophenyl)ethyl)-2-methyl-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (**6**)*

*5.1.1.1. 5-Bromo-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (**18**)*

To a solution of 5-bromo-1H-pyrrolo[2,3-b]pyridine **17** (5 g, 25 mmol) in N,N-Dimethylformamide (DMF, 40 mL) Sodium hydride (NaH, 0.91 g, 37 mmol) was added at 0 °C and stirred at 25 °C for 20 min. To the mixture Benzenesulfonyl chloride (5.37 g, 30 mmol) was added dropwise at 0 °C and stirred at 25 °C for 1 h. The mixture was quenched with Ammonium chloride (NH_4Cl) solution, extracted with Bichloromethane (DCM, 50 mL × 2). The organic phase was washed with water (50 mL × 2), brine (50 mL × 2), dried over Sodium sulfate (Na_2SO_4). After filtering, the organic phase was concentrated to give compound **18** as a white solid (8.5 g, 99.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H, Ar-H), 8.18-8.16 (d, J = 7.8 Hz, 2H, Ar-H), 7.96 (s, 1H, Ar-H), 7.74-7.73 (m, J = 4.0 Hz, 1H, Ar-H), 7.59-7.50 (t, J = 7.4 Hz, 1H, Ar-H), 7.47-7.38 (t, 2H, Ar-H), 6.55-6.54 (d, J = 4.0 Hz, 1H, Ar-H).

*5.1.1.2. 5-Bromo-2-methyl-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (**19**)*

To a solution of diispropylamine (2.52 g, 24.91 mmol) in anhydrous Tetrahydrofuran (THF,

50 mL) n-BuLi (2 M, 9.25 mL, 23.13 mmol) was added dropwise at -78 °C and stirring at -78 °C under N_2 for 30 min. To the mixture a solution of compound **18** (3 g, 8.90 mmol) in anhydrous THF (20 mL) was added and stirred at -78 °C for 40 min. To the mixture a solution of compound MeI (3.78 g, 26.69 mmol) in anhydrous THF (20 mL) was added at -78 °C and stirred 25 °C for 2 h. The mixture was quenched with sat. NH_4Cl solution and extracted with Ethyl acetate (EtOAc, 100 mL × 3). The organic phase was washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography on silica (PE:EA = 40:1) to give compound **19** as a white solid (2.0 g, 64.1% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.36 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 8.13-8.05 (m, 2H, Ar-H), 7.72-7.70 (t, J = 7.5 Hz, 1H, Ar-H), 7.63-7.59 (t, J = 7.8 Hz, 2H, Ar-H), 6.56 (s, 1H, Ar-H), 2.71 (s, 3H, CH3). ESI-MS m/z: 351.11, 353.11 $(M + H)^+$.

*5.1.1.3. 5-Bromo-2-methyl-1H-pyrrolo[2,3-b]pyridine (**20**)*

To a solution of compound **19** (1.3 g, 3.7 mmol) in Methynol (MeOH, 85 mL) 2 M of Sodium hydroxide (NaOH) solution (22 mL) was added. After refluxing for 2 h, the mixture was extracted with EtOAc (100 mL × 3). The organic phase was washed with brine (30 mL × 2), dried over Na_2SO_4, concentrated to give compound **20** as a white solid (600 mg, 76.9% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.69 (s, 1H, NH), 8.13 (s, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 6.14 (s, 1H, Ar-H), 2.40 (s, 3H, CH3). ESI-MS m/z: 211.16, 213.16 $(M + H)^+$.

*5.1.1.4. 5-Bromo-3-(1-(2,6-dichloro-3-fluorophenyl)ethyl)-2-methyl-1H-pyrrolo[2,3-b]pyridine (**22**)*

To a solution of compound **20** (450 mg, 2.13 mmol) in DCM (10 mL) Trifluoromethanesulfonic acid (TfOH, 1.28 g, 8.53 mmol) and 1-(2,6-Dichloro-3-fluorophenyl)ethanol (1.63 g, 8.53 mmol) was added dropwise. After stirring at 25 °C under N_2 for 16 h, the mixture was quenched with sat. Sodium bicarbonate ($NaHCO_3$) solution, extracted with DCM (100 mL × 3). The organic phase was washed with water (20 mL × 2), brine (20 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated and purified by column chromatography on silica (DCM:MeOH = 200:1) to give **22** as a white solid (650 mg, 75.8% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.66 (s, 1H, NH), 8.12 (s, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.54-7.50 (dd, J = 8.9, 5.1 Hz, 1H, Ar-H), 7.39-7.34 (t, J = 8.7 Hz, 1H, Ar-H),

5.15-5.12 (q, J = 7.4 Hz, 1H, CH), 2.18 (s, 3H, CH3), 1.86-1.83 (d, J = 7.5 Hz, 3H, CH3).

*5.1.1.5. tert-Butyl 4-(4-(3-(1-(2,6-dichloro-3-fluorophenyl)ethyl)-2-methyl-1H-pyrrolo[2,3-b]pyridin-5-yl)-1H-pyrazol-1-yl)piperidine-1-carboxylate (**23**)*

To a mixture of compound **22** (450 mg, 1.12 mmol), compound **16** (633 mg, 1.68 mmol) and Potassium carbonate (K_2CO_3, 464 mg, 3.357 mmol) in Dioxane (16 mL) and H_2O (4 mL) Dichloro(1,1'-bis(diphenylphosphino)ferrocene)palladium (II) ($Pd(dppf)Cl_2$, 91.3 mg, 0.11 mmol) was added under N_2. After stirring at 100 °C under N_2 for 12 h, the mixture was diluted with water (20 mL), extracted with EtOAc (20 mL × 3). The organic phase was washed with brine (20 mL × 2), dried over Na_2SO_4, concentrated and purified by column chromatography on silica (DCM:MeOH = 100:1) to give compound **23** as a white solid (320 mg, 50% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.33 (s, 1H, NH), 8.37-8.29 (m, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.54-7.50 (m, 1H, Ar-H), 7.39-7.32 (t, J = 8.7 Hz, 1H, Ar-H), 5.21-5.15 (q, J = 7.5 Hz, 1H, CH), 4.42-4.31 (m, 1H, CH), 4.20-3.96 (m, 4H, CH2), 2.99-2.78 (m, 4H, CH2), 2.15 (s, 3H, CH3), 1.93-1.92 (d, J =7.5 Hz, 3H, CH3), 1.43 (s, 9H, C(CH3)3).

