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Discovery of novel pyrrolo[2,3-b]pyridine derivatives bearing 1,2,3-triazole moiety as c-Met kinase inhibitors

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ABSTRACT

A series of novel pyrrolo[2,3-b]pyridine derivatives bearing 1,2,3-triazole moiety were designed, synthesized, and evaluated for their c-Met kinase inhibitory activities and antiproliferative activities against 4 cancer cell lines (HT-29, A549, MCF-7, and PC-3) in vitro. Most compounds showed moderate to excellent potency, with the most promising analog **34** showing a c-Met IC_{50} value of 1.68 nM. Structure–activity relationship studies indicated that electron-withdrawing groups (X = CF_3, R^1 = F, R^2 = 4-F) were required to decrease the higher electron density on the 5-atom linker to a proper degree to improve the inhibitory activity.

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Receptor tyrosine kinases (RTKs) play crucial roles in signal transduction pathways and cellular processes, and many are implicated in cancer.[1] c-Met [the receptor for hepatocyte growth factor/scatter factor, (HGF/SF)][2] belongs to a subfamily of RTKs which composed of an extracellular α chain and a membrane-spanning β chain connected through a disulfide bond.[3,4] c-Met has been shown to be frequently amplified or overexpressed in various cancers, including brain, colorectal, gastric, lung, head, neck, and stomach cancers. Both amplifications/overexpression are associated with poor clinical outcomes, underscoring the importance of increased c-Met signaling in these cancer types.[5–8] Therefore, c-Met shows high potential as a therapeutic target for human cancer.

Recently, significant progress has been made in the development of small-molecule c-Met inhibitors, resulting in the marketing of cabozantinib[9] (**1**, a small molecule that inhibits the activity of multiple tyrosine kinases, including c-Met, RET, and VEGFR-2/KDR, marketed in 2012),[10] and several candidates currently under clinical trials, such as foretinib (**2**, GSK1363089), compounds **3** and **4**.[11–13] According to the structure characteristics of these inhibitors, general structure which contained four parts: moiety A, B, C, and D can be summarized (Fig. 1).

Many structure types of these derivatives were included judging from moiety A, such as substituted quinoline, substituted pyridine, thieno[2,3-b]pyridine, and pyrrolo[2,3-b]pyridine series (**1**–**2**, **3**, **4**, and **5**, respectively). However, the main modification of these different series of derivatives was focused on moiety C (a 5-atom linker) which has two obvious structural characteristics. One is the '5 atoms regulation', which means six chemical bonds distance existing between moiety B and moiety D; the other is the linker containing hydrogen, oxygen, and nitrogen atoms which could form hydrogen-bond donor or acceptor.[14,15]

1,2,3-Triazole fragment was widely used as a building block in the design of anticancer agents. For example, compounds **6**, 7, **8** (Fig. 2) displayed a multitude of biological activities.[16–18] In this work, 1,2,3-triazole was introduced to moiety C to form the 5-atom linker, because the three nitrogen atoms in 1,2,3-triazole as the hydrogen-bond acceptor have high ability to form hydrogen-bonding interactions with c-Met. Pyrrolo[2,3-b]pyridine was used as the moiety A. Substituted phenyl ring was reserved as the moiety B and D. Small substituent X, R^1, and R^2 were introduced to investigate their effects on activity of the target compounds. Accordingly, we designed a novel series of pyrrolo[2,3-b]pyridine derivatives bearing 1,2,3-triazole moiety (Fig. 3).

