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# Novel 5-(benzyloxy)pyridin-2(1*H*)-one derivatives as potent c-Met inhibitors

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ABSTRACT

A series of novel 5-(benzyloxy)pyridin-2(1*H*)-ones were designed, synthesized and biologically evaluated for c-Met inhibition. Various amides and benzoimidazoles at C-3 position were investigated. A potent compound **12b** with a c-Met $IC_{50}$ of 12 nM was identified. This compound exhibited potent inhibition of EBC-1 cell associated with c-Met constitutive activation and showed high selectivity for c-Met than other tested 11 kinases. The binding model **12b** with c-Met was disclosed by docking analysis.



c-Met is a unique member of receptor tyrosine kinase (RTK) expressed in both normal and malignant cells. It is a cell surface receptor for hepatocyte growth factor (HGF), a pleiotropic cytokine that conveys a unique combination of pro-migratory, anti-apoptotic and mitogenic signals.[1] Aberrant c-Met signalling has been identified in various human cancers. Moreover, both c-Met over-expression and *MET* amplification have been associated with poor clinical outcomes of cancers. Of particular note, HGF/c-Met signaling is responsible for resistance to other cancer therapies.[2] Without a doubt, c-Met has become an attractive target for cancer therapy. In the past decade, a plethora of efforts have been devoted to explore the effective means to interrupt the abnormal c-Met pathway. Small molecule inhibitors are an important class of therapeutic techniques targeting c-Met.

To date, a respectable number of c-Met inhibitors have already been reported.[2,3] A well-known compound, crizotinib (Fig. 1B, **1**), developed by scientists at Pfizer displayed c-Met inhibition with a $K_i$ of 2 nM.[4] The cocrystal structure of crizotinib (Fig. 1A) disclosed its aminopyridine formed bidentate hydrogen bonds with the hinge of c-Met, 2,6-dichloro-3-fluorobenzyloxy fragment involved a π–π interaction with activation loop residue Tyr1230 and 4-(1*H*-pyrazol-1-yl)piperidine reached out into the solvent. Xcovery subsequently reported a series of pyridazin-3-amines as c-Met inhibitors,[5a] in which X376 (Fig. 1B, **2**) with a c-Met $IC_{50}$ of 0.69 nM was identified.[5b] Apart from the bidentate hydrogen bonding fashion, a single hydrogen bond interaction with the hinge region of c-Met was also proved effective. For example, 6-benzyloxyquinoline analogue (Fig. 1B, **4**), of which quinoline nitrogen H-bonded with the Met 1160 residue of the hinge region, exhibited c-Met inhibition at 23 nM.[6] Similarly, researchers at Sanofi demonstrated that 6-benzyloxybenzo[*d*]thiazole derivatives (Fig. 1B, **3**) were potent c-Met inhibitors ($IC_{50}$ <100 nM).[7] The remarkable discrepancies of these structures lie in the fragments interactive with the hing region of c-Met.

The less potency of compound **4** than crizotinib and X376 might be ascribed to the quinoline core only providing one hydrogen bond interactive with the hinge of c-Met. On the basis of the pharmacophore model of compound **4**, a novel pyridin-2(1*H*)-one scaffold was designed (Fig. 2). We envisaged that the pyridin-2(1*H*)-one scaffold might deliver bidentate hydrogen bonding with the hinge, in which the carbonyl oxygen can act as a hydrogen bond acceptor and NH as a donor. The bidentate hydrogen bonding might improve the c-Met potency. Meanwhile, the 2,6-dichloro-3-fluorobenzyloxy group was maintained owing to its potential π–π interaction with the residue Tyr 1230. The side chain R was expected to extend to the solvent accessible region. Herein, we disclosed our efforts to synthesis and biological evaluation of the novel 5-(benzyloxy)pyridin-2(1*H*)-one derivatives against c-Met.

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† These authors contributed equally to this study.