*5.1.1.6. 3-(1-(2,6-Dichloro-3-fluorophenyl)ethyl)-2-methyl-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (**6**)*

To a solution of compound **23** (250 mg, 0.44 mmol) in DCM (15 mL) Trifluoroacetic acid (TFA, 8 mL) was added. After stirring at 25 °C for 24 h, the mixture was diluted with $NaHCO_3$ solution, extracted with DCM (20 mL × 3). the organic phase was washed with brine (20 mL × 3), dried over Na_2SO_4, concentrated and purified by column chromatography on silica (DCM:MeOH = 6:1) to give compound **6** as a white solid (100 mg, 48.5% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.36 (s, 1H, NH), 8.70-8.58 (s, 1H, NH), 8.34 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.55-7.49 (m, 1H, Ar-H), 7.39-7.34 (t, J = 8.7 Hz, 1H, Ar-H), 5.20-5.18 (q, J = 7.6 Hz, 1H, CH), 4.56-4.48 (m, 1H, CH), 3.46-3.39 (m, 2H, CH2), 3.15-3.05 (m, 2H, CH2), 2.28-2.16 (m, 4H, CH2), 2.16 (s, 3H, CH3), 1.92-1.90 (d, J = 7.5 Hz, 3H, CH3). ^{13}C NMR (400 MHz, DMSO-d6) δ 158.67, 156.01, 146.83, 143.12, 139.16, 136.29, 134.80,

130.54, 129.60, 125.38, 123.04, 121.46, 120.37, 119.25, 115.83, 108.98, 55.63, 42.50, 35.47, 29.12, 17.51, 12.84. ESI-MS m/z: 472.37 $(M + H)^+$.

*5.1.2. Synthesis of target compound 2-Chloro-3-(1-(2,6-dichloro-3-fluorophenyl)ethyl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (**7**)*

*5.1.2.1. 3,3,5-Tribromo-1H-pyrrolo[2,3-b]pyridin-2(3H)-one (**25**)*

To a solution of compound 5-Bromo-1H-pyrrolo[2,3-b]pyridine (3.0 g, 15.2 mmol) in tert-Butanol (t-BuOH, 15 mL) and H_2O (15 mL) Bromine (Br_2, 2 mL) was added dropwise. The mixture was stirred at 25 °C for 1 h at dark place. The reaction mixture was extracted with DCM (50 mL × 3). The organic phase was washed with water (30 mL × 2), brine (30 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give compound **25** as a red solid (5.64 g, 99.7% yield). ESI-MS m/z: 370.92, 372.91 $(M + H)^+$.

*5.1.2.2. 5-Bromo-1H-pyrrolo[2,3-b]pyridin-2(3H)-one (**26**)*

To a solution of compound **25** (5.64 g, 15.2 mmol) in Acetic acid (AcOH, 100 mL) Zn power (9.88 g, 0.15 mmol) was added. After stirring at 25 °C under N_2 for 3 h, the mixture was distilled to remove excess AcOH and extracted with DCM (100 mL). The organic phase was washed with water (50 mL × 2), brine (50 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated and purified by column chromatography on silica (DCM:MeOH = 150:1) to give compound **26** as an off-white solid (2.75 g, 84.8% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.15 (s, 1H, NH), 8.17 (s, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 3.59 (s, 2H, CH2). ESI-MS m/z: 211.01, 213.04 $(M - H)^-$.

*5.1.2.3. 5-Bromo-2-chloro-1H-pyrrolo[2,3-b]pyridine (**27**)*

A solution of compound **26** (1.3 g, 6.1 mmol) in $POCl_3$ (9.35 g, 61 mmol, 6 mL) was refluxed for 4 h. After concentrated, the mixture was diluted with DCM (20 mL) and quenched with water 20 mL). To the cooled mixture 1M NaOH solution was added to adjust pH to 7 - 8, extracted with DCM (50 mL × 4), the organic phase was washed with water (30 mL × 2), brine(30 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give compound

27 as a pale yellow solid (1.4 g, 99.1% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.76 (s, 1H, NH), 8.27 (s, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H). ESI-MS m/z: 231.17, 233.17 $(M + H)^+$.

*5.1.2.4. 5-Bromo-2-chloro-3-(1-(2,6-dichloro-3-fluorophenyl)ethyl)-1H-pyrrolo[2,3-b]pyridine (**28**)*

To a solution of compound **27** (400 mg, 1.73 mmol) in DCM (40 mL) Trifluoromethanesulfonic acid (1.04 g, 6.92 mmol) and 1-(2,6-Dichloro-3-fluorophenyl)ethanol (1.45 g, 6.92 mmol) was added dropwise. After stirring at 25 °C under N_2 for 16 h, the mixture was quenched with sat. $NaHCO_3$ solution, extracted with DCM (50 mL × 3). The organic phase was washed with water (50 mL × 2), brine (50 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated and purified by column chromatography on silica (DCM:MeOH = 200:1) to give crude compound **28** as a pale yellow solid (500 mg, 68.5% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.15 (s, 1H, NH), 8.03 (s, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.54-7.50 (dd, J = 8.8, 5.1 Hz, 1H, Ar-H), 7.41-7.37 (t, J = 8.7 Hz, 1H, Ar-H), 5.54-5.49 (q, J = 7.6 Hz, 1H, CH), 1.91-1.85 (d, J = 7.6 Hz, 3H, CH3). ESI-MS m/z: 420.84, 422.93 $(M - H)^-$.

*5.1.2.5. tert-Butyl4-(4-(2-chloro-3-(1-(2,6-dichloro-3-fluorophenyl)ethyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)-1H-pyrazol-1-yl)piperidine-1-carboxylate (**29**)*

To a solution of compound **28** (350 m g, 0.83 mmol) in Dioxane (16 mL) and H_2O (4 mL) compound **16** (468.8 mg, 1.24 mmol), K_2CO_3 (343.4 mg, 2.48 mmol) and then $Pd(dppf)Cl_2$ (67.6 mg, 0.08 mmol) was added under N_2. After stirring at 100 °C under N_2 for 12 h, the mixture was diluted with water (150 mL), extracted with EtOAc (50 mL × 3). The organic phase was washed with brine (50 mL × 2), dried over Na_2SO_4, concentrated and purified by column chromatography on silica (DCM:MeOH = 100:1) to give crude compound **29** as a pale yellow solid (119 mg, 24.2% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.36 (s, 1H, NH), 8.46 (s, 1H, Ar-H), 8.29 (s, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.55-7.50 (m, 1H, Ar-H), 7.41-7.35 (t, J = 8.7 Hz, 1H, Ar-H), 5.22-5.16 (q, J = 7.6 Hz, 1H, CH), 4.39-4.33 (m, 1H, CH), 4.09-4.01 (m, 2H, CH2), 2.95-2.88 (m, 2H, CH2), 2.05-1.99 (m, 4H, CH2), 1.95 (d, J = 7.6 Hz, 5H, Ch2,CH3), 1.43 (S, 9H,

C(CH3)3). ESI-MS m/z: 591.84 $(M - H)^-$.

5.1.2.6.

