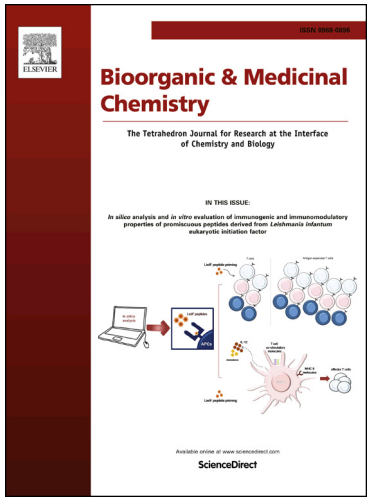


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# Synthesis and bioevaluation study of novel *N*-methylpicolinamide and thienopyrimidine derivatives as selectivity c-Met kinase inhibitors

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**Abstract:** Four series of *N*-methylpicolinamide moiety and thienopyrimidine moiety bearing pyridazinone were designed and synthesized and evaluated for the $IC_{50}$ values against three cancer cell lines (A549, HepG2 and MCF-7) and some selected compounds were further evaluated for the activity against c-Met, Flt-3, VEGFR-2, c-Kit and EGFR kinases. Three compounds (**35**, **39** and **43**) showed more active than positive control Foretinib against A549, HepG2 and MCF-7 cell lines. The most promising compound **43** showed superior activity against A549, HepG2 and MCF-7, with the $IC_{50}$ values of 0.58 ± 0.15 μM, 0.47 ± 0.06 μM and 0.74 ± 0.12 μM, which were 3.73-5.39 fold more activity than Foretinib, respectively. The experiments of enzyme-based showed that **43** restrain the c-Met selectively, with the $IC_{50}$ values of 16 nM, which showed equal activity to Foretinib (14 nM) and better than the compound **5** (90 nM). Moreover, AO and Annexin V/PI staining and docking studies were carried out.

**Keywords:** *N*-methylpicolinamide; Thienopyrimidine; Pyridazinone; c-Met; Bioevaluation



† These authors contribute equally to this work

## 1. Introduction

Receptor tyrosine kinases (RTKs) play a significant roles in signal transduction pathways and the treatment of various types of cancers.[1] c-Met [the receptor for hepatocyte growth factor/scatter factor, (HGF/SF)][2] and members of the vascular endothelial growth factor receptor (VEGFR)[3] family1 including VEGFR1 (Flt-1), VEGFR2 (KDR) and VEGFR3 (Flt-4) are among the RTKs under investigation as potential targets for the development of small molecule cancer therapeutics. Among these kinases, the c-Met kinase is one of hot spot in the anticancer field.

c-Met inhibitors are multitarget compounds which can inhibit the c-Met kinase selectively and used 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, Sorafenib, **4**, **5** and **6** (Fig.1 ). [5-8]

Many researches showed that nearly all the Cabozantinib derivatives show excellent activity and contain two obvious structural characteristics: HD/HR (hydrogen bond donor and hydrogen bond receptor) and HI (hydrophobic interaction) can be summarized (Fig.1). And the main modification of these different series of derivatives was focused on HD/HR moiety which could form hydrogen-bond donor or receptor.[9] What's more, the HI moiety may form hydrophobic bond with c-Met and bound to c-Met nicely.

In our previous research, a series of pyrrolo[2,3-*b*]pyridine derivatives (compound **7**, Fig.1) were designed and synthesized as potent c-Met inhibitors.[10] Most of these compounds exhibited potent activity, especially the most promising compound **7** with the $IC_{50}$ values in the nanomole level. The SARs and docking study exhibited that

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6-oxo-pyridazinone may be beneficial to the *in vitro* activity.

In this work, further modification was concentrated on pyrrolo[2,3-*b*]pyridine moiety which belonged to the HD/HR. Firstly, according to our previous work, the 6-oxo-pyridazinone scaffold of compound **7** was retained. And inspired by Sorafenib, the pyrrolo[2,3-*b*]pyridine moiety was replaced with *N*-methylpicolinamide to study the effects of increasing the molecular flexible to the compounds activity, and the first series compounds **24-29** were yield. Then, inspired by the compound **5** and compound **6**, the thieno[3,2-*d*]pyrimidine was introduced to investigate the effects of increasing the number of hydrogen donor and receptor to activity and the second series compounds (**30-37** and **38-44**) were prepared. Consequently, thieno[3,2-*d*]pyrimidine was replaced by thieno[2,3-*d*]pyrimidine to study the effects on the position of S atom to activity, and the third series compounds (**45-51** and **52-58**) were prepared. What's more, according to the SARs of the first three series compounds, we found that the 4-(2-fluoro-4-phenoxy)thieno[3,2-*d*]pyrimidine moiety was a privileged structure. Thus, the privileged structure was retained, and then the 4-oxo-pridazinone was introduced into the HD moiety the fourth series compounds 59-63 was obtained. As a result, four series of compounds **24-29**, **30-37**, **38-44**, **45-51**, **52-58** and **59-63** were synthesized. And the design strategy for all target compounds was described in Fig.2.

Herein we disclosed the synthesis and antitumor activity of *N*-methylpicolinamide moiety and thienopyrimidine moiety bearing pyridazinone 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 and docking studies were presented within this paper as well.