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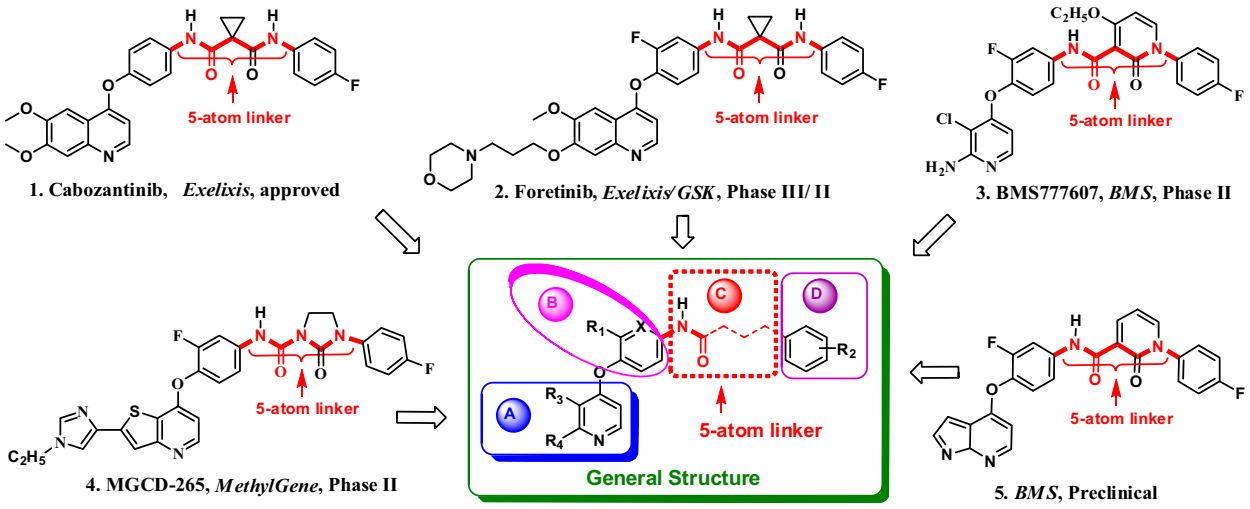


Fig. 1. The representative c-Met kinase inhibitors and the corresponding general structure.

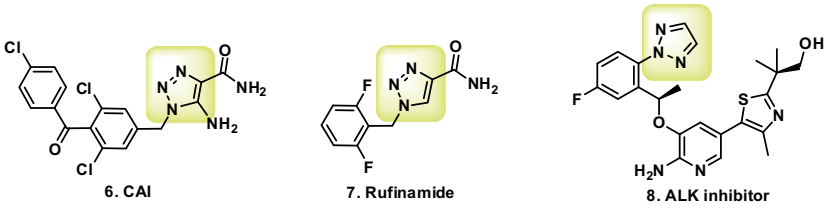


Fig. 2. Anticancer agents bearing 1,2,3-triazole fragments.

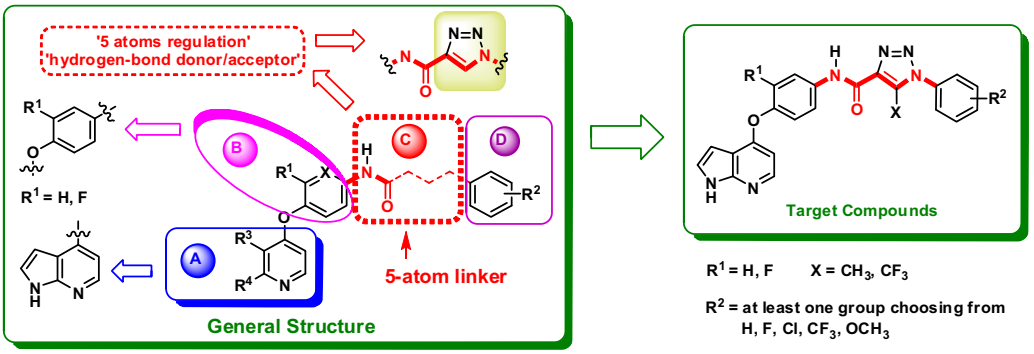


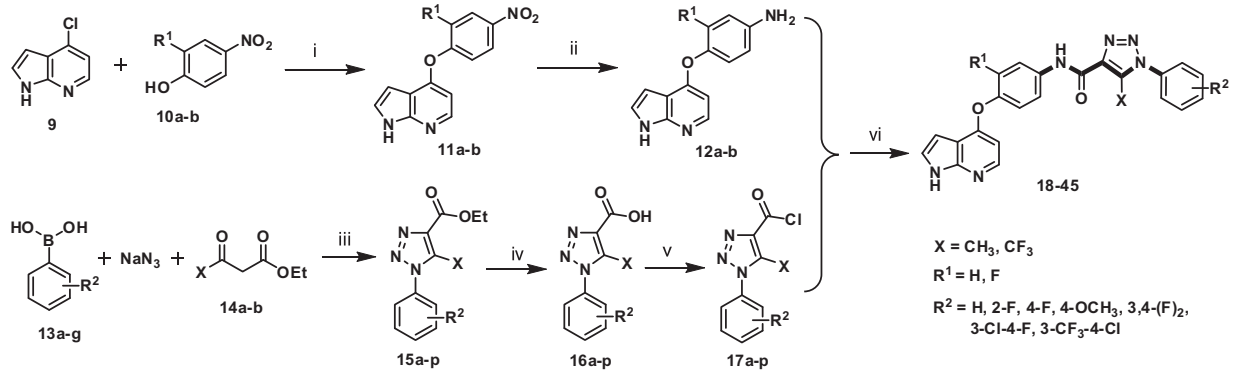
Fig. 3. Design strategy for the pyrrolo[2,3-b]pyridine derivatives bearing 1,2,3-triazole moiety.

