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Design and synthesis of novel substituted naphthyridines as potential c-Met kinase inhibitors based on MK-2461

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ABSTRACT

Two series of novel 1,5-naphthyridine and 1,6-naphthyridine derivatives were designed and synthesized based on the c-Met kinase inhibitor MK-2461 under the guidance of scaffold hopping strategy. All were tested on c-Met kinase and in vitro anti-tumor activities against Hela and A549 cell lines. The results indicated that 1,6-naphthyridine was a more promising c-Met inhibitory structure core compared with 1,5-naphthyridine. Among them, **26b** and **26c** showed the best enzymic and cytotoxic activities. The western blot experiments implied that the cytotoxic activity of **26c** might be partially through suppressing the phosphorylation of c-Met kinase.

c-Met is confirmed as the only high affinity receptor tyrosine kinase (RTK) that binds with the hepatocyte growth factor (HGF).[1] c-Met/HGF widely exists in the development of mammalian cells, but its expression level is very low in normal tissues.[2] Tumor biopsies reveal that c-Met overexpresses and HGF widely exists in solid cancer.[3] Binding of HGF to c-Met results in the autophosphorylation of Tyr1234/1235 in activation loop, which activates the downstream pathways involving promoting cell growth, inhibiting cell apoptosis, changing cytoskeleton function and increasing the metastasis.[4,5] A number of small-molecule c-Met inhibitors have been reported and marketed (Fig. 1).[6–11]

Among them, MK-2461 was developed by Merck and identified as an ATP-competitive c-Met inhibitor. It is efficacious in preclinical animal models of tumor suppression and its phase I/II study in patients with advanced solid tumors has also been completed recently.[8] As the fused tricyclic core of MK-2461 is unfavourable to its water solubility and pharmacokinetic profile, two series of 1,5-naphthyridine and 1,6-naphthyridine (Fig. 2) in which the heptatomic ring is omitted compared with MK-2461, have been designed under the guidance of scaffold hopping strategy in medicinal chemistry. Based on the structure–activity relationship (SAR) studies of MK-2461, the nitrogen of its ring A forms a key hydrogen bond with NH of Met1160 main chain in the hinge, so ring A is preserved in the target scaffolds. The scaffolds still remain planarity, meanwhile N atom at 5- or 6-position of ring B could simulate the hydrogen bond between the atom O of carbonyl and water in the original heptatomic ring of MK-2461. The SAR studies of MK-2461 revealed that substitution of phenyl group at 3-position with 1-methyl-4-pyrazolyl slightly increases the inhibitory activity of enzyme but greatly improves the cytotoxic activity,[8] so the pyrazolyl groups are reserved as designed. The studies on the SAR of these naphthyridines might identify the simplified pharmacophore and provide useful information for this class of potent c-Met inhibitor. Herein we report the synthesis of these substituted naphthyridines and their preliminary c-Met inhibitory results as well as the antiproliferative activities in cancer cells.

The synthesis of scaffold **1** was accomplished by a seven-step sequence, as illustrated in Scheme 1. In the Skraup reaction to prepare 1,5-naphthyridine **4**, *m*-NO_2PhSO_3Na was used as an oxidant,[12] which displayed a higher yield (45%) and a better reproducibility than I_2.[13] After bromination and oxidation,[14] compound **6** was obtained in a relatively low yield of 37% for 2 steps, which was attributed to the low regioselectivities of these reactions. Then compound **7** was obtained in 62% yield by the reaction of **6** with TsCl under the condition of K_2CO_3. Compound **9** was prepared in 83% yield by Suzuki reaction of **7** and **8** under the catalysis of $Pd(PPh_3)_4$ with Cs_2CO_3 as the alkali. K_2CO_3 and CsF were also examined under the same condition but showed extremely poor conversions. Compound **9** could be converted to **10** by refluxing in $POCl_3$ in 68% yield. In the workup procedure, the pH of the

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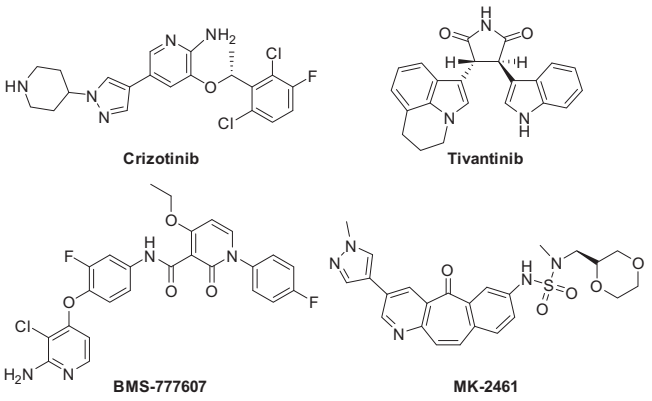


Figure 1. Representatives of c-Met inhibitors.