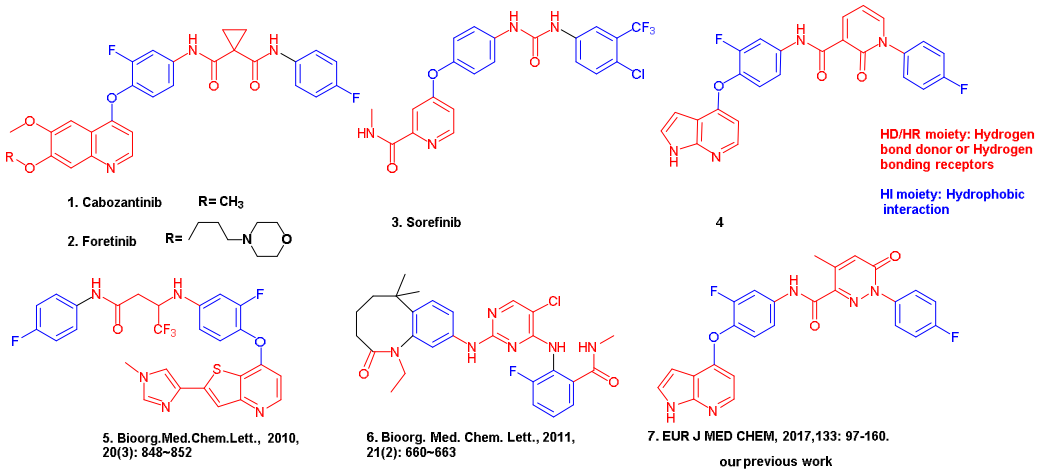



**Fig. 1** Structures characteristic of small-molecule tyrosine kinases inhibitors

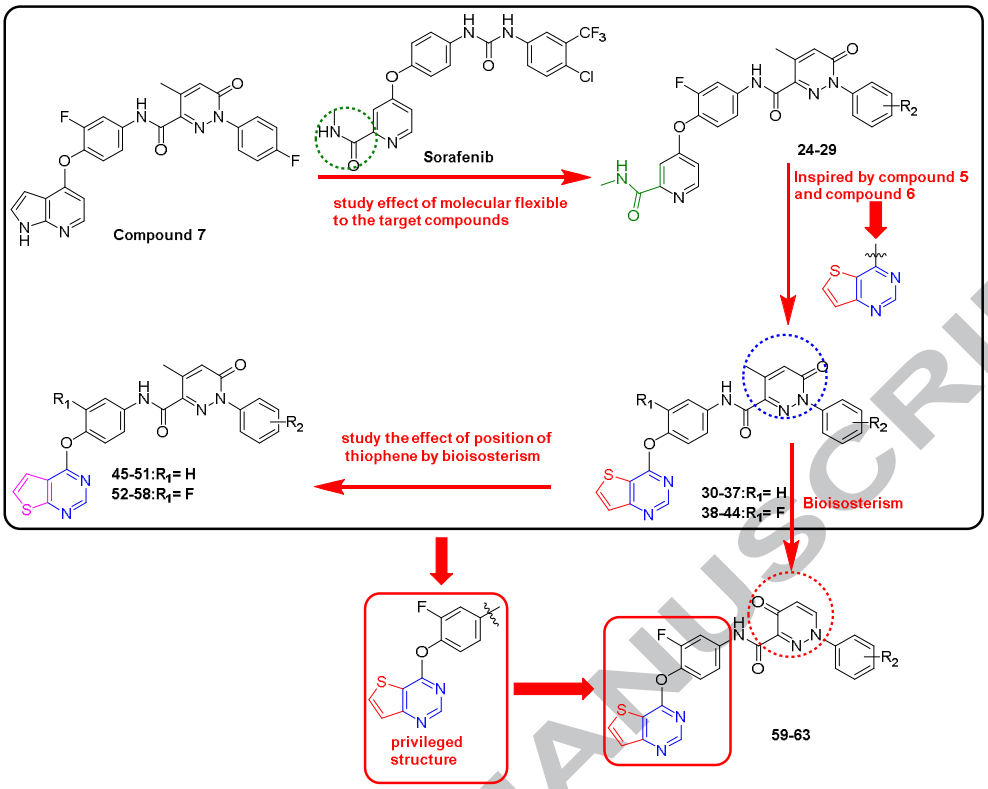


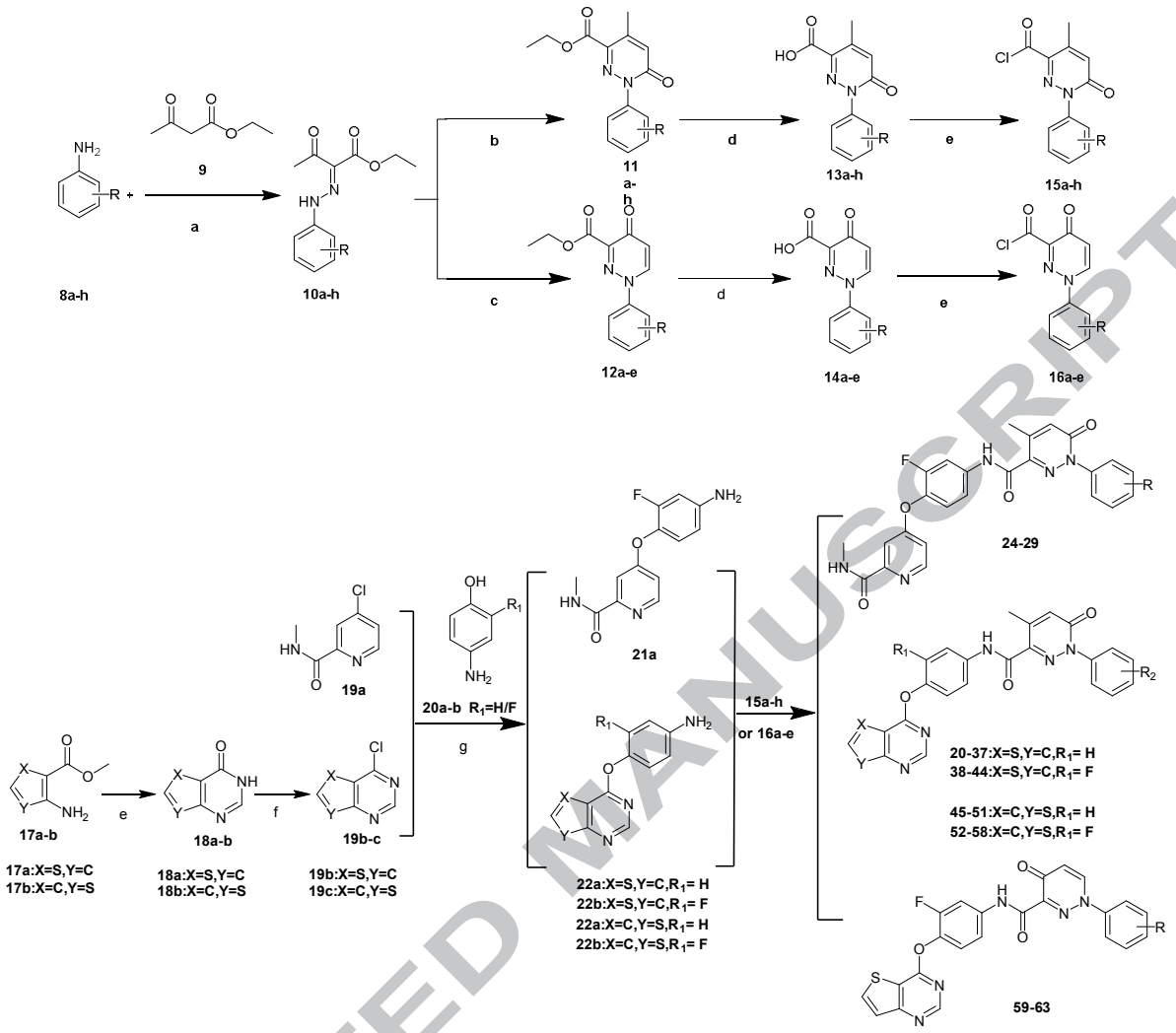

**Fig. 2** Structures and design strategy for target compounds **24-63**.

## 2. Chemistry

The preparations of target compounds **24-63** were described in scheme 1

Substituted anilines **8a-h** were diazotized and then reacted with ethyl-acetoacetate to get compounds **10a-h**, which condensed with DMF-DMA or ethyl (triphenylphosphoranylidene)acetate to yield compounds **11a-h** and **12a-e** with the absence of different catalyst, respectively. Following by hydrolysis reaction and chlorination reaction, **15a-h** and **16a-e** were obtained.

The key intermediates **19b-c** were synthesized from methyl 2-amino-2,3-dihydrothiophene-3-carboxylate and methyl 3-amino-2,3-dihydrothiophene-2-carboxylate **17a-b** *via* cyclization and chlorination with formamidine acetate and phosphorus oxychloride, respectively. Then, intermediates **21a**, **22a-b** and **23a-b** were obtained by substitution reaction with 4-aminphenol or 2-fluoro-4-aminphenol **20a-b**. Finally, substitution reaction of amides **21a**, **22a-b** and **23a-b** with the carbonyl chloride **15a-h/16a-e**. promoted by DIPEA in dichloromethane at room temperature to obtain target compounds **24-63**, respectively.




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

Reagents and conditions: (**a**) sodium nitrite, EtOH, ethyl acetoactate, 0 ℃, 30 minute; (**b**) ethyl (triphenylphosphoranylidene)acetate, $Et_2NH$, 85℃, 6 h; (**c**) DMF-DMA, 100 ℃, reflux, 6-10 h; (**d**) sodium carbonate, ethanol/$H_2O$ (5:1), 80℃, 3-5 h; (**e**) oxalyl chloride, DMF, $CH_2Cl_2$, rt, 5 minute; (**f**) Formamidine acetate, EtOH, 110℃, 2h, 90%; (**g**) phosphorus oxychloride, DMF, 110℃, 1h, 95%; (**h**) sodium carbonate, sodium hydroxide, tetrahydrofuran, 1,4-dioxane/$H_2O$ (5:1), 80℃, 1 h; (**i**) DIPEA, $CH_2Cl_2$, rt, 0.5 h.

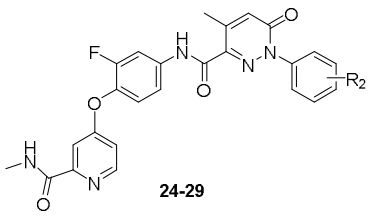
## 3. Results and discussion

### *3.1. Biological evaluation*

Taking Foretinib as reference compounds, the target compounds **24-63** were evaluated for the cytotoxicity against three cancer cell lines (A549, HepG2 and MCF-7) by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay. Compounds **35**, **39** and **43** were further evaluated for the activity against

c-Met, Flt-3, VEGFR-2, c-Kit and EGFR kinases. The results expressed as $IC_{50}$ values were summarized in Tables 1-6 and the values were the average of at least two independent experiments.

**Table 1.** Structures and activity of target compounds **24-29**



**24-29**

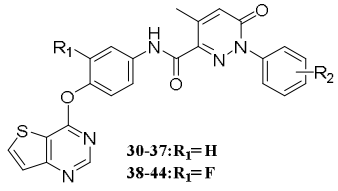
| Compounds | $R_2$ | $IC_{50}$[a] (μM) | | |
|---|---|---|---|---|
| | | A549 | HepG2 | MCF-7 |
| **24** | 2-$CF_3$ | NA | NA | 35.74 ± 1.53 |
| **25** | 2-F-4-Br | 7.93 ± 1.73 | 2.83 ± 0.45 | 9.36 ± 1.28 |
| **26** | 2-$OCF_3$ | NA | NA | NA |
| **27** | 3-F | 9.54 ± 1.89 | 10.21 ± 1.19 | 6.41 ± 0.92 |
| **28** | 4-$CH_3$ | 11.47 ± 1.51 | 10.64 ± 1.06 | 8.38 ± 0.93 |
| **29** | 2-F | NA | NA | NA |
| **Foretinib**[b] | - | 3.13 ± 0.67 | 2.27 ± 0.25 | 2.76 ± 0.53 |

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

[b]Used as a positive control.

[c] NA: Not active ($IC_{50}$ > 40 μM).

**Table 2.** Structures and activity of target compounds **30-44**



**30-37:$R_1$=H**
**38-44:$R_1$=F**

| Compounds | $R_1$ | $R_2$ | $IC_{50}$[a] (μM) | | |
|---|---|---|---|---|---|
| | | | A549 | HepG2 | MCF-7 |
| **30** | H | 2-$CF_3$ | 8.85 ± 1.38 | 9.23 ± 1.25 | 5.55 ± 0.86 |
| **31** | H | 2-F-4-Br | 7.93 ± 1.73 | 10.64 ± 1.06 | 9.36 ± 1.28 |
| **32** | H | 2-$OCF_3$ | 10.14 ± 1.18 | 24.99 ± 1.43 | 23.79 ± 1.49 |
| **33** | H | 3-F | 9.54 ± 1.89 | 10.21 ± 1.19 | 6.41 ± 0.92 |
| **34** | H | 4-$CH_3$ | 11.47 ± 1.18 | 24.99 ± 1.43 | 23.79 ± 1.49 |
| **35** | H | 4-F | **2.36 ± 0.23**[d] | **2.11 ± 0.47** | **4.4 ± 0.63** |
| **36** | H | 3,4-diF | 8.18 ± 1.38 | 9.23 ± 1.25 | 5.55 ± 0.86 |

| | | | | | |
|---|---|---|---|---|---|
| **37** | H | 2,5-diC | 16.4 ± 1.16 | 17.92 ± 1.62 | 11.93 ± 1.06 |
| **38** | F | 2-$CF_3$ | 4.68 ± 0.86 | 9.38 ± 1.62 | 6.02 ± 0.94 |
| **39** | F | 2-F-4-Br | **2.98 ± 0.73** | **3.35 ± 0.77** | **1.55 ± 0.26** |
| **40** | F | 2-$OCF_3$ | 5.72 ± 0.62 | 15.66 ± 1.45 | 11.49 ± 1.13 |
| **41** | F | 3-F | 5.22 ± 0.89 | 7.81 ± 1.16 | 9.05 ± 1.07 |
| **42** | F | 4-$CH_3$ | 5.89 ± 0.89 | 17.25 ± 1.43 | 7.25 ± 1.51 |
| **43** | F | 4-F | **0.58 ± 0.15** | **0.47 ± 0.06** | **0.74 ± 0.12** |
| **44** | F | 3,4-diF | 4.59 ± 1.28 | 6.93 ± 0.89 | 6.9 ± 1.11 |
| **Foretinib**[b] | | - | 3.13 ± 0.67 | 2.27 ± 0.25 | 2.76 ± 0.53 |

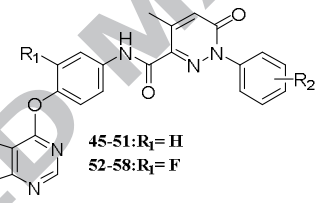
[a]The values an average of two separate determinations.

