Letters

Discovery and Optimization of Triazolopyridazines as Potent and Selective Inhibitors of the c-Met Kinase†

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Abstract: Tumorigenesis is a multistep process in which oncogenes play a key role in tumor formation, growth, and maintenance. MET was discovered as an oncogene that is activated by its ligand, hepatocyte growth factor. Deregulated signaling in the c-Met pathway has been observed in multiple tumor types. Herein we report the discovery of potent and selective triazolopyridazine small molecules that inhibit c-Met activity.

The receptor tyrosine kinase, c-Met, and its natural ligand, hepatocyte growth factor (HGF[a]), are involved in cell proliferation, migration, and invasion and are essential for normal embryonic development.[1] However, when deregulated, the c-Met/HGF pathway leads to tumorigenesis and metastasis.[2] The overexpression of c-Met and/or HGF, the amplification of the MET gene, and mutations in the c-Met kinase domain have been linked to human cancers.[3] Recently it has been shown that MET amplification occurs as a resistance mechanism in some lung cancer patients that were initially responsive to gefitinib.[4] Inhibition of c-Met activity in cell lines that reproduce this resistance mechanism restored sensitivity to gefitinib. For these reasons, c-Met small molecule kinase inhibitors have been sought for therapeutic intervention.

Inhibition of the tyrosine kinase activity by an ATP-competitive small molecule is a pharmacologically attractive method that has been demonstrated for other tyrosine kinases.[5] One limitation to small molecule kinase inhibitors is the difficulty of obtaining specificity for the desired enzyme. The aim of the present work was to develop a potent, selective, ATP-competitive orally bioavailable small molecule inhibitor of c-Met.[6]

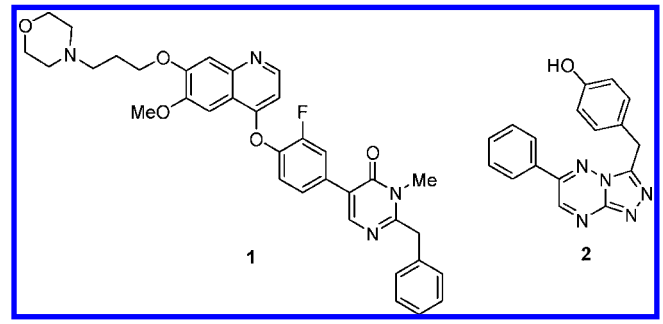
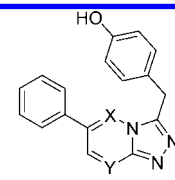


Figure 1. Reported c-Met inhibitors.



Compound	X	Y	c-Met IC_{50} (nM)
3a	N	CH	120 ± 18
3b	CH	N	610 ± 77
3c	CH	CH	2100 ± 760

Figure 2. Analogues of triazolotriazine **2**.

Recently, we disclosed the structure of pyrimidinone **1** as a potent (IC_{50} = 10 nM) c-Met inhibitor (Figure 1).[7] In an ongoing effort to design novel inhibitors of the c-Met enzyme, we were intrigued by a report from Sugen in which they showed that a series of triazolotriazines of low molecular weight were potent c-Met inhibitors.[8] They reported that a representative example, triazolotriazine **2**, was shown to inhibit c-Met activity with an IC_{50} of 6 nM.

Intrigued by the low molecular weight and unknown binding mode of triazolotriazine **2** to c-Met, three structurally relevant novel compounds were prepared and evaluated for their potency against the c-Met enzyme (Figure 2). Since triazolopyridazine **3a** had the greatest activity and was exquisitely selective against other kinases,[9] it was investigated further.

The cocrystal structure of **3a** bound to the unphosphorylated c-Met kinase domain revealed a bent "U-shaped" binding mode with the inhibitor wrapped around Met1211 (Figure 3). A direct hydrogen bond is formed between the backbone NH of Met1160 (linker) and the phenol-O with a distance of 3.0 Å. A second hydrogen bond is mediated by a water molecule and bridges the backbone carbonyl of Met1160 and the phenol-H. Other notable interactions include a π-stacking interaction between the triazolopyridazine core and Tyr1230 and a hydrogen bonding interaction between N1 of the inhibitor and the backbone NH of Asp1222.