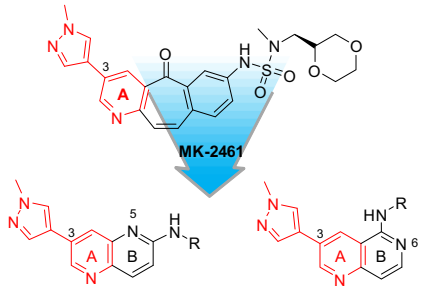


Figure 2. Design of 1,5-naphthyridine and 1,6-naphthyridine scaffolds.

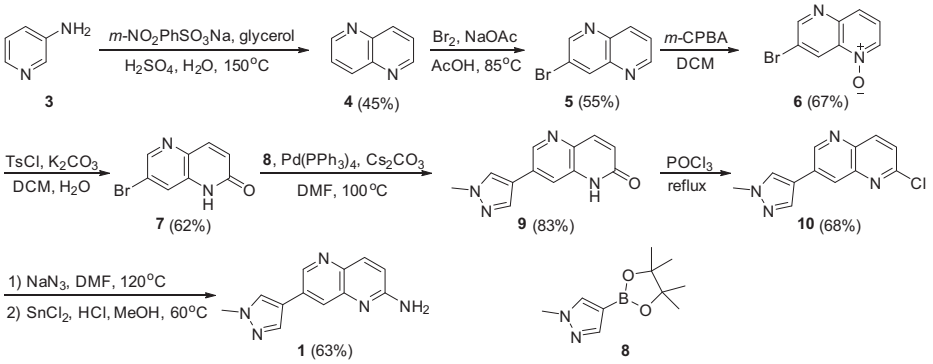
reaction mixture should be neutralized carefully around 7.0 with aqueous NaOH, in case product **10** hydrolyzed to **9**. The mixture of compound **10** and 25% aqueous ammonia was stirred at 120 °C using a microwave reaction device, but few product was obtained.[15] Finally, the azidation of **10** with NaN_3 followed by reduction with $SnCl_2$[16] gave 1,5-naphthyridine scaffold **1** in 63% yield for 2 steps. So far, the synthesis of compound **1** was accomplished via 7 steps synthetic route with commercial available 3-aminopyridine and glycerol as starting materials in 3.3% overall yield.

As shown in Scheme 2, the preparation of scaffold **2** followed a different synthetic route with **1** using 1,1,3,3-tetramethoxypropane and 2-cyanoacetamide as starting materials. The compounds **11**–**13** were synthesized according to the literature procedures[17] in a yield of 26% for 3 steps. The conversion of **13** to **15** was firstly tried by Sonogashira reaction directly, but achieved a yield as low as 50% since the 5-Br was a competitive position. However, after regioselective replacement of Cl to a more active I,[18] the transformation of **14** to **15** exhibited a much higher yield of 93% (from **13** to **15**, 70% yield in 2 steps). After addition of the triple bond, the aldehyde acetal **16** was obtained in 68% yield. Compound **17** was produced by oxidation of the cyano group, followed by cyclization to get compound **18** in 52% yield for 2 steps.[19] Afterwards, the preparation of **2** from **18** was the same as that of **1** from **7**. Thus, the synthesis of novel scaffold **2** was achieved in 2.9% overall yield via 11 steps synthetic route.