[b]Used as a positive control.

[c] NA: Not active ($IC_{50}$ > 40 μM).

[d]the data of bold showed more activity than Foretinib

**Table 3.** Structures and activity of target compounds **45-58**



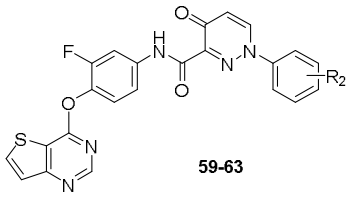

| Compounds | $R_1$ | $R_2$ | $IC_{50}$[a] (μM) | | |
|---|---|---|---|---|---|
| | | | A549 | HepG2 | MCF-7 |
| **45** | H | 2-$CF_3$ | 19.93 ± 1.52 | NA | NA |
| **46** | H | 2-F-4-Br | 12.77 ± 1.28 | 6.3 ± 0.84 | 15.2 ± 1.29 |
| **47** | H | 2-$OCF_3$ | 15.50 ± 1.36 | 14.44 ± 1.37 | 11.04 ± 1.15 |
| **48** | H | 3-F | 16.40 ± 1.76 | 18.51 ± 1.63 | 27.18 ± 2.78 |
| **49** | H | 4-$CH_3$ | NA[c] | NA | NA |
| **50** | H | 4-F | 7.05 ± 1.02 | 6.46 ± 0.85 | 20.96 ± 0.84 |
| **51** | H | 3,4-diF | 13.50 ± 2.39 | 16.77 ± 1.92 | 16.12 ± 2.27 |
| **52** | F | 2-$CF_3$ | 16.28 ± 1.38 | 14.65 ± 1.20 | 13.8 ± 1.25 |
| **53** | F | 2-F-4-Br | 11.47 ± 1.24 | 13.84 ± 1.18 | 13.25 ± 1.22 |
| **54** | F | 2-$OCF_3$ | NA | NA | NA |
| **55** | F | 3-F | 19.51 ± 1.46 | 24.45 ± 1.42 | 17.32 ± 1.35 |
| **56** | F | 4-$CH_3$ | NA | NA | NA |
| **57** | F | 4-F | 2.62 ± 0.59 | 12.47 ± 1.09 | 12.27 ± 1.32 |
| **58** | F | 3,4-diF | 15.70 ± 2.73 | NA | 20.56 ± 1.96 |
| **Foretinib**[b] | | - | 3.13 ± 0.67 | 2.27 ± 0.25 | 2.76 ± 0.53 |

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

[b]Used as a positive control.

[c] NA: Not active ($IC_{50}$ > 40 μM).

**Table 4.** Structures and activity of target compounds **59-63**



**59-63**

| Compounds | $R_2$ | $IC_{50}$[a] (μM) | | |
|---|---|---|---|---|
| | | A549 | HepG2 | MCF-7 |
| **59** | 4-F | 8.23 ± 1.34 | 9.16 ± 1.51 | 5.72 ± 0.69 |
| **60** | 3-Cl-4-F | 9.65 ± 1.45 | 10.64 ± 1.24 | 6.63 ± 0.43 |
| **61** | 4-Br | 7.84 ± 1.25 | 10.73 ± 1.16 | 9.62 ± 1.72 |
| **62** | H | 19.73 ± 1.16 | 13.52 ± 1.31 | 12.38 ± 0.73 |
| **63** | 4-$OCH_3$ | 11.62 ± 1.04 | 24.25 ± 1.83 | 23.15 ± 1.73 |
| **Foretinib**[b] | - | 3.13 ± 0.67 | 2.27 ± 0.25 | 2.76 ± 0.53 |

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

[b]Used as a positive control.

**Table 5.** c-Met kinase inhibitory activity of selected compounds **35**, **39** and **43**.

| Compounds No. | $R_1$ | $R_2$ | $IC_{50}$[a] (nM) c-Met |
|---|---|---|---|
| **35** | H | 4-F | 48 |
| **39** | F | 2-F-4-Br | 32 |
| **43** | F | 4-F | 19 |
| **Compound 5**[c] | - | - | 90 |
| **Foretinib**[b] | - | - | 14 |

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

[b]Used as a positive control.

[c]the data of c-Met kinase activity is from Gaudette 's work[7]

**Table 6.**Tyrosine kinases inhibitory activity of **43**

| Kinase | Enzyme $IC_{50}$ (nM) |
|---|---|
| c-Met | 19 |
| Flt-3 | 950 |
| VEGFR-2 | 450 |
| c-Kit | 3700 |
| EGFR | 6900 |

As showed in Tables 1-6, many compounds displayed excellent anticancer activity against three cancer cells lines (A549, HepG2 and MCF-7) with potency from single-digit μM to nanomole range. Among all the compounds, three selected compounds (**35**, **39** and **43**) showed better activity against A549, HepG2 and MCF-7 cells lines compared with the positive drug Foretinib. The most promising compound **43** exhibited the best activity against A549, HepG2 and MCF-7 cell lines with the $IC_{50}$ values of 0.58 ± 0.15 μM, 0.47 ± 0.06 μM and 0.74 ± 0.12 μM, which were 3.73**-**5.39 fold more activity than the lead drug Foretinib, respectively. The results claimed that the thieno[3,2-*d*]pyrimidine derivatives modified with 6-oxo-pyridazinone moiety was a privileged scaffold for anticancer activity.

What's more, $R_1$ group of compounds sbstituted with F atom showed more activity than no substituent against A549 and HepG2 cell lines. The trend also could be observed in compounds **24**-**29**, **30**-**44** and so on.

Furthermore, different $R_2$ substituent of the aryl group also affected the cytotoxicity of target compounds. In general, it's seem to be that target compounds with electron drawing groups(EWGs) have a significant impact on the *in vitro* activity. For example, the compounds substituted with F, Cl and Br showed more activity than no substituent or the electron donating groups (EDGs). Moreover, the F atom on the 4-C position showed the best activity, such as compounds **35**, **43**, **57** and so on. The results suggested that the F atom on the 4-C position could improve the compounds activity against HepG2 significantly, with $IC_{50}$ values of 0.47 ± 0.06 μM.

Activity against c**-**Met kinase of compounds **35**, **39** and **43** was further carried out in this paper. As shown in Table 5, we can easily find that the compounds **39** and **43** are more active than compound **35**, especially compound **43** with $IC_{50}$ value of 19 nM against c-Met kinase. The results prompt us that the target compounds

may be a series of c-Met kinase inhibitors. Furthermore, compound **43** exhibited medium inhibitory effects against Flt-3 ($IC_{50}$ = 950 nM), VEGFR-2 ($IC_{50}$ = 450 nM), c-Kit ($IC_{50}$ = 3700 nM) and EGFR ($IC_{50}$ = 6900 nM), which was 59.4-, 23.7-, 194.8- and 431.3- fold lower than that of c-Met, respectively (Table 6). These data indicated that compound **43** could inhibit the c-Met kinase selectively.

### *3.2. Morphologic changes of HepG2 cells under inverted microscopy and fluorescence microscopy*

In order to investigate the target compound whether could induce the apoptosis of HepG2, acridine orange (AO) was carried out. As show in the Fig. 3, the control group cell (Fig. 3a) was treated with nothing and showed normal HepG2 cell shape. But in the Fig. 3b, the cell shape was abnormal with cell shrinkage, chromatin condensation after 0.47 μM concentration of compound **43** acted on the HepG2 cells. It claimed that the compounds **43** could induce apoptosis of HepG2 cells.

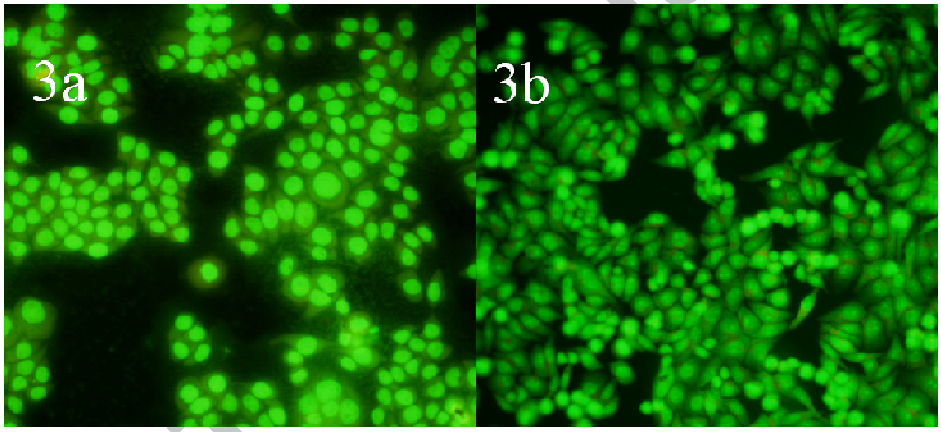



**Fig. 3** Morphologic changes of HepG2 cells under inverted microscopy and fluorescence microscopy

### *3.3. Apoptosis result was carried out by flow cytometry*

In order to further reveal the mechanism of apoptosis of HepG2 induced by the compound **43**,the experiment of Annexin V/PI staining was carried out ( Fig. 4). Compared to the control group, compound **43** could induce the late apoptotic significantly, with 21.01% late apoptotic and only 13.89% early apoptotic treated with 0.94 μM. And the same trend was also observed in other concentration. In addition, the number of apoptotic and dead cells increased with increasing concentration of the compound. Therefore, it can be concluded that the target compound **43** could induce late apoptosis and in a concentration- dependent manner.

