DR WUFU ZHU (Orcid ID : 0000-0001-6075-0450)

Article type : Research Article

# Discovery of novel pyrrolopyrimidine/pyrazolopyrimidine derivatives bearing 1,2,3-triazole moiety as c-Met kinase inhibitors

Linxiao Wang[1,†], Xiaobo Liu[1,†], Yongli Duan[1], Xiaojing Li[2],Bingbing Zhao[1], Caolin Wang[1], Zhen Xiao[1], Pengwu Zheng, Qidong Tang[1,*],Wufu Zhu[1,*]

[1]*Jiangxi Provincial Key Laboratory of Drug Design and Evaluation,School of Pharmacy,Jiangxi Science & Technology Normal University,Nanchang 330013,P.R. China*

[2]*College of Service,Naval University of Academy of PLA,Tianjin 300450,PR China*

***Correspondence author.**

Tel/Fax: +86 791 8380-2393;

E-mail address:zhuwf@jxstnu.edu.cn, zhuwufu-1122@163.com (W. Zhu).tangqidongcn@126.com

**Abstract:** Six series of pyrrolo[2,3-*d*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine derivatives bearing 1,2,3-triazole moiety were designed, synthesized, and some bio-evaluation were also carried out. As a result, four points can be summarized: Firslty, some of compounds exhibited excellent cytotoxicity activity and selectivity with the $IC_{50}$ values in single-digit μM level. Especially, the most promising compound **16d** showed equal activity to lead compound Foretinib against A549, HepG2 and MCF-7 cell lines, with the $IC_{50}$ values of 4.79 ± 0.82 μM, 2.03 ± 0.39 μM and 2.90 ± 0.43 μM, respectively. Secondly, The SARs and docking studies indicated that the *in vitro* antitumor activity of pyrrolo[2,3-*d*]pyrimidine derivatives bearing 1,2,3-triazole moiety was superior to the pyrazolo[3,4-*d*]pyrimidine derivatives bearing 1,2,3-triazole moiety. Thirdly, Three selected compounds (**16d**, **18d** and **20d**) were further evaluated for inhibitory activity against the c-Met kinase, and the 16d could inhibit the c-Met kinase selectively by experiments of enzyme-based selectivity. What's more, **16d** could induce apoptosis of HepG2 cells and inhibitor the cell cycle of HepG2 on G2/M phase by acridine orange (AO) staining and cell cycle

experiments, respectively.

**Keywords:** Pyrrolopyrimidine/pyrazolopyrimidine, 1,2,3-triazole, Antitumor activity, Apoptosis and Cell cycle, c-Met, Docking Study[1]

## 1. Introduction

c-Met, the receptor tyrosine kinase encoded by MET proto oncogene located on chromosome 7b and 7q21–q31 that encoded a 1408 amino acid transmembrane glycoprotein[1] . c-Met is expressed in both normal and malignant cells which is an epithelial/endothelial cell surface transmembrane receptor with specificity for hepatocyte growth factor (HGF) or also known as scatter factor (SF) [1-3].

c-Met inhibitors, a class of small molecules which can inhibit the enzymatic activity of the c-Met kinase and have therapeutic application in the treatment of various types of cancers[4]. Cabozantinib, the first small-molecule c-Met inhibitor, was approved by FDA on November 29, 2012. In recent years, many Cabozantinib derivatives were reported, such as Foretinib, compounds **3** and **4** (The structures were shown in Fig.1[5-7]).

Many researches showed that nearly all the Cabozantinib derivatives show excellent activity and contain two obvious structural characteristics ( Foretinib was used as a template): moiety A and C were belonged to the HD/HR (Hydrogen bond donor or Hydrogen bond receptor) structure, while the moiety B and D were belong to HI (Hydrophobic interaction) structure (Fig.1, Fig.2). And many bioactivity structure have been reported that introduced into the moiety A, such as substituted quinoline, substituted pyridine and pyrrolo[2,3-*b*]pyridine series (**1**-**2**, **3** and **4**, respectively). But, nearly all the Cabozantinib derivatives reserve the moiety C (a 5-atom linker) which has two obvious structural characteristics. One is the '5 atoms regulation', which means six chemical bonds distance existing between moiety B and moiety D; the other one is that the 5-atom linker containing HR/HD structure which could form hydrogen-bond with the c-Met. [8-9] Furthermore, the moiety B and moiety D may form hydrophobic bond bound to c-Met preferably.

In our previous research, several series of pyrrolo[2,3-*b*]pyridine derivatives (compounds **5** and **6**, Fig.1) were designed and synthesized as potent c-Met inhibitors according to the '5 atom regulation'.[10-11]

† These authors contribute equally to this work

*Corresponding author. Tel./fax: +86 791 83802393.

E-mail address: zhuwf@jxstnu.edu.cn, zhuwufu-1122@163.com (W. Zhu), tangqidong@126.com

Most of these compounds exhibited potent activity, especially the most promising compound **6** with the $IC_{50}$ values in the nanomole level. What's more, the triazole also contain other boiactivity, such as anti-vaccinia, adenovirus and HIV-1 inhibitors and can be prepared in a high yield and without azide isolation or purification**.**[12-17] These results show that 1,2,3-triazole moiety may be beneficial to the *in vitro* activity.

In continue to our previous work and inspired by compound **6**, further modification was concentrated on pyrrolo[2,3-*b*]pyridine moiety. Firstly, reserving the 1,2,3-triazole scaffold of compound **6** and replacing the pyrrolo[2,3-*b*]pyridine moiety with pyrrolo[2,3-*d*]pyrimidine moiety to study the effects of increasing the hydrogen receptor to the compounds activity and the first two serials compounds (**15a-f** and **16a-f** ) were prepared. And then the methyl group was introduced to *N*-7 position of pyrrolo[2,3-*d*]pyrimidine moiety to investigate the effects of electron donor effect to the target compounds and the second two series of compounds were obtained. Thus, four series of target compounds **15a-f**, **16a-f**, **17a-f** and **18a-f** were achieved. Furthermore, pyrrolo[2,3-*d*]pyrimidine moiety was further modified with pyrazolo[3,4-*d*]pyrimidine to study the effects of pyrrolopyrimidine /pyrazolopyrimidine scaffold to activity. As a result, the latter two series of compounds **19a-f** and **20a-f** were prepared. And the design strategy for all target compounds was described in Fig.2.

Herein we disclosed the synthesis and antitumor activity of pyrrolo[2,3-*d*]pyrimidine or pyrazolo[3,4-*d*]pyrimi -dine bearing 1,2,3-triazole moiety against A549 (human lung cancer), HepG2 (human liver cancer), MCF-7 (human breast cancer) cancer cell lines, and c-Met kinase. Moreover, experiments of enzyme-based selectivity, acridine orange single staining, cell cycle and docking studies were presented within this paper as well.

(**Fig.1 should be here**)

(**Fig. 2 should be here**)

## 2. Materials and Methods

### *2.1 chemistry*

#### *2.1.1 Reagents and materials*

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were 4 uncorrected. NMR spectra were performed using Bruker 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LCMS (Agilent, Palo Alto, CA, USA). All the materials

were obtained from commercial suppliers and used without purification, unless otherwise specified.TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). All the materials were obtained from commercial suppliers and used without purification, unless otherwise specified.

*2.1.2 Synthesis*

**General procedure for the key intermediate 9a-f**

4-Aminophenol or 2-fluoro-4-aminophenol **8a-b** (0.1mol) which was dissolved in tetrahydrofuran (50ml), was added to a 1,4-dioxane/$H_2O$ (50ml, 5:1) solution of compounds **7a-c** (0.1mol), sodium carbonate and hydrogen sodium at 80℃ for 2 hours. Then the solution was concentrated in vacuum and washed with water, filtered to give a solid .

**4-((1*H*-pyrrolo[3,4-*d*]pyrimidine-4-yl)oxy)aniline (9a)**

Light yellow solid; Yield: 61%; M.P.: 186-188℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.09 (s, 1H), 8.26 (s, 1H), 7.39 – 7.33 (m, 1H), 6.87 (d, *J* = 8.7 Hz, 2H), 6.59 (d, *J* = 8.7 Hz, 2H), 6.23 (dd, *J* = 3.3, 1.7 Hz, 1H), 5.04 (s, 2H) ; MS (ESI) m/z(%):227.09 $[M+H]^+$.

**3-Fluoro-4-((1*H*-pyrrolo[3,4-*d*]pyrimidine-4-yl)oxy)aniline (9b)**

Light yellow solid; Yield: 56%; M.P.: 189-191℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.17 (s, 1H), 8.26 (s, 1H), 7.43 (s, 1H), 6.99 (t, *J* = 8.9 Hz, 1H), 6.44 (dd, *J* = 8.7, 5.4 Hz, 3H), 5.34 (s, 2H) ; MS (ESI) m/z(%):245.08$[M+H]^+$.

**4-((1-Methyl-1*H*-pyrrolo[3,4-*d*]pyrimidine-4-yl)oxy)aniline (9c)**

Light yellow solid; Yield: 58%; M.P.: 193-195℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.41 (s, 1H), 7.53 (d, *J* = 3.5 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 2H), 6.69 (d, *J* = 8.7 Hz,2H), 6.34 (d, *J* = 3.5 Hz, 1H), 5.17 (s, 2H), 3.89 (s,3H) ; MS (ESI) m/z(%):241.10 $[M+H]^+$.

**3-Fluoro-4-((1-methyl-1*H*-pyrrolo[3,4-*d*]pyrimidine-4-yl)oxy)aniline (9d)**

Light yellow solid; Yield: 59%; M.P.: 194-197℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.50 (t, *J* = 3.2 Hz, 1H), 8.47 (s, 1H), 8.32 (d, *J* = 9.0 Hz, 1H), 7.89 (t, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 3.5 Hz,1H), 6.82 (d, *J* = 3.5 Hz,1H), 5.27 (s, 2H), 3.96 (s,3H) ; MS (ESI) m/z(%):259.10 $[M+H]^+$.

**4-((1-Methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)aniline(9e)**

Light yellow solid; Yield: 55%; M.P.: 201-202℃;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.54 (s, 1H), 7.67 (s, 1H), 6.96 (d, *J* = 8.7 Hz,2H), 6.64 (d, *J* = 8.7 Hz, 2H), 5.20 (s, 2H), 4.02 (s, 3H) ;MS (ESI) m/z(%):242.10 $[M+H]^+$.