2-Chloro-3-(1-(2,6-dichloro-3-fluorophenyl)ethyl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (7)

To a solution of compound **29** (110 mg, 0.185 mmol) in DCM (15 mL) TFA (4mL) was added and stirred at 25 °C for 24 h. The mixture was neutralized with $NaHCO_3$ solution, extracted with DCM (20 mL × 3). The organic phase was washed with brine (30 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give a residue which was purified by column chromatography on silica (DCM:MeOH = 6:1) to give compound **7** as a white solid (46 mg, 50.5% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.47 (s, 1H, Ar-H), 8.38-8.33 (s, 1H, NH), 8.24 (s, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 7.58-7.47 (m, 1H, Ar-H), 7.41-7.36 (t, J = 8.6 Hz, 1H, Ar-H), 5.22-5.16 (q, J = 7.6 Hz, 1H, CH), 4.35-4.25 (m, 1H, CH), 3.18-3.12 (m, 2H, CH2), 2.78-2.70 (m, 2H, CH2), 2.08-2.01 (m, 2H, CH2), 1.98-1.90 (m, 5H, CH2, CH3). ^{13}C NMR (400 MHz, DMSO-d6) δ 158.60, 156.01, 145.85, 142.13, 140.96, 136.51, 132.86, 130.52, 129.72, 128.49, 125.78, 123.58, 121.61, 120.67, 120.29, 116.06, 109.56, 55.64, 42.58, 35.14, 29.13, 17.04. ESI-MS m/z: 492.38, 494.37 $(M + H)^+$.

*5.1.3. Synthesis of target compound 3-((2,6-Dichloro-3-fluorophenyl)sulfinyl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (**8**)*

*5.1.3.1. 2,6-Dichloro-3-fluorobenzenethiol (**31**)*

To a solution of 2,4-Dichloro-1-fluorobenzene (8.0 g, 48.5 mmol) in THF (63 mL) n-BuLi (23.27 mL, 2.5 M, 43.6 mmol) was added dropwise at -78 °C and stirred for 1.5 h. To the mixture Sulfur (3.1 g, 72.7 mmol) was added at -78 °C and stirring for 2 h. To the resulting mixture 10% Hydrochloric acid (HCl) was added to adjust pH to 5 - 6. The resulting mixture was extracted with EtOAc (200 mL × 3), the combined organic phase was washed with brine (100 mL × 2), dried over Na_2SO_4. After filtered, the organic phase was concentrated to dry to give compound **31** as yellow oil (11.4 g, 99.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.19 (m, 1H, Ar-H), 6.95-6.89 (t, 1H, Ar-H), 4.69 (s, 1H, SH).

*5.1.3.2. 5-Bromo-3-iodo-1H-pyrrolo[2,3-b]pyridine (**33**)*

To a solution of 5-Bromo-1H-pyrrolo[2,3-b]pyridine (2.0 g, 10.2 mmol) in Acetone (20 mL) NIS (2.7 g, 12.0 mmol) was added at 25 °C and stirred for 1 h. The mixture was concentrated and then extracted with EtOAc (30 mL × 2), the organic phase was washed with sat. Sodium thiosulfate ($Na_2S_2O_3$) solution (30 mL × 2), brine (30 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give compound **33** as an off-white solid (2.9 g, 88.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H, NH), 8.35 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 7.43 (s, 1H, Ar-H).

*5.1.3.3. 5-Bromo-3-iodo-1-(4-methoxybenzyl)-1H-pyrrolo[2,3-b]pyridine (**34**)*

To a solution of compound **33** (2.9 g, 9.0 mmol) in DMF (20 mL) K_2CO_3 (3.7 g, 26.8 mmol) and 1-(Chloromethyl)-4-methoxybenzene (PMBCl, 1.7 g, 10.8 mmol) was added. After stirring at 25 °C for 4 h, the mixture was diluted with water (50 mL), extracted with EtOAc (20 mL × 3), the organic phase was washed with brine (50 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated and purified by column chromatography on silica (PE:EA = 30:1) to give compound **34** as a white solid (3.6 g, 90.3% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.21-7.17 (d, J = 8.6 Hz, 2H, Ar-H), 6.88-6.84 (t, J = 8.0 Hz, 2H, Ar-H), 5.37 (s, 2H, CH2), 3.78 (s, 3H, CH3).

5.1.3.4.

*5-Bromo-3-((2,6-dichloro-3-fluorophenyl)thio)-1-(4-methoxybenzyl)-1H-pyrrolo[2,3-b]Pyridine (**35**)*

To a solution of compound **34** (3.6 g, 8.1 mmol) in Isopropanol (i-PrOH, 50 mL) compound **31** (1.9 g, 9.7 mmol), K_2CO_3 (2.2 g, 16.2 mmol), Cuprous iodide (CuI, 1.5 g, 8.1 mmol) and ethylene glycol (1.0 g, 16.2 mmol) was added. After stirring at 100 °C under N_2 for 5 h, the mixture was filtered and washed with EtOAc (100 mL). The organic phase was washed with ammonia water (50 mL × 2), brine (50 mL × 2). The organic phase was dried over Na_2SO_4, concentrated and purified by column chromatography on silica (PE:EA = 100:1) to give compound **35** as a white solid (2.5 g, 70.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H,

Ar-H), 8.19 (s, 1H, Ar-H), 7.55 (s, 1H, Ar-H), 7.35-7.30 (m, 1H, Ar-H), 7.19-7.13 (m, 2H, Ar-H), 7.06-7.00 (m, 1H, Ar-H), 6.87-6.80 (m, 2H, Ar-H), 5.36 (s, 2H, CH2), 3.79 (s, 3H, CH3).

*5.1.3.5. tert-Butyl 4-(4-(3-((2,6-dichloro-3-fluorophenyl)thio)-1-(4-methoxybenzyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)-1 H-pyrazol-1-yl)piperidine-1-carboxylate (**36**)*

To a solution of compound **35** (2.5 g, 4.9 mmol), Cesium carbonate (Cs_2CO_3, 4.8 g, 14.7 mmol), compound **16** (2.6 g, 6.8 mmol) in Dioxane (16 mL) and H_2O (4 mL) Tetrakis(triphenylphosphine)platinum ($Pd(Ph_3P)_4$, 170 mg, 0.15 mmol) was added under N_2. After stirring at 80 °C under N_2 for 12 h, the mixture was diluted with water (30 mL), extracted with EtOAc (20 mL × 3). The organic phase was washed with brine (30 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated and purified by column chromatography on silica (PE:EA = 10:1) to give compound **36** as an off-white solid (1.4 g, 41.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H, Ar-H), 8.12-8.08 (m, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.50 (s, 1H, Ar-H), 7.36-7.30 (m, 1H, Ar-H), 7.21-7.16 (m, 2H, Ar-H), 7.07-7.01 (m, 1H, Ar-H), 6.88-6.80 (m, 2H, Ar-H), 5.36 (s, 2H, CH2), 4.33-4.26 (m, 3H, CH, CH2), 3.79 (s, 3H, CH3), 2.89-2.81 (m, 2H, CH2), 2.15-2.09 (m, 2H, CH2), 1.95-1.89 (m, 2H, CH2), 1.49 (s, 9H, C(CH3)3).