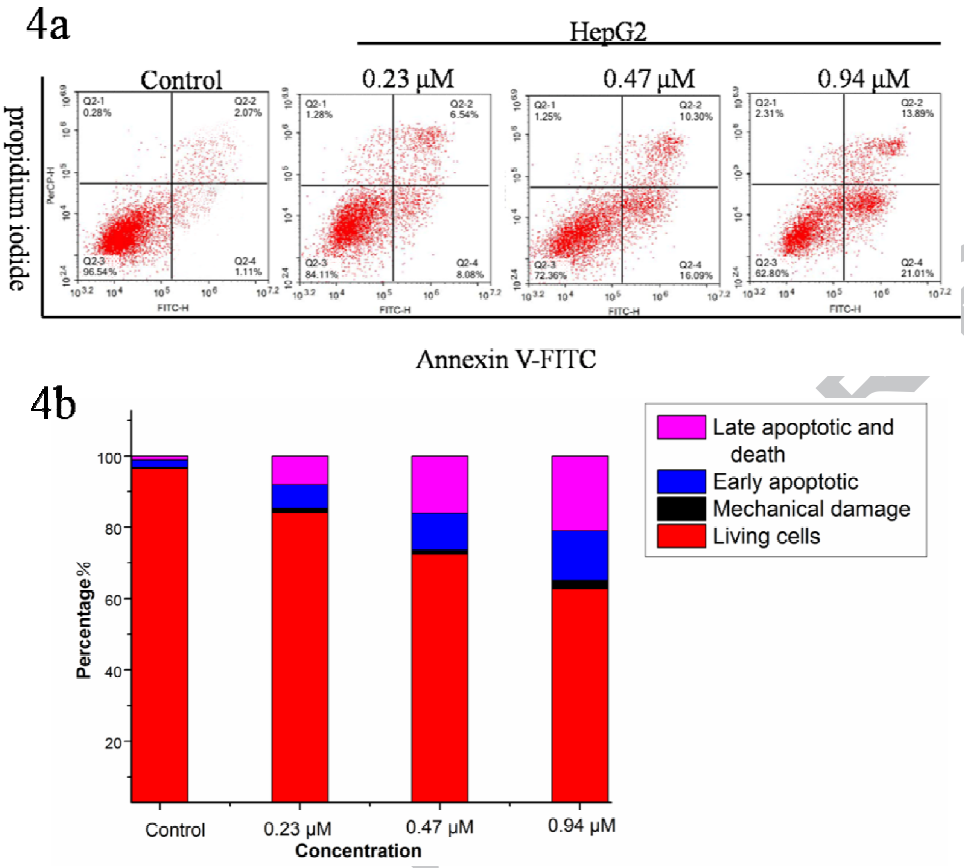



**Fig. 4** Annexin V (x-axis) versus PI (y-axis) analyses show in the Fig.4a and 4b was summarized by a drawing Fig.4b.

### *3.4. Molecular docking study*

To explore the binding modes of target compounds with the active site of c-Met, molecular docking simulation studies were carried out by using SYBYL 6.9.1 (Tripos, St. Louis, MO, USA), pymol 1.8.6 and ChemDraw. And basing on the *in vitro* inhibition results, we selected compound **43**, our best c-Met inhibitor in this study, as the ligand example, and the structure of c-Met was selected as the docking model (PDB ID code: 3LQ8 ). [13] The binding modes of compound **43** and c-Met were depicted in Fig.5. Visual inspection of the pose of compound **43** into c-Met binding site revealed that compound **43** was tightly embedded into the active binding pocket. In the binding mode, compound **43** was potently bound to the active binding site of c-Met *via* three hydrogen bonds and two pi–pi interactions. The N atoms of thieno[3,2-*d*]pyrimidine moiety and the O atom formed one hydrogen bond with the ASP 1222, respectively. And the O atom of 6-oxo-pyridazinone contact with MET 1160 *via* one hydrogen bond; contribute to the hydrogen bonding interaction together, being a probable explanation for its nice activity. On the other hand, the two substituted aryl group formed one pi–pi interaction

with PHE 1223 and TYR 1159 respectively. In general, these results of the molecular docking study showed that compound **43** may be a potential inhibitor of c-Met.

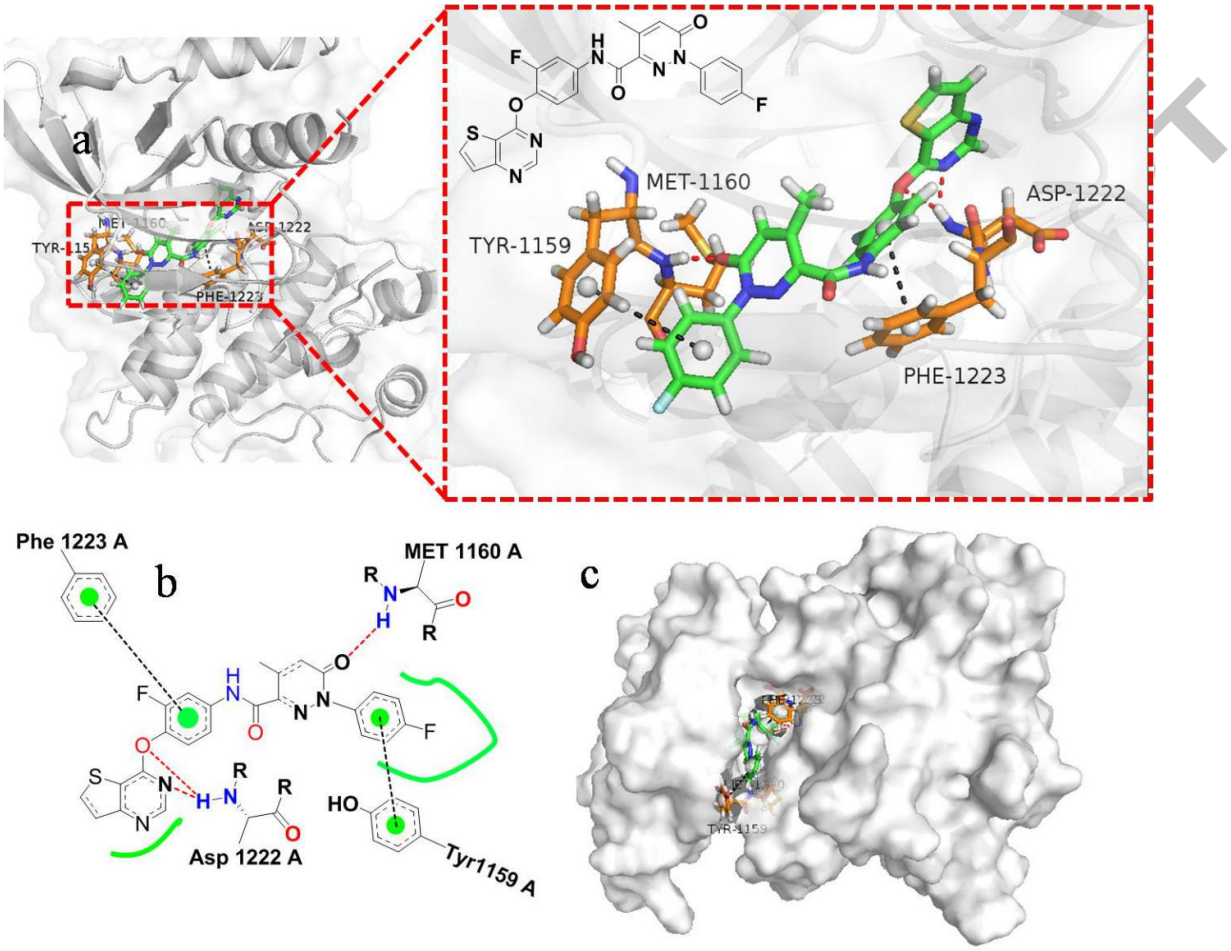



**Fig. 5 .** The 3D interaction map between the **43** and c-Met, the hydrogen bonds was colored with red and the pi-pi bonds was colored with black, respectively (Fig.5a). 2D interaction map between the **43** and c-Met, the hydrogen bonds was colored with red and the pi-pi bonds was colored with black (Fig.5b). **43** occupied the activity package of protein, and the hydrogen bonds was colored with red while the pi-pi interaction was colored with black(Fig.5c).

## 4. Conclusions

In summary, we designed and synthesized four series of *N*-methylpicolinamide moiety and thienopyrimidine moiety bearing pyridazinone and evaluated for the $IC_{50}$ values against three cancer cell lines (A549, HepG2 and MCF-7) and some selected compounds were further evaluated for the activity against c-Met, Flt-3, VEGFR-2,

c-Kit and EGFR kinases. Three compounds (**35**, **39** and **43**) showed more active than positive control Foretinib against one or more cell lines. The most promising compound **43** showed superior activity against A549, HepG2 and MCF-7, with the $IC_{50}$ values of 0.58 ± 0.15 μM, 0.47 ± 0.06 μM and 0.74 ± 0.12 μM, which were 3.73-5.39 fold more activity than the lead drug Foretinib, respectively. The experiments of enzyme-based showed that **43** could inhibitor the c-Met selectively, with the $IC_{50}$ values of 19 nM, which show equal activity to Foretinib and better than the compound **5**. Moreover, SARs and docking studies indicated that thieno[3,2-*d*]pyrimidine derivatives bearing 6-oxo-pyridazinone were favorable to the activity. Especially, the 4-C position of aryl group substituted with F atom show the best activity. What's more, According to the result of AO single staining and Annexin V/PI staining, it's claimed that the **43** could induce late apoptosis of HepG2 cells by a concentration-dependent manner.