Our previous crystallographic analysis of pyrimidinone **1** revealed a strikingly different mode of binding to the c-Met active site (Figure 4). Instead of an overall bent shape,

† Cocrystal structures of c-Met with **3a** and **4** have been deposited in the Protein Data Bank with access codes 3CCN and 3CD8, respectively.

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[a] Abbreviations: ATP, adenosine triphosphate; HGF, hepatocyte growth factor; Met1211/1260, methionine 1211/1260; Tyr1230, tyrosine 1230; Asp1222, aspartic acid 1222; NADPH, nicotinamide adenine dinucleotide phosphate; HATU, *N,N,N′,N′*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate.

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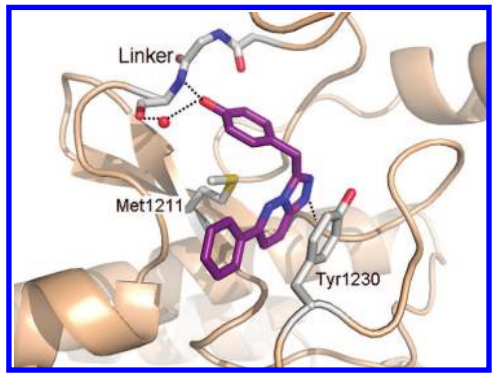


Figure 3. Cocrystal structures of triazolopyridazine **3a** and c-Met.

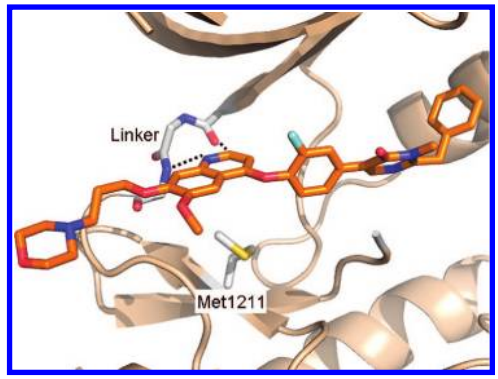


Figure 4. Cocrystal structures of pyimidone **1** and c-Met.

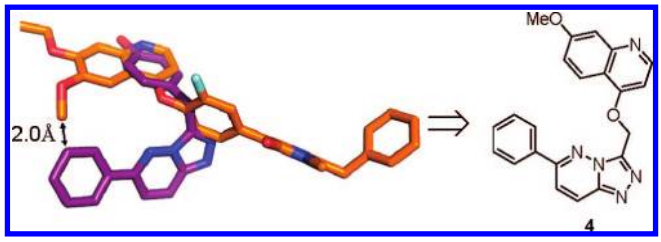
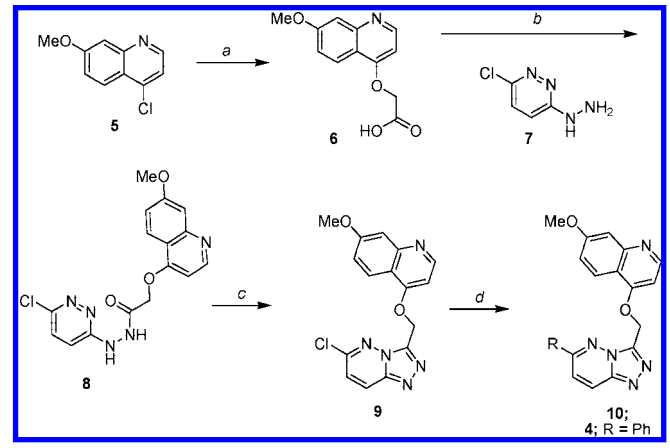