**3-Fluoro-4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)aniline(9f)**

Light yellow solid; Yield: 58%; M.P.: 202-205℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.46 (s, 1H), 7.99 (s, 1H), 6.98 (t, *J* = 8.9 Hz, 1H), 6.42 (d, *J* = 10.8 Hz, 1H), 6.35 (d, *J* = 8.7 Hz, 1H), 5.36 (s, 2H), 3.95 (s, 3H) ;MS (ESI) m/z(%):259.11 $[M+H]^+$.

**General procedure for preparation of compounds 12a-f.**

The intermediates 1-aryl-5-methyl (or trifluoromethyl)-1,4,5-trisubstituted-1,2,3-triazoles **12a-f** were synthesized in high yield by a one-pot three-component reaction of arylboronic acids **10a-g**, sodium azide,and active methylene ketones, such as ethyl acetoacetate (or ethyl 4,4,4-trifluoroacetoacetate) **11a-b** in the presence of $Cu(OAc)_2$ and piperidine using a DMSO/$H_2O$ (10:1) mixture as solvent. [14-15] The reaction was monitored by TLC until completed. Finally the solution was filtered and washed with a plenty of water to give a white solid.

**General procedure for preparation of 13a-f**

Appropriate intermediate **12a-f** (0.11 mol), sodium carbonate (0.2 mol) were dissolved in Ethanol/$H_2O$ (50ml, 5:1) ,and heated to 80℃ for 5-6 hour. Subsequently, the mixture solution acidified to pH 2-3 to yield the compounds **13a-f**, respectively.

**General procedure for the key intermediate 14a-f**

The compounds **13a-f**（0.001 mol）and appropriate DMF (0.0001 mol) were dissolved in dichloromethane, then appropriate oxalyl chloride was added slowly and monitored by TLC. The solution was used for next step without further purification.

**General procedure for the preparation of target compounds 15a-f, 16a-f, 17a-f, 18a-f, 19a-f and 20a-f.**

A solution of phenylpyrdazinone carbonyl chloride **14a-f** (0.82 mmol) in dichloromethane (10 mL) was added drop-wise to a solution of aniline **9a-f** (0.41 mmol) and diisopropylethylamine (0.49 mmol) in dichloromethane (10 mL) in an ice bath. Upon completion of the addition, the reaction mixture was removed from the ice bath and placed in room temperature for 30 min and monitored by TLC. The mixture was concentrated in vacuum to yield **15a-f**, **16a-f**, **17a-f**, **18a-f**, **19a-f** and **20a-f** which were recrystallized by isopropanol.

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-1-(3-chloro-4-fluorophenyl)-5-(trifluoromethyl)-1*H*- 1,2,3-triazole-4-carboxamide (15a)**

Yield: 62%; M.P.: 206–207℃; ESI-MS m/z:518.06 $[M+H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.11（s，1H）,11.13 (s, 1H), 8.33 (s, 1H), 8.21 (dd, *J* = 6.4, 2.4 Hz, 1H), 7.89 (d, *J* = 9.0 Hz, 2H), 7.86 – 7.83 (m, 1H), 7.76 (t, *J* = 8.9 Hz, 1H), 7.51 (d, *J* = 3.4 Hz, 1H), 7.26 (d, *J* = 8.9 Hz, 2H), 6.51 (d, *J* = 3.4 Hz, 1H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 161.66, 160.00, 159.97, 157.46, 156.39, 152.45, 150.05, 148.96, 146.53, 142.09, 135.27, 132.12, 129.20, 127.95, 122.18(2C), 121.81(2C), 118.02, 117.79, 104.71, 97.37.

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-1-(3,4-difluorophenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide (15b)**

Yield: 64%; M.P.: 206–208℃; ESI-MS m/z:501.10 $[M+H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.11（s，1H）,11.13 (s, 1H), 8.33 (s, 1H), 8.10 (t, *J* = 7.7 Hz, 2H), 7.80 (dd, *J* = 18.5, 9.1 Hz, 2H), 7.72 (s, 1H), 7.51 (d, *J* = 3.4 Hz, 1H), 7.26 (d, *J* = 8.8 Hz, 2H), 6.51 (d, *J* = 3.4 Hz, 1H).

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-1-(4-chlorophenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide (15c)**

Yield: 62%; M.P.: 203–205℃; ESI-MS m/z:500.07 $[M+H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.14（s，1H）,11.11 (s, 1H), 8.34 (s, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.82 – 7.74 (m, 4H), 7.51 (d, *J* = 3.3 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.50 (d, *J* = 3.3 Hz, 1H) .

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-1-(4-fluorophenyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide (15d)**

Yield: 65%; M.P.: 207–208℃; ESI-MS m/z:429.14 $[M+H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.21（s，1H）,10.78 (s, 1H), 8.45 (s, 1H), 8.03 (d, *J* = 8.9 Hz, 2H), 7.86 (dd, *J* = 8.8, 4.9 Hz, 2H), 7.62 (dd, *J* = 10.6, 6.8 Hz, 3H), 7.34 (d, *J* = 8.9 Hz, 2H), 6.59 (d, *J* = 3.4 Hz, 1H), 3.93 (s, 3H), 2.60 (s, 3H).

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-1-phenyl-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**15e**)

Yield: 63%; M.P.: 210–211℃; ESI-MS m/z:411.15 $[M+H]^+$ ; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.13（s，1H）,10.78 (s, 1H), 8.45 (s, 1H), 8.03 (d, *J* = 8.9 Hz, 2H), 7.76 (d, *J* = 6.9 Hz, 5H), 7.62 (d, *J* = 3.4 Hz, 1H), 7.34 (d, *J* = 8.9 Hz, 2H), 6.59 (d, *J* = 3.5 Hz, 1H), 2.69 (s,3H).

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1-(2-(trifluoromethyl)phenyl)-1*H*- 1,2,3-triazole-4-carboxamide** (**15f**)

Yield: 67%; M.P.: 213–215℃; ESI-MS m/z:533.11 $[M+H]^+$ ; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.12（s，1H）, δ 11.30 (s, 1H), 8.45 (s, 1H), 8.25 (d, *J* = 7.2 Hz, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 8.17 – 8.08 (m, 2H), 8.01 (d, *J* = 8.9 Hz, 2H), 7.63 (d, *J* = 3.4 Hz, 1H), 7.38 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 3.5 Hz, 1H).

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)-3-fluorophenyl)-1-(3-chloro-4-fluorophenyl)-5-(trifluoro- methyl)-1*H*-1,2,3-triazole-4-carboxamide** (**16a**)

Yield: 71%; M.P.: 209–211℃; ESI-MS m/z: 536.06 $[M+H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.14（s，1H）,11.26 (s, 1H), 8.27 (s, 1H), 8.06 (d, *J* = 10.2 Hz, 1H), 7.88 (d, *J* = 12.8 Hz, 1H), 7.79 – 7.72 (m, 1H), 7.67 (d, *J* = 6.2 Hz, 2H), 7.51 (d, *J* = 3.5 Hz, 1H), 7.45 – 7.40 (m, 1H), 6.58 (d, *J* = 3.5 Hz, 1H)

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)-3-fluorophenyl)-1-(3,4-difluorophenyl)-5-(trifluoro-methyl)-1*H*-1,2,3-triazole-4-carboxamide (16b)**

Yield: 67%; M.P.: 213–214℃; ES I-MS m/z: 519.09 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ12.28 (s, 1H),11.26 (s, 1H), 8.27 (s, 1H), 8.05 (t, *J* = 8.9 Hz, 1H), 7.88 (d, *J* = 12.6 Hz, 1H), 7.76 – 7.70 (m, 1H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 3.5 Hz, 1H), 7.44 – 7.40 (m, 1H), 6.58 (d, *J* = 3.4 Hz, 1H).

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)-3-fluorophenyl)-1-(4-chlorophenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide (16c)**

Yield: 72%; M.P.: 211–212℃; ES I-MS m/z:518.06 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.29 (s, 1H), 11.27 (s, 1H), 8.29 (s, 1H), 7.93 (d, *J* = 12.9 Hz, 1H), 7.82 – 7.75 (m, 4H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.51 (t, *J* = 2.9 Hz, 1H), 7.45 (t, *J* = 8.9 Hz, 1H), 6.61 (s, 1H)

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)-3-fluorophenyl)-1-(4-fluorophenyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide (16d)**

Yield: 58%; M.P.: 218–219℃; ESI-MS m/z: 447.13 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) $^1$H NMR (400 MHz, ) δ 12.27 (s, 1H), 10.83 (s, 1H), 8.29 (s, 1H), 7.97 (d, *J* = 12.8 Hz, 1H), 7.76 (d, *J* = 8.8 Hz, 1H), 7.66 (m, 4H), 7.50 (s, 1H), 7.40 (t, *J* = 8.9 Hz, 1H), 6.59 (s, 1H), 2.58 (s, 3H).; $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 160.43, 159.18, 154.22, 152.02, 151.79, 149.45, 137.47, 136.75, 134.73, 129.58, 129.20, 129.09(2C), 124.95(2C), 123.62, 116.20, 108.15, 103.69, 101.01, 96.70, 8.97.

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)-3-fluorophenyl)-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide (16e)**

Yield: 69%; M.P.: 214–216℃; ES I-MS m/z: 429.14 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.28 (s, 1H), 10.84 (s, 1H), 8.29 (s, 1H), 7.98 (d, *J* = 13.3 Hz, 1H), 7.76 (d, *J* = 9.2 Hz, 1H), 7.70 – 7.57 (m, 5H), 7.50 (s, 1H), 7.40 (t, *J* = 9.0 Hz, 1H), 6.60 (s, 1H), 2.58 (s, 3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 161.76, 160.81, 158.59, 153.54, 152.40, 149.94, 142.85, 141.29, 136.96, 129.68, 127.91(2C), 125.57, 124.46, 116.47, 115.52(2C), 108.34, 104.02, 101.50, 97.77, 9.46.