## 5. Experimental

### 5.1 Chemistry

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. Yields were not optimized. 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. Yields were not optimized.

### 5.2 General procedure for preparation of compounds 10a-h

Appropriate substituted anilines **8a-h** (0.101 mol) was added to the mixture solution of hydrogen chloride/water (1:1, 10 ml), sodium nitrite (0.1mol) and stirred for 5 minute at 0□, respectively. Then the mixture was added to the ethanol solution which ammonium acetate (1 mol) and ethyl acetoacetate (0.1 mol) were dissolved in. The reaction was monitored by TLC until completed. Finally the solution was filtered and washed with a plenty of water to give a yellow solid.

### 5.3 General procedure for preparation of 11a-h

Intermediate **10a-h** (0.105 mol), ethyl (triphenylphosphoranylidene) acetate (0.15 mol) and $Et_2NH$ (0.1 mol) were dissolved in DMSO, heated to 85□ from room temperature and stirred for 4 hours. After the solution cooled to the

room temperature, it was poured to water, extracted with dichloromethane, washed with brine (50ml×3), dried with anhydrous sodium sulfate, then the organic layer was concentrated in vacuum to give the product **11a-h**.

**5.4 Preparation of compounds 12a-e**

The yellow solid **10a-h** (0.115 mol) was dissolve in the DMF-DMA (50 ml), heated from 0□ to 100□ and stirred for 5-6 hour. After the reaction was completed, poured the mixture to the petroleum ether and filtered to obtain the compounds **12a-e** .

**5.5 General procedure for preparation of 13a-h and 14a-e**

Appropriate intermediate **11a-h** (0.11 mol) or **12a-e** (0.104 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-h** and **14a-e**, respectively.

**5.6 General procedure for the key intermediate 15a-h and 16a-e**

The compounds **13a-h** or **14a-e** (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.

**5.7 General procedure for the key intermediate 18a-b**

2-Aminothiophene-3-carboxylate or methyl 3-aminothiophene-2-carboxylate **17a-b** (0.1 mol), formamidine acetate (0.15 mol) were dissolved in ethanol (100 mL), and heated to 100℃ for 2h. The reaction was monitored by TLC until completed. Finally the solution was cooled and pour into ice water stirred for 0.5 hour to give a gray solid.

**5.8 General procedure for the key intermediate 19a-b**

Thieno[2,3-*d*]pyrimidin-4(3H)-one or thieno[3,2-*d*]pyrimidin-4(3H)-one 18a-b (0.101 mol) was dissolved in toluene, and phosphorus oxychloride (3 mL), DMF (0.05 mL) was added slowly. Then the solution was heated to 110℃ and stirring for 1hour. The reaction was monitored by TLC until completed. The solution was cooled to rt and pour into ice water slowly to obtain a white solid.

**5.9 General procedure for the key intermediate 21a, 22a-b and 23a-b**

4-Aminophenol or 2-fluoro-4-aminphenol **20a-b** (0.1mol) which was dissolved in tetrahydrofuran (50ml), was added to a 1,4-dioxane/$H_2O$ (50ml, 5:1) solution of compounds **19a-c**, 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 .

**5.10. General procedure for the preparation of target compounds 24-29, 30-37, 38-44, 45-51, 52-58 and 59-63.**

A solution of phenylpyrdazinone carbonyl chloride **15a-h** or **16a-e** (0.82 mmol) in dichloromethane (10 mL) was added drop-wise to a solution of aniline **21a** or **22a-b** or **23a-b** (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 **24-29**, **30-37**, **38-44**, **45-51**, **52-58** and **59-63** which were recrystallized by isopropanol.

5.10.1 *N*-(3-fluoro-4-((2-(methylcarbamoyl)pyridin-4-yl)oxy)phenyl)-4-methyl-6-oxo-1-(2-(trifluoromethyl)phenyl)-1,6-dihydropyridazine-3-carboxamide (24)

This compound was obtained as pale yellow solid in 67% yield; ESI-MS m/z: 541.14$[M+H]^+$. $^1$HNMR (400 MHz, DMSO-$d_6$) δ 12.32 (s, 1H), 8.85 (s, 1H), 8.82 (d, *J* =8.7Hz, 1H), 8.54 (d, *J* = 5.7 Hz, 1H), 8.08-8.00 (m, 1H), 7.99-7.94 (m, 1H), 7.94-7.90 (m, 1H), 7.87 (d, *J* = 7.1Hz, 1H), 7.56 (d, *J* = 8.2Hz, 1H), 7.50-7.43 (m, 1H), 7.41 (s, 1H), 7.21(s, 1H), 2.79 (d, *J* = 4.6 Hz, 3H), 2.10 (s, 3H).

5.10.2 1-(4-Bromo-2-fluorophenyl)-*N*-(3-fluoro-4-((2-(methylcarbamoyl)pyridin-4-yl)oxy)phenyl)-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxamide (25)

This compound was obtained as pale yellow solid in 68% yield;ESI-MS m/z: 569.05$[M+H]^+$. $^1$HNMR (400 MHz, DMSO-$d_6$) δ 10.85 (s, 1H), 8.82 (d, *J* = 4.7 Hz, 1H), 8.54 (d, *J* =5.6 Hz, 1H), 7.97-7.88 (m, 1H), 7.85 (d, *J* =9.9 Hz, 1H), 7.68 (m, 2H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.45 (t, *J* =9.0 Hz, 1H), 7.37 (d, *J* = 2.5 Hz, 1H), 7.21 (s, 1H), 7.11(s, 1H), 2.78 (d, *J* = 4.6 Hz, 3H),2.42 (s, 3H).

5.10.3 *N*-(3-fluoro-4-((2-(methylcarbamoyl)pyridin-4-yl)oxy)phenyl)-4-methyl-6-oxo-1-(2-(trifluoromethoxy)phenyl)-1,6-dihydropyridazine-3-carboxamide (26)

This compound was obtained as pale yellow solid in 75% yield; ESI-MS m/z: 557.13$[M+H]^+$. $^1$HNMR (400 MHz,

DMSO-$d_6$) δ 12.22 (s, 1H), 8.96-8.73 (m, 2H), 8.54 (d, *J* = 5.5Hz, 1H), 8.08-8.00 (m, 1H), 7.89 (d, *J* = 7.5Hz, 1H), 7.72 (dd, *J* = 21.3, 7.1Hz, 3H), 7.56 (d, *J* = 9.1 Hz, 1H), 7.46 (dt, *J* = 7.8, 9.1 Hz, 2H), 7.22 (s, 1H), 2.79 (d, *J* = 4.7 Hz, 3H),2.04 (s, 3H).

5.10.4 *N*-(3-fluoro-4-((2-(methylcarbamoyl)pyridin-4-yl)oxy)phenyl)-1-(3-fluorophenyl)-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxamide (27)

This compound was obtained as pale yellow solid in 76% yield; ESI-MS m/z: 491.14[M+H]$^+$. $^1$HNMR (400 MHz, DMSO-$d_6$) δ 10.87 (s, 1H), 8.82 (d, *J* = 4.6 Hz, 1H), 8.54 (d, *J* = 5.5 Hz, 1H), 7.95 (m, 1H), 7.94-7.90 (d, *J* = 12.9 Hz, 1H), 7.70 (d, *J* = 7.1Hz, 1H), 7.63 (d, *J* = 8.1Hz, 2H), 7.59-7.52 (m, 1H), 7.46 (t, *J* = 9.0 Hz, 1H), 7.39 (s, 1H), 7.33 (t, *J* = 8.1 Hz, 1H), 7.27-7.19 (m, 1H), 7.09 (s, 1H), 2.78 (d, *J* = 4.5 Hz, 3H),2.41(s, 3H).

5.10.5 *N*-(3-fluoro-4-((2-(methylcarbamoyl)pyridin-4-yl)oxy)phenyl)-4-methyl-6-oxo-1-(p-tolyl)-1,6-dihydropyridazine-3-carboxamide (28)

This compound was obtained as pale yellow solid in 73% yield; ESI-MS m/z: 487.17[M+H]$^+$. $^1$HNMR (400 MHz, DMSO-$d_6$) δ 10.83 (s, 1H), 8.82 (d, *J* = 4.6 Hz, 1H), 8.54 (d, *J* = 5.7 Hz, 1H), 7.94 (d, *J* = 12.9 Hz, 1H), 7.67-7.50 (m, 3H), 7.46 (t, *J* = 9.0 Hz, 1H), 7.40-7.26 (m, 3H), 7.22(t, *J* = 2.7 Hz, 1H), 2.78 (d, *J* = 4.5 Hz, 3H),2.37 (s, 3H).

5.10.6 *N*-(3-fluoro-4-((2-(methylcarbamoyl)pyridin-4-yl)oxy)phenyl)-1-(2-fluorophenyl)-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxamide (29)

This compound was obtained as pale yellow solid in 75% yield; ESI-MS m/z: 491.14[M+H]$^+$. $^1$HNMR (400 MHz, DMSO-$d_6$) δ 10.86 (s, 1H), 8.82 (d, *J* = 4.7 Hz, 1H), 8.51 (dd, *J* = 19.4, 5.7 Hz, 1H), 7.93 (d, *J* = 12.8 Hz, 1H), 7.69 (t, *J* = 7.4 Hz, 1H), 7.63-7.52 (m, 2H), 7.45 (t, *J* = 8.4 Hz, 2H), 7.38 (t, *J* = 12.4 Hz, 2H), 7.26-7.16 (m, 1H), 7.11 (s, 1H), 2.79 (d, *J* = 4.8 Hz, 3H),2.43 (s, 3H).