The target compounds synthesized were evaluated for their c-Met kinase activities and antiproliferative activities against 4 cancer cell lines included the HT-29, A549, MCF-7, and PC-3. Apparent growth inhibition on c-Met and the 4 cancer cell lines was observed for most of the compounds. The most promising compound **34** were chosen to screen against 5 other tyrosine kinases (Flt-3, PDGFR-β, KDR, c-Kit, and EGFR) to examine its selectivity on c-Met. Docking analysis of compounds with c-Met was performed using compound **34**.

The key intermediates 4-(1*H*-pyrrolo[2,3-b]pyridin-4-yloxy) anilines **12a–b** was prepared by coupling 4-chloro-1*H*-pyrrolo [2,3-*b*]pyridine **9** with 4-nitrophenol or 2-fluoro-4-nitrophenol **10a–b**, followed by reduction of the nitro group of intermediate **11a–b** using zinc metal as shown in Scheme 1.[19] The intermediates 1-aryl-5-methyl (or trifluoromethyl)-1,4,5-trisubstituted-1,2,3-triazoles **15a–p** were synthesized in high yield by a one-pot three-component reaction of arylboronic acids **13a–g**, sodium azide, and active methylene ketones, such as ethyl acetoacetate (or ethyl 4,4,4-trifluoroacetoacetate) **14a–b** in the presence of $Cu(OAc)_2$ and piperidine using a DMSO/H_2O (10:1) mixture as solvent.[20] Simple procedures such as hydrolysis and acyl chlorination were used to convert ethyl 5-methyl(or trifluoromethyl)-1-substitutedphenyl-1*H*-1,2,3-triazole-4-carboxylate **15a–p** to the corresponding acyl chloride **17a–p**; the reactions proceeded with 20% H_2SO_4 and thionyl chloride, respectively. Reaction of anilines **12a–b** with acyl chloride **17a–p** promoted by DIPEA in dichloromethane at room temperature yielded the target compounds **18–45**.[21]

The c-Met enzymatic assays of all synthesized compounds were evaluated using homogeneous time-resolved fluorescence (HTRF) assay.[22] The results expressed as the half-maximal inhibitory concentration (IC_{50}) values presented in Table 1; the mean values of experiments performed in triplicate are shown.

As illustrated in Table 1, these novel pyrrolo[2,3-b]pyridine derivatives bearing 1,2,3-triazole moiety were found to be active



Scheme 1. Reagents and conditions: (i) *i*-Pr_2NEt, microwave, 200 °C, NMP, 42–44%; (ii) Zn, NH_4Cl, MeOH/THF, rt, overnight, 77–80%; (iii) $Cu(OAc)_2$, DMSO/H_2O (10:1), Piperidine, rt to 80 °C, 24 h, 88–92%; (iv) 20% H_2SO_4, 100 °C, 5–10 h, 72–76%; (v) $SOCl_2$, reflux, 6 h, 92–94%; (vi) appropriate aniline, carbonyl chloride, DIPEA, 0 °C, 1 h, rt, 7–10 h, 56–61%.