Figure 5. Overlay of structures **1** and **3a** and proposed hybrid **4**.

pyrimidinone **1** adopts an extended conformation. In addition, **1** utilizes a quinoline to bind to Met1160 instead of a phenol. The cocrystal structures of c-Met with **1** and **3a** were aligned to see how the inhibitors are situated relative to one another in the c-Met binding pocket (Figure 5). The alignment of the two structures shows that the quinoline of **1** and the phenol of **3a** occupy the same area of the protein and they both make a donor−acceptor interaction with Met1160. We sought to capitalize on this overlap and design a novel c-Met inhibitor through the formation of a hybrid structure that contains the triazolopyridazine core of **3a** and the quinoline portion of **1**. Because the C-6 methoxy group on the quinoline was only 2.0 Å away from the C-6 phenyl group on **3a**, we omitted it from the hybrid product. The outcome of this exercise was the formation of triazolopyridazine quinoline **4**, which was an efficient inhibitor of the c-Met enzyme with good cellular activity (Table 1, entry 1).

The cocrystal structure of **4** and c-Met confirmed that **4** binds the way it was envisioned (Figure 6). On the basis of the cocrystal structure, we rationalized that modifications of the C-6 phenyl group on the triazolopyridazine core could modulate the π-stacking interactions with Tyr1230 allowing for increased potency. For this reason, aromatic and heteroaromatic groups

Scheme 1[a]



[a] Reagents and conditions: (a) glycolic acid, KOH, DMSO, 160°C, then HCl to pH ~3; (b) HATU, $^{i}Pr_2NEt$, DMF, 50°C; (c) *p*-TsOH, MeOH, 55°C. (d) **4**, **10a**−**l**, **10n**: $RB(OH)_2$, Pd (0), $^{t}Bu_3P$ or dppf, DMF or dioxane, Δ. **10m**: $RSnMe_3$, Pd(0), X-Phos, dioxane, Δ.

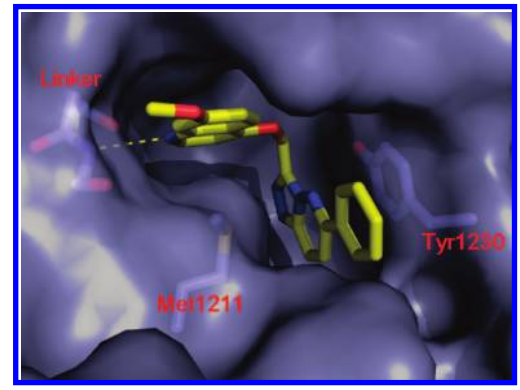


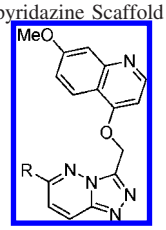
Figure 6. Cocrystal structures of triazolopyridazine **4** and c-Met.

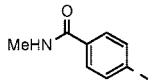
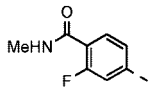
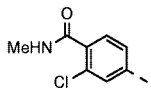
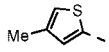
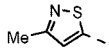
were directly attached to the triazolopyridazine core of the molecule. In addition to the cocrystal structure, metabolite identification revealed that the C6-phenyl ring was prone to metabolism. Incubation of triazolopyridazine **4** with rat and human liver microsomes in the presence of NADPH qualitatively yielded C6-phenylarene oxidation products as the major metabolites. With the proof of concept in hand, an effort to explore the SAR around **4** was initiated.

On the basis of the above rationale, we necessitated a general method that allowed for the rapid preparation of C-6 aryl analogues. The synthesis began by treating 4-chloro-7-methoxyquinoline **5** with glycolic acid in the presence of KOH to afford substituted acetic acid derivative **6** (Scheme 1). HATU coupling of acid **6** and 1-(6-chloropyridazin-3-yl)hydrazine **7** yielded hydrazide **8**, which was dehydratively cyclized under mildly acidic conditions. Triazolopyridazine **9** contained an activated triazolopyridazine chloride, which was an effective coupling partner in a variety of palladium catalyzed reactions affording analogues **10**.

