Bioorganic & Medicinal Chemistry Letters 22 (2012) 6368–6372

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Multisubstituted quinoxalines and pyrido[2,3-*d*]pyrimidines: Synthesis and SAR study as tyrosine kinase c-Met inhibitors

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ARTICLE INFO

Article history:
Received 9 July 2012
Revised 7 August 2012
Accepted 20 August 2012
Available online 27 August 2012

Keywords:
c-Met kinase
3,5-Diamino-7-trifluoroquinoline
hERG
Antitumor activity
Structure–activity relationship (SAR)

ABSTRACT

Two series of new analogues were designed by replacing the quinoline scaffold of our earlier lead **2** (zgwatinib) with quinoxaline and pyrido[2,3-*d*]pyrimidine frameworks. Moderate c-Met inhibitory activity was observed in the quinoxaline series. Among the pyrido[2,3-*d*]pyrimidine series, compounds **13a–c** possessing an O-linkage were inactive, whilst the N-linked analogues **15a–c** retained c-Met inhibitory potency. Highest activity was observed in the 3-nitrobenzyl analog **15b** that showed an IC_{50} value of 6.5 nM. Further structural modifications based on this compound were undergoing.

Similar to most of the receptor tyrosine kinases (RTK), the hepatocyte growth factor (HGF) c-Met is a regulator of many critical cellular processes including embryological development, cell growth, differentiation, neovascularization and tissue regeneration.[1,2] Aberrantly high expression of HGF/c-Met has been implicated in a variety of solid tumors.[3–9] Therefore, c-Met has emerged as an attractive molecular target and inhibition of HGF/c-Met signaling pathway has shown great therapeutic benefit as novel cancer therapy.[10–15] Among the large number of c-Met-targeting small molecules reported recently,[16–18] crizotinib (**1**, PF-02 341066, Fig. 1) has been the only one approved by FDA as a first-in-class c-Met inhibitor antitumor drug. However, this drug is indeed a dual inhibitor with equal potency at both c-Met and ALK (anaplastic lymphoma kinase) kinases.[19] Therefore, a direct correlation between the clinical anti-cancer benefit and the c-Met potency is dampened by the polypharmacy profile, and highly selective and potent c-Met inhibitors are emergently needed as probes to validate clinical efficacy of the c-Met targeting strategy.

We recently reported[20,21] a series of highly potent c-Met inhibitors structurally featured by multisubstituted quinolines. One of these compounds, 3-(4-methylpiperazin-1-yl)-*N*-(3-nitrobenzyl)-7-(trifluoromethyl)quinolin-5-amine (**2**)[20], also named zgwatinib (Fig. 1), displayed high c-Met potency both at enzymatic (0.93 nM) and cellular (230 nM) levels, thereafter was selected for earlier preclinical investigations. Meanwhile, a series of triazolo[4,3-*b*]pyridazine analogues were designed[21] by merging the 3-piperazinyl-7-trifluoromethylquinoline core of **2**[20] and the triazolopyridazine core of **3**,[15] another potent c-Met inhibitor reported by Amgen, into one molecule to improve the memberane permeability and then eradicate the discrepancy between the enzymatic and cellur potency of **2** (Fig. 2). Unfortunately, these new triazolo[4,3-*b*]pyridazin-3-ylmethanamines **4**[21] showed much reduced inhibitory effects to c-Met enzyme.

As a continuation of our study toward the identification of potent c-Met inhibitors, we decided to take advantage of the widely-used quinoxazoline skeleton as a privileged scaffold of RTK inhibitors and modifiy it with the three key substutients of compound **4** or lead **2**, thereby designing a new series of quinoxaline analogues **I** (Fig. 2). Meanwhile, replacement of the quinoline core **2** with a pyrido[2,3-*d*]pyrimidine artwork and concurrent incorporation of the two side chains of **1** led to another series of new analogues **II** (Fig. 2). Herein, in this report we disclose the synthesis and c-Met inhibition study of these two series of new analogues.

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http://dx.doi.org/10.1016/j.bmcl.2012.08.075

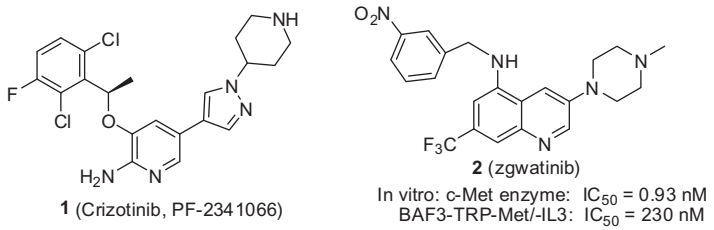


Figure 1. Marketed c-Met inhibitor **1** and our earlier reported compound **2**.

