Bioorganic Chemistry 65 (2016) 146–158

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

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

Na Liu [a,1], Yanfen Wang [a,1], Gongchao Huang [b,1], Conghui Ji [b], Wei Fan [a], Haitao Li [b], Ying Cheng [b], Hongqi Tian [c,*]

[a] School of Biological Engineering, Tianjin University of Science and Technology, #29, 13th Avenue, TEDA, Tianjin 300457, China
[b] Tianjin Binjiang Pharma, Inc., #238 Baidi Road, Nankai District, Tianjin 300192, China
[c] Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Sciences & Peking Union Medical College, #238 Baidi Road, Nankai District, Tianjin 300192, China

ARTICLE INFO

Article history:
Received 11 January 2016
Revised 25 February 2016
Accepted 27 February 2016
Available online 2 March 2016

Keywords:
c-Met inhibitor
Azaindole
Azaindazole
Synthesis
Biological evaluation

ABSTRACT

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

1. Introduction

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

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

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

* Corresponding author.
E-mail address: tianhongqi@irm-cams.ac.cn (H. Tian).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.bioorg.2016.02.009
0045-2068/

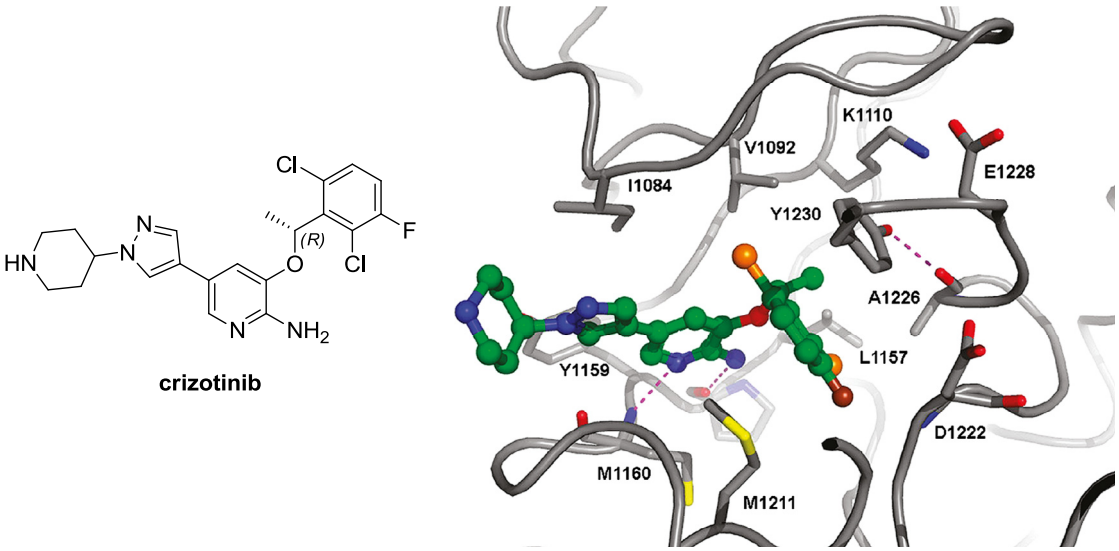

Fig. 1. Cocrystalline structure of Crizotinib combined with c-Met.

and 3-fluoro elements on the 3-benzyloxy group point toward the NH of D1222 of the activation loop, indicating that there may be beneficial electrostatic interactions. The methylene linker allows the two critical binding elements to wrap around M1211 and form hydrogen bonds with the backbone of the hinge and π-stacking interactions with Y1230. The R-methyl group is used to rigidify the benzyl group, and also make favorable hydrophobic interactions with the residues of V1092, L1157, K1110, and A1108 in the pocket. The 5-pyrazol-4-yl group is bound through the narrow lipophilic tunnel surrounded by I1084 and Y1159 and provides the inhibition of c-Met. The terminal piperidine ring that appears to serve as a solubilizing group, attached to the N1 position of the pyrazol-4-yl, extends out into the solvent surrounding the kinase hinge segment. Bringing together all of these groups in Crizotinib compound, results in a strong inhibition of c-Met.

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

2. Results and discussion

The results of this study included the design, synthesis, and characterization of the five new compounds, following the replacement of the aminopyridine in Crizotinib with the closely related 1H-pyrrolo[2,3-*b*]pyridine or 1H-pyrazolo[3,4-*b*]pyridine. The results also included the design, characterization and biological evaluation of the five new compounds directed against c-Met, following the replacement of the linkage between the 7-azaindole or 7-azaindazole and the 2,6-dichloro-3-fluorophenyl with a methylene, sulfoxide or cyclopropyl. The mode of interactions between Crizotinib and c-Met kinase was reported previously by J. Jean Cui and her co-authors in Ref. [8] (Fig. 1). The new derivatives, taking Crizotinib and compound 1 as a lead scaffold, were expected to have the similar binding mode. The NH and ring nitrogen of 7-azaindole or 7-azaindazole in new compounds were expected to result in two hydrogen bonds with the backbone CO of P1158 and NH of M1160 of the kinase hinge region, similar to the 2-aminopyridine of Crizotinib. The halogenated phenyl group was maintained owing to its potential co-planar π-interaction with the residue Y1230 of the activation loop. Both the 2-chloro and 3-fluoro elements on the phenyl group were maintained due to the electrostatic interactions with the backbone NH of D1222. The pyrazole group at the C5 position of the 2-aminopyridine was maintained due to its compact size and its ability to modulate physicochemical properties. The terminal piperidine ring, which attached to the N1 position of the pyrazol-4-yl, was maintained to serve as a solubilizing group.

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

Further optimization resulted from the introduction of 7-azaindazole and replacement of the linkage between the 7-azaindole and 7-azaindazole and the 2,6-dichloro-3-fluorophenyl. Following replacement of the aminopyridine in Crizotinib with 7-azaindole and 7-azaindazole, the methylene