The SAR commenced by the introduction of fluorine on the phenyl ring in an effort to block the observed metabolic activation and modulate π-stacking interactions with the hope of increased potency (Table 1). Both meta and para substitutions (**10a/b**) were potent in the enzyme assay with a substantial shift in the cellular assay. The ortho-substituted fluorine analogue **10c** was not as well tolerated by the c-Met enzyme. In addition, both the 3,4-difluoro **10d** and the 3,5-difluoro **10e** analogues were potent against c-Met enzyme activity but again showed a

Table 1. SAR of Triazolopyridazine Scaffold against c-Met.



		c-Met IC_{50} (nM)[a]	
Compound	R	Biochemical[b]	Cellular[c]
4		9 ± 2	46 ± 11
10a		12 ± 3	42 ± 9
10b		7 ± 1	20 ± 7
10c		67 ± 4	400 ± 51
10d		9 ± 5	26 ± 10
10e		7 ± 1	14 ± 5
10f		4 ± 1	7 ± 2
10g		6 ± 0.3	16 ± 2
10h		3 ± 0.2	3 ± 0.7
10i		1 ± 0.1	2 ± 1
10j		2 ± 0.5	6 ± 1
10k		4 ± 0.1	29 ± 13
10l		1 ± 0.1	2 ± 0.3
10m		3 ± 0.1	2 ± 0.3
10n		29 ± 8	132 ± 6

[a] $n \geq 2$. [b] Inhibition of kinase activity. [c] Inhibition of HGF-mediated c-Met phosphorylation in PC3 cells. See *Supporting Information*.

shift in the cellular assay. Interestingly, the trifluoro analogue **10f** was potent in the enzyme and cellular assay with IC_{50} < 10 nM. The cocrystal structure of **4** also indicated that the para position of the C-6 phenyl substituent projected toward solvent; therefore, polar functionality was incorporated at this position. Initially it was found that the *p*-methylbenzamide **10g** was well tolerated and potent in the cellular assay. The potency of **10g** could be improved to low single-digit nanomolar in the cellular assay by incorporation of a fluorine or chlorine atom ortho to the amide (**10h** or **10i**, respectively).

In addition to substituted phenyl rings, five-membered heterocycles were also explored. It was found that the 2-thiophenyl analogue **10j** was much more potent than the corresponding 2-furanyl analogue **10n**. Although the 2- and 3-thiophenyl analogues **10j/k** were virtually equipotent in the enzyme assay, **10j** was significantly more potent in the cellular assay at 6 nM. Incorporation of a single methyl group at the thiophene 4 position (**10l**) showed an increase in potency in the enzyme and cellular assays. Methylisothiazole **10m** was prepared to optimize the pharmacokinetic properties of thiophene **10l** while still maintaining cellular activity at 2 nM.

The pharmacokinetic profile of selected analogues was evaluated (Table 2). Compound **4**, trifluoro analogue **10f**, and methylisothiazole analogue **10m** had desirable pharmacokinetics. Chlorobenzamide analogue **10i** was intrinsically stable in liver microsomes and yet was rapidly cleared from the plasma compartment in vivo.[10] Methylthiophene **10l** was metabolically unstable and rapidly cleared in vivo. Interestingly, substitution of the original triazolopyridazine **4** with three fluorine atoms did not improve the microsomal clearance; yet the in vivo clearance was markedly improved. Although **4** and **10m** had a higher bioavailability, the overall exposure of **10f** was higher. Analogue **10f** possessed the best overall profile (PK/potency) and was a candidate for our mouse pharmacodynamic assay.

Compound **10f** was screened against a panel of tyrosine and serine/threonine kinases. Impressively, **10f** was found to be highly selective for c-Met over a variety of kinases (>10 μM against KDR, Lck, Src, IGF1R, Btk, Tie2, p38, Jnk2, CDK5, Erk1, PKBα, PKAα, Msk1, Jak2, Abl, cKit, Aur2).