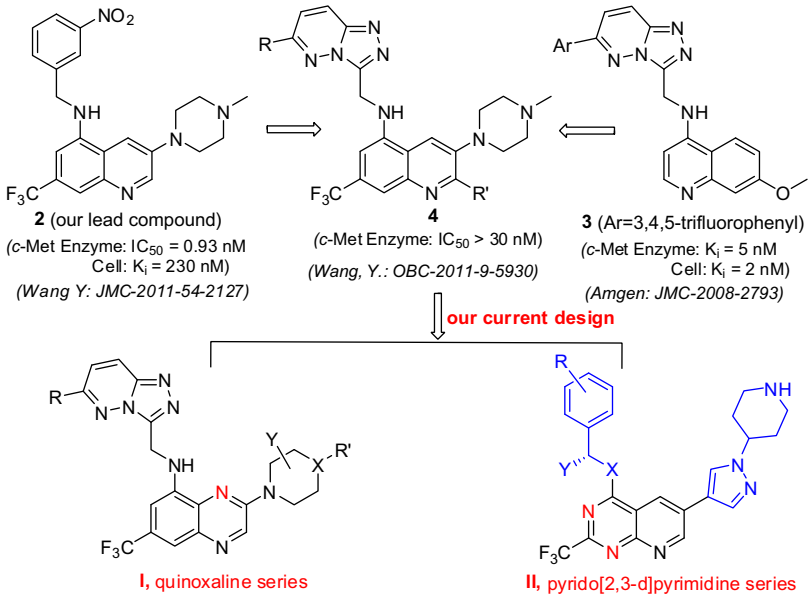
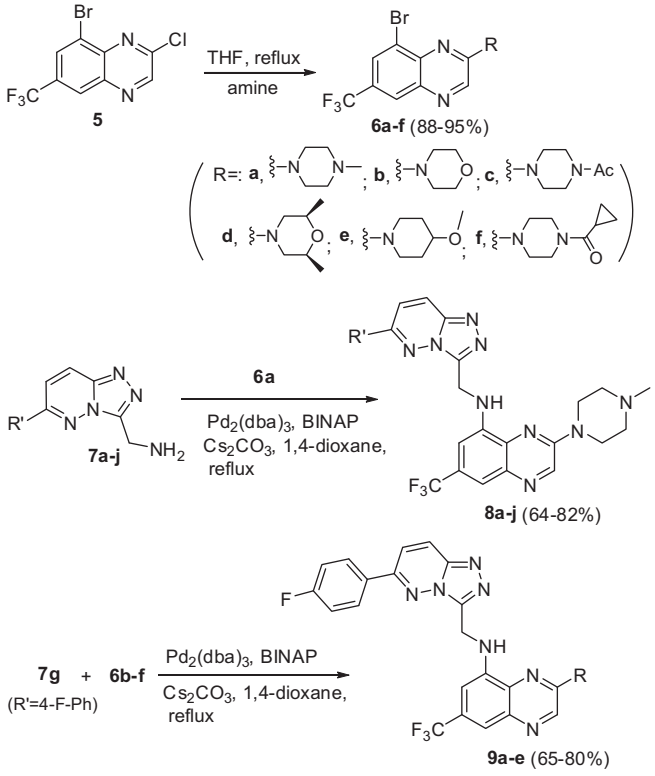


Figure 2. Our new compounds design.



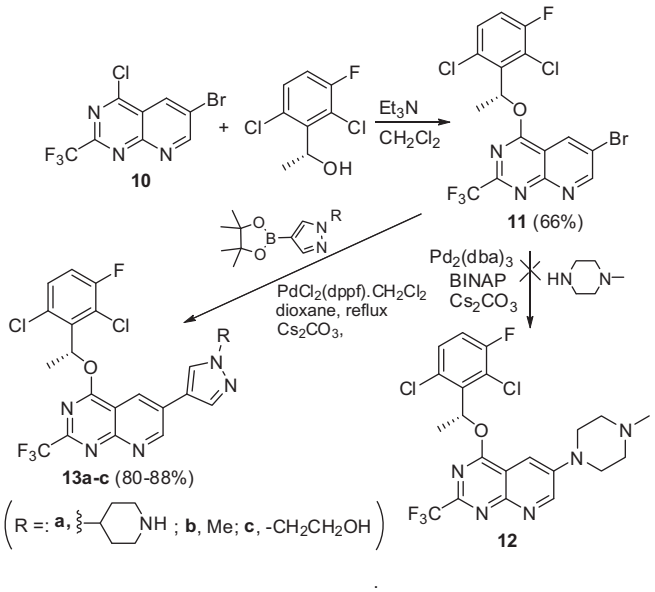
Scheme 1. Synthesis of compounds **8a–j** and **9a–e** (Series **I**).