***N*-(4-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)-3-fluorophenyl)-5-(trifluoromethyl)-1-(2-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (16f)**

Yield:65%; M.P.: 217–219℃; ES I-MS m/z: 551.10 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.40 (s, 1H), 11.44 (s, 1H), 8.41 (s, 1H), 8.25 (d, *J* = 7.6 Hz, 1H), 8.21 (d, *J* = 7.5 Hz, 1H), 8.17 – 8.08 (m, 2H), 8.05 (d, *J* = 12.9 Hz, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.63 (s, 1H), 7.57 (t, *J* = 8.7 Hz, 1H), 6.72 (s, 1H).

**1-(3-Chloro-4-fluorophenyl)-*N*-(4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoro-methyl)-1*H*-1,2,3-triazole-4-carboxamide** (**17a**)

Yield: 59%; M.P.: 213–215℃; ES I-MS m/z: 532.08 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.13 (s, 1H), 8.33 (s, 1H), 8.21 (dd, *J* = 6.4, 2.4 Hz, 1H), 7.89 (d, *J* = 9.0 Hz, 2H), 7.86 – 7.83 (m, 1H), 7.76 (t, *J* = 8.9 Hz, 1H), 7.51 (d, *J* = 3.4 Hz, 1H), 7.26 (d, *J* = 8.9 Hz, 2H), 6.51 (d, *J* = 3.4 Hz, 1H), 3.82 (s, 3H).

**1-(3,4-Difluorophenyl)-*N*-(4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**17b**)

Yield: 63%; M.P.: 217–219℃; ES I-MS m/z: 515.12 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.13 (s, 1H), 8.33 (s, 1H), 8.10 (t, *J* = 7.7 Hz, 2H), 7.80 (dd, *J* = 18.5, 9.1 Hz, 1H), 7.72 (s, 1H), 7.51 (d, *J* = 3.4 Hz, 1H), 7.26 (d, *J* = 8.8 Hz, 2H), 6.51 (d, *J* = 3.4 Hz, 1H), 3.82 (s, 3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 161.66, 160.10, 156.40, 152.45, 150.05, 148.95, 142.11, 135.27, 131.60, 129.27, 124.45, 122.20(2C), 121.78(2C), 118.63, 118.44, 117.06, 116.85, 104.69, 101.49, 97.37, 39.90, 31.18.

**1-(4-Chlorophenyl)-*N*-(4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**17c**)

Yield: 65%; M.P.: 220–221℃; ES I-MS m/z: 514.09 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.11 (s, 1H), 8.34 (s, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.82 – 7.74 (m, 4H), 7.51 (d, *J* = 3.3 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.50 (d, *J* = 3.3 Hz, 1H), 3.82 (s, 3H).

**1-(4-Fluorophenyl)-5-methyl-*N*-(4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-1*H*-1,2,3-triazole-4-carboxamide** (**17d**)

Yield: 71%; M.P.: 218–219℃; ES I-MS m/z: 443.15 $[M+H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.78 (s, 1H), 8.45 (s, 1H), 8.03 (d, *J* = 8.9 Hz, 2H), 7.86 (dd, *J* = 8.8, 4.9 Hz, 2H), 7.62 (dd, *J* = 10.6, 6.8 Hz, 3H), 7.34 (d, *J* = 8.9 Hz, 2H), 6.59 (d, *J* = 3.4 Hz, 1H), 3.93 (s, 3H), 2.60 (s, 3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 161.26, 160.82, 158.92, 152.01, 149.59, 148.00, 137.43, 135.32, 131.18, 128.69, 127.53(2C), 121.41(2C), 121.16(2C), 116.27(2C), 104.24, 101.00, 96.92, 30.67, 8.86.

**5-Methyl-*N*-(4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide** (**17e**)

Yield: 69%; M.P.: 215–218℃; ES I-MS m/z: 425.16 [M+H] $^{+}$;$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 10.78 (s, 1H), 8.45 (s, 1H), 8.03 (d, *J* = 8.9 Hz, 2H), 7.76 (d, *J* = 6.9 Hz, 5H), 7.62 (d, *J* = 3.4 Hz, 1H), 7.34 (d, *J* = 8.9 Hz, 2H), 6.59 (d, *J* = 3.5 Hz, 1H), 3.93 (s, 3H), 2.69 (s,3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 161.75, 159.45, 152.43, 150.08, 148.38, 138.17, 137.66, 135.86, 135.25, 129.69(2C), 129.19, 125.43(2C), 122.55, 121.97(2C), 121.63(2C), 104.68, 97.40, 39.49, 31.18, 9.46.

***N*-(4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1-(2-(trifluoromethyl)**

**phenyl)-1*H*-1,2,3-triazole-4-carboxamide** (**17f**)

Yield: 72%; M.P.: 224–225℃; ES I-MS m/z: 547.12 [M+H] $^{+}$;$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 11.29 (s, 1H), 8.45 (s, 1H), 8.25 (d, *J* = 7.2 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.15 – 8.08 (m, 2H), 8.01 (d, *J* = 8.9 Hz, 2H), 7.63 (d, *J* = 3.4 Hz, 1H), 7.38 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 3.5 Hz, 1H), 3.93 (s, 3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 161.16, 155.61, 152.00, 149.58, 148.60, 141.49, 134.67, 133.80, 132.38, 131.53, 129.52, 128.77, 127.20, 124.76, 123.38, 121.65(2C), 121.44(2C), 119.53, 104.24, 101.00, 96.89, 30.68.

**1-(3-Chloro-4-fluorophenyl)-*N*-(3-fluoro-4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifl--uoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**18a**)

Yield: 65%; M.P.: 213–214℃; ES I-MS m/z: 550.07 [M+H] $^{+}$;$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 11.26 (s, 1H), 8.27 (s, 1H), 8.16 (dd, *J* = 6.5, 2.5 Hz, 1H), 7.88 (dd, *J* = 12.7, 2.2 Hz, 1H), 7.83 – 7.77 (m, 1H), 7.70 (dd, *J* = 16.2, 7.7 Hz, 2H), 7.51 (d, *J* = 3.5 Hz, 1H), 7.39 (t, *J* = 8.8 Hz, 1H), 6.58 (d, *J* = 3.5 Hz, 1H), 3.77 (s, 3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 160.34, 159.50, 157.00, 156.11, 154.27, 152.03, 149.41, 141.25, 136.08, 135.17, 131.60, 129.16, 128.70, 127.36, 123.88, 117.51, 117.28, 116.43, 108.55, 108.32, 103.68, 96.68, 30.74.

**1-(3,4-Difluorophenyl)-*N*-(3-fluoro-4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**18b**)

Yield: 63%; M.P.: 214–216℃; ES I-MS m/z: 533.11 [M+H] $^{+}$;$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 11.26 (s,

1H), 8.27 (s, 1H), 8.05 (t, *J* = 8.9 Hz, 1H), 7.88 (d, *J* = 12.6 Hz, 1H), 7.76 – 7.70 (m, 1H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 3.5 Hz, 1H), 7.39 (t, *J* = 8.9 Hz, 1H), 6.58 (d, *J* = 3.4 Hz, 1H), 3.74 (s, 3H).

**1-(4-Chlorophenyl)-*N*-(3-fluoro-4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoro-**

**methyl)-1*H*-1,2,3-triazole-4-carboxamide** (**18c**)

Yield: 71%; M.P.: 217–218℃; ES I-MS m/z: 532.08 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.24 (s, 1H), 8.27 (s, 1H), 7.88 (d, *J* = 12.4 Hz, 1H), 7.73 (m, 3H), 7.70 – 7.64 (m, 2H), 7.51 (d, *J* = 3.5 Hz, 1H), 7.39 (t, *J* = 8.9 Hz, 1H), 6.58 (d, *J* = 3.4 Hz, 1H), 3.77 (s, 3H).

***N*-(3-fluoro-4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-1-(4-fluorophenyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide** (**18d**)

Yield: 66%; M.P.: 219–220℃; ES I-MS m/z: 461.14 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.88 (s, 1H), 8.34 (s, 1H), 7.98 (dd, *J* = 13.0, 2.3 Hz, 1H), 7.79 – 7.72 (m, 2H), 7.56 (d, *J* = 3.5 Hz, 1H), 7.52 (dd, *J* = 11.9, 5.5 Hz, 3H), 7.40 (t, *J* = 8.9 Hz, 1H), 6.63 (d, *J* = 3.5 Hz, 1H), 3.83 (s, 3H), 2.56 (s, 3H).

***N*-(3-fluoro-4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide** (**18e**)

Yield: 67%; M.P.: 218–221℃; ES I-MS m/z: 443.15 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.87 (s, 1H), 8.34 (s, 1H), 7.98 (dd, *J* = 13.0, 2.2 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.73 – 7.61 (m, 5H), 7.56 (d, *J* = 3.5 Hz, 1H), 7.41 (t, *J* = 9.0 Hz, 1H), 6.63 (d, *J* = 3.5 Hz, 1H), 3.83 (s, 3H), 2.58 (s, 3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 160.43, 159.18, 154.22, 152.02, 151.79, 149.45, 137.47, 136.75, 134.73, 129.58, 129.20(2C), 129.09, 124.95(2C), 123.62, 116.18, 108.07, 103.69, 101.00, 96.70, 30.74, 8.97.

***N*-(3-fluoro-4-((7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1-(2-( trifluoromethyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide** (**18f**)

Yield: 69%; M.P.: 221–224℃; ES I-MS m/z: 565.11 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.36 (s, 1H), 8.34 (s, 1H), 8.12 (dd, *J* = 14.0, 7.4 Hz, 2H), 8.07 – 7.90 (m, 3H), 7.74 (d, *J* = 8.1 Hz, 1H), 7.56 (s, 1H), 7.46 (t, *J* = 8.8 Hz, 1H), 6.64 (d, *J* = 3.4 Hz, 1H), 3.83 (s, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 160.34, 155.82, 154.25, 152.04, 151.82, 149.43, 141.12, 136.07, 135.36, 135.24, 133.80, 132.41, 131.82, 131.49,

129.51, 129.18, 127.21, 123.88, 116.56, 108.69, 108.46, 103.69, 96.68, 30.75.