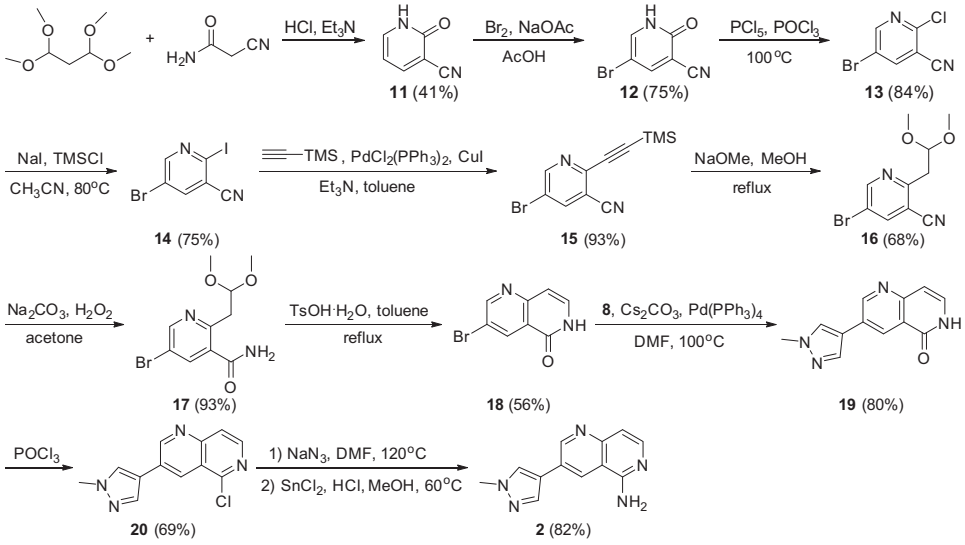
With scaffolds **1** and **2** in hand, we subsequently proceeded to the functionalization of their amino groups using MK-2461 as a reference. Three series of 1,5-naphthyridine derivatives **21**–**23** and 1,6-naphthyridine derivatives **25**–**27**, including acylated, alkylated and sulfonylated derivatives, were designed and synthesized according to the theory of bioisosterism (Scheme 3). The acylation of the scaffolds **1** and **2** with various acyl chlorides containing alkyl, cycloalkyl, phenyl and heterocyclic groups afforded the target compounds **21** and **25**. Unexpectedly, only di-acylated products **24a**–**e** were attained from 1,6-naphthyridine scaffold **2**, so alcoholysis of **24a**–**e** was subsequently carried out to get mono-acylated products **25a**–**e**. Compounds **21** and **25** were refluxed in THF with reductant $LiAlH_4$ for about 3 h to achieve the alkylated derivatives **22** and **26**, respectively. It is worth mentioning that the sulfonylation of **1** and **2** did not occur by heating at 120 °C, as sulfochlorides were less active than acylchlorides. The reaction proceeded efficiently under the condition of microwave at 120 °C for 1 h to give **23** and **27** in good yields.

The preliminary c-Met inhibitory activity of these target compounds and their antiproliferative activities in cancer cells were evaluated and listed in Table 1. The compounds were tested at a single concentration of 10 μM and defined as effective which inhibit over half of the c-Met kinase at that concentration. Most of the 1,6-naphthyridine derivatives (**2**, **25a**–**c**, **26a**–**c**, **27h** and **27j**) were effective, while a minority of the 1,5-naphthyridine derivatives (**1**, **21b** and **22a**–**d**) were active. Therefore it can be inferred that the alkylamino side chain at the 5-position was beneficial to the inhibition of c-Met kinase.

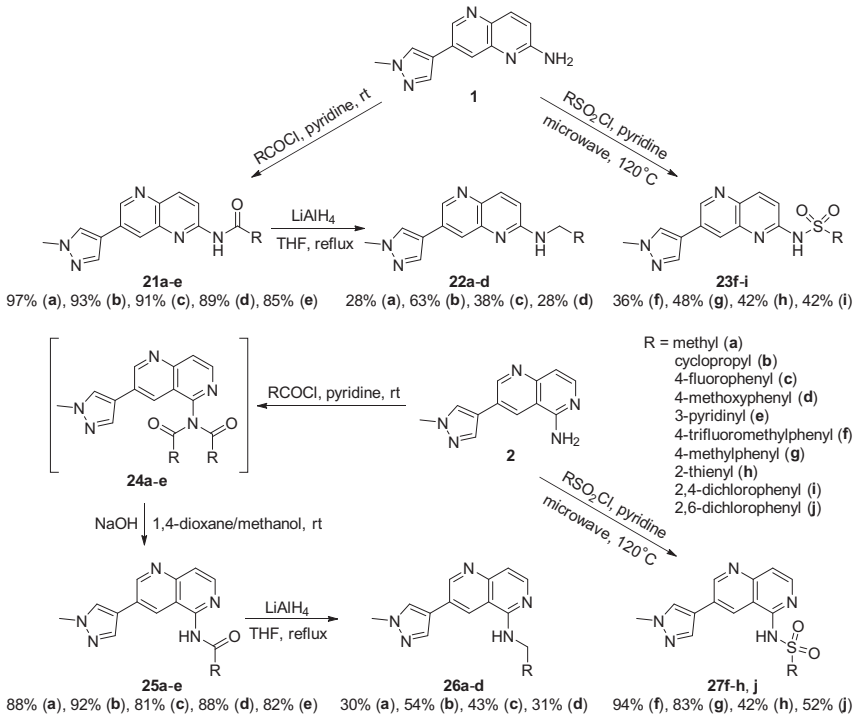
The compounds were further tested on the Hela and A549 cell lines (Table 1). Hela is a cervical cancer cell line and A549 is a lung cancer cell line, both of which could express high level of c-Met. 1,6-Naphthyridine derivatives performed better than 1,5-naphthyridine derivatives on both enzymic and cytotoxic activities, indicating that 1,6-naphthyridine skeleton could be a potential c-Met inhibitory structure core. Among 1,5-naphthyridine derivatives, only **21d** had cytotoxic activities in Hela and A549 cell lines, however **21d** could merely inhibit around 30% of the c-Met kinase, indicating that it might follow a different mechanism of action. Among 1,6-naphthyridine derivatives, **26b**, **26c** and **27h** showed cytotoxic activities with IC_{50} in the range of 5–10 μM. It was noting