The synthesis of compound series **I** was started from 3-chloro-5-bromo-7-trifluoromethylquinoxaline (**5**)[22] which was prepared by following a literature procedure. As described in Scheme 1, treatment of **5** with variant amines provided 3-amino-quinoxalines **6a–f** in 88–95% yields. $Pd_2(dba)_3$-catalyzed *C–N* coupling[20] of **6a** with (6-substituted-[1,2,4]triazolo[4,3-*b*]pyridazin-3-yl)methanamines **7a–j** afforded **8a–j** in 64–82% yields. Meanwhile, coupling of 4-fluorophenyl substituted [1,2,4]triazolo[4,3-*b*]pyridazin-3-ylmethanamine **7g** with bromides **6b–f** yielded N-substituted piperazines **9a–e** in 65–80% yields.

Table 1
c-Met activity of compounds **8a–j**[a]

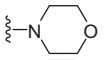
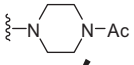
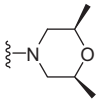
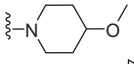
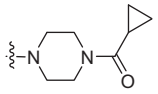
Compd	R′	IC_{50}[a] (nM)
8a	H	2500 ± 67.0
8b	MeO	2700 ± 317
8c	2-Thienyl	659 ± 129
8d	3-Thienyl	441 ± 52.5
8e	2-Furyl	409 ± 183
8f	Ph	1980 ± 328
8g	4-F-Ph	626 ± 97.2
8h	3-F-Ph	385 ± 84.7
8i	3-Cl-Ph	257 ± 40.3
8j	1-Me-pyrazol-4-yl	924 ± 179
2[20]	—	0.93 ± 0.18
4[21]	R=R′=H	330

[a] In vitro kinase assays were performed with the indicated purified recombinant c-Met kinase domains, IC_{50}s were calculated by Logit method from the results of at least three independent tests with six concentrations each.



Scheme 2. Synthesis of compounds **13a–c.**

Table 2
c-Met activity of compounds **9a–e**[a]

Compd	R–	IC_{50}[a] (nM)
9a		485 ± 5.5
9b		309 ± 19.3
9c		539 ± 15.3
9d		879 ± 95.5
9e		486 ± 48.4

[a] In vitro kinase assays were performed with the indicated purified recombinant c-Met kinase domains, IC_{50}s were calculated by Logit method from the results of at least three independent tests with six concentrations each.

The c-Met enzymatic activity of compounds **8a–j** was shown in Table 1. Compared to compounds **4**[21], most of the newly synthetic compounds **8a–j** displayed reduced activity, except compounds **8d**, **8e** and **8g–i** that showing only slightly decreased or equally potent c-Met inhibitory activity. The substituted phenyl derivatives **8g–i** had higher potency than the non-substituted phenyl analog **8f**. [1,2,4]Triazolo[4,3-*b*]pyridazine analogues (**8c–j**) with a larger C6-substituent were more potent than non-substituted congener **8a** or analogues with a small C6-substituent (**8b, 8f**).

Table 3
c-Met activity of selected compounds[a]

Compd	IC_{50}[a] (nM)	Compd	IC_{50}[a] (nM)
13a	>10 μM	**15a**	441 ± 110
13b	>10 μM	**15b**	6.5 ± 0.2
13c	>10 μM	**15c**	>1000