**1-(3-Chloro-4-fluorophenyl)-*N*-(4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**19a**).

Yield: 57%; M.P.: 221–225℃; ES I-MS m/z: 533.08 $[M+H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.17 (s, 1H), 8.54 (s, 1H), 8.25 – 8.19 (m, 1H), 8.17 (s, 1H), 7.93 (d, *J* = 8.9 Hz, 2H), 7.89 – 7.83 (m, 1H), 7.76 (t, *J* = 8.9 Hz, 1H), 7.34 (d, *J* = 8.9 Hz,2H), 4.04 (s, 3H).

**1-(3,4-Difluorophenyl)-*N*-(4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**19b**)

Yield: 64%; M.P.: 223–224℃; ES I-MS m/z: 516.11 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.10 (s, 1H), 8.47 (s, 1H), 8.10 (s, 1H), 8.04 (dd, *J* = 10.0, 7.0 Hz, 1H), 7.86 (d, *J* = 8.7 Hz, 2H), 7.73 (q, *J* = 9.1 Hz, 1H), 7.65 (s, 1H), 7.27 (d, *J* = 8.7 Hz, 2H), 3.97 (s, 3H).

**1-(4-Chlorophenyl)-*N*-(4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**19c**)

Yield: 65%; M.P.: 225–226℃; ES I-MS m/z: 515.09 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.08 (s, 1H), 8.47 (s, 1H), 8.10 (s, 1H), 7.85 (d, *J* = 8.9 Hz, 2H), 7.72 (dd, *J* = 8.2, 2.8 Hz, 4H), 7.27 (d, *J* = 8.7 Hz, 2H), 3.97 (s, 3H).

**1-(4-Fluorophenyl)-5-methyl-*N*-(4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-1*H*-1,2,3-triazole-4-carboxamide** (**19d**)

Yield: 69%; M.P.: 224–226℃; ES I-MS m/z: 444.15 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.63 (s, 1H), 8.47 (s, 1H), 8.05 (s, 1H), 7.89 (d, *J* = 8.9 Hz, 2H), 7.68 (dd, *J* = 8.8, 5.0 Hz, 2H), 7.44 (t, *J* = 8.7 Hz, 2H), 7.23 (d, *J* = 8.9 Hz, 2H), 3.97 (s, 3H).

**5-Methyl-*N*-(4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide** (**19e**).

Yield: 65%; M.P.: 226–228℃; ES I-MS m/z: 426.16 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.62 (s, 1H), 8.48 (s, 1H), 8.04 (s, 1H), 7.89 (d, *J* = 9.0 Hz, 2H), 7.62 – 7.51 (m, 5H), 7.23 (d, *J* = 8.9 Hz, 2H), 4.01 (s, 3H).

***N*-(4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1-(2-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide** (**19f**).

Yield: 66%; M.P.: 227–229℃; ES I-MS m/z: 548.12 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.14 (s, 1H), 8.48 (s, 1H), 8.10 – 8.00 (m, 2H), 7.94 (p, *J* = 7.5 Hz, 3H), 7.88 – 7.84 (m, 2H), 7.28 (d, *J* = 9.0 Hz, 2H), 3.97 (s, 3H).

**1-(3-Chloro-4-fluorophenyl)-*N*-(3-fluoro-4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**20a**).

Yield: 67%; M.P.: 227–228℃; ES I-MS m/z: 551.07 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.38 (s, 1H), 8.58 (s, 1H), 8.42 (s, 1H), 8.24 (dd, *J* = 6.6, 2.6 Hz, 1H), 8.01 (dd, *J* = 12.7, 2.4 Hz, 1H), 7.89 (q, *J* = 4.2 Hz, 1H), 7.82 – 7.74 (m, 2H), 7.54 (t, *J* = 8.8 Hz, 1H), 4.09 (s, 3H).

**1-(3,4-Difluorophenyl)-*N*-(3-fluoro-4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**20b**)

Yield: 65%; M.P.: 225–227℃; ES I-MS m/z: 534.10 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.37 (s, 1H), 8.57 (s, 1H), 8.41 (s, 1H), 8.13 (s, 1H), 7.99 (d, *J* = 12.3 Hz, 1H), 7.79 (dt, *J* = 17.7, 9.4 Hz, 3H), 7.53 (t, *J* = 8.9 Hz, 1H), 4.08 (s, 3H).

**1-(4-Chlorophenyl)-*N*-(3-fluoro-4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide** (**20c**)

Yield:68%; M.P.: 230–231℃; ES I-MS m/z: 533.08 $[M+H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.35 (s, 1H), 8.58 (s, 1H), 8.41 (s, 1H), 8.11 (s, 1H), 7.99 (d, *J* = 12.8 Hz, 1H), 7.84 – 7.69 (m, 4H), 7.53 (t, *J* = 8.9 Hz, 1H), 4.08 (s, 3H).

***N*-(3-fluoro-4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-1-(4-fluorophenyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide** (**20d**).

Yield: 67%; M.P.: 223–225℃; ES I-MS m/z: 462.14 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.94 (s, 1H), 8.58 (s, 1H), 8.39 (s, 1H), 8.04 (d, *J* = 12.8 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.79 – 7.73 (m, 1H), 7.51 (dt, *J* = 17.6, 8.8 Hz, 3H), 4.07 (s, 3H), 2.58 (s, 3H).

***N*-(3-fluoro-4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide** (**20e**).

Yield: 72%; M.P.: 226–228℃; ES I-MS m/z: 444.15 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.94 (s, 1H), 8.58 (s, 1H), 8.40 (s, 1H), 8.04 (d, *J* = 13.0 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.68 (s, 5H), 7.49 (t, *J* = 8.6 Hz, 1H), 4.08 (s, 3H), 2.60 (s, 3H).

***N*-(3-fluoro-4-((1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)phenyl)-5-(trifluoromethyl)-1-(2-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide** (**20f**)

Yield: 67%; M.P.: 228–231℃; ES I-MS m/z: 566.11 $[M+H]^+$;$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.42 (s, 1H), 8.58 (s, 1H), 8.41 (s, 1H), 8.14 (dd, *J* = 13.9, 7.5 Hz, 2H), 8.07 – 7.97 (m, 3H), 7.78 (d, *J* = 9.0 Hz, 1H), 7.54 (t, *J* = 8.9 Hz, 1H), 4.08 (s, 3H).

*2.2 Biology*

*2.2.1 Cytotoxicity assay in vitro*

The cytotoxic activities of target compounds (**15a-f**, **16a-f**, **17a-f**, **18a-f**, **19a-f** and **20a-f**) were evaluated with A549, HepG2 and MCF-7cell lines by the standard MTT assay *in vitro*, with compounds c-MET inhibitors Foretinib as positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately $4\times10^3$ 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 test compounds at 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 5μg/mL and incubated with cells at 37°C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All the compounds were tested three times in each of the cell lines. The results expressed as inhibition rates or $IC_{50}$ (half-maximal inhibitory concentration) were the averages of two determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

*2.2.2 Tyrosine kinases assay in vitro*

The selected compounds ( **16d**, **18d** and **20d** ) are tested for their activity against one or several tyrosine kinases (c-Met, Flt-3, VEGFR-2, c-Kit and EGFR kinases) through the mobility shift assay.[18,19] (The rationale of measuring the activity of different types of tyrosine kinases : the tyrosine kinase can catalyze the phosphorylation of the substrate, and this process requires the involvement of ATP. Then the activity of the sample enzyme was estimated by making a standard curve after measuring the radioactivity of the reaction product**.)** All kinase assays were performed in 96-well plates in a 50 μL reaction volume. The kinase buffer contains 50 mM HEPES, pH 7.5, 10 mM MgCl 2, 0.0015% Brij-35 and 2 mM DTT. The stop buffer contains 100 mM HEPES, pH 7.5, 0.015% Brij-35, 0.2% Coating Reagent #3 and 50 mM EDTA. Dilute the compounds to 500μM by 100% DMSO, then transfer 10 μL of compound to a new 96-well plate as the intermediate plate, add 90μL kinase buffer to each well. Transfer 5 μL of each well of the intermediate plate to 384-well plates. The following amounts of enzyme and substrate were used per well: kinase base buffer, FAM-labeled peptide, ATP and enzyme solution. Wells containing the substrate, enzyme, DMSO without compound were used as DMSO control. Wells containing just the substrate without enzyme were used as low control. Incubate at room temperature for 10 min. Add 10μL peptide solution to each well. Incubate at 28℃ for specified period of time and stop reaction by 25 μL stop buffer. At last collect data on Caliper program and convert conversion values to inhibition values. Percent inhibition = (max - conversion)/(max - min) × 100. ‘max’ stands for DMSO control, ‘min’ stands for low control.

*2. 3 Acridine orange (AO) single staining*

The cancer cell apoptotic of target compounds **16d** were evaluated with HepG2 cancer cell lines by acridine orange (AO) single staining. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately $2\times10^3$ cells, suspended in MEM medium, were plated onto each well of a 24-well plate and incubated in 5% $CO_2$ at 37°C for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 12 h. Fresh acridine orange was added to each well at a terminal concentration of 10μg/mL and keep in dark place to stained for 15 min. Then, the staining cells washed three times with PBS and placed under fluorescence microscopy to observe cell morphology.

*2. 4 Analyzing of cell cycle*

HepG2 cells were seeded in 16-well plates at a density of $1 \times 10^6$ cells/well in DMEM, then treated with 2.03 μM concentration of **16d** and treated with 2.42 μM concentration of Foretinib for 12 h, respectively. Cultured cells were stained with propidium iodide (PI) in the dark at 4 ℃ for 30 min and analyzed by ACEA NovoCyte™.[20]

*2. 5 Docking studies*

For docking purposes, the three-dimensional structure of the c-Met (PDB code: 3LQ8) was obtained from RCSB Protein Data Bank.[7]Hydrogen atoms were added to the structure allowing for appropriate ionization at physiological pH. The protonated state of several important residue were adjusted by using AutoDock vina 1.1.2 in favor of forming reasonable hydrogen bond with the ligand. And using AutoDock vina 1.1.2 to produce twenty ligand conformation, the best molecular conformation was used as a ligand. Molecular docking analysis was carried out by the Discover Studio 2.5 Visualization to explore the binding model for the active site of c-Met with its ligand. All atoms located within the range of 5.0 Å from any atom of the cofactor were selected into the active site, and the corresponding amino acid residue was, therefore, involved into the active site if only one of its atoms was selected. Other default parameters were adopted in the AutoDock calculations. All calculations were performed on Silicon Graphics workstation.