Scheme 1. Synthesis of 1,5-naphthyridine scaffold **1**.



Scheme 2. Synthesis of 1,6-naphthyridine scaffold **2**.



Scheme 3. Synthesis of 1,5-naphthyridine derivatives (**21**–**23**) and 1,6-naphthyridine derivatives (**25**–**27**).

that amide as the connection of side chain at 5-position in **25a**–**e** totally lost the cytotoxic activities. Consistent with the results of c-Met inhibitory activities, the series of **26** displayed stronger cytotoxicity than the series of **25** and **27**. A possible explanation was that **26b** and **26c** had flexible alkylamino side chains which were more suitable for their induced fit interaction with the hydrophobic region of c-Met kinase. Moreover, the target molecules with rigid connecting side chains showed poor water and lipid solubility. Therefore, **26b** and **26c** were regarded as the most promising candidates as potential c-Met inhibitors among these target molecules.

Further, compound **26c** was selected to determine the inhibitory effect on phosphorylation of c-Met kinase using Western blot analysis. Hela cells were treated with **26c** at different concentrations (1.25–20 μM) or DMSO as negative control for 24 h. Then, the cells were harvested, lysed and subjected to Western blot analysis with the antibodies of phospho-Met (Tyr1234/1235) and Met, in which the level of actin served as loading control. Indeed, as shown in Figure 3, the phosphorylation of c-Met was suppressed by **26c** in a concentration dependent way, whereas the Met expression level was not obviously influenced. These results implied that the cytotoxic activity of **26c** might be, at least partially, through repressing c-Met kinase activation.

Based on the X-ray co-crystal structure of MK-2461 analog bound to the c-Met kinase domain (PDB: 3R7O[8]), we established the binding mode of new scaffold inhibitor **26c** by Schrodinger

Table 1
The inhibitory activities of 1,5-naphthyridine and 1,6-naphthyridine derivatives on c-Met kinase, Hela and A549 cell lines

Compd	c-Met (%) (10 μM)	Hela IC_{50} (μM)	A549 IC_{50} (μM)	Compd	c-Met (%) (10 μM)	Hela IC_{50} (μM)	A549 IC_{50} (μM)
1	57.1	>50	>50	**2**	73.7	40.8	33.4
21a	47.8	>50	>50	**25a**	55.4	>50	>50
21b	74.6	>50	>50	**25b**	60.1	>50	>50
21c	17.6	>50	>50	**25c**	55.0	>50	>50
21d	30.4	4.9	6.4	**25d**	49.8	>50	>50
21e	45.5	>50	>50	**25e**	48.7	>50	>50
22a	61.1	>50	>50	**26a**	59.2	>50	40.7
22b	64.0	>50	>50	**26b**	61.4	7.4	8.9
22c	61.4	46.6	>50	**26c**	73.5	9.2	5.2
22d	58.6	>50	>50	**26d**	38.1	42.2	>50
23f	47.4	>50	>50	**27f**	18.6	>50	>50
23g	39.3	>50	45.5	**27g**	29.3	>50	>50
23h	44.2	38.4	39.5	**27h**	57.0	17.4	9.4
23i	36.2	>50	>50	**27j**	64.4	>50	>50
Crizotinib	100% @50 nM	—	—	Cisplatin	—	3.3	20.4