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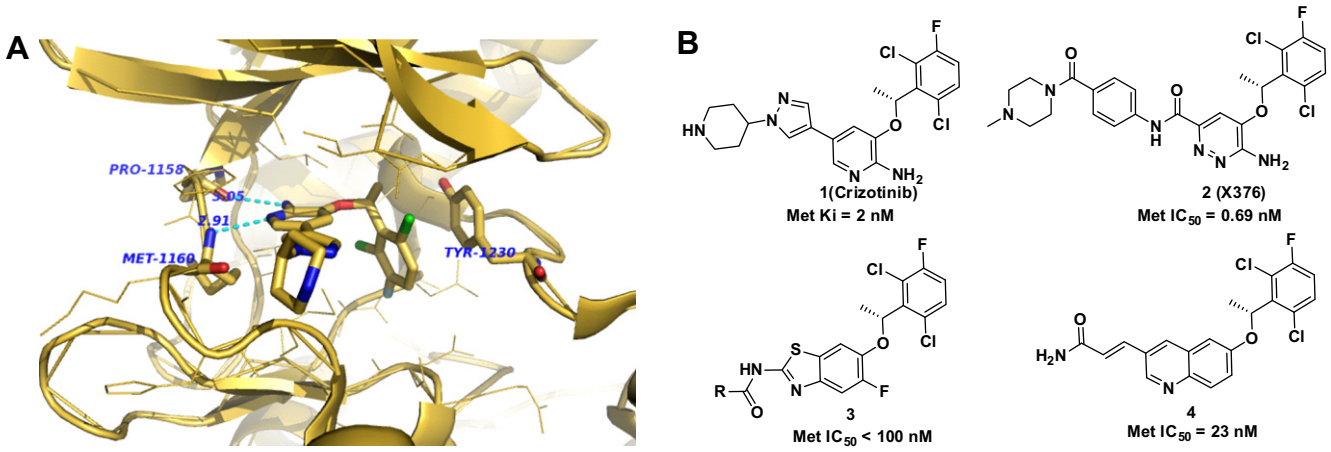



**Figure 1.** (A) Cocrystal structure of crizotinib bound to c-Met. (B) Selected examples of c-Met kinase inhibitors.

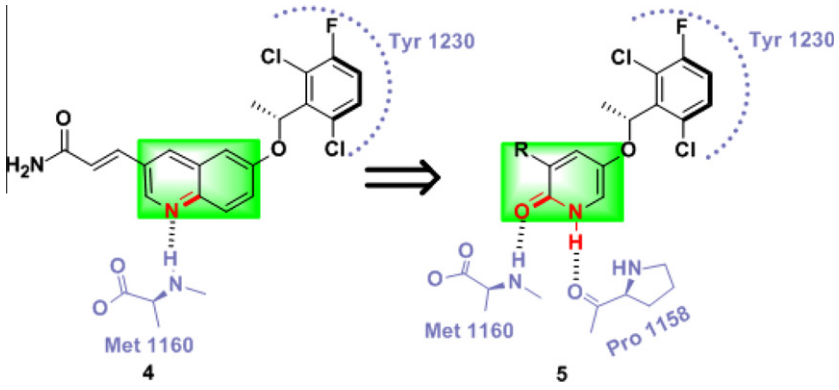
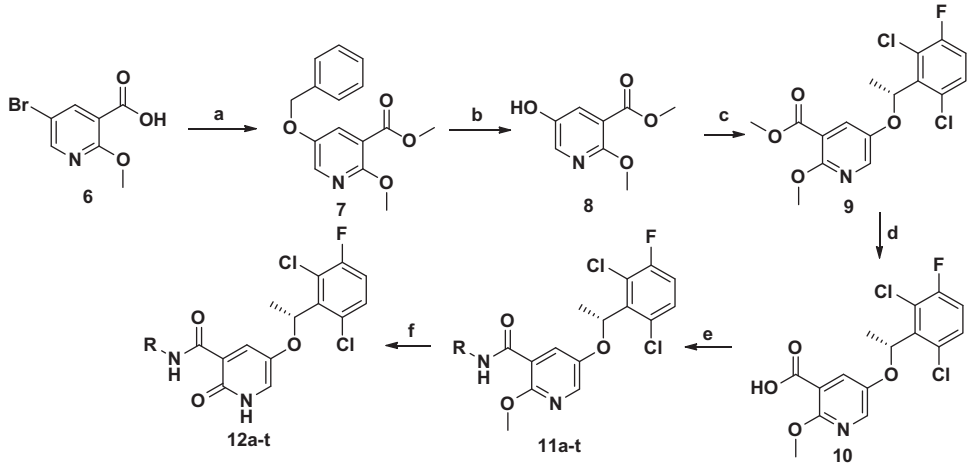
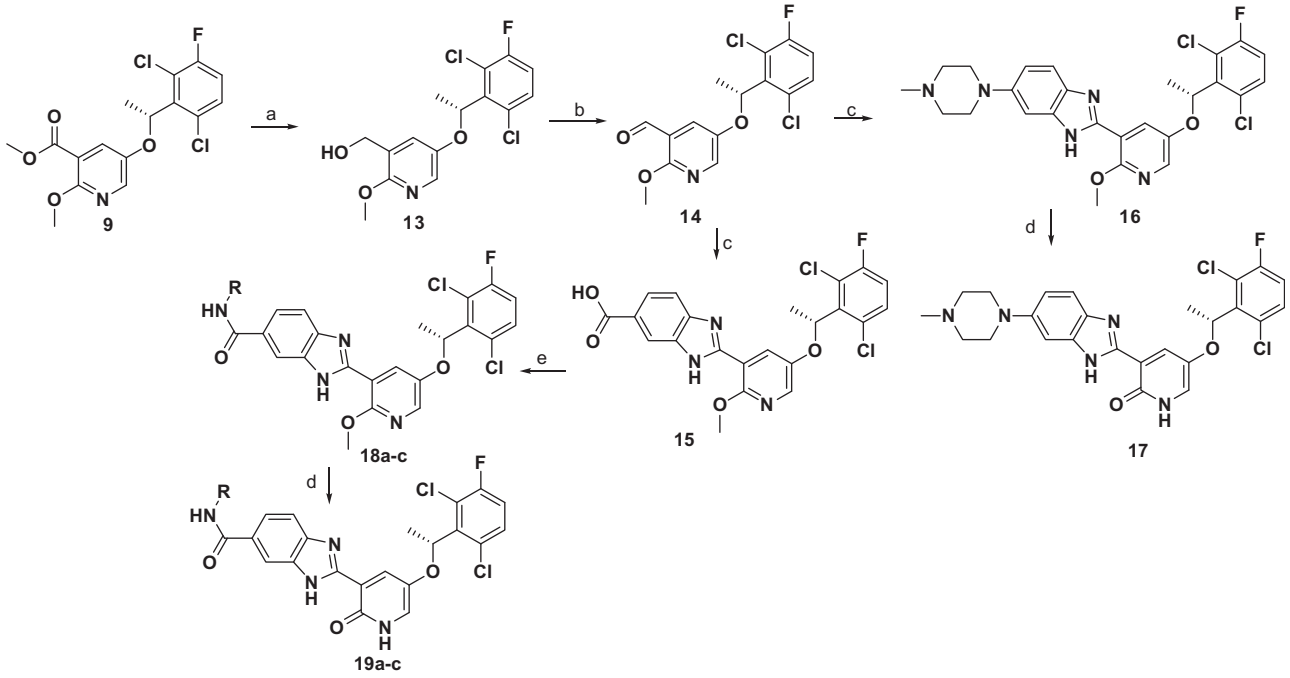