5.10.7 4-Methyl-6-oxo-*N*-(4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1-(2-(trifluoromethyl)phenyl)-1,6-dihydropyridazine-3-carboxamide (30)

This compound was obtained as pale yellow solid in 73% yield; M.P.: 201.0-203.0 □; ESI-MS m/z: 523.09[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.31 (s, 1H), 8.98 (s, 1H), 8.81 (s, 1H), 8.58 (d, *J* = 5.4 Hz, 1H), 8.15 (d, *J* = 7.7 Hz, 1H), 8.08 (d, *J* = 7.3 Hz, 1H), 8.05 – 7.95 (m, 2H), 7.92 (d, *J* = 8.8 Hz, 2H), 7.78 (d, *J* = 5.3 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 2.21 (s, 3H).

5.10.8 1-(4-Bromo-2-fluorophenyl)-4-methyl-6-oxo-*N*-(4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1,6-dihydropyridazine-3-carboxamide (31)

This compound was obtained as pale yellow solid in 75% yield; M.P.: 204.0-205.0 □; ESI-MS m/z: 553.00[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.77 (s, 1H), 8.80 (s, 1H), 8.57 (d, *J* = 5.2 Hz, 1H), 7.94 (d, *J* = 9.5 Hz, 1H), 7.89 (d, *J* = 8.6 Hz, 2H), 7.83 – 7.71 (m, 3H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.20 (s, 1H), 2.51 (s, 3H).

5.10.9 4-Methyl-6-oxo-*N*-(4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1-(2-(trifluoromethoxy)phenyl)-1,6-dihydropyridazine-3-carboxamide (32)

This compound was obtained as pale yellow solid in 64% yield; M.P.: 207.0-208.0 □; ESI-MS m/z: 539.09[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.20 (s, 1H), 8.97 (s, 1H), 8.81 (s, 1H), 8.58 (d, *J* = 5.4 Hz, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.8 Hz, 2H), 7.89 – 7.83 (m, 2H), 7.79 (t, *J* = 6.3 Hz,2H), 7.47 (d, *J* = 8.8 Hz, 2H), 2.21 (s, 3H).

5.10.10 1-(3-fluorophenyl)-4-methyl-6-oxo-*N*-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1,6-dihydro-pyridazine-3-carboxamide (33)

This compound was obtained as pale yellow solid in 65% yield; M.P.: 206.0-207.0 □; ESI-MS m/z: 473.10[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.77 (s, 1H), 8.80 (s, 1H), 8.58 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 8.8 Hz, 2H), 7.82 – 7.77 (m, 2H), 7.70 (dt, *J* = 15.8, 7.3 Hz, 2H), 7.48 – 7.40 (m, 3H), 7.19 (s, 1H), 2.51 (s, 3H).

5.10.11 4-Methyl-6-oxo-*N*-(4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1-(*p*-tolyl)-1,6-dihydropyridazine-3-carb-oxamide (34)

This compound was obtained as pale yellow solid in 59% yield; M.P.: 207.0-209.0 □; ESI-MS m/z: 469.12[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.74 (s, 1H), 8.80 (s, 1H), 8.58 (d, *J* = 5.4 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.78 (d, *J* = 5.3 Hz, 1H), 7.69 (d, *J* = 8.2 Hz,2H), 7.44 (t, *J* = 7.6 Hz,4H), 7.15 (s, 1H), 2.49 (s, 3H), 2.48 (s, 3H).

5.10.12 1-(4-Fluorophenyl)-4-methyl-6-oxo-*N*-(4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1,6-dihydro-pyridazine-3-carboxamide (35)

This compound was obtained as pale yellow solid in 77% yield; M.P.: 209.0-211.0 □; ESI-MS m/z: 473.10[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.66 (s, 1H), 8.70 (s, 1H), 8.47 (d, *J* = 5.2 Hz, 1H), 7.84 – 7.76 (m, 4H),

7.68 (d, *J* = 5.3 Hz, 1H), 7.42 – 7.33 (m, 4H), 7.07 (s, 1H), 2.41 (s, 3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 168.99, 163.19, 162.49, 161.62, 160.05, 158.64, 152.85, 148.12, 142.77, 141.73, 137.02, 135.80, 129.60, 127.89, 127.80, 127.29, 122.24(2C), 121.41(2C), 118.40, 115.50, 115.28, 18.11..

5.10.13 1-(3,4-Difluorophenyl)-4-methyl-6-oxo-*N*-(4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1,6-dihydro-pyridazine-3-carboxamide (36)

This compound was obtained as pale yellow solid in 76% yield; M.P.: 205.0-207.0 □; ESI-MS m/z: 491.09[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.67 (s, 0H), 8.71 (s, 1H), 8.47 (d, *J* = 5.3 Hz,1H), 8.02 – 7.93 (m, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.68 (d, *J* = 5.4 Hz, 2H), 7.64 – 7.56 (m, 1H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.08 (s, 1H), 2.42 (s, 3H).

5.10.14 1-(2,4-Dimethylphenyl)-4-methyl-6-oxo-*N*-(4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1,6-dihydro-pyridazine-3-carboxamide (37)

This compound was obtained as pale yellow solid in 56% yield; M.P.: 209.0-211.0 □; ESI-MS m/z: 483.14[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.57 (s, 1H), 8.71 (s, 1H), 8.49 – 8.46 (m, 1H), 7.80 (d, *J* = 6.7 Hz, 2H), 7.69 – 7.68 (m, 1H), 7.35 (d, *J* = 6.9 Hz,2H), 7.25 (d, *J* = 17.2 Hz, 2H), 7.06 (s, 1H), 2.43 (s, 3H), 2.34 (s, 3H), 2.09 (s, 3H).

5.10.15 *N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1-(2-(trifluoromethyl)phenyl)-1,6-dihydropyridazine-3-carboxamide (38)

This compound was obtained as pale yellow solid in 64% yield; M.P.: 210.0-212.0 □; ESI-MS m/z: 541.08[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.29 (s, 1H), 8.88 (s, 1H), 8.72 (s, 1H), 8.51 (d, *J* = 5.4 Hz, 1H), 8.04 (d, *J* = 7.8 Hz, 1H), 8.01 – 7.90 (m, 4H), 7.87 (t, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 5.4 Hz, 1H), 7.58 – 7.48 (m, 2H), 2.09 (s, 3H).

5.10.16 1-(4-Bromo-2-fluorophenyl)-*N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxamide (39)

This compound was obtained as pale yellow solid in 54% yield; M.P.: 213.0-215.0 □; ESI-MS m/z: 570.99[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.80 (s, 1H), 8.71 (s, 1H), 8.51 (d, *J* = 5.4 Hz, 1H), 7.85 (t, *J* = 11.3 Hz, 2H), 7.70 (d, *J* = 5.4 Hz, 1H), 7.65 (t, *J* = 10.0 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 1H), 7.53 – 7.47 (m, 1H), 7.10 (s, 1H),

2.41 (s,3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 169.77, 162.80, 162.14, 158.92, 155.09, 153.30, 152.63, 150.57, 148.12, 143.55, 141.81, 137.67, 135.41, 130.34, 128.42, 124.84, 123.26, 118.69, 118.50, 117.84, 117.16, 116.05, 109.19,18.74.

5.10.17 *N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1-(2-(trifluoromethoxy)phenyl)-1,6-dihydropyridazine-3-carboxamide (40)

This compound was obtained as pale yellow solid in 58% yield; M.P.: 214.0-215.0 □; ESI-MS m/z: 557.08[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.31 (s, 1H), 8.98 (s, 1H), 8.84 (s, 1H), 8.63 (d, *J* = 5.3 Hz, 1H), 8.06 (d, *J* = 11.9 Hz, 1H), 7.99 (d, *J* = 8.1 Hz, 1H), 7.90 – 7.74 (m, 4H), 7.68 – 7.60 (m, 2H), 2.21 (s, 3H).

5.10.18 *N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1-(3-fluorophenyl)-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxamide (41)

This compound was obtained as pale yellow solid in 64% yield; M.P.: 217.0-218.0 □; ESI-MS m/z: 491.09[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.92 (s, 1H), 8.83 (s, 1H), 8.63 (d, *J* = 5.2 Hz, 1H), 8.00 (d, *J* = 11.8 Hz, 1H), 7.84 – 7.77 (m, 2H), 7.75 – 7.59 (m, 4H), 7.44 (t, *J* = 10.3 Hz, 1H), 7.20 (s, 1H), 2.52 (s, 3H).

5.10.19 *N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1-(p-tolyl)-1,6-dihydropyridazine-3-carboxamide (42)

This compound was obtained as pale yellow solid in 71% yield; M.P.: 211.0-212.0 □; ESI-MS m/z: 487.11[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.89 (s, 1H), 8.82 (s, 1H), 8.63 (d, *J* = 5.3 Hz, 1H), 7.99 (d, *J* = 13.1 Hz, 1H), 7.82 (d, *J* = 5.3 Hz, 1H), 7.69 (d, *J* = 8.2 Hz, 3H), 7.62 (t, *J* = 8.3 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.15 (s, 1H), 2.50 (s, 3H), 2.48 (s, 3H).

5.10.20 *N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxamide (43)

This compound was obtained as pale yellow solid in 75% yield; M.P.: 213.0-215.0 ℃;ESI-MS m/z: 491.09[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.84 (s, 1H), 8.73 (s, 1H), 8.53 (d, *J* = 5.3 Hz, 1H), 7.91 (d, *J* = 12.6 Hz, 1H), 7.81 – 7.71 (m, 3H), 7.60 (d, *J* = 8.8 Hz, 1H), 7.53 (t, *J* = 8.6 Hz, 1H), 7.38 (t, *J* = 8.6 Hz, 2H), 7.08 (s,1H), 2.42 (s,3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 168.78, 161.83, 161.35, 159.62, 158.16, 152.31, 142.39, 140.77, 136.72, 136.49, 134.29, 129.21, 127.43 (2C), 127.34 (2C), 123.85, 117.72, 116.14, 115.05, 114.83, 108.18, 107.95, 17.72.