**3. Results and discussion**

*3.1 Chemistry*

The key intermediates **9a-f** were synthesized from 4-cholor-1-substituted-pyrrolo[2,3-*d*]pyrimdine **7a-b** or 4-cholor-1-methyl-pyrrolo[2,3-*d*]pyrimidine **7c** *via* substitution reaction with 4-aminophenol or 2-fluoro-4-aminophenol **8a-b** as show in Scheme 1.

The intermediates 1-aryl-5-methyl (or trifluoromethyl)-1,4,5-trisubstituted-1,2,3-triazoles **12a-f** were synthesized in high yield by a one-pot three-component reaction of arylboronic acids **10a-g**, sodium azide,and active methylene ketones, such as ethyl acetoacetate (or ethyl 4,4,4-trifluoroacetoacetate) **11a-b** in the presence of $Cu(OAc)_2$ and piperidine using a DMSO/$H_2O$ (10:1) mixture as solvent. [12] Following by hydrolysis reaction and chlorination reaction, **13a-f** and **14a-f** were obtained. Finally, substitution reaction of amides **9a-d** and **9e-f** with carbonyl-chloride **14a-f** promoted by DIPEA in dichloromethane at room temperature to obtain the target compounds **15a-f**, **16a-f**, **17a-f**, **18a-f**, **19a-f** and **20a-f**, respectively.

(**Scheme 1 should be here**)

*3.2 Biology*

Taking Foretinib as reference compound, the target compounds (**15a-f**, **16a-f**, **17a-f**, **18a-f**, **19a-f** and **20a-f**) were evaluated for the cytotoxicityagainst three cancer cell lines A549, HepG2 and MCF-7 by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay. The selected compounds ( **16d**, **18d** and **20d** ) are tested for their activity against one or several tyrosine kinases (c-Met, Flt-3, VEGFR-2, c-Kit and EGFR kinases) through the mobility shift assay. The results expressed as $IC_{50}$ values were summarized in Tables 1-3 and the values are the average of at least two independent experiments.

**Table 1.** Activity of target compounds **15a-f**, **16a-f**, **17a-f**, **18a-f**, **19a-f** and **20a-f**.

| Compounds No. | $R_1$ | Z | $R_2$ | $IC_{50}$[a] (μM) | | |
|---|---|---|---|---|---|---|
| | | | | A549 | HepG2 | MCF-7 |
| **15a** | **H** | **$CF_3$** | **3-F-4-Cl** | 27.54 ± 2.68 | 56.84 ± 4.71 | NA |
| **15b** | **H** | **$CF_3$** | **3-F-4-F** | 13.36 ± 2.34 | 14.13 ± 3.26 | NA |
| **15c** | **H** | **$CF_3$** | **4-Cl** | 19.76 ± 1.98 | 23.63 ± 3.14 | 32.06 ± 2.47 |
| **15d** | **H** | **$CH_3$** | **4-F** | 12.53 ± 1.22 | 6.20 ± 0.98 | 4.71 ± 0.67 |
| **15e** | **H** | **$CH_3$** | **H** | 32.21 ± 2.28 | 37.91 ± 2.92 | NA |
| **15f** | **H** | **$CF_3$** | **2-$CF_3$** | NA | 12.54 ± 2.41 | 39.34 ± 3.27 |
| **16a** | **F** | **$CF_3$** | **3-F-4-Cl** | 10.53 ± 2.12 | 27.44 ± 3.83 | NA |
| **16b** | **F** | **$CF_3$** | **3-F-4-F** | 5.11 ± 0.43 | 6.82 ± 0.97 | 49.28 ± 4.67 |
| **16c** | **F** | **$CF_3$** | **4-Cl** | 7.56 ± 1.17 | 11.41 ± 2.32 | 22.5 ± 2.79 |
| **16d** | **F** | **$CH_3$** | **4-F** | 4.79 ± 0.82 | 2.03 ± 0.39 | 2.90 ± 0.43 |
| **16e** | **F** | **$CH_3$** | **H** | 12.32 ± 1.22 | 18.30 ± 2.76 | NA |
| **16f** | **F** | **$CF_3$** | **2-$CF_3$** | 31.92 ± 3.56 | 8.05 ± 1.07 | 27.61 ± 3.81 |
| **17a** | **H** | **$CF_3$** | **3-F-4-Cl** | 42.35 ± 2.26 | NA | NA |
| **17b** | **H** | **$CF_3$** | **3-F-4-F** | 21.74 ± 2.21 | 30.32 ± 3.28 | NA |

| | | | | | | |
|---|---|---|---|---|---|---|
| **17c** | **H** | **$CF_3$** | **4-Cl** | 25.44 ± 1.74 | 46.68 ± 3.32 | NA |
| **17d** | **H** | **$CH_3$** | **4-F** | 20.37 ± 1.36 | 7.83 ± 0.87 | 9.48±1.52 |
| **17e** | **H** | **$CH_3$** | **H** | 46.37 ± 2.64 | 45.64 ± 2.84 | NA |
| **17f** | **H** | **$CF_3$** | **2-$CF_3$** | NA | 35.62 ± 3.54 | NA |
| **18a** | **F** | **$CF_3$** | **3-F-4-Cl** | 15.74 ± 1.83 | 31.21 ± 2.56 | NA |
| **18b** | **F** | **$CF_3$** | **3-F-4-F** | 11.93 ± 1.36 | 9.84 ± 1.17 | NA |
| **18c** | **F** | **$CF_3$** | **4-Cl** | 8.92 ± 1.52 | 16.47 ± 2.18 | 34.51 ± 3.86 |
| **18d** | **F** | **$CH_3$** | **4-F** | 7.54 ± 1.27 | 5.37 ± 1.68 | 4.73 ± 1.02 |
| **18e** | **F** | **$CH_3$** | **H** | 23.25 ± 1.58 | 20.18 ± 2.26 | NA |
| **18f** | **F** | **$CF_3$** | **2-$CF_3$** | 51.32 ± 3.85 | 14.61 ± 1.37 | 38.27 ± 3.68 |
| **19a** | **H** | **$CF_3$** | **3-F-4-Cl** | NA | NA | NA |
| **19b** | **H** | **$CF_3$** | **3-F-4-F** | 33.56 ± 2.75 | NA | NA |
| **19c** | **H** | **$CF_3$** | **4-Cl** | 42.36 ± 2.93 | NA | NA |
| **19d** | **H** | **$CH_3$** | **4-F** | 25.22 ± 2.68 | 11.25 ± 1.27 | 14.83 ± 1.54 |
| **19e** | **H** | **$CH_3$** | **H** | NA | NA | NA |
| **19f** | **H** | **$CF_3$** | **2-$CF_3$** | NA | NA | NA |
| **20a** | **F** | **$CF_3$** | **3-F-4-Cl** | 22.68 ± 1.52 | NA | NA |
| **20b** | **F** | **$CF_3$** | **3-F-4-F** | 19.62 ± 1.83 | 15.83 ± 2.61 | NA |
| **20c** | **F** | **$CF_3$** | **4-Cl** | 15.52 ± 2.08 | 24.27 ± 2.63 | 49.34 ± 3.92 |
| **20d** | **F** | **$CH_3$** | **4-F** | 10.34 ± 2.18 | 9.72 ± 1.25 | 6.43 ± 1.76 |
| **20e** | **F** | **$CH_3$** | **H** | 42.26 ± 2.79 | 30.22 ± 2. 31 | NA |
| **20f** | **F** | **$CF_3$** | **2-$CF_3$** | NA | 30.47 ± 2.66 | NA |
| **Foretinib**[b] | | | **-** | 3.71 ± 0.41 | 2.42 ± 0.25 | 4.89 ± 0.62 |

[a]The values are an average of two separate determinations.

[b]Used as a positive control.

[c] NA: Not active ($IC_{50}$> 60 μM).

**Table 2.** c-Met kinase inhibitory activity of selected compounds**16d, 18d** and **20d**.

| Compounds No. | $IC_{50}$[a] (μM) |
|---|---|
| | c-Met |
| **16d** | 0.79 ± 0.11 |
| **18d** | 1.23 ± 0.13 |
| **20d** | 1.67 ± 0.19 |
| **Foretinib**[b] | 0.019 ± 0.003 |

[a]The values are an average of two separate determinations.

[b]Used as a positive control.

**Table 3** Inhibition of tyrosine kinases by compound **16d**

| Kinase | Enzyme $IC_{50}$ (μM) |
|---|---|
| Flt-3 | 5.23 ± 0.35 |
| VEGFR-2 | 27.6 ± 1.25 |
| c-Kit | 20.2 ± 1.42 |
| EGFR | >100 |

As showed in Tables 1-3, some of compounds displayed excellent anticancer activity against three cancer cells lines (A549, HepG2 and MCF-7) with single-digit μM. According to the Table 1-3, we could know that the first four series of compounds (**15a-f**, **16a-f**, **17a-f** and **18a-f**) showed more activity than the other two series of compounds (**19a-f** and **20a-f**). And The most promising compound **16d** exhibited the best activity against A549, HepG2 and MCF-7 cell lines with the $IC_{50}$ values of 4.79 ± 0.82 μM, 2.03 ± 0.39 μM and 2.90 ± 0.43 μM, which were equal to the lead drug Foretinib, respectively. The results suggested that the pyrrolo[2,3-b]pyrimidine moiety was a privileged scaffold for anticancer activity.

What's more, those compounds without substituents (Y = H) at *N*-7 position of pyrrolopyrimidines/ pyrazolopyrimidines moiety show more activity than that substituted with -$CH_3$ group. That's why the first and second series compounds (**15a-f** and **16a-f**) were more activity than that of the third and fourth series

(**17a-f** and **18a-f**).