**Figure 2.** Design of the pyridin-2(1*H*)-one scaffold.



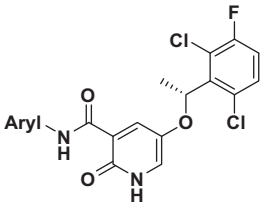

**Scheme 1.** Reagents and conditions: (a) benzyl alcohol, CuI, 1,10-phenanthroline, $Cs_2CO_3$, toluene, 110 °C; oxalyl chloride, DMF, $CH_2Cl_2$, 0–55 °C, MeOH, rt, 43% yield; (b) Pd/C, $H_2$, MeOH, 91% yield; (c) (*S*)-1-(2,6-dichloro-3-fluorophenyl)ethanol, DIAD, $PPh_3$, toluene, 0 °C to rt, 85% yield; (d) LiOH, THF/MeOH/$H_2O$ (2/1/1, v/v/v), rt, 95% yield; (e) amines, HATU, DIPEA, DMF, 0 °C to rt, 40–87% yield; (f) TMSCl, NaI, $CH_3CN$, rt, 46–90% yield.

The construction of 5-(benzyloxy)pyridin-2(1*H*)-one scaffold was described in Scheme 1. According to previous research work,[5a] a series of amide chains were initially installed at C-3 position of the 5-(benzyloxy)pyridin-2(1*H*)-one scaffold. Our synthesis began with commercial available 5-bromo-2-methoxynicotinic acid. C–O coupling of 5-bromo-2-methoxynicotinic acid **6** with benzyl alcohol,[8] followed by esterification of the resulting acid, afforded methyl ester **7** in 43% overall yield. The hydroxyl group was smoothly installed by debenzylation of the intermediate **7**. Treatment of the methyl 5-hydroxy-2-methoxynicotinate **8** with (*S*)-1-(2,6-dichloro-3-fluorophenyl)ethanol under Mitsunobu conditions gave the key intermediate **9** in 85% yield.[9] Hydrolysis of the ester **9** followed by coupling with a series of amines deliver **11a–t** (40–87% yield). Finally, compounds **12a–t** were obtained in