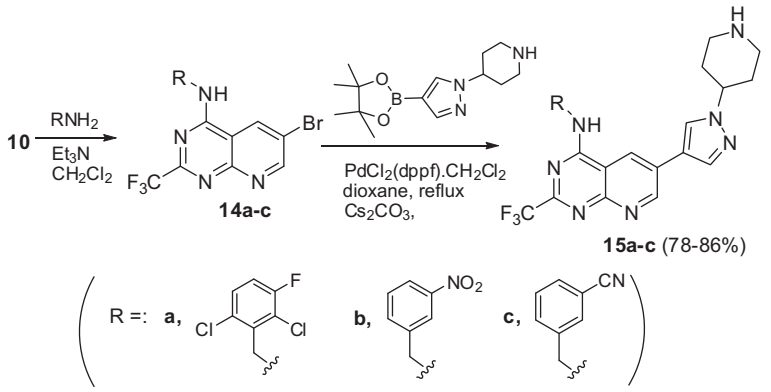
[a] IC_{50}s were calculated by Logit method from the results of at least three independent tests with six concentrations each and expressed as mean ± S.D.

Compared to compound **8g**, all the analogues **9a–e** bearing diversely substituted piperazine or morpholine fragments showed moderate, but statistically similar activity. The IC_{50} values of these compounds varied in the range of 300–900 nM. In comparison to compounds **9a** and **9c**, or **9b** and **9e**, higher activity was observed on compounds with less steric effects.

Since the quinoxaline series did not give improved activity, we then turned our attention to the pyrido[2,3-*d*]pyrimidine core. Initially, we wanted to incorporate only the (*R*)-1-(2,6-dichloro-3-fluorophenyl)ethoxy component of **1** to make compound **12** (Scheme 2). Quite disappointingly, the coupling of N-methylpiperazine with bromide **11** (prepared from 6-bromo-4-chloro-2-(trifluoromethyl)pyrido[2,3-*d*]pyrimidine[23] in 66% yield) under variant conditions, including $Pd_2(dba)_3$/BINAP, did not occur. To our delight, Suzuki coupling[24] of **11** with 1-substituted pyrazo-4-ylboranes under $PdCl_2(dppf)\cdot CH_2Cl_2/Cs_2CO_3$ went through smoothly. Compounds **13a–c** bearing both (*R*)-1-(2,6-dichloro-3-fluorophenyl) ethoxy and 1-substituted-1*H*-pyrazol-4-yl moieties of **1** were obtained in 80–88% yields.

Unfortunately, all the three pyrido[2,3-*d*]pyrimidine analogues **13a–c** did not show noticeable enzymatic inhibition to c-Met even at 10 μM concentrations (Table 3). To determine the role of the oxygen-linkage between the substituted benzyl and the pyrido[2,3-*d*]pyrimidine core in **13a**, corresponding N-linked analog **15a** was prepared in 70% yield by treating chloride **10** with corresponding amine followed by Suzuki coupling under $PdCl_2(dppf)\cdot CH_2Cl_2/Cs_2CO_3$ system[24] (Scheme 3).

To our delight, moderate activity was retained in the N-linked compound **15a** with an IC_{50} value of 441 nM, comparable to that of quinoxaline series in Tables 1 and 2 and our earlier reported compounds **4**, yet still much less potent than our initial lead **2**. Switching the 2,6-dichloro-3-fluorophenyl to 3-nitrophenyl delivered compound **15b** that showed significantly improved



Scheme 3. Synthesis of compounds **15a–c**.

