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Original article

Design, synthesis and molecular docking studies of some novel spiro[indoline-3, 4′-piperidine]-2-ones as potential c-Met inhibitors

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Deregulation of receptor tyrosine kinase c-Met has been reported in human cancers and is considered as an attractive target for small molecule drug discovery. In this study, a series of spiro[indoline-3, 4′-piperidine]-2-ones were designed, synthesized and evaluated as novel c-Met inhibitors. The results showed that the majority of the compounds exhibited significant inhibitory effect on c-Met with IC_{50} values of 0.0147–17 μM in TR-FRET-based assay and IC_{50} values of 1.56–1400 μM in cell-based assay. Furthermore, our docking experiments verified the results and explained the molecular mechanism of eminent activities to c-Met.

1. Introduction

c-Met is a tyrosine kinase receptor for hepatocyte growth factor (HGF). Both c-Met and HGF are expressed in different tissues [1]. Binding of HGF to the extracellular domain of c-Met can cause multimerization of the receptor and phosphorylation of tyrosine residues. c-Met/HGF signaling is essential for normal cell proliferation, migration, angiogenesis and tissue regeneration [2]. In addition, aberrant c-met/HGF signaling plays a major role in tumorgenesis invasion and metastasis in many human tumors [3]. The mutation and over-expression of c-Met proto-oncogene and/or HGF have been detected in different types of malignant solid tumors and correlated with advanced stages and poor prognosis [4]. Therefore, c-Met has recently become an attractive therapeutic target for cancer therapy. One way to block c-Met signaling is by inhibiting phosphorylation of the tyrosine kinase c-Met domain with small molecular inhibitors [5].

In our previous study, we discovered the spiro[indoline-3, 4′-piperidine]-2(1*H*)-ones **1** and **2** as potent c-Met inhibitors (Fig. 1) [6]. In an ongoing effort to design novel and selective inhibitors of the c-Met enzyme, we were intrigued by filed applications from *Pfizer* [7] and *Janssen* [8], in which they claimed that a series of 1-(quinolin-6-ylmethyl)-3*H*-[1,2,3]triazolo[4,5-*b*] pyrazine derivatives and 3-(quinolin-6-ylmethyl)-3*H*-[1,2,4]triazolo[4,3-*b*]pyridazin derivatives were potent and selective c-Met inhibitors. Two representative examples, PF-04217903 **3** and JNJ-33377605 **4** (Fig. 1), are in Phase I clinical trials.

Intrigued by the low molecular weight and unknown binding mode of PF-04217903 and JNJ-33377605 to c-Met and our previous study, we introduced the spiro[indoline-3, 4′-piperidine]-2-ones to the 1-(quinolin-6-ylmethyl)-3*H*-[1,2,3] triazolo[4,5-*b*]pyrazine and the 3-(quinolin-6-ylmethyl)-3*H*-[1,2,4]triazolo[4,3-*b*] pyridazine and designed structurally relevant novel compounds **5a**–**5b** and **6a**–**6b**(Fig. 2). Based on the principle of bioisosterism, we replaced the [1,2,3]triazolo[4,5-*b*]pyrazine and 3*H*-[1,2,4]triazolo[4,3-*b*] pyridazine with 3*H*-[1,2, 3]triazolo[4,5-*d*]pyrimidine and 7*H*-purine to obtain other new compounds **5c**–**5d** and **6c**–**6d** (Fig. 2). We synthesized these compounds, investigated their inhibitions on c-Met, verified the results and explained the molecular mechanism of eminent activities to c-Met through docking experiments.

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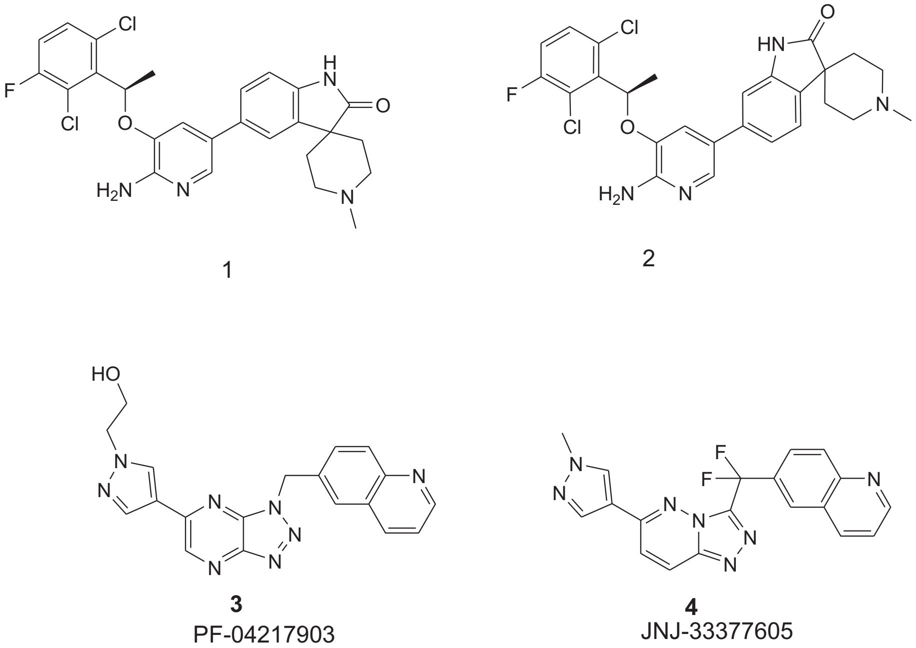

1

2

3
PF-04217903

4
JNJ-33377605

Fig. 1. Reported c-Met inhibitors.