**Scheme 2.** Reagents and conditions: (a) $LiAlH_4$, THF, −5 to 0 °C, 84% yield; (b) Dess–Martin periodinane, $NaHCO_3$, $CH_2Cl_2$, rt, 70% yield; (c) diamines, aq $NaHSO_3$, EtOH, reflux. For **15**, 81% yield; for **16**, 70% yield; (d) TMSCl, NaI, $CH_3CN$, rt, 64–85% yield; (e) amines, HATU, DIPEA, DMF, 0 °C to rt, 77–87% yield.

**Table 1**
SAR about heteroaryl/aryl amides at C-3 position



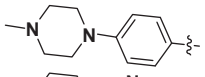
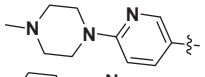
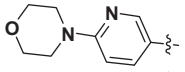
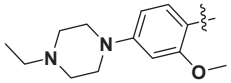
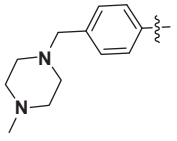
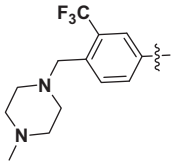
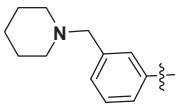
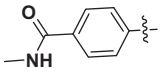
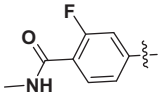
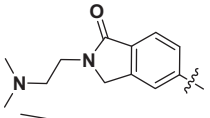
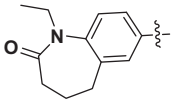
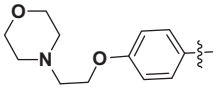
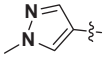
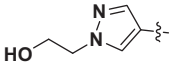
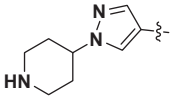
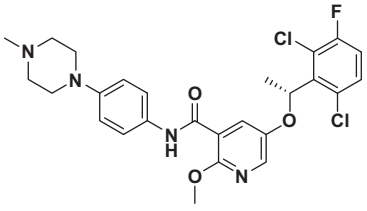
| Compound | Aryl | c-Met $IC_{50}$ (nM) | EBC-1[a] $IC_{50}$ (μM) |
|---|---|---|---|
| **12a** |  | 8 ± 1 (μM) | ND[c] |
| **12b** |  | 12 ± 3 | 2.2 ± 0.5 |
| **12c** |  | 31 ± 11 | 3.9 ± 0.7 |
| **12d** |  | 78 ± 1 | ND[c] |
| **12e** |  | NA[b] | ND[c] |
| **12f** |  | 24 ± 8 | 1.1 ± 0.2 |
| **12g** |  | 152 ± 53 | ND[c] |

**Table 1** (*continued*)

| Compound | Aryl | c-Met $IC_{50}$ (nM) | EBC-1[a] $IC_{50}$ (μM) |
|---|---|---|---|
| **12h** |  | 76 ± 14 | ND[c] |
| **12i** |  | 48 ± 2 | ND[c] |
| **12j** |  | 142 ± 27 | ND[c] |
| **12k** |  | 23 ± 4 | 2.6 ± 0.3 |
| **12l** |  | 75 ± 19 | ND[c] |
| **12m** |  | 27 ± 1 | 5.9 ± 0.8 |
| **12n** |  | 30 ± 8 | 9.8 ± 2.4 |
| **12o** |  | 27 ± 2 | 1.1 ± 0.3 |
| **12p** |  | 14 ± 3 | 1.0 ± 0.2 |
| **11b** |  | NA[b] | ND[c] |

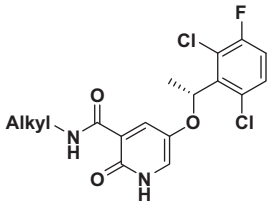
[a] EBC-1: human non-small-cell lung cancer cell line that expresses elevated levels of constitutively active c-Met.
[b] NA: not active at 1.0 μM.
[c] ND: not determined.