Then, $R_1$ group ($R_1$= H/F) were introduced to C-2 position of aminophenoxy moiety to investigate the effect of electron withdrawing effect to the target compounds activity. The result revealed that the aminophenoxy moiety substituting with substituent ($R_1$= F) was benefit to the activity of the target compounds (**16a-f**) compared with H-atom substituent (**15a-f**). What's more,, the same trend also was observed in compounds **18a-f**/**17a-f** and **20a-f**/**19a-f**.

Furthermore, different substituents at aryl group also affected the cytotoxicity of target compounds. In general, it's seem to be that target compounds with electron drawing groups (EWGs) show better *in vitro* activity, such as compounds **15b**, **15d**, **16b-d**, **18b-d** and so on. Among these compounds, the EWGs on the 4-C position of aryl was more preferred, such as compounds **15d**, **16c-d**, **18c-d** and so on. Especially, the compound **16d** substituted with F atom on the 4-C position of aryl showed the best activity.

Activity against c-Met kinase of compounds **16d (**$IC_{50}$ = 0.79 ± 0.11 μM), **18d** ($IC_{50}$ = 1.23 ± 0.13 μM) and **20d** ($IC_{50}$ = 1.67 ± 0.19 μM**)** was further carried out in this paper to investigate the *in vitro* activity of these compounds. According to the results of Table 2, we could easily find that the three selected compounds showed moderate to excellent inhibitory on c-Met kinase. Compounds **16d** showed more active than the compounds **18d** and **20d**, with $IC_{50}$ value of 0.79 μM against c-Met kinase.

In order to examine whether compound **16d** was a selectivity c-Met inhibitor or not, experiments of enzyme-based selectivity was carried out to meansure the another four tyrosine kinases (Flt-3, VEGFR-2, c-Kit and EGFR ). As shown in Table 3, compound **16d** exhibited medium inhibitory effects against Flt-3 ($IC_{50}$ = 5.23 ± 0.35 μM),VEGFR-2 ($IC_{50}$ = 27.6 ± 1.25 μM), c-Kit ($IC_{50}$ = 20.2 ± 1.42 μM) and EGFR ($IC_{50}$ >100 μM), which was 5.9-, 31.0-, 22.7- and 112.3- fold lower than that of c-Met. These data indicated that compound **16d** could inhibit the c-Met kinase selectively.

*3.3 Acridine orange (AO) single staining*

As showed in the Figure.3, the control group cell (Fig. 3a) was stained with acridine orange (AO) and shape of the cell was full and the edge was clear, which treated with nothing. But in the Fig. 3b, the cell shape was abnormal with cell shrinkage, chromatin condensation and the DNA fragments were stained with Orange after 2.03 ± 0.39 μM concentration of compound **16d** acted on the HepG2 cells. It claimed that the compounds **16d** could induce apoptosis of HepG2 cells.

(**Fig. 3 should be here**)

*3.4 Analyzing of cell cycle*

The cell growth inhibitory potency of the tested compounds prompted us to evaluate their effects on the cell mitosis. For cell cycle progression analysis, the effect of the **16d** at $IC_{50}$ concentrations on cell cycle progression was investigated in HepG2 cells *via* flow cytometry. As shown in Fig.4, **16d** arrested efficiently the cell cycle progression in G2/ M phase, with increasing the percentage of cells in G2/M phase in a $IC_{50}$ concentration (2.03 μM ) from 26.85 to 49.09 compared with Foretinib (2.42 μM). And the index of Coefficient of Variation (CV) G1 and G2 are below the 7% which show that the data is reliable.

(**Fig. 4 should be here**)

*3.5 Docking studies*

*3.5.1 Binding modes of target compounds with c-Met*

To explore the binding modes of target compounds with the active site of c-Met, molecular docking simulation studies were carried out by using AutoDock vina 1.1.2 and analyzed by Discover Studio 2.5 Visualization. The three selected compounds (**16d**, **18d** and **20d**) occupy the ATP site of c-Met kinase and all can form hydrogen bond with c-Met in the pyrrolo[2,3-*d*]pyrimidines moiety and 1,2,3-triazole moiety (Fig. 5a). And basing on the *in vitro* inhibition results, we selected compound **16d**, our best c-Met inhibitor in this study, as the ligand example, and the c-Met protein was selected as the docking model (PDB ID code: 3LQ8 ).The binding modes of compound **16d** and c-Met were depicted in Fig.5b, Fig.5c. Visual inspection of the pose of compound **16d** into c-Met binding site revealed that compound **16d** was tightly embedded into the active binding pocket . In the binding mode, compound **16d** is potently bound to the active binding site of c-Met *via* four hydrogen bond and one pi–pi interactions. The nitrogen atom and hydrogen atom of pyrrolo[2,3-*d*]pyrimidines moiety formed two hydrogen bonds with MET A： 1160; the aminophenoxy moiety can form a pi-pi interaction with PHE A: 1223; the two nitrogen atom of 1,2,3-triazole moiety *via* two hydrogen bond interaction with ASP A: 1222; the aryl group is firmly embedded in the hydrophobic pocket of the c-Met kinase so that show better activity. Contributing to the hydrogen bond, pi-pi and hydrophobic interaction, it may be a probable explanation for its nice activity. In general, these results of the molecular docking study showed that 1,2,3-triazole derivatives bearing pyrrolo[2,3-*d*]pyrimidine moiety could act synergistically to interact with the active binding site of c-Met, suggested that compound **16d** may be a potential inhibitor of c-Met.

(**Fig. 5 should be here**)

*3.5.2 Analysis of the interaction between c-Met and selected compounds **16d**, **18d** and **20d***

To explore the strong and weak of activity of target compounds with c-Met, the binding modes of

three selected compounds (**16d**, **18d** and **20d**) were labeled with distances between the c-Met and target compounds, and the results were analyzed by Discover Studio Visualization 2.5 which described in Fig.6a, Fig.6b and Fig.6c. What's more, all the information was summarized in the Fig.6d. In the bonding modes, the distance between the **16d** and c-Met is shorter than **18d** and **20d**, which claimed that the interaction between **16d** and c-Met is stronger than **18d** and **20d**. And it may be a reasonable explanation why the **16d** show the best activity among all the target compounds.

(**Fig. 6 should be here**)

## 4. Conclusion

In summary, we designed and synthesized six series of pyrrolopyrimidine and pyrazolopyrimidine derivatives bearing 1,2,3-triazole moiety and evaluated for the $IC_{50}$ values against three cancer cell lines (A549, HepG2 and MCF-7). Some selected compounds (**16d**, **18d** and **20d**) were further evaluated for the activity against c-Met, Flt-3,VEGFR-2, c-Kit and EGFR kinases. The most promising compound **16d** showed equal activity to lead compound Foretinib against A549, HepG2, MCF-7 cell lines, with the $IC_{50}$ values of 4.79 ± 0.82μM, 2.03 ± 0.39 μM and 2.90 ± 0.43 μM, respectively. SARs and docking studies indicated that pyrrolo[2,3-*d*]pyrimidine moiety may be a privileged structure compared with pyrazolo[3,4-*d*]pyrimidine .And EWGs on the aryl group could increase the activity of compounds, especially compound **16d** which the 4-position of the aryl group substituted with F atom show the best activity. What's more, **16d** could induce apoptosis of HepG2 cells and inhibitor the cell cycle of HepG2 on G2/M phase by acridine orange (AO) staining and Cell cycle experiments, respectively. And further study found that **16d** could inhibitor the c-Met kinase selectively by the experiment of enzyme-based selectivity. According to these results, it's claimed that the pyrrolo[2,3-*d*]pyrimidine bearing 1,2,3-triazole moiety may be a privileged scaffold and compound **16d** may be a potential inhibitor of c-Met.

## 5. Acknowledgments

We gratefully acknowledge the generous support provided by The National Natural Science Funds of China (No. 21662014,81460527), Outstanding Youth Foundation of Jiangxi, Natural Science Foundation of Jiangxi, China (20171BCB23078), Natural Science Foundation of Jiangxi, China (20171ACB21052 & 20171BAB215073 & 20161BAB215216), Science and Technology Project Founded by the Education Department of Jiangxi Province (GJJ160787), Graduate Students' Science and Technology Innovation Project of Jiangxi Science & Technology Normal University (YC2016-X16).

**Conflict of interest**

The authors declare that there are no conflicts of interests.

**References**

[1] Park M, Dean M, Kaul K, *et al*. Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors[*J*]. *Proceedings of the National Academy of Sciences*, 1987, 84(18): 6379-6383.

[2] Naldini L, Weidner K M, Vigna E, *et al*. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor[*J*]. *The EMBO journal*, 1991, 10(10): 2867.

[3] Bottaro D P, Rubin J S. Identification of the Hepatocyte Growth Factor Receptor as the c-met Porto-Oncogene Product[*J*]. *Science*, 1991, 251(4995): 802.

[4] Liu X, Newton R C, Scherle P A. Development of c-MET pathway inhibitors[*J*]. *Expert opinion on investigational drugs*, 2011, 20(9): 1225-1241.

[5] Kataoka Y, Mukohara T, Tomioka H, *et al*. Foretinib (GSK1363089), a multi-kinase inhibitor of MET and VEGFRs, inhibits growth of gastric cancer cell lines by blocking inter-receptor tyrosine kinase networks[*J*]. *Investigational new drugs*, 2012, 30(4): 1352-1360.

[6] Cecchi F, Rabe D C, Bottaro D P. Targeting the HGF/Met signalling pathway in cancer[*J*]. *European journal of cancer*, 2010, 46(7): 1260-1270.

[7] Kim K S, Zhang L, Schmidt R, *et al*. Discovery of pyrrolopyridine− pyridone based inhibitors of Met kinase: Synthesis, X-ray crystallographic analysis, and biological activities[*J*]. *Journal of medicinal chemistry*, 2008, 51(17): 5330-5341

[8] Li S, Huang Q, Liu Y, *et al*. Design, synthesis and antitumour activity of bisquinoline derivatives connected by 4-oxy-3-fluoroaniline moiety[*J*]. *European journal of medicinal chemistry*, 2013, 64: 62-73.

[9] Li S, Zhao Y, Wang K, *et al*. Discovery of novel 4-(2-fluorophenoxy) quinoline derivatives bearing 4-oxo-1, 4-dihydrocinnoline-3-carboxamide moiety as c-Met kinase inhibitors[*J*]. *Bioorganic & medicinal chemistry*, 2013, 21(11): 2843-2855.