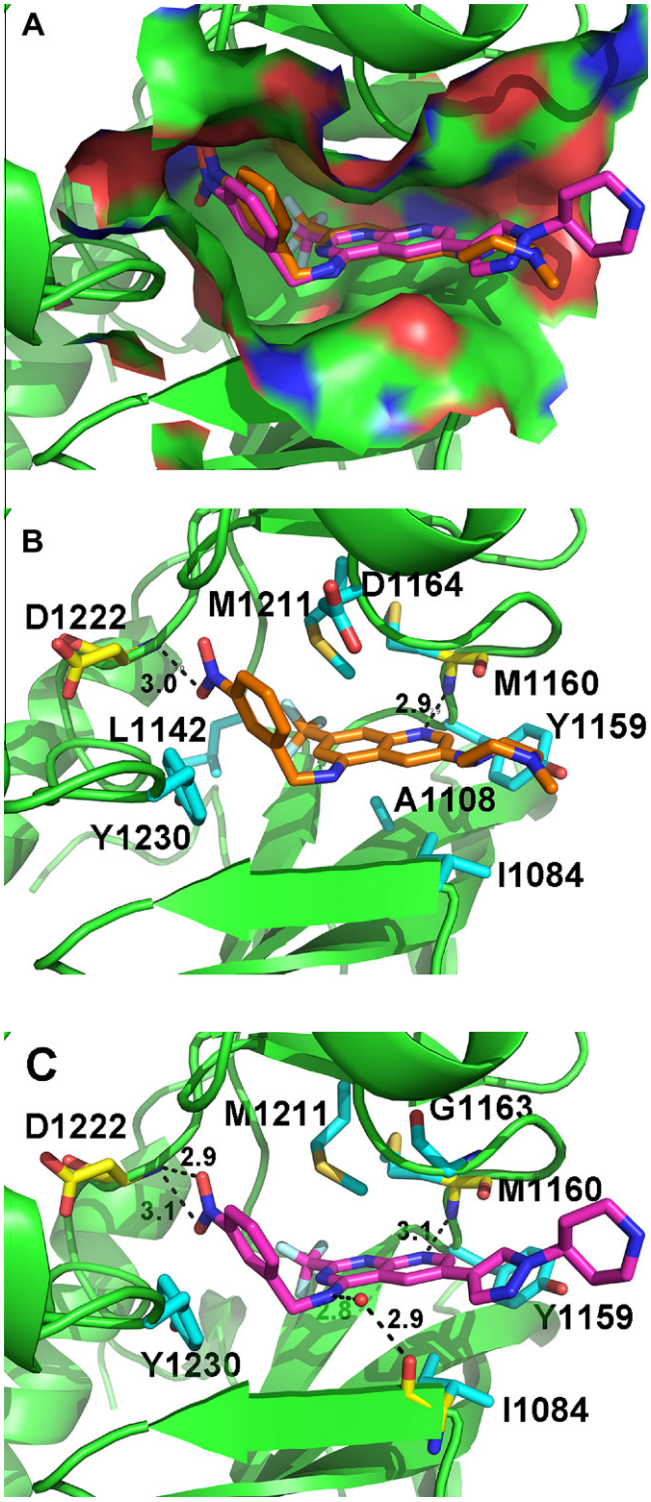


Figure 3. Co-crystal structures of lead **2** (4GG5) and new compound **15b** (4GG7) in complex with the kinase domain of c-Met. (A) Molecular surface of the binding pockets for lead **2** (brown) and compound **15b** (magenta). (B, C) The interactions of lead **2** (B) and compound **15b** (C) with residues as well as water molecules in complex structures. The residues which formed hydrophobic interactions with the inhibitor are colour in cyan and those H-bond to the inhibitors are colour in yellow. The black dash lines represent the H-bonds and the length of each H-bond is labeled. The hydrophobic and H-bonds interactions were measure by the program LIGPLOT27.

c-Met activity. It has an IC_{50} value of 6.5 nM, only five-fold less potent than our lead **2**. Other aryl (e.g. 3-cyanophenyl, **15c**) substituted analogs were much less potent. This result is in agreement to our earlier observation[20,25] that the nitro group in lead **2** made key interactions with c-Met enzyme.

Fortunately, we were able to obtain the co-crystal structures of lead **2** and compound **15b** bound to the c-Met active domain by following a literature procedure[27] (Fig. 3). As shown in Figure 3A, both compounds bound to the kinase in a similar manner.[28] The compounds formed both hydrophobic and H-bond interactions with the kinase. The pyridyl *N*-atom in both the quinoline and pyrido[2,3-*d*]pyrimidine cores formed a H-bond to Met^{1160} in the hinge region of the kinase, the CF_3 and 3-nitrobenzylamino groups occupied the hydrophobic pockets. It is of note that the nitro group formed one or two H-bonds with Asp^{1222} accounting for the observed importance of this group to the c-Met potency. The piperazine in **2** or piperidine in **15b** positioned towards solvent. Therefore, further work will focus on more modification in this region to generate compounds with more c-Met potency and better druglikeness.

In summary, we have designed two series of new analogues[26] bearing quinoxaline and pyrido[2,3-*d*]pyrimidine frameworks as the core skeletons to replace the quinoline scaffold in our earlier lead **2** (zgwatinib). Moderate c-Met inhibitory activity was observed in the quinoxalines series of **8a–j** and **9a–e**. Among the pyrido[2,3-*d*]pyrimidine series, compounds **13a–c** possessing an O-linkage were inactive, whilst the N-linked analogues **15a–c** retained c-Met inhibitory potency. The 3-nitrobenzyl analog **15b** showed the highest activity with an IC_{50} value of 6.5 nM. Further structural modifications based on this compound are undergoing.