*5.1.3.6. 3-((2,6-Dichloro-3-fluorophenyl)thio)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]py ridine (**37**)*

To a solution of compound **36** (1.4 g, 2.1 mmol) in DCM (10 mL) TFA (5 mL) and concentrated Sulfuric acid (con. H_2SO_4, 1 mL) was added. After stirring at 25 °C for 2 h, to the mixture ammonia water was added to adjust pH to 7 - 8, extracted with DCM (20 mL × 2). The organic phase was washed with sat. $NaHCO_3$ solution (30 mL × 2), brine (30 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give compound **37** as a tan solid (761.5 mg, 78.4% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1H, Ar-H), 8.43-8.39 (m, 1H, Ar-H), 8.15-8.11 (m, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 7.53 (s, 1H, Ar-H), 7.37-7.31 (m, 1H, Ar-H), 7.09-7.05 (m, 1H, Ar-H), 4.50-4.45 (m, 1H, CH), 3.73-3.67 (m, 2H,

CH2), 3.22-3.17 (m, 2H, CH2), 2.49-2.41 (m, 4H, CH2).

5.1.3.7.

*3-((2,6-Dichloro-3-fluorophenyl)sulfinyl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (**8**)*

To a solution of compound **37** (761.5 mg, 1.6 mmol) in DCM (10 mL) m-CPBA (334.0 mg, 1.8 mmol) was added and stirred at 0 °C for 2 h. The mixture was quenched with sat. $Na_2S_2O_3$ solution, extracted with DCM (10 mL × 2). The organic phase was washed with sat. $NaHCO_3$ solution (30 mL × 2), brine (30 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated and purified by column chromatography on silica (DCM:MeOH = 10:1) to give compound **8** as an off-white solid (608.0 mg, 77.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H, Ar-H), 8.32 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.99-7.97 (d, 2H, Ar-H), 7.67-7-65 (d, 2H, Ar-H), 4.56-4.51 (m, 1H, CH), 3.43-3.35 (m, 2H, CH2), 3.13-3.07 (m, 2H, CH2), 2.27-2.14 (m, 4H, CH2). ^{13}C NMR (400 MHz, DMSO-d6) δ 158.74, 156.25, 147.74, 142.84, 139.34, 136.57, 132.02, 131.95, 130.38, 129.44, 126.04, 122.88, 120.73, 119.69, 117.35, 112.64, 55.76, 42.59, 29.18. ESI-MS m/z: 478.23, 480.22 $(M + H)^+$.

*5.1.4. Synthesis of target compound 3-(1-(2,6-Dichloro-3-fluorophenyl)ethyl)-2-methyl-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridine (**9**)*

*5.1.4.1. 5-Bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine (**39**)*

To a solution of 5-Bromo-1H-pyrazolo[3,4-b]pyridine (2.0 g, 10.1 mmol) in DMF (20 mL) Potassium hydroxide (KOH, 1.2 g, 21.4 mmol) was added at 25 °C. After stirring for 10 min, Iodine (2.8 g, 11.1 mmol) was added and stirred at 25 °C for 4 h. The resulting mixture was diluted with water (30 mL), extracted with EtOAc (20 mL × 3). The organic phase was washed with sat. $Na_2S_2O_3$ solution (30 mL × 2), brine (30 mL × 2), dried over Na_2SO_4, and concentrated to give compound **39** as a brown solid (2.8 g, 85.6% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 14.30 (s, 1H, NH), 8.65 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H).

*5.1.4.2. 5-Bromo-3-iodo-1-(4-methoxybenzyl)-1H-pyrazolo[3,4-b]pyridine (**40**)*

To a solution of compound **39** (2.8 g, 8.6 mmol) in DMF (20 mL) K_2CO_3 (3.6 g, 26.0 mmol) and 1-(Chloromethyl)-4-methoxybenzene (1.7 g, 10.9 mmol) was added and stirred at 25 °C for 4 h. The mixture was diluted with water (50 mL), extracted with EtOAc (20 mL × 3). The organic phase was washed with brine (50 mL × 3), dried over Na_2SO_4. After filtering, the organic layer was concentrated and purified by column chromatography on silica (PE:EA = 100:1) to give compound **40** as a white solid (3.6 g, 90.3% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.58 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.35-7.31 (d, J = 8.4 Hz, 2H, Ar-H), 6.84-6.80 (d, J = 8.8 Hz, 2H, Ar-H), 5.60 (s, 2H, CH2), 3.76 (s, 3H, CH3).

5.1.4.3.

5-Bromo-3-((2,6-dichloro-3-fluorophenyl)thio)-1-(4-methoxybenzyl)-1H-pyrazolo[3,4-b]pyridine ***(41)***

To a solution of compound **40** (3.6 g, 8.1 mmol) in i-PrOH (50 mL) compound 31 (1.9 g, 9.7 mmol), K_2CO_3 (2.2 g, 16.2 mmol), CuI (1.5 g, 8.1 mmol) and ethylene glycol (1.0 g, 16.2 mmol) was added. The mixture was stirred at 100 °C under N_2 for 5 h. The resulting mixture was filtered and washed with EtOAc (50 mL), the combined organic phase was washed with ammonia water (50 mL × 2), brine (50 mL × 2). The organic phase was dried over Na_2SO_4, concentrated and purified by column chromatography on silica (PE:EA = 100:1) to give compound **41** as a white solid (2.5 g, 70.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.40-7.36 (d, J = 4.8 Hz, 1H, Ar-H), 7.28-7.23 (d, J = 8.4 Hz, 2H, Ar-H), 7.16-7.12 (d, J = 7.6 Hz, 1H, Ar-H), 6.81-6.77 (d, J = 8.4 Hz, 2H, Ar-H), 5.57 (s, 2H, CH2), 3.76 (s, 3H, CH3).