[10] Zhu W, Wang W, Xu S, et al. Design, synthesis, and docking studies of phenylpicolinamide derivatives bearing 1H-pyrrolo [2, 3-*b*] pyridine moiety as c-Met inhibitors[*J*]. *Bioorganic &*

*medicinal chemistry*, 2016, 24(4): 812-819.

[11] Tang Q, Wang L, Tu Y, *et al*. Discovery of novel pyrrolo [2, 3-*b*] pyridine derivatives bearing 1, 2, 3-triazole moiety as c-Met kinase inhibitors[*J*]. *Bioorganic & medicinal chemistry letters*, 2016, 26(7): 1680-1684.

[12] Zhang J, Jin G, Xiao S, *et al*. Novel synthesis of 1, 4, 5-trisubstituted 1, 2, 3-triazoles via a one-pot three-component reaction of boronic acids, azide, and active methylene ketones[*J*]. *Tetrahedron*, 2013, 69(10): 2352-2356.

[13] Chen Z, Cox B D, Garnier-Amblard E C, *et al*. Synthesis and anti-HCV activity of a series of β-D-2'-deoxy-2'-dibromo nucleosides and their corresponding phosphoramidate prodrugs[*J*]. *Bioorganic & Medicinal Chemistry Letters*, 2017.

[14] Kang D, Zhang H, Zhou Z, *et al*. First discovery of novel 3-hydroxy-quinazoline-2, 4 (1H, 3H)-diones as specific anti-vaccinia and adenovirus agents via 'privileged scaffold'refining approach[*J*]. *Bioorganic & medicinal chemistry letters*, 2016, 26(21): 5182-5186.

[15] Kim S. Getting the most out of PubChem for virtual screening[*J*]. *Expert opinion on drug discovery*, 2016, 11(9): 843-855.

[16] Wang X, Huang B, Liu X, *et al*. Discovery of bioactive molecules from CuAAC click-chemistry-based combinatorial libraries[*J*]. *Drug Discovery Today*, 2016, 21(1): 118-132.

[17] Fang Z, Kang D, Zhang L, *et al*. Synthesis and Biological Evaluation of a Series of 2‐((1‐substituted‐1H‐1, 2, 3‐triazol‐4‐yl) methylthio)‐6‐(naphthalen‐1‐ylmethyl) pyrimidin‐4 (3H)‐one As Potential HIV‐1 Inhibitors[*J*]. *Chemical biology & drug design*, 2015, 86(4): 614-618.

[18] Wu C, Wang M, Tang Q, *et al*. Design, synthesis, activity and docking study of sorafenib analogs bearing sulfonylurea unit[*J*]. *Molecules*, 2015, 20(10): 19361-19371.

[19] Zhu W, Liu Y, Zhai X, *et al*. Design, synthesis and 3D-QSAR analysis of novel 2-hydrazinyl-4-morpholinothi*eno* [3, 2-*d*]pyrimidine derivatives as potential antitumor agents[*J*]. *European journal of medicinal chemistry*, 2012, 57: 162-175.

[20] Zhang P, Wu Y, Dai Q, *et al*. p38-MAPK signaling pathway is not involved in osteogenic differentiation during early response of mesenchymal stem cells to continuous mechanical strain. *Mol Cell Biochem*. 2013 Jun;378(1-2): 19-28.

**Legends to the figures and schemes**

**Fig. 1.** Structures of small-molecule c-Met inhibitors

**Fig. 2.** Structures and design strategy for target compounds **15a-f**, **16a-f**, **17a-f**, **18a-f**, **19a-f** and **20a-f**

**Scheme 1.** Synthetic route of target compounds

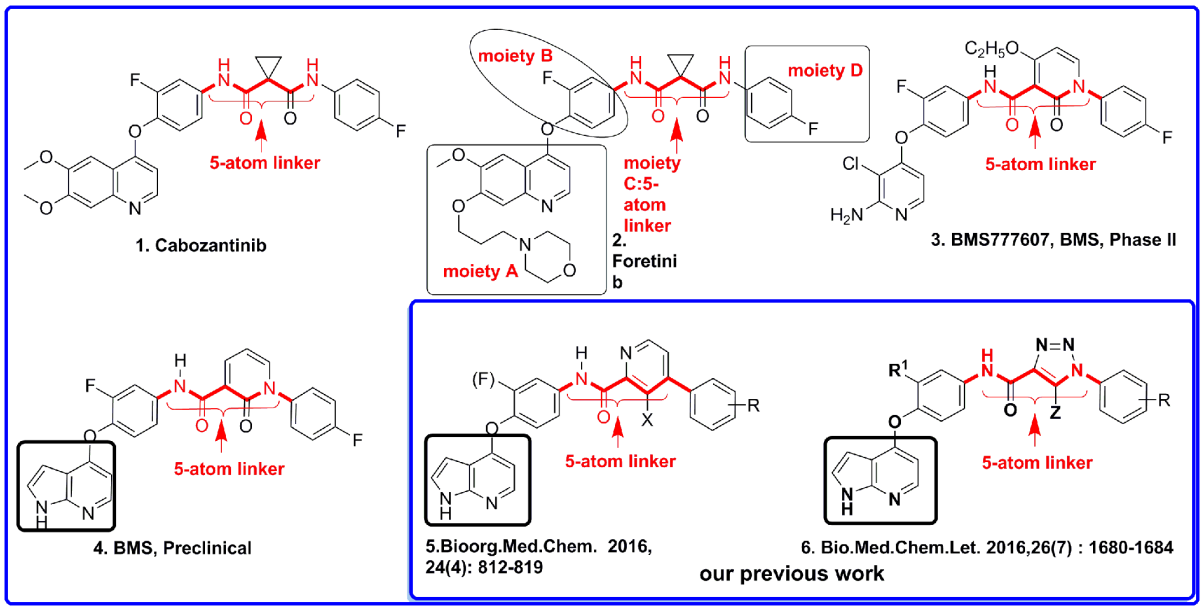
**Reagents and conditions**: (**a**) sodium carbonate, sodium hydroxide, tetrahydrofuran , 1,4-dioxane/$H_2O$ (5:1), 80℃, 1 h; (**b**) Cu(OAc) 2 , DMSO/H 2 O (10:1),Piperidine, rt to 80 ℃, 24 h, 88–92%; (**c**) sodium carbonate , ethanol/$H_2O$(5:1), 80℃， 3-5 h; (**d**) oxalyl chloride , DMF, $CH_2Cl_2$ , rt, 5 minute; (**e**) DIPEA, $CH_2Cl_2$ , rt,0.5 h.

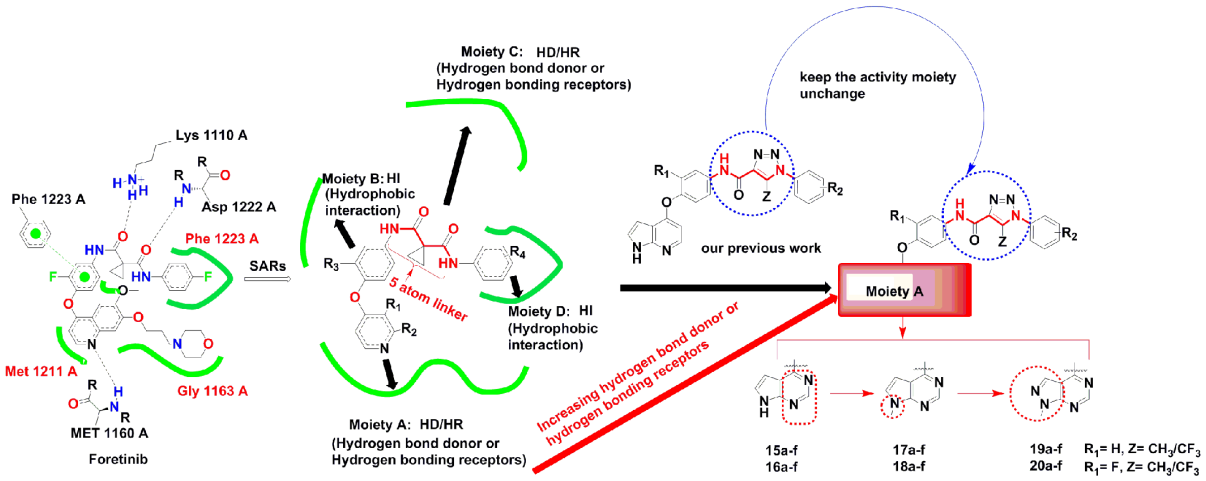
**Fig. 3. Morphologic changes of** HepG2 **cells under inverted microscopy and fluorescence microscopy**

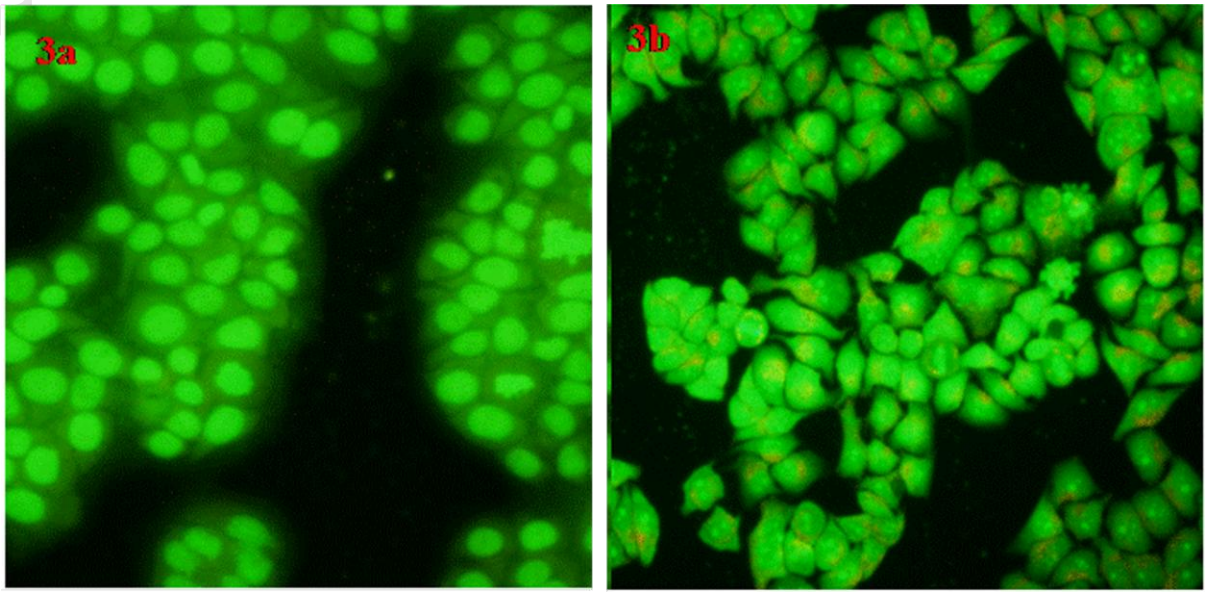
**Fig. 4 .** Identification of apoptotic stage of HepG2 by cell cycle. Cell cycle experiments were carried out to verify the duration of apoptosis of cancer cells under the action of $IC_{50}$ concentration of Foretinib (Fig.4a) and **16d** (Fig.4b).