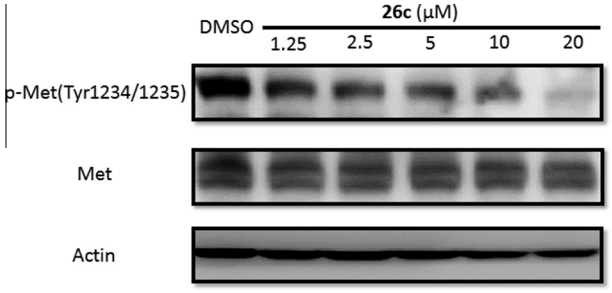


Figure 3. Effects of **26c** on the phosphorylation of c-Met kinase and its expression level in Hela cells.

10.1. As shown in Figure 4, the 1,6-naphthyridine derivative **26c** binds to the ATP binding pocket of the activated (phosphorylated) c-Met kinase domain in a 'U-shape' conformation. In particular, the N atom of A ring forms a key hydrogen bond with the hinge backbone NH of Met1160. In this orientation, the pyrazole moiety extends along the hinge and faces to the solvent accessible region. The H atom of amino group at 5-positon is involved in a water-mediated hydrogen bond to the carboxyl oxygen of side-chain Asp1164, and the *p*-fluorophenyl tail falls into the hydrophobic pocket. All these interactions contribute to the tight binding and greatly enhance the inhibitory potency of **26c**. Compared with MK-2461, **26c** lacks a side chain hydrogen bonding interaction with the c-Met, so further structural modifications will be focused on the substituents with hydrogen-bonding donor/acceptor.

In conclusion, two series of novel 1,5-naphthyridine and 1,6-naphthyridine derivatives were designed and synthesized based on c-Met kinase inhibitor MK-2461, and the preliminary inhibitory activities on c-Met and the antiproliferative activities in cancer cells were evaluated. Among 28 derivatives, **26b** and **26c** were identified as the promising c-Met kinase inhibitor candidates with obvious cytotoxic activities against Hela and A549 cell lines. Additionally, Western blotting results showed that phosphorylation levels of c-Met at Tyr1234/1235 were dose-dependently decreased by **26c** in Hela cells. The SAR outlined in the current study will definitely facilitate the further structural modification of these naphthyridine derivatives as potential anti-cancer agents by targeting c-Met kinase.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.05.082.

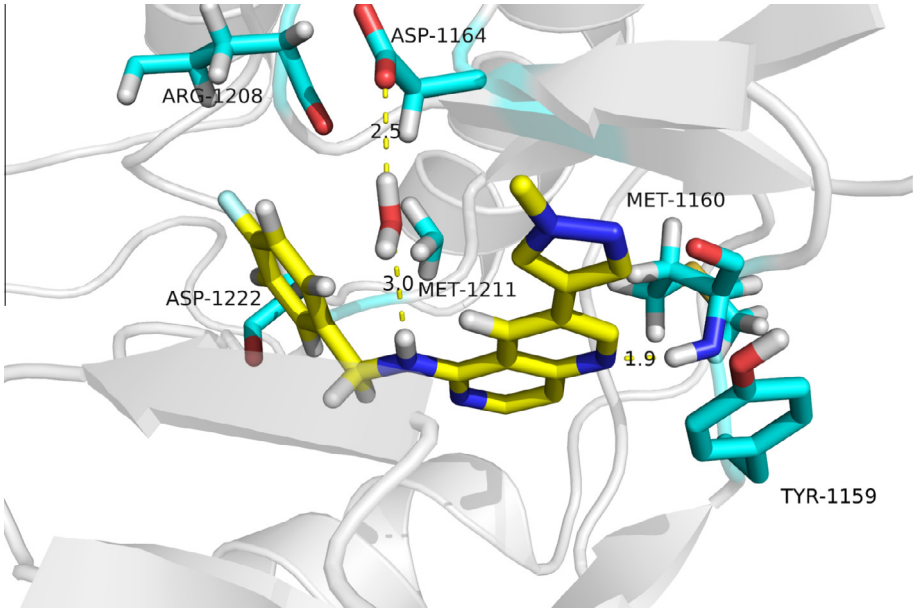

Figure 4. Proposed binding mode of **26c** in the ATP binding pocket of the c-Met kinase domain.

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