Table 1
c-Met kinase activities of the target compounds **18–45**

Compd.	X	R^1	R^2	c-Met IC_{50} (nM)
18	CH_3	F	H	16.82 ± 1.16
19	CH_3	F	2-F	14.34 ± 1.22
20	CH_3	F	4-F	12.78 ± 1.42
21	CH_3	F	4-OCH_3	24.14 ± 1.42
22	CH_3	F	3,4-$(F)_2$	21.63 ± 1.27
23	CH_3	F	3-Cl-4-F	29.27 ± 1.34
24	CH_3	F	3-CF_3-4-Cl	37.71 ± 1.84
25	CH_3	H	H	19.36 ± 1.51
26	CH_3	H	2-F	16.25 ± 1.03
27	CH_3	H	4-F	14.74 ± 0.42
28	CH_3	H	4-OCH_3	25.42 ± 1.25
29	CH_3	H	3,4-$(F)_2$	21.49 ± 0.32
30	CH_3	H	3-Cl-4-F	22.33 ± 1.27
31	CH_3	H	3-CF_3-4-Cl	42.56 ± 1.91
32	CF_3	F	H	**7.25 ± 0.59**[a]
33	CF_3	F	2-F	**5.24 ± 0.51**
34	CF_3	F	4-F	**1.68 ± 0.36**
35	CF_3	F	4-OCH_3	12.56 ± 0.35
36	CF_3	F	3,4-$(F)_2$	11.26 ± 1.23
37	CF_3	F	3-Cl-4-F	15.69 ± 1.46
38	CF_3	F	3-CF_3-4-Cl	18.15 ± 1.38
39	CF_3	H	H	11.37 ± 1.31
40	CF_3	H	2-F	10.73 ± 1.62
41	CF_3	H	4-F	**6.39 ± 0.43**
42	CF_3	H	4-OCH_3	16.38 ± 1.85
43	CF_3	H	3,4-$(F)_2$	21.27 ± 1.72
44	CF_3	H	3-Cl-4-F	19.39 ± 1.64
45	CF_3	H	3-CF_3-4-Cl	21.26 ± 1.78
Foretinib				**1.92 ± 0.21**

[a] Bold values show the IC_{50} values of the target compounds lower than 10 nM.

against c-Met kinase with IC_{50} values ranging from 1.68 to 42.56 nM; 4 of them (**34**[23], IC_{50} = 1.68 nM; **33**, IC_{50} = 5.24 nM; **41**, IC_{50} = 6.39 nM; **32**, IC_{50} = 7.25 nM) showed high potency with IC_{50} values in single-digit nanomole range, which indicated that it's a good design strategy to use pyrrolo[2,3-b]pyridine as moiety A and introduce 1,2,3-triazole fragment to moiety C to form the 5-atom linker.

According to the data shown in Table 1, enzymatic assays data showed a preference for activity when the X group on 1,2,3-triazole fragment was trifluoromethyl instead of methyl, indicating that the introduction of electron-withdrawing groups (EWGs, such as CF_3) on the 5-atom linker had a positive effect. For example, the activity of compound **39** (IC_{50} = 11.37 nM; X = CF_3, R^1 = H, R^2 = H) was higher than compound **25** (IC_{50} = 19.36 nM; X = CH_3, R^1 = H, R^2 = H).

Moreover, compounds substituted with fluoro atom (R^1 group) on moiety B are more active than that of substituted with hydrogen atom, which indicated that the EWGs (such as F) on moiety B benefited to the potency. For example, the activity of compound **32** (IC_{50} = 7.25 nM; X = CF_3, R^1 = F, R^2 = H) was higher than compound **39** (IC_{50} = 11.37 nM; X = CF_3, R^1 = H, R^2 = H), and the same trend was observed in compounds **18**/**25**, **19**/**26**, **20**/**27** and so on.

Further studies were performed to examine the effect of different substituents R^2 on the phenyl ring (moiety D) on potency. The mono-electron-withdrawing groups (mono-EWGs) introduced to the phenyl ring increased the c-Met inhibitory efficacy, while incorporation of the mono-EWGs at 4-position of the phenyl showed a higher preference. For example, compound **32**, with no substituent on the phenyl ring, showed a c-Met IC_{50} value 7.25 nM. Introduction of mono-EDGs at 4-position of the phenyl (**34**, R^2 = 4-F, IC_{50} = 1.68 nM, increased 4.3-fold) increased the inhibitory efficacy to a greater extent than that of mono-EDGs at 2-position of the phenyl (**36**, R^2 = 2-F, IC_{50} = 5.24 nM, increased 1.4-fold). This was further confirmed by introduction of these 2 types of groups in compounds **19**/**20**, **26**/**27**, and **40**/**41**. However, incorporation of mono-donating groups (mono-EDGs) and double electron-withdrawing groups (double-EWGs) showed opposite trend in potency of the compounds, such as **21** (R^2 = 4-OCH_3, IC_{50} = 24.14 nM), **22** (R^2 = 3,4-$(F)_2$, IC_{50} = 21.63 nM), **23** (R^2 = 3-Cl-4-F, IC_{50} = 29.27 nM) and so on. Similarly to our previous study, regulating electron density on the 5-atom linker to a proper degree was a key factor in improving the potency.[21] The 1,2,3-triazole ring, which is part of the 5-atom linker, has higher electron density because of the three nitrogen atoms. Therefore, EWGs (X = CF_3, R^1 = F, R^2 = 4-F) were required to decrease the higher electron density on the 5-atom linker to a proper degree to improve the inhibitory activity.