2. Results and discussions

2.1. Chemistry

The preparation of target compounds **5a**–**5d** and **6a**–**6d** was described in Scheme 1. Compounds (**7a**–**7d**) were prepared according to references [6–8].

The pinacol borane esters **8** and **9** were synthesized by reaction of halide **10** or **11** and bis(pinacolato)diboron under basic conditions in 100% yields, while **10** and **11** were synthesized using commercially available 5-bromo-1,3-dihydro-2*H*- indole-2-one(**12**) or 6-bromo-1,3-dihydro-2*H*- indole-2-one(**13**)as a material with the yield of 78% and 82% (Scheme 2).

The reactions (7a)–(7d) of with **8** or **9** gave **5a**–**5d** and **6a**–**6d** with yields of 67%, 78%, 70%, 71%, 72%, 75%, 76%, and 72% respectively *via Suzuki coupling* reaction. This coupling reaction with pinacol borane esters gave good yields under basic conditions using DMF/H_2O as a solvent and Pd $(PPh_3)_4$ as a catalyst [9]. The present synthesis provided a range of applicability.

All the products were characterized by ^{1}H NMR, ^{13}C NMR, elemental analyses and ESI-MS.

2.2. Evaluation of biological activity

The results of anti-c-Met enzyme activities *in vitro* showed that all the title compounds were active against c-Met enzyme, in which **5a**–**5b** and **6a**–**6b** had more potent activities against the c-Met enzyme, in which **6a** had the best inhibitory effect with IC_{50} of 0.0147 μM, **5c**–**5d** and **6c**–**6d** had lower inhibitory effect on c-Met enzyme (Table 1). The IC_{50} values derived from *in vitro* screening studies revealed that all the compounds posed significant inhibition on MKN-45 cell lines (Table 1). In accordance with the results of c-Met enzyme, Compounds **5a**, **5b**, **6a**, and **6b** showed higher inhibition on MKN-45 cell lines with IC_{50} values of 4.77, 5.32, 1.56 and 3.8 μM, respectively. The IC_{50} values of the compounds revealed that 3*H*-[1,2,3]triazolo[4,5-*b*]pyrazine, 3*H*-[1,2,4]triazolo [4,3-*b*] pyridazine in 5-position and 6-position of spiro[indoline-3, 4′-piperidine]-2-ones increased the activity.

2.3. Docking studies

In order to validate the docking reliability, the ligand SGX523 was removed from the active site and docked back into the binding pocket. The root mean square deviation (RMSD) between the predicted conformation and the actual conformation from the crystal structure of ligand was 0.619Å, which is smaller than the resolution of X-ray crystallography, 1.8Å. It indicated that the parameter set for the Surflex-dock simulation was reasonable to reproduce the X-ray structure. Therefore the simulation method and the parameter set could be extended to study the binding conformations of the other inhibitors.

In general, all the compounds assumed a ‘U-shaped’ binding mode (shown in Fig. 3), which is the typical character of selectivity [10]. The quinoline moiety engaged the hinge region with a single typical hydrogen bond between the N of quinoline and the back bone carbonyl of Met1160, which was highly characteristic of all compounds bound to ATP binding site in kinase domains. Triazolopyridazine, as an electron deficient group, was sandwiched in between Tyr1230 and Met1211 through a π–π stacking interaction. In addition, the hydrogen bond was important to the binding affinity [11]. Two nitrogen atoms of the triazolopyridine in compound **6a** formed two hydrogen bonds with the back bone NH of Asp1222 (shown in Fig. 4), so it had higher affinity and lower IC_{50} value than the **6b**, **6c** and **6d**, which only one hydrogen bond generated. Moreover, the hydrogen bonds with the Asp1222 would stabilize the conformations with lower energy [12]. The binding mode of our compounds differed completely from the similar spiroindolinones reported by P. Eastwood [13]. As p38 alpha MAP kinases inhibitors, their compounds adopted an opposite orientation. The hinge region formed two hydrogen bonds with the carbonyl moiety of the indolin-2-one through Met109 and Gly110, and the spirocyclic ring pointed out towards the solvent surface neighboring the hinge region. While in our compounds, the nitrogen atom of quinoline formed the hydrogen bond with hinge region through Met1160, and the spirocyclic pyridine ring also pointed out towards the solvent surface after “U-shape” folding, however, the solvent surface was far away from the hinge region