5.10.21 1-(3,4-Difluorophenyl)-*N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1,6-dih

ydropyridazine-3-carboxamide (44)

This compound was obtained as pale yellow solid in 78% yield; M.P.: 219.0-220.0 □; ESI-MS m/z: 509.08[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.83 (s, 1H), 8.73 (s, 1H), 8.53 (d, $J$ = 5.4 Hz, 1H), 8.01 – 7.86 (m, 2H), 7.77 – 7.63 (m, 3H), 7.60 (d, $J$ = 10.2 Hz,1H), 7.54 (t, $J$ = 8.6 Hz, 1H), 7.10 (s, 1H), 2.43 (s, 3H).

5.10.22 4-Methyl-6-oxo-*N*-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(2-(trifluoromethyl)phenyl)-1,

6-dihydropyridazine-3-carboxamide (45)

This compound was obtained as pale yellow solid in 73% yield; M.P.: 201.0-203.0 □; ESI-MS m/z: 523.09[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.19 (s, 1H), 8.86 (s, 1H), 8.61 (s, 1H), 8.03 (d, $J$ = 7.4 Hz, 1H), 7.95 (d, $J$ = 5.6 Hz, 2H), 7.93 – 7.84 (m, 2H), 7.79 (d, $J$ = 8.7 Hz, 2H), 7.65 (d, $J$ = 5.8 Hz, 1H), 7.32 (d, $J$ = 8.7 Hz, 3H), 2.09 (s, 3H).

5.10.23 1-(4-Bromo-2-fluorophenyl)-4-methyl-6-oxo-*N*-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1,

6-dihydropyridazine-3-carboxamide (46)

This compound was obtained as pale yellow solid in 75% yield; M.P.: 204.0-205.0 □; ESI-MS m/z: 553.00[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.64 (s, 1H), 8.60 (s, 1H), 7.95 (d, $J$ = 6.0 Hz, 1H), 7.83 (d, $J$ = 8.3 Hz, 1H), 7.77 (d, $J$ = 9.4 Hz, 2H), 7.65 (d, $J$ = 4.2 Hz, 3H), 7.30 (d, $J$ = 6.9 Hz, 2H), 7.09 (s, 1H), 2.39 (s, 3H).

5.10.24 4-Methyl-6-oxo-*N*-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(2-(trifluoromethoxy)phenyl)-1,

6-dihydropyridazine-3-carboxamide (47)

This compound was obtained as pale yellow solid in 64% yield; M.P.: 207.0-208.0 □; ESI-MS m/z: 539.09 [M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.08 (s, 1H), 8.85 (s, 1H), 8.61 (s, 1H), 7.95 (d, $J$ = 5.9 Hz, 1H), 7.87 (d, $J$ = 7.2 Hz, 1H), 7.79 (d, $J$ = 8.5 Hz, 2H), 7.73 (d, $J$ = 7.1 Hz,2H), 7.67 (t, $J$ = 8.1 Hz, 2H), 7.32 (d, $J$ = 8.3 Hz, 2H), 2.09 (s, 3H).

5.10.25 1-(3-Fluorophenyl)-4-methyl-6-oxo-*N*-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1,6-dihydro-

pyridazine-3-carboxamide (48)

This compound was obtained as pale yellow solid in 65% yield; M.P.: 206.0-207.0 □; ESI-MS m/z: 473.10[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.64 (s, 1H), 8.60 (s, 1H), 7.96 (d, $J$ = 3.5 Hz, 1H), 7.79 (d, $J$ = 8.1 Hz, 2H),

7.72 – 7.53 (m, 4H), 7.31 (d, *J* = 5.1 Hz, 3H), 7.08 (s, 1H), 2.39 (s,3H).

5.10.26 4-Methyl-6-oxo-*N*-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(*p*-tolyl)-1,6-dihydropyridazine-3-carboxamide (49)

This compound was obtained as pale yellow solid in 59% yield; M.P.: 207.0-209.0 □; ESI-MS m/z: 469.12[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.61 (s, 1H), 8.60 (s, 1H), 7.96 (d, *J* = 4.8 Hz, 1H), 7.79 (d, *J* = 7.9 Hz, 2H), 7.65 (d, *J* = 5.1 Hz, 1H), 7.58 (d, *J* = 4.1 Hz, 2H), 7.31 (d, *J* = 1.2 Hz, 4H), 7.03 (s, 1H), 2.38 (s, 3H), 2.36 (s, 3H).

5.10.27 1-(4-Fluorophenyl)-4-methyl-6-oxo-*N*-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1,6-dihydro-pyridazine-3-carboxamide (50)

This compound was obtained as pale yellow solid in 77% yield; M.P.: 209.0-211.0 □; ESI-MS m/z: 473.10[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.57 (s, 1H), 8.52 (s, 1H), 7.88 (d, *J* = 5.9 Hz, 1H), 7.70 (t, *J* = 9.7 Hz, 4H), 7.58 (d, *J* = 5.8 Hz, 1H), 7.29 (t, *J* = 8.7 Hz, 2H), 7.23 (d, *J* = 8.6 Hz, 2H), 6.98 (s, 1H), 2.41 (s, 3H).

5.10.28 1-(3,4-Difluorophenyl)-4-methyl-6-oxo-*N*-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1,6-dihydro-pyridazine-3-carboxamide (51)

This compound was obtained as pale yellow solid in 76% yield; M.P.: 205.0-207.0 □; ESI-MS m/z: 491.09[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.57 (s, 1H), 8.52 (s, 1H), 7.90 – 7.84 (m, 2H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.60 – 7.48 (m, 3H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.00 (s, 1H), 2.32 (s, 3H).

5.10.29 *N*-(3-fluoro-4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1-(2-(trifluoromethyl)phenyl)-1,6-dihydropyridazine-3-carboxamide (52)

This compound was obtained as pale yellow solid in 64% yield; M.P.: 210.0-212.0 □; ESI-MS m/z: 541.08[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.29 (s, 1H), 8.87 (s, 1H), 8.64 (s, 1H), 8.03 (d, *J* = 7.9 Hz, 1H), 8.01 (d, *J* = 6.0 Hz, 1H), 7.98 – 7.91 (m, 3H), 7.87 (t, *J* = 7.7 Hz, 1H), 7.71 (d, *J* = 5.9 Hz, 1H), 7.54 – 7.46 (m, 2H), 2.09 (s, 3H).

5.10.30 1-(4-Bromo-2-fluorophenyl)-*N*-(3-fluoro-4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxamide (53)

This compound was obtained as pale yellow solid in 54% yield; M.P.: 213.0-215.0 □; ESI-MS m/z: 570.99[M+H]

$^{+}$. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 10.79 (s, 1H), 8.62 (s, 1H), 8.01 (d, *J* = 5.8 Hz, 1H), 7.84 (t, *J* = 9.9 Hz, 2H), 7.72 – 7.60 (m,3H), 7.55 (d, *J* = 8.8 Hz, 1H), 7.47 (t, *J* = 8.8 Hz, 1H), 7.10 (s, 1H), 2.40 (s, 3H).

5.10.31 *N*-(3-fluoro-4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1-(2-(trifluoromethoxy)phenyl) -1,6-dihydropyridazine-3-carboxamide (54)

This compound was obtained as pale yellow solid in 58% yield; M.P.: 214.0-215.0 □; ESI-MS m/z: 557.08[M+H] $^{+}$. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 12.19 (s, 1H), 8.86 (s, 1H), 8.64 (s, 1H), 8.01 (d, *J* = 5.9 Hz, 1H), 7.94 (d, *J* = 11.7 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.70 (mz, 4H), 7.53 – 7.46 (m, 2H), 2.09 (s, 3H).

5.10.32 *N*-(3-fluoro-4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(3-fluorophenyl)-4-methyl-6-oxo-1,6-dihydro pyridazine-3-carboxamide (55)

This compound was obtained as pale yellow solid in 64% yield; M.P.: 217.0-218.0 □; ESI-MS m/z: 491.09[M+H] $^{+}$. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 10.80 (s, 1H), 8.63 (s, 1H), 8.01 (d, *J* = 5.9 Hz, 1H), 7.88 (d, *J* = 12.5 Hz, 1H), 7.73 – 7.65 (m, 2H), 7.63 – 7.53 (m, 3H), 7.49 (t, *J* = 8.7 Hz, 1H), 7.32 (t, *J* = 8.2 Hz, 1H), 7.08 (s, 1H), 2.40 (s, 3H).

5.10.33 *N*-(3-fluoro-4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1-(p-tolyl)-1,6-dihydropyridazi ne-3-carboxamide (56)

This compound was obtained as pale yellow solid in 71% yield; M.P.: 211.0-212.0 □; ESI-MS m/z: 487.11[M+H] $^{+}$. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 10.76 (s, 1H), 8.62 (s, 1H), 8.00 (d, *J* = 5.9 Hz, 1H), 7.87 (d, *J* = 14.4 Hz, 1H), 7.70 (d, *J* = 5.9 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 3H), 7.47 (t, *J* = 8.8 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.03 (s, 1H), 2.38 (s,1H), 2.36 (s,3H).

5.10.34 *N*-(3-fluoro-4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-4-methyl-6-oxo-1,6-dihydro pyridazine-3-carboxamide (57)

This compound was obtained as pale yellow solid in 75% yield; M.P.: 213.0-215.0 □; ESI-MS m/z: 491.09[M+H] $^{+}$.$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 10.72 (s, 1H), 8.56 (s, 1H), 7.93 (s, 1H), 7.81 (d, *J* = 12.7 Hz,1H), 7.69 (dt, *J* = 8.8, 4.1 Hz, 2H), 7.64 (d, *J* = 5.7 Hz, 1H), 7.50 (d, *J* = 9.0 Hz, 1H), 7.41 (t, *J* = 8.6 Hz, 1H), 7.30 (t, *J* = 9.0 Hz, 2H), 6.99 (s, 1H), 2.42 (s, 3H).