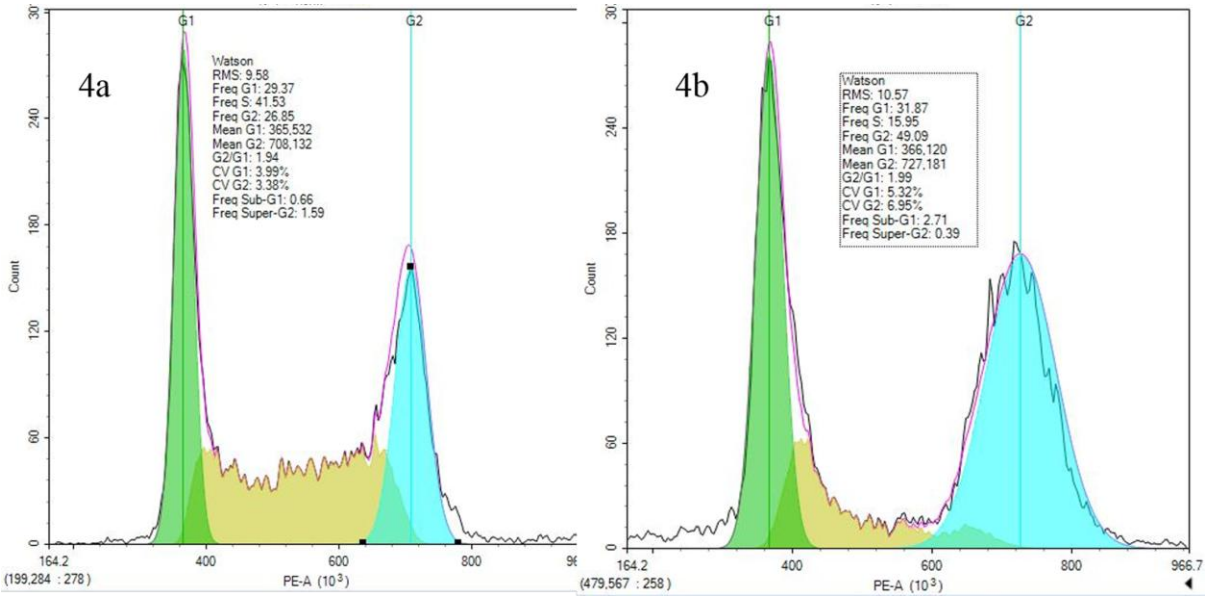
**Fig. 5** The protonated state of several important residue were adjusted by using AutoDock vina v1.02 in favor of forming reasonable hydrogen bond with the ligand and Molecular docking analysis was carried out by the Discover Studio Visualization to explore the binding model for the active site of c-Met with its ligand. Docking simulations show that the three selected compounds (**16d**, **18d** and **20d**) occupy the ATP site of c-Met kinase (Fig.5a). Then We employed 3D interaction map (Figure.5b) , 2D diagram (Figure.5c : Foretinib was used as a template) to display the interaction between **16d** and the targeted protein(3LQ8). In the binding model, compound **16d** is nicely bound to c-Met via four hydrogen bond and one pi–pi interaction.

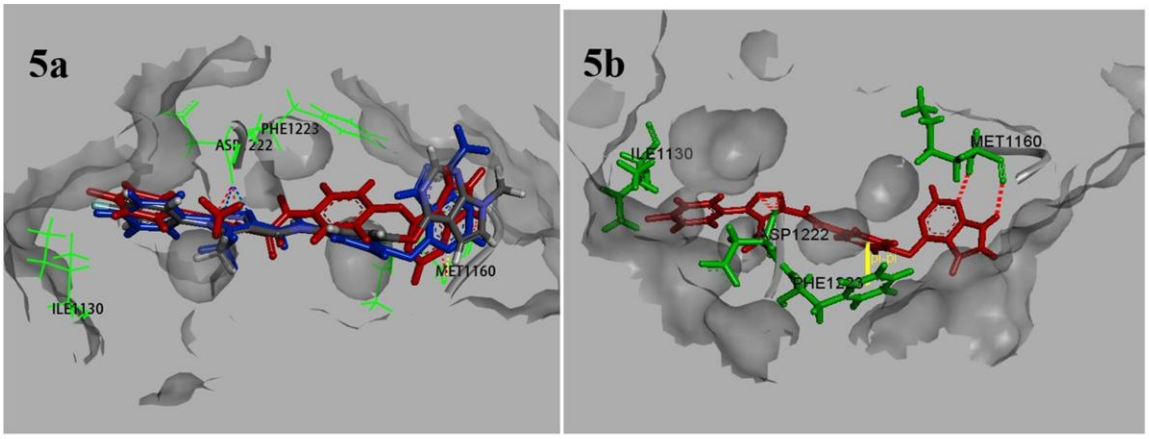
**Fig. 6** The binding modes of three selected compounds (**16d**, **18d** and **20d**) were labeled with distances between the c-Met and target compounds and analysed by Discover Studio 2.5, which described in Fig.6a, Fig.6b and Fig.6c and all the information were summarized in the Fig.6d.

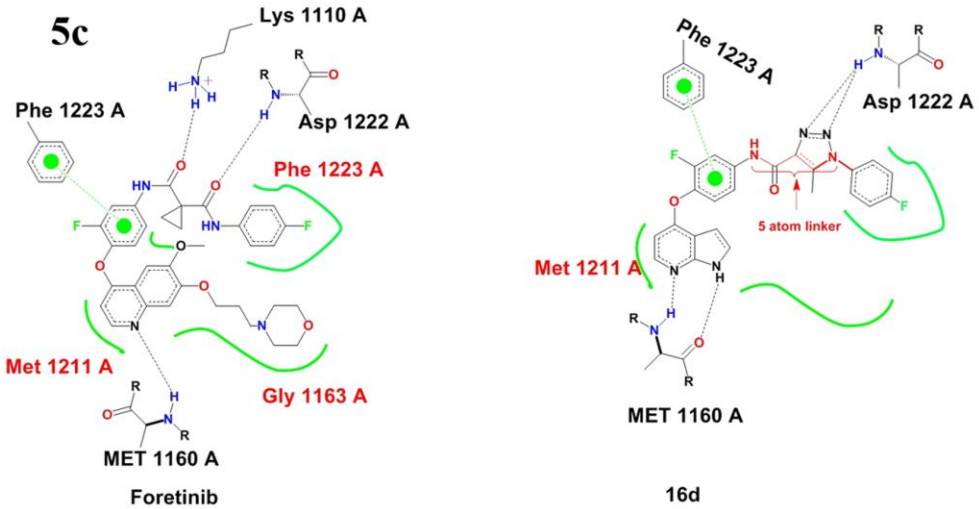


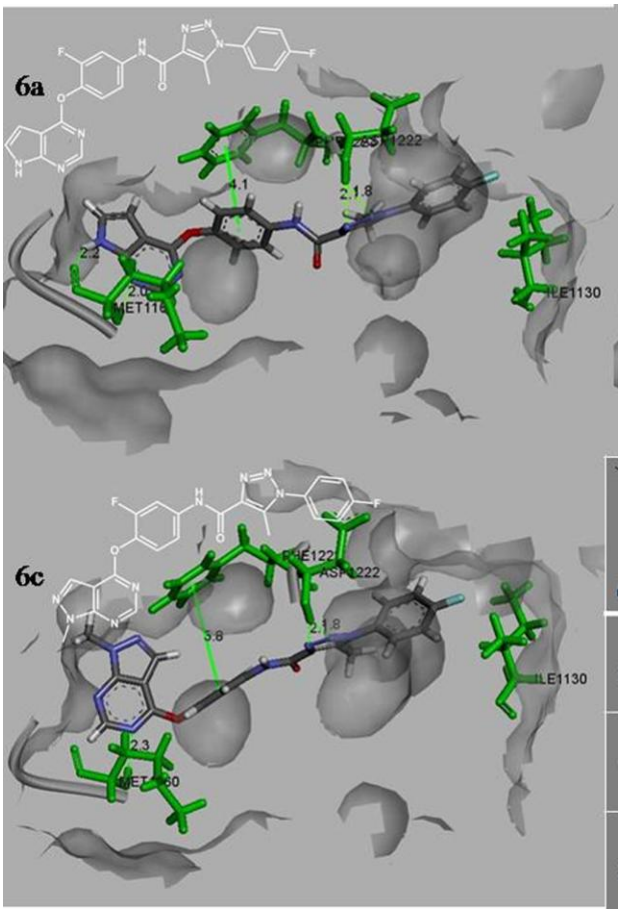








| 6d compounds \ residues | | MET 1160 | | PHE 1223 | ASP1222 | |
|---|---|---|---|---|---|---|
| 16d | number | 2 | | 1 | 2 | |
| | distance | 2.0 | 2.2 | 4.1 | 2.1 | 1.8 |
| 18d | number | 1 | | 1 | 2 | |
| | distance | 2.3 | | 5.6 | 2.1 | 1.8 |
| 20d | number | 1 | | 1 | 2 | |
| | distance | 2.3 | | 5.8 | 2.1 | 1.8 |