46–90% yield by selective demethylation of **11a–t** in the presence of TMSCl/NaI.[10]

To further investigate of SAR at C-3 position of the 5-(benzyloxy)pyridin-2(1*H*)-one scaffold, a series of benzoimidazole analogs were prepared as illustrated in Scheme 2. Reduction of the ester **9** with $LiAlH_4$ afforded the corresponding alcohol **13** in 84% yield. Oxidation of the alcohol **13** was achieved with Dess–Martin periodinane to give aldehyde **14** in 70% yield. Next, condensation of **14** with diamines furnished the benzoimidazole intermediates **15** and **16**.[11] Demethylation of **15** smoothly gave the compound **17** in 85% yield. Compounds **19a–c** were prepared in a similar manner as **12a–t**.

Initially, a series of aryl/heteroaryl amides at C-3 position were biological evaluated against c-Met. The results were summarized in Table 1. The unsubstituted phenyl amide analog **12a** displayed weak c-Met inhibition at 8 μM. Installation of the 4-(*N*-methylpiperazine) group (**12b**) on the phenyl of compound **12a** dramatically improved c-Met inhibition and showed an enzymatic $IC_{50}$ of 12 nM and moderate EBC-1 cell $IC_{50}$ of 2.2 μM, which suggested a polar tail had notable impact on c-Met binding. Replacement of phenyl ring (**12b**) with pyridyl group (**12c** and **12d**) resulted in 2- to 6-fold loss of enzymatic inhibition. Interestingly, introduction of a methoxy group adjacent to the amide (**12e**) completely abolished activity, suggesting a coplanar geometric arrangement of the pyridone core with the aniline aromatic ring seems to be preferred. Compared with **12b**, the enzymatic activity was slightly weak with the introduction of 4-[4-(methylpiperazin-1-yl)-methyl]phenyl group (**12f**) but substantially decreased with the incorporation of an additional $CF_3$ group (**12g**). Appending a 3-(piperidin-1-ylmethyl group (**12h**) led to a sixfold drop in potency. Incorporation of benzamides (**12i** and **12j**) and cyclic benzamides (**12k** and **12l**) was also investigated. However, there was no significant gain in both enzymatic and cellular activity. Pyrazole fragment is usually used to modulate physical chemical properties in c-Met inhibitors, presumably due to its lower *c* log*P* and higher PSA.[3] Appendage of pyrazoly amides at the C-3 position of the pyridone exhibited enzymatic inhibition with $IC_{50}$ ranging from 30 to 14 nM. It seemed that introduction of the polar tails onto the pyrazole (**12o** and **12p**) was more preferred over alkyl substituted pyrazole (**12n**) for activity at cellular level. Compound **12p** displayed c-Met enzymatic inhibition as **12b**, but showed better cellular potency. It is worth noting that the carbonyl oxygen of the pyridin-2(1*H*)-one contributed significantly to the c-Met binding affinity because block of oxygen with methyl group (**11b**) resulted in a sharp loss of activity.

**Table 2**
SAR about alkyl amides at C-3 position



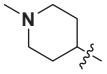
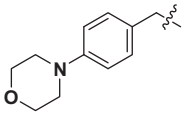
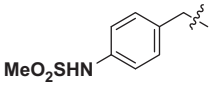
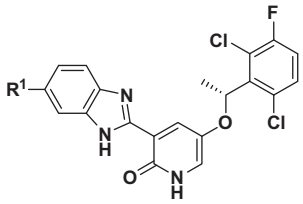
| Compound | Alkyl | c-Met $IC_{50}$ |
|---|---|---|
| **12q** |  | 0.32 ± 0.10 |
| **12r** |  | <50%@10 μM |
| **12s** |  | <50%@10 μM |
| **12t** |  | <50%@1 μM |

**Table 3**
SAR about benzoimidazoles at C-3 position



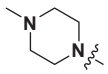
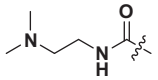
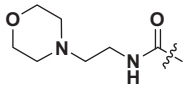
| Compound | $R^1$ | c-Met $IC_{50}$ (μM) |
|---|---|---|
| **17** |  | 0.13 ± 0.03 |
| **19a** |  | 0.14 ± 0.04 |
| **19b** |  | 0.33 ± 0.01 |
| **19c** |  | 0.36 ± 0.12 |