5.1.4.4. *tert-Butyl 4-(4-(3-((2,6-dichloro-3-fluorophenyl)thio)-1-(4-methoxybenzyl)-1H-pyrazolo[3,4-b]pyridin-5-yl)-1H-pyrazol-1-yl)piperidine-1-carboxylate* ***(42)***

To a solution of compound **41** (2.5 g, 4.9 mmol) in Glycol dimethyl ether (DME, 16 mL) and H_2O (4 mL) compound **16** (2.6 g, 6.8 mmol), Cs_2CO_3 (4.8 g, 14.7 mmol) and then $Pd(Ph_3P)_4$ (170 mg, 0.15 mmol) was added under N_2. After stirring at 80 °C under N_2 for 12 h, the mixture was diluted with water (30 mL), extracted with EtOAc (20 mL × 3). The organic phase was washed with brine (20 mL × 3), dried over Na_2SO_4, concentrated and purified by column chromatography

on silica (PE:EA = 25:1) to give compound **42** as an off-white solid (1.6 g, 47.8% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.65 (s, 1H, Ar-H), 7.82 (s, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.39-7.35 (dd, J= 4.8 Hz, 1H, Ar-H), 7.31-7.26 (d, J= 8.8 Hz, 2H, Ar-H), 7.15-7.11 (t, 1H, Ar-H), 6.82-6.76 (m, J= 8.8 Hz, 2H, Ar-H), 5.61 (s, 2H, CH2), 4.39-4.26 (m, 3H, CH, CH2), 3.76 (s, 3H, CH3), 2.95-2.88 (m, 2H, CH2), 2.19-2.16 (m, 2H, CH2), 2.01-1.92 (m, 2H, CH2), 1.49 (s, 9H, C(CH3)3).

5.1.4.5.

*3-((2,6-Dichloro-3-fluorophenyl)thio)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrazolo[3,4-b]pyridine (**9**)*

To a solution of compound **42** (1.6 g, 2.3 mmol) in DCM (20 mL) TFA (5 mL) and con. H_2SO_4 (1 mL) was added. After stirring at 25 °C for 2 h, to the mixture ammonia water was added to adjust pH to 7 - 8, extracted with DCM (20 mL × 2). The organic phase was washed with sat. $NaHCO_3$ solution (20 mL × 2), brine (20 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give compound **9** as an off-white solid (870.5 mg, 80.3% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.89 (s, 1H, Ar-H), 8.38 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 8.02 (s, 1H), 7.75-7.68 (m, 1H, Ar-H), 7.65-7.58 (m, 1H, Ar-H), 4.57-4.47 (m, 1H, CH), 3.38-3.32 (m, 2H, CH2), 3.10-3.03 (m, 2H, CH2), 2.30-2.17 (m, 4H, CH2). ^{13}C NMR (400 MHz, DMSO-d6) δ 158.53, 156.05, 151.55, 148.55, 136.76, 135.50, 131.97, 130.25, 127.41, 126.25, 123.72, 122.98, 119.47, 119.16, 115.24, 55.97, 42.65, 29.34. ESI-MS m/z: 463.21, 465.21 $(M + H)^+$.

*5.1.5. Synthesis of target compound 3-(1-(2,6-Dichloro-3-fluorophenyl)cyclopropyl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrazolo[3,4-b]pyridine (**10**)*

*5.1.5.1. 2-(2,6-Dichloro-3-fluorophenyl)ethanol (**44**)*

To a solution of 2,4-Dichloro-1-fluorobenzene (165.0 g, 1.0 mol) in THF (500 mL) n-BuLi (420 mL, 2.5 M, 1.05 mol) was added at -78 °C. After stirring at -78 °C for 1 h, Oxirane (44.0 g, 1.0 mmol) was added and stirred at -78 °C for 2 h. The mixture was quenched with sat. NH_4Cl solution, extracted with EtOAc (200 mL × 3). The organic phase was washed with water (100 mL × 2), brine (100 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to

give compound **44** as a colorless liquid (156.7 g, 75.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 1H, Ar-H), 7.02-6.97 (m, 1H, Ar-H), 3.89-3.84 (m, 2H, CH2), 3.29-3.24 (m, 2H, CH2).

*5.1.5.2. 2-(2,6-Dichloro-3-fluorophenyl)acetic acid (**45**)*

To a solution of compound **44** (156.7 g, 750.0 mmol) and 2,2,6,6-Tetramethylpiperidine N-oxide (TEMPO, 129.0 g, 825.0 mmol) in phosphate buffer (PH = 6.5, 500 mL) Sodium chlorite solution was added dropwise at 0 °C while stirring. After stirring at 25 °C for 3 h, to the mixture HCl was added to adjust pH to 2-3, extracted with EtOAc (200 mL × 3). The organic phase was washed with water (100 mL × 2), brine (100 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give compound **45** as a white solid (150.5 g, 90.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.36 (m, 1H, Ar-H), 7.19-7.14 (m, 1H, Ar-H), 4.02 (s, 2H, CH2).

*5.1.5.3. 2-(2,6-Dichloro-3-fluorophenyl)acetamide (**46**)*

A solution of compound **45** (150.5 g, 674.9 mmol) in $SOCl_2$ (100 mL) was refluxed for 1 h. The mixture was distilled to remove excessive $SOCl_2$, the residue was redissolved in DCM and added dropwise to ammonia water (300 mL) at 0 °C. After stirring at 25 °C for 3 h, the mixture was filtered and washed with water (100 mL × 2), dried to give compound **46** as a white solid (142.3 g, 95.0% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.58 (s, 1H, NH), 7.54-7.50 (m, 1H, Ar-H), 7.42-7.37 (m, 1H, Ar-H), 7.09 (s, 1H, NH), 3.80 (s, 2H, CH2).

*5.1.5.4. 2-(2,6-Dichloro-3-fluorophenyl)acetonitrile (**47**)*

To a solution of compound **46** (142.3 g, 641.0 mmol) in Pyridine (1000 mL) $SOCl_2$ (380 g, 3.2 mmol) was added dropwise at 0 °C. After stirring at 0 °C for 2 h, the mixture was quenched with cooled water, extracted with petroleum ether (200 mL × 3). The organic phase was washed with sat. Copper(II) sulfate ($CuSO_4$) solution (100 mL × 2), brine (100 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give compound **47** as an off-white solid (85.9 g, 65.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.30 (m, 1H, Ar-H), 7.11-7.06 (m, 1H, Ar-H), 4.08 (s, 2H, CH2).

*5.1.5.5. 1-(2,6-Dichloro-3-fluorophenyl)cyclopropanecarbonitrile (**48**)*

To a solution of compound **47** (85.9 g, 421.1 mmol) in DMF (500 mL) NaH (60% purity, 42.1 g, 1.05 mol) was added at 0 °C. After stirring at 25 °C for 1 h, 1,2-Dibromoethane (95 g, 505 mmol) was added and stirred at 25 °C for 3 h. The mixture was quenched with sat. NH_4Cl solution, extracted with EtOAc (200 mL × 3). The organic phase was washed with water (100 mL × 2), brine (100 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated and purified by column chromatography on silica (PE:EA = 100:1) to give compound **48** as an off-white solid (83.1 g, 85.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.32 (m, 1H, Ar-H), 7.17-7.13 (m, 1H, Ar-H), 1.98-1.94 (m, 2H, CH2), 1.50-1.46 (m, 2H, CH2).