The cytotoxic activities of all target compounds **18–45** have been evaluated against the HT-29 and A549 cell lines using the MTT assay.[24] Some potent compounds were further evaluated against the MCF-7 and PC-3 cell lines. The results expressed as IC_{50} values are shown in Table 2 as the mean values of triplicate experiments.

As illustrated in Table 2, all target compounds showed moderate to excellent cytotoxic activity against different cancer cells with potencies in the single-digit μM range. The IC_{50} values of the most promising compound **34** were 0.029, 0.082, 0.11, and 1.52 μM against the HT29, A549, MCF-7, and PC-3 cell lines, respectively. Examination of the structure-activity relationships (SARs) indicated that these analogs had similar SARs as summarized in the c-Met kinase level: (a) the target compounds showed excellent selectivity toward HT29 cell line; (b) the 1,2,3-triazole ring, which was a part of the 5-atom linker, required EWGs (such as F and CF_3) to decrease the electron density; (c) the number

Table 2
Cytotoxic activities of compounds **18**–**45** against HT-29, A549, MCF-7 and PC-3 cancer cell lines in vitro

Compd	X	R^1	R^2	IC_{50} (μM) ± SD			
				HT29	A549	MCF-7	PC-3
18	CH_3	F	H	0.29 ± 0.03	0.36 ± 0.02	0.35 ± 0.02	2.34 ± 0.04
19	CH_3	F	2-F	**0.18 ± 0.02**[a]	0.39 ± 0.02	0.42 ± 0.02	3.24 ± 0.09
20	CH_3	F	4-F	**0.12 ± 0.03**	**0.15 ± 0.01**	0.17 ± 0.03	4.35 ± 0.12
21	CH_3	F	4-OCH_3	0.59 ± 0.04	0.71 ± 0.04	ND[b]	ND
22	CH_3	F	3,4-$(F)_2$	0.61 ± 0.03	0.57 ± 0.03	0.82 ± 0.02	6.54 ± 0.11
23	CH_3	F	3-Cl-4-F	1.06 ± 0.04	0.73 ± 0.03	ND	ND
24	CH_3	F	3-CF_3-4-Cl	1.12 ± 0.05	2.37 ± 0.06	ND	ND
25	CH_3	H	H	0.35 ± 0.03	0.42 ± 0.02	0.32 ± 0.04	7.26 ± 0.15
26	CH_3	H	2-F	0.36 ± 0.05	0.32 ± 0.02	0.28 ± 0.02	3.45 ± 0.28
27	CH_3	H	4-F	**0.18 ± 0.03**	0.22 ± 0.03	0.39 ± 0.05	3.45 ± 0.32
28	CH_3	H	4-OCH_3	1.16 ± 0.10	2.42 ± 0.18	ND	ND
29	CH_3	H	3,4-$(F)_2$	1.07 ± 0.06	2.26 ± 0.12	ND	ND
30	CH_3	H	3-Cl-4-F	2.18 ± 0.12	0.98 ± 0.07	6.31 ± 0.15	12.59 ± 0.13
31	CH_3	H	3-CF_3-4-Cl	3.29 ± 0.11	2.83 ± 0.15	8.41 ± 0.12	10.92 ± 0.15
32	CF_3	F	H	**0.091 ± 0.003**	0.22 ± 0.04	1.15 ± 0.11	2.32 ± 0.11
33	CF_3	F	2-F	**0.073 ± 0.004**	**0.19 ± 0.02**	0.28 ± 004	2.13 ± 0.12
34	CF_3	F	4-F	**0.029 ± 0.002**	**0.082 ± 0.003**	**0.11 ± 0.02**	1.52 ± 0.14
35	CF_3	F	4-OCH_3	0.29 ± 0.04	0.36 ± 0.04	0.51 ± 0.05	1.85 ± 0.16
36	CF_3	F	3,4-$(F)_2$	0.32 ± 0.003	0.35 ± 0.02	0.59 ± 0.03	2.48 ± 0.12
37	CF_3	F	3-Cl-4-F	0.63 ± 0.03	0.82 ± 0.03	ND	ND
38	CF_3	F	3-CF_3-4-Cl	1.05 ± 0.12	1.29 ± 0.10	ND	ND
39	CF_3	H	H	**0.19 ± 0.02**	0.29 ± 0.01	0.26 ± 0.04	3.57 ± 0.12
40	CF_3	H	2-F	**0.12 ± 0.03**	0.21 ± 0.03	0.29 ± 0.03	3.69 ± 0.14
41	CF_3	H	4-F	**0.093 ± 0.006**	**0.18 ± 0.02**	0.21 ± 0.04	2.83 ± 0.16
42	CF_3	H	4-OCH_3	0.29 ± 0.04	0.46 ± 0.05	0.58 ± 0.03	4.85 ± 0.11
43	CF_3	H	3,4-$(F)_2$	0.52 ± 0.03	0.79 ± 0.03	0.69 ± 0.05	7.31 ± 0.14
44	CF_3	H	3-Cl-4-F	1.02 ± 0.04	1.12 ± 0.09	ND	ND
45	CF_3	H	3-CF_3-4-Cl	1.53 ± 0.11	2.34 ± 0.13	ND	ND
Foretinib				0.26 ± 0.03	0.49 ± 0.08	6.25 ± 0.36	0.89 ± 0.12