5.10.35 1-(3,4-Difluorophenyl)-*N*-(3-fluoro-4-(thieno[2,3-*d*]pyrimidin-4-yloxy)phenyl)-4-methyl-6-oxo-1,6-

dihydropyridazine-3-carboxamide (58)

This compound was obtained as pale yellow solid in 78% yield; M.P.: 219.0-220.0 □; ESI-MS m/z: 509.08[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.72 (s, 1H), 8.55 (s, 1H), 7.93 (d, *J* = 5.9 Hz,1H), 7.87 (dd, *J* = 11.0, 8.0 Hz, 1H), 7.80 (d, *J* = 12.6 Hz, 1H), 7.63 (d, *J* = 5.9 Hz, 1H), 7.59 – 7.46 (m, 1H), 7.41 (t, *J* = 8.7 Hz, 3H), 7.01 (s, 1H), 2.41 (s, 3H).

5.10.36 *N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydropyridazine -3-carboxamide (59)

This compound was obtained as pale yellow solid in 61% yield; M.P.: 223.0-224.0 □; ESI-MS m/z: 477.07[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.04 (s, 1H), 8.98 (d, *J* = 8.0 Hz, 1H), 8.72 (s, 1H), 8.51 (d, *J* = 5.6 Hz, 1H), 7.95 (d, *J* = 12.6 Hz, 1H), 7.89 – 7.81 (m, 2H), 7.71 (d, *J* = 5.5 Hz, 1H), 7.49 (dt, *J* = 17.0, 9.6 Hz, 4H), 6.93 (d, *J* = 7.8 Hz, 1H).

5.10.37 1-(3-Chloro-4-fluorophenyl)-*N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-oxo-1,4-dihydro pyridazine-3-carboxamide (60)

This compound was obtained as pale yellow solid in 64% yield; M.P.: 220.0-221.0 □; ESI-MS m/z: 511.03[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.94 (s, 1H), 9.01 (d, *J* = 7.8 Hz, 1H), 8.73 (s, 1H), 8.52 (d, *J* = 5.4 Hz, 1H), 8.13 (s, 1H), 7.95 (d, *J* = 12.5 Hz, 1H), 7.86 (s, 1H), 7.71 (d, *J* = 6.5 Hz, 2H), 7.53 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 7.8 Hz, 1H).

5.10.38 1-(4-Bromophenyl)-*N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-oxo-1,4-dihydro-

pyridazine-3-carboxamide (61)

This compound was obtained as pale yellow solid in 66% yield; M.P.: 225.0-226.0 □; ESI-MS m/z: 538.99[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.96 (s, 1H), 9.01 (d, *J* = 7.8 Hz, 1H), 8.74 (d, *J* = 12.1 Hz, 1H), 8.52 (d, *J* = 5.5 Hz, 1H), 7.95 (d, *J* = 12.3 Hz, 1H), 7.80 (q, *J* = 8.6 Hz, 4H), 7.71 (d, *J* = 5.6 Hz, 1H), 7.53 (d, *J* = 9.5 Hz, 2H), 6.93 (d, *J* = 7.9 Hz, 1H).

5.10.39 *N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-oxo-1-phenyl-1,4-dihydropyridazine-3-

carboxamide (62)

This compound was obtained as pale yellow solid in 67% yield; M.P.: 220.0-221.0 □; ESI-MS m/z: 459.08[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.04 (s, 1H), 9.02 (d, *J* = 7.8 Hz, 1H), 8.73 (s, 1H), 8.52 (d, *J* = 5.5 Hz, 1H), 8.00 – 7.92 (m, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 5.4 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 2H), 7.53 (d, *J* = 8.9 Hz, 3H), 6.93 (d, *J* = 7.8 Hz, 1H).

5.10.40 *N*-(3-fluoro-4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)-4-oxo-1-(*p*-tolyl)-1,4-dihydropyridazine-3-carboxamide (63)

This compound was obtained as pale yellow solid in 67% yield; M.P.: 222.0-223.0□; ESI-MS m/z: 473.10[M+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.15 (s, 1H), 8.94 (d, *J* = 7.7 Hz, 1H), 8.73 (s, 1H), 8.52 (d, *J* = 5.5 Hz, 1H), 7.96 (d, *J* = 12.4 Hz, 1H), 7.71 (dd, *J* = 7.2, 4.6 Hz, 3H), 7.53 (q, *J* = 10.4, 9.5 Hz, 2H), 7.15 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 7.8 Hz, 1H), 3.83 (s, 3H).

### *5.11. Cytotoxicity assay in vitro*

The cytotoxic activities of target compounds (**24**-**29**, **30**-**37**, **38**-**44**, **45**-**51**, **52**-**58** and **59**-**63**) were evaluated with A549, HepG2, MCF-7 and PC-3 cell lines by the standard MTT assay *in vitro*, with compounds c-MET inhibitors Foretinib as positive control.[14] 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 of 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.

### *5.12. Tyrosine kinases assay in vitro*

The selected compounds ( **35**, **39** and **43**) 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[8,9]. 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 500 μM 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 10 μL 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.

### *5.13. Annexin V-FITC staining*

After digestion, the cells were centrifuged at 1000 rpm for 5 min. The supernatant was abandoned and the cells were collected, gently resuspended with PBS and counted. 5-10 million resuspended cells were centrifuged at 1000 rpm for 5 min. Then the supernatant was abandoned and 195 μl of Annexin V-FITC binding solution was added to gently resuspend the cells. Afterwards, 5 μl of Annexin V-FITC and 10 μl of propidium iodide staining solution were added and the solution was mixed gently. Finally, the solution was incubated in dark and in room temperature (20-25℃) for 10-20 min and tested by flow cytometry.[15]

### *5.14. Docking studies*

For docking purposes, the three-dimensional structure of the c-Met (PDB code: 3LQ8) was obtained from RCSB Protein Data Bank. Hydrogen atoms were added to the structure allowing for appropriate ionization at physiological pH and the water was removed. The protonated state of several important residue were adjusted and docking was carried out by using SYBYL 6.9.1 in favor of forming reasonable hydrogen bond with the ligand. And using pymol 1.8.6 to analyzing the results. Molecular docking of **43** into the 3D c-Met complex structure (PDB code: 3LQ8) was carried out using the SYBYL 6.9.1 (Tripos, St. Louis, MO, USA) and analyzing the results by the pymol 1.8.6. Displaying the hydrogen bond, p-pi interaction bond, surface and cartoon model to show the relationship between the 43 and c-Met(Fig 5a). According to the 3D figure, the 2D figure was carried out by chemdraw (Fig 5b). And from the Fig 5C, we can know that the compound **43** occupied the package of the c-Met.

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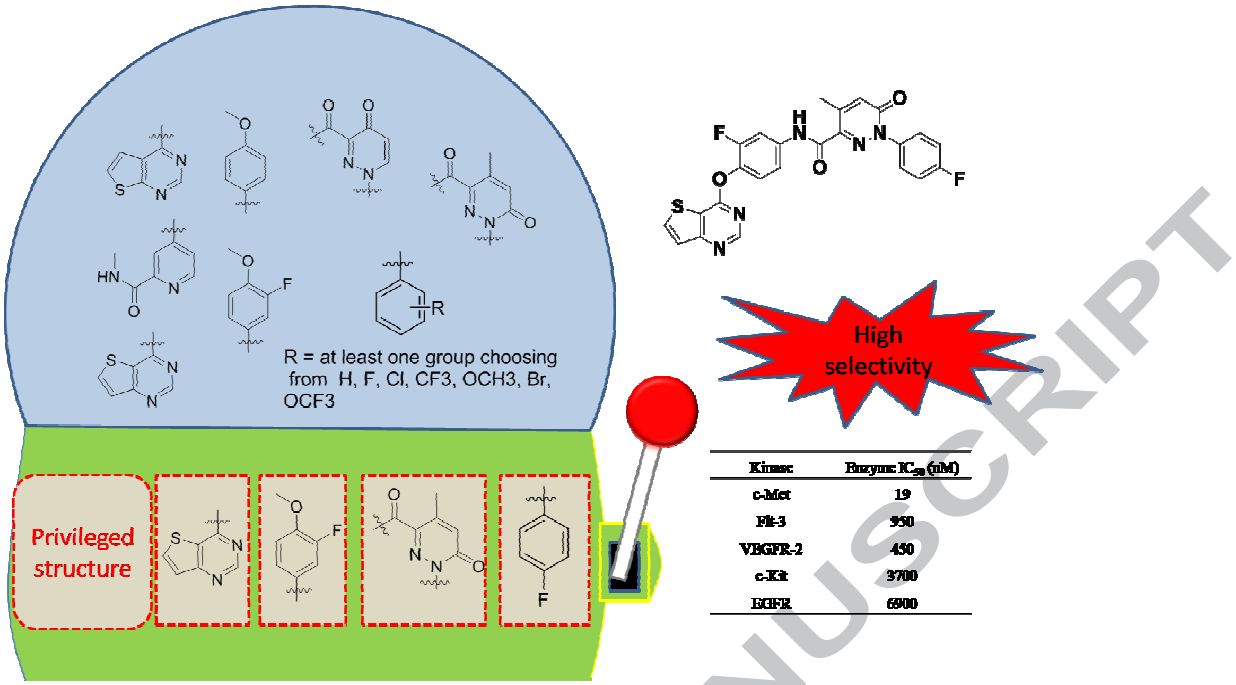


Four series of *N*-methylpicolinamide moiety and thienopyrimidine moiety bearing pyridazinone were designed and synthesized Moreover, antitumour activity, enzyme-based selectivity, AO staining, Annexin V/PI staining and docking study were carried out.