*5.1.5.6. 1-(2,6-Dichloro-3-fluorophenyl)cyclopropanecarbaldehyde (**49**)*

To a solution of compound **48** (10.0 g, 43.47 mmol) in anhydrous Toluene (100 mL) Di-isobutylaluminum hydride (DIBAL-H, 1M, 65.2 mL, 65.2 mmol) was added dropwise and stirred at -78 °C under N_2 for 1 h. The mixture was quenched with 1N of HCl at 0 °C and stirred for 30 min, extracted with DCM. The organic phase was washed with brine, dried over Na_2SO_4, concentrated to give compound **49** as an off-white solid (7.5 g, 74.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H, CHO), 7.33-7.30 (m, 1H, Ar-H), 7.13-7.09 (m, 1H, Ar-H), 1.92-1.89 (m, 2H, CH2), 1.61-1.57 (m, 2H, CH2).

*5.1.5.7. (5-Bromo-2-fluoropyridin-3-yl)(1-(2,6-dichloro-3-fluorophenyl)cyclopropyl)methanol (**50**)*

To a solution of 5-Bromo-2-fluoropyridine (6.2 g, 35.4 mmol) in anhydrous THF (50 mL) Lithium bis(trimethylsilyl)amide (LiHMDS, 1M, 48.3 mL, 48.3 mmol) was added dropwise and stirred at -78 °C under N_2 for 30 min. To the mixture a solution of **49** (7.5 g, 32.2 mmol) in anhydrous THF (20 mL) was added and stirred at -78 °C for 1 h. The mixture was quenched with sat. NH_4Cl solution at 0 °C, extracted with EtOAc (100 mL × 2). The organic phase was washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography on silica (PE:EA = 50:1) to give compound **50** as an off-white solid (11.0 g, 83.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17-8.15 (m, 1H, Ar-H), 7.67-7.65 (m, 1H, Ar-H), 7.30-7.18 (m, 1H, Ar-H), 7.08-7.02 (m, 1H, Ar-H), 4.95-4.92 (m, 1H, OH), 2.73-2.66 (m, 1H, CH), 1.53-1.01 (m, 4H, C(CH2)2).

*5.1.5.8. (5-Bromo-2-fluoropyridin-3-yl)(1-(2,6-dichloro-3-fluorophenyl)cyclopropyl)methanone (**51**)*

To a solution of compound **50** (11.0 g, 26.9 mmol) in DCM (50 mL) Pyridinium chlorochromate (PCC, 11.6 g, 53.8 mmol) was added and stirred at 25 °C for 4 h. The mixture was concentrated and purified by column chromatography on silica (PE:EA = 50:1) to give compound **51** as an off-white solid (10.6 g, 96.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H, Ar-H), 7.76-7.73 (m, 1H, Ar-H), 7.31-7.25 (m, 1H, Ar-H), 7.11-7.04 (m, 1H, Ar-H), 2.18-7.15 (m, 2H, CH2), 1.69-1.65 (m, 2H, CH2).

*5.1.5.9. 5-Bromo-3-(1-(2,6-dichloro-3-fluorophenyl)cyclopropyl)-1H-pyrazolo[3,4-b]pyridine (**52**)*

To a solution of compound **51** (10.6 g, 26.0 mmol) in Ethanol (EtOH, 100 mL) 50% Hydrazine hydrate (15.6 g, 156 mmol) was added and refluxed for 10 h. The mixture was distilled to remove excess EtOH and diluted with water. The formed solid was filtered and washed with water. The resulting solid was purified by column chromatography on silica (PE:EA = 15:1) to give compound **52** as an off-white solid (5.8 g, 55.3% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (s, 1H, NH), 8.49 (s, 1H, Ar-H), 7.53 (s, 1H, Ar-H), 7.42-7.37 (m, 1H, Ar-H), 7.20-7.16 (m, 1H, Ar-H), 2.10-2.04 (m, 2H, CH2), 1.59 (m, 2H, CH2).

*5.1.5.10. tert-butyl 4-(4-(3-(1-(2,6-Dichloro-3-fluorophenyl)cyclopropyl)-1H-pyrazolo[3,4-b]pyridin-5-yl)-1H-pyrazol-1-yl)piperidine-1-carboxylate (**53**)*

To a solution of compound **52** (5.8 g, 14.5 mmol), Cs_2CO_3 (14.2 g, 43.5 mmol), compound **16** (8.24 g, 21.8 mmol) in dioxane (75 mL) and H_2O (25 mL) $Pd(Ph_3P)_4$ (508 mg, 0.44 mmol) was added. After stirring at 80 °C under N_2 for 12 h, the mixture was diluted with water (50 mL), extracted with EtOAc (50 mL × 3). The organic phase was washed with brine (50 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated and purified by column chromatography on silica (DCM:MeOH = 25:1) to give compound **53** as a pale yellow solid (5.4 g, 65.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.63 (s, 1H, NH), 8.61 (s, 1H, Ar-H), 7.63 (s, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.42-7.38 (m, 2H, Ar-H), 7.20-7.16 (m, 1H, Ar-H), 4.31-4.25 (m, 4H,

CH2), 2.93-2.89 (m, 3H, CH, CH2), 2.17-1.99 (m, 2H, CH2), 1.98-1.93 (m, 2H, CH2), 1.61-1.57 (m, 2H, CH2), 1.49 (s, 9H, C(CH3)3).

5.1.5.11.

*3-(1-(2,6-Dichloro-3-fluorophenyl)cyclopropyl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-1H-pyrazolo[3,4-b]pyridine (**10**)*

To a solution of compound **53** (2.7 g, 4.8 mmol) in DCM (20 mL) TFA (5 mL) was added. After stirring at 25 °C for 2 h, the mixture was added ammonia water to adjust pH to 7 - 8, extracted with DCM (20 mL × 2). The organic phase was washed with sat. $NaHCO_3$ solution (30 mL × 2), brine (30 mL × 2), dried over Na_2SO_4. After filtering, the organic phase was concentrated to give compound **10** as an off-white solid (1.3 g, 27.5% yield). ^{1}H NMR (400 MHz, MeOD) δ 8.66 (s, 1H, Ar-H), 7.99 (m, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.56-7.53 (m, 1H, Ar-H), 7.40-7.34 (m, 2H, Ar-H), 4.37-4.29 (m, 1H, CH), 3.24-3.20 (m, 2H, CH2), 2.82-2.75 (m, 2H, CH2), 2.15-2.10 (m, 4H, CH2), 2.02-1.92 (m, 2H, CH2), 1.62-1.57 (m, 2H, CH2). ^{13}C NMR (400 MHz, DMSO-d6) δ 158.44, 155.99, 151.90, 147.43, 145.09, 140.24, 136.45, 133.17, 129.99, 125.34, 124.59, 121.70, 119.49, 117.33, 111.55, 56.00, 42.65, 29.40, 24.85, 20.75. ESI-MS m/z: 472.36 $(M + H)^+$.