[a] Bold values show the IC_{50} values of the target compounds lower than 0.2 μM.
[b] ND: not determined.

Table 3
Inhibition of tyrosine kinases by compound **34**

Kinase	Enzyme IC_{50} (nM)
Flt-3	2.06
PDGFR-β	4.57
KDR	86.25
c-Kit	324.58
EGFR	582.17

and electronic property of substituents (R^2 group) on the phenyl ring (moiety D) were key factors in improving the potency.

To examine the selectivity of compound **34** on c-Met over other kinases, this compound was screened against 5 other tyrosine kinases (Table 3). Compared with its high potency against c-Met (IC_{50} = 1.68 nM), **34** also exhibited high inhibitory effects against Flt-3 (IC_{50} = 2.06 nM) and PDGFR-β (IC_{50} = 4.57 nM). Moreover, compound **34** showed inhibitory effects against KDR, c-Kit and

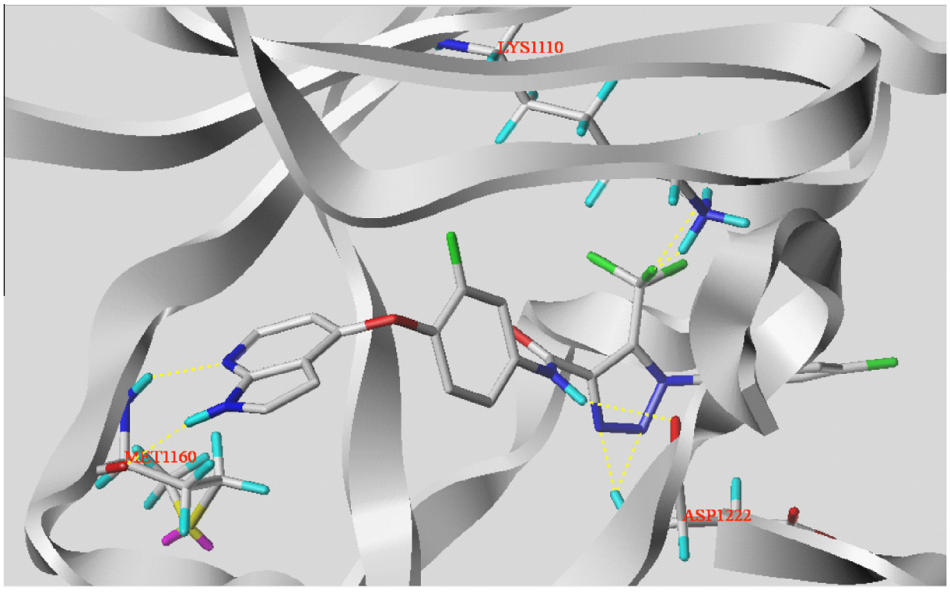