**Table 4**
Enzyme inhibitory activity of **12b** on various kinases

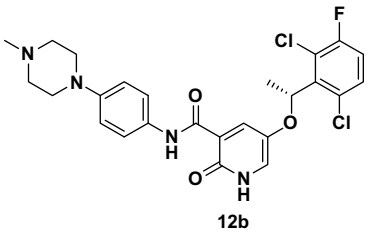
| Kinase | $IC_{50}$ (nM) |
|---|---|
| c-Met | 12 ± 3 |
| RON | 446 ± 26 |
| ABL | 281 ± 105 |
| Tyro-3 | >1000 |
| Flt-1 | >1000 |
| KDR | >1000 |
| PDGFR-α | >1000 |
| RET | >1000 |
| EGFR | >1000 |
| ErbB2 | >1000 |
| ErbB4 | >1000 |
| EGFR/T790M/L858R | >1000 |
| FGFR1 | >1000 |

Next, a series of alkyl amides at C-3 position were explored. Table 2 illustrates the enzymatic activity. Generally, the alkyl amides were not well accommodated at the position. Tetrahydropyranyl amide analog (**12q**) displayed c-Met inhibition with an $IC_{50}$ of 0.33 μM. However, derivative bearing a 1-methylpiperidin-4-yl group (**12r**) was significantly less active. Incorporation of benzyl amides (**12s** and **12t**) was also proved ineffective in c-Met enzymatic assay (inactive at 10 μM).

Although various aryl/heteroaryl/alkyl amides at C-3 position were investigated and some compounds showed promising enzymatic inhibition, no remarkable cellular potency was observed presumably due to low cell permeability. We envisaged bioisosteric replacement of the polar phenyl amide with less polar benzoimidazole might be beneficial for the cellular potency. With this in mind, a series of benzoimidazole analog were synthesized and evaluated for c-Met inhibition (Table 3). Compared with the parent compound **12b**, the derivative **17** was notably less active in enzymatic assay. It was similarly observed for analogue **19a** inferior to compound **12i**. These results suggest benzoimidazole substitution at C-3 position might be not well tolerated.

To examine whether compound **12b** is a selective c-Met inhibitor, the kinase selectivity of **12b** was assessed by screening against c-Met family member, RON, along with other 11 tyrosine kinases (Table 4). In contrast to its high potency against c-Met ($IC_{50}$ = 12 ± 3 nM), **12b** showed more than 20-fold less potency against RON, ABL, and barely inhibited kinase activity against the other 10 tested tyrosine kinases ($IC_{50}$ >1 μM).

Compound **12b** has been proved to be the most potent and selective c-Met inhibitors in our research, docking simulation was further applied to explore its binding mode in the atomic level (Fig. 3). Here, the co-crystal structure of PF-02341066 with c-Met[4]



12b

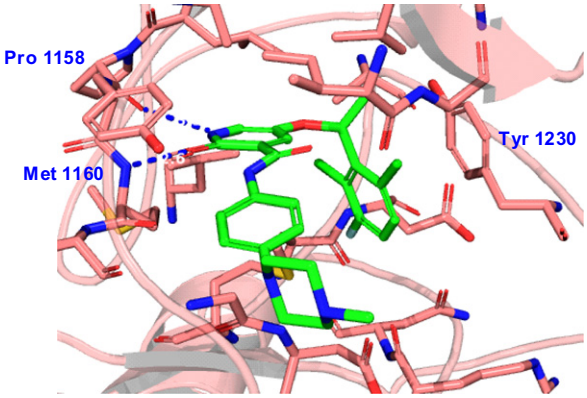



**Figure 3.** A proposed structure of **12b** bound to c-Met.

was selected as the model (PDB ID code: 2WGJ) by employing AutoDock4.2 software. The result found the binding model of **12b** with c-Met was similar to that of PF-02341066. As expected in our original design, the pyridone core is responsible for hydrogen bonding with the hinge region of c-Met. The carbonyl and NH of the pyridone core form hydrogen bonds with the residue Met 1160 and Pro 1158, respectively. The 2,6-dichloro-3-fluorobenzyloxy fragment extends to inside pocket involving a π–π interaction with the residue Tyr 1230. The amide side chain faces to the solvent accessible region.

To conclude, a series of novel 5-(benzyloxy)pyridin-2(1*H*)-ones were designed, synthesized and biologically evaluated for c-Met inhibition. The carbonyl oxygen of the pyridin-2(1*H*)-one was demonstrated to be an important factor for c-Met binding affinity. In addition, various amide and benzoimidazole analogs were explored. A potent compound **12b** with an $IC_{50}$ of 12 nM was identified. This compound exhibited potent inhibition of EBC-1 cell associated with c-Met constitutive activation and showed high selectivity for c-Met than other tested 11 kinases. Further optimization for improvement of the cellular potency and in vivo evaluation are undertaken in our lab.

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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.2013.02.037.

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