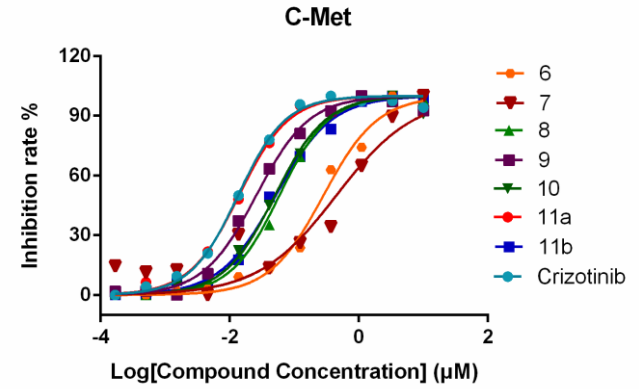
5.2. Kinase Assays

5.2.1. C-Met Z-LYTE Assay

The c-Met kinase activity of five target compounds and three positive compounds were evaluated using standard Z-LYTE Assays (fluorescence resonance energy transfer, FRET).

The test compounds or reference controls were dissolved in dimethyl sulfoxide (DMSO) as stock solution. The DMSO solution was serial diluted (duplicated with 3-fold dilution) to make that the concentrations were from 10 μM to 0.17 nM in 11 doses. The enzyme reaction mixture of c-Met Z-LYTE assay contained 2.5 nM c-Met, 1 μM Peptide substrate (Tyr 6) and 17 μM ATP in a buffer containing 50 mM HEPES (pH 7.0), 10 mM $MgCl_2$, 0.01% Brij-35, 1 mM EGTA at a final volume of 10 μL. The enzyme reaction were carried out at room temperature in black Proxiplate 384-Plus plate (PerkinElmer) for 90 min. 5 μL of the detection reagents A (Invitrogen) was added at final concentrations of 64-fold dilution. The plates were incubated at room temperature for 60 min and then read in the Envision plate reader (PerkinElmer).

Graphs corresponding to the % of inhibition of c-Met activity by the different compound concentrations were presented in Graph 1. Using the curve and the GraphPad Prism software, the 50% inhibition concentration (IC_{50}) of each compound against c-Met kinase activity was calculated.



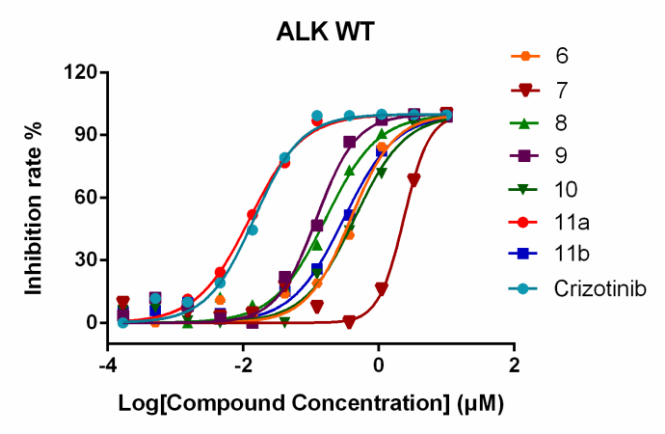
Graph 1. The curves corresponding to the percentage of c-Met kinase inhibition according to the concentrations of the different compounds.

5.2.2. ALK HTRF Assays

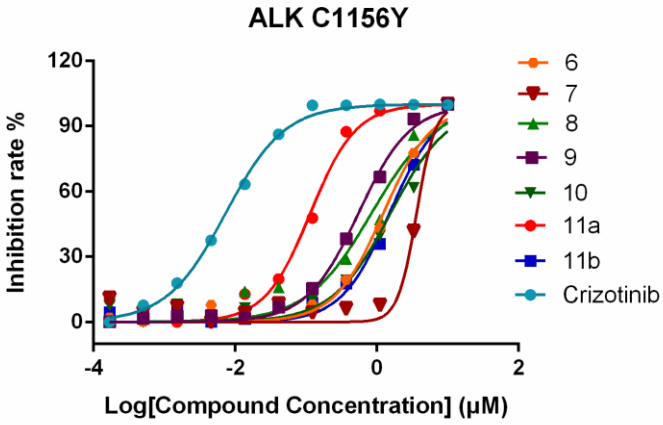
The ALK kinase activity of five target compounds and three positive compounds were evaluated using standard homogeneous time-resolved fluorescence (HTRF) assay (High Fluorescence Resonance Energy Transfer).

The test compounds or reference controls were dissolved in DMSO as stock solution. The DMSO solution was serial diluted (duplicated with 3-fold dilution) to make that the concentrations were from 10 μM to 0.17 nM in 11 doses. The enzyme reaction mixture of ALK WT/ ALK C1156Y/ ALK L1196M for HTRF assay contained 0.5 nM ALK WT/ 0.15 nM ALK C1156Y/ 0.15 nM ALK L1196M, 1 μM biotin-TK peptide, and 30 μM ATP in a kinase reaction buffer containing 50 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 0.01% BSA, 0.1 mM Orthovanadate at a final volume of 10 μL. The enzyme reaction were carried out at room temperature in white Proxiplate 384-Plus plate (PerkinElmer) for 90 min. 10 μL of the detection reagents contained 6.7 nM TK-Antibody and 16.67 nM XL665 in a detection buffer containing 50 mM HEPES (pH 7.5), 0.8 M KF, 20 mM EDTA, 0.01% BSA were added. The plates were incubated at room temperature for 60 min and then read using the Envision plate reader.

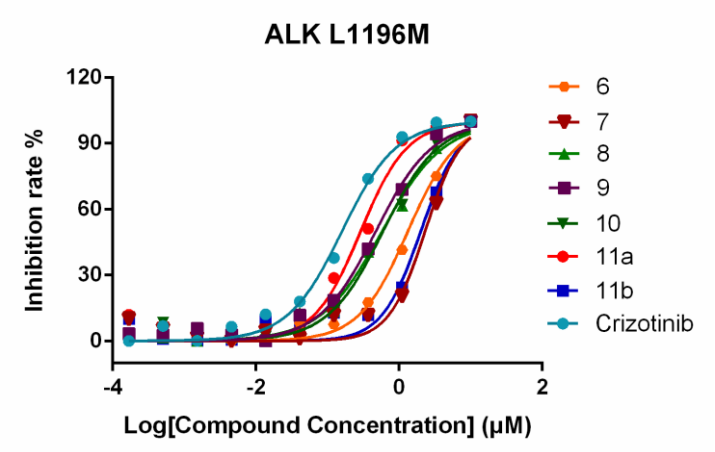
Graphs corresponding to the % of inhibition of ALK WT, ALK C1156Y and ALK L1196M activity by the different compound concentrations were presented in Graphs 2-4. Using the curve and the GraphPad Prism software, the IC_{50} of each compound against ALK kinase activity was calculated.