Acknowledgments

The authors are grateful to Grants from the Chinese National Science Foundation for the Distinguished Young Scholars Program (81125021 for A.Z.) and for the Innovation Research Group (810 21062 for M.G.), and Grants from National Science & Technology Major Project on Key New Drug Creation and Manufacturing Program (2012ZX09103-101-035, 2010ZX09401-401, 2012ZX093010 01-007). Supports from Shanghai Commission of Science and Technology (10JC1417100, 10dz1910104), the Hundred Talents Project of Chinese Academy of Sciences (to Y.X.), and the National Natural Science Foundation of China (81102461) and National Program on Key Basic Research Project of China (2012CB910704) are also highly appreciated.

Supplementary data

Supplementary data (general procedure and spectral data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.075.

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26. Analytic data for representative compounds. Compound **8i:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.16 (d, J = 9.6 Hz, 1H), 7.95 (s, 1H), 7.84 (d, J = 9.6 Hz, 1H), 7.52 (m, 4H), 7.10 (s, 1H), 6.60 (s, 1H), 4.19(d, J = 6.6 Hz, 2H), 2.76 (t, J = 3.9 Hz, 4H), 1.51 (t, J = 4.2 Hz, 4H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.6, 151.1, 147.7, 142.7, 141.1, 136.5, 134.7, 134.3, 134.2, 131.3, 130.5, 129.2, 127.3, 126.9 (q, J = 33 Hz), 124.5, 124.4, 119.2, 113.3, 113.3, 101.0, 53.4, 46.1, 43.7, 43.4, 37.3; EI-MS (m/z) 553 (M^+); HRMS calcd for $C_{26}H_{23}ClF_3N_9$ [M^+]: 553.1717, found: 553.1721.; Compound **15b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (d, J = 1.8 Hz, 1H), 8.67 (d, J = 1.8 Hz, 1H), 8.15 (s, 1H), 7.92 (s, 2H), 7.75 (s, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.34 (t, J = 7.8 Hz, 1H), 4.79 (s, 2H), 4.17 (d, J = 11.7 Hz, 1H), 3.10 (d, J = 12.9 Hz, 2H), 2.64 (t, J = 11.7 Hz, 2H), 2.04 (d, J = 11.4 Hz, 2H), 1.89–1.68 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.5, 155.7, 154.9, 154.6, 153.4, 147.8, 139.7, 136.0, 134.7, 129.1, 128.1, 126.6, 124.7, 123.0, 122.1, 120.7, 117.9, 117.9, 110.1, 59.3, 44.6, 44.2, 32.6; EI-MS (m/z) 498 (M^+); HRMS calcd for $C_{23}H_{21}CF_3N_8O_2$ [M^+]: 498.1740, found: 498.1743.
27. (a) Wang, W.; Marimuthu, A.; Tsai, J.; Kumar, A.; Krupka, H. I.; Zhang, C.; Nguyen, H.; Tabrizizad, M.; Luu, C.; West, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 3563; (b) Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. *Protein Eng.* **1995**, *8*, 127.
28. *Protein purification and crystallization*. Production of the kinase domain (1038–1346aa) of recombinant human c-Met followed the protocols of Wang[27a] with certain modifications. The cDNA fragment was cloned into the vector pET28a and the protein was co-expressed with catYopH subcloned in pET15b (164–468AA)2. The expressed c-Met kinase domain was passed through a Ni-NTA column (Qiagen) and further purified by QHP ion exchange column (GE) which eluted with 25 mM Tris pH 8.5, 100 mM NaCl, 10% glycerol, 1 mM DTT. The protein was concentrated to about 10 mg/mL for further crystallization.Cocrystallization of the c-Met kinase domain with compound **2** was carried out by mixing a solution of the protein-ligand complex with an equal volume of precipitant solution (100 mM HEPES pH 7.5, 8% isopropanol, 3 mM TCEP, 16% PEG4K). The protein-ligand complex was prepared by adding the compound to the protein solution to a final concentration of 1 mM of **2**. Cocrystallization utilized the vapour-diffusion method in hanging drops. The same cocrystallization protocol was also utilized for compound **15b** and the precipitant solution includes 100 mM Tris pH 7.5, 15% glycerol, 12% MPD, 5% isopropanol, 14% PEG5KMME. Crystals were flash frozen in liquid nitrogen in the presence of well solution supplemented with 25% glycerol.