The inhibition of HGF-mediated c-Met phosphorylation in mouse liver was evaluated. **10f** was administered to mice by oral gavage (3, 10, 30 mg/kg). Six hours postdose, human HGF was injected iv to phosphorylate c-Met in the liver. The livers were harvested, and c-Met phosphorylation was quantified. Oral treatment of **10f** led to a dose-dependent inhibition of HGF-mediated c-Met phosphorylation with an approximate ED_{90} of 30 mg/kg and a corresponding plasma concentration of 6.7 μM (Figure 7).

In summary, through the use of structural biology we were able to devise a novel inhibitor of c-Met (**4**, IC_{50} = 9 nM). Although numerous potent analogues were prepared, analogue **10f** possessed the most desirable profile. Furthermore, it was determined that **10f** was a potent inhibitor of HGF-mediated c-Met phosphorylation in a mouse pharmacodynamic assay. The inhibition of c-Met phosphorylation in this pharmacodynamic model and the exquisite c-Met selectivity warrant future studies

Table 2. Pharmacokinetic Profile of Selected Compounds

	Cl,[a] μL/min/mg						
compd	RLM	MLM	Cl, L/h/kg	V_{ss}, L/kg	$T_{1/2}$, h	$AUC_{0-\infty}$,[d] ng·h/mL	F,[d] %
4	131	122	0.37[c]	0.38[c]	1.0[c]	2517	43
10f	190	156	0.058[b]	0.152[b]	3.5[b]	7840	22
10i	77	61	6.0[c]	3.8[c]	0.7[c]	ND	ND
10l	>1800	653	3.7[c]	1.0[c]	0.3[c]	ND	ND
10m	420	173	0.24[b]	0.35[b]	2.58[b]	5100	59

[a] In vitro (RLM = rat liver microsomes; MLM = mouse liver microsomes). In vivo experiments were carried out with male Sprague−Dawley rats (n = 3). [b] iv, 0.25 mg/kg (DMSO). [c] iv, 0.5 mg/kg (DMSO). [d] po, 2 mg/kg.

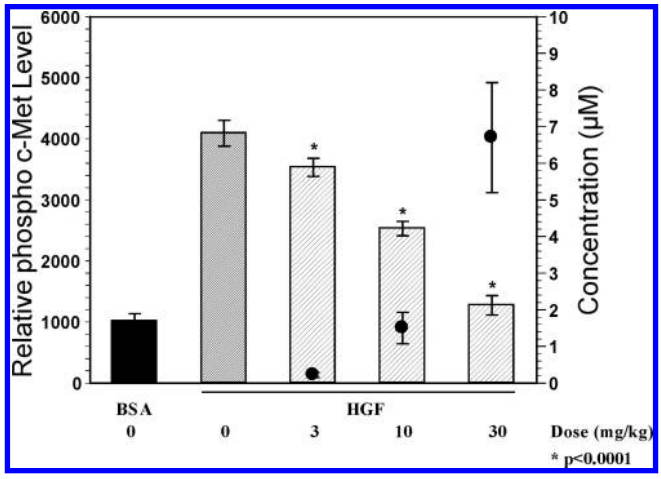


Figure 7. Effect of **10f** on HGF-mediated c-Met phosphorylation at 6 h (black circles correspond to plasma concentrations of **10f**).

for this series of triazolopyridazines in cancer disease models. These studies will be reported in due course.

Supporting Information Available: Analytical data and experimental protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) >25 μM against all kinases tested, including KDR, IGF1R, Tie2, Lck, Jak3, BTK, p38α, PKBα, PKAα, Aur1/2, Abl.

(10) A qualitative bile-duct cannulated study in male Sprauge−Dawley rats with this compound revealed that parent and metabolites were being excreted in the bile after 8 h. Further details will be discussed elsewhere.

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