Graph 2. The curves corresponding to the percentage of ALK WT kinase inhibition according to the concentrations of the different compounds.



Graph 3. The curves corresponding to the percentage of ALK C1156Y kinase inhibition according to the concentrations of the different compounds.



Graph 4. The curves corresponding to the percentage of ALK L1196M kinase inhibition according to the concentrations of the different compounds.

5.3. Cell Inhibition Assays

The 50% inhibition concentration (IC_{50}) of five target compounds and three positive compounds were tested on two cancer cell lines of the human gastric cancer cell line MKN-45 and the human non-small-cell lung cancer (NSCLC) cell line EBC-1 using standard CellTiter-Glo (CTG) assay.

5.3.1. Materials, Reagents and Equipment:

The human gastric cell line MKN-45 and the human NSCLC cell line EBC-1 (CrownBio Bioscicence); CellTiter-Glo Luminescent Cell Viability Assay (Promega, Stored at -20 °C); Culture media and FBS (Gibco-Life Technologies); 96-Well Flat Clear Bottom Black TC-Treated Microplate (Corning); EnVision Multi Label Reader (PerkinElmer); CO_2 Water Jacketed Incubator (Therma); Reverse microscope (Chongguang XDS-1B, Chongqing Guangdian Corp, China).

5.3.2. Methods:

The cells during the logarithmic growth period were harvested and counted using Countstar Automated Cell Counter. The cell concentration was adjusted with cell culture medium. MKN-45 was cultured in RPMI 1640 medium and supplemented with 10% fetal bovine serum (FBS); EBC-1 was cultured in MEM medium and supplemented with 10% fetal bovine serum (FBS); All the cells were cultured at 37 °C in 5% CO_2 and 95% humidity. 90 μL cell suspensions were added to 96-well plates with the designated density. The plates were incubated overnight in humidified incubator at 37 °C in 5% CO_2 and 95% humidity. The test compounds or reference controls were

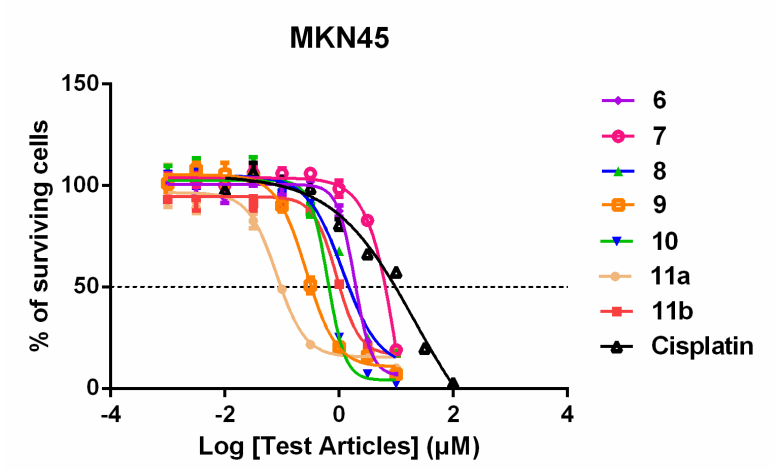
dissolved in DMSO or PBS as stock solution. 1000× solution of test article (10 mM) was prepared using DMSO. The DMSO solution was serial diluted 100× fold with culture medium or PBS to 10× working solution. 10× reference control solution was prepared with culture medium or PBS. 10 µL drug solution (10×) was dispensed to each well (triplicated for each concentration). (DMSO final concentration in culture medium is 0.1% [v/v]). The plates were cultured for 72 h. The plates and its contents were equilibrated at room temperature for approximately 30 min. CellTiter-Glo Reagent equal to the volume of cell culture medium presented in each well was added. The plates and its contents were mixed for 5 min on an orbital shaker to induce cell lysis. The plates were incubated at room temperature for 20 min to stabilize luminescent signal. The luminescence was recorded using EnVision Multi Label Reader.

5.3.3. Data Analysis:

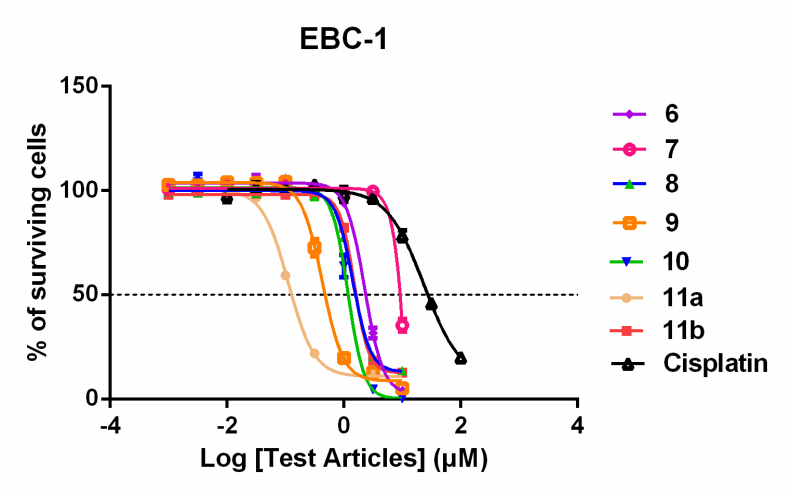
Finally, the percentage of surviving cells following treatment by the different compounds was calculated as follows:

% of surviving cells = $(Lum_{test\ article} - Lum_{medium\ control}) / (Lum_{cell\ control} - Lum_{medium\ control}) \times 100$.

The curves corresponding to the percentage of surviving cells according to the concentrations of each compound were presented in Graphs 5 and 6. The compound concentration that inhibited 50% of the cell growth (IC_{50}) was calculated using the software of GraphPad Prism. The graphical curves were fitted using a nonlinear regression model with a sigmoidal dose response.



Graph 5. The curves corresponding to the percentage of surviving MKN-45 cells according to the concentrations of the different compounds.



Graph 6. The curves corresponding to the percentage of surviving EBC-1 cells according to the concentrations of the different compounds.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://

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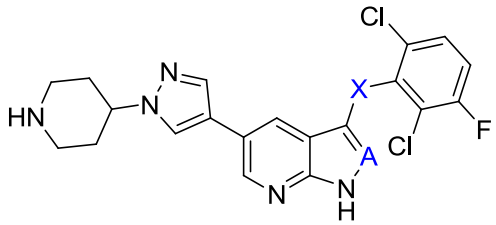
Graphical abstract



A = C-Me, C-Cl, CH, N.

X = CH-Me, S=O, S, $C(CH_2)_2$

Highlights

- Novel 7-azaindole and 7-azaindazole derivatives were designed and synthesized.
- Compound **9** displayed strong c-Met kinase, MKN-45 cell and EBC-1 cell inhibition.
- The synthesis methods of 7-azaindole and 7-azaindazole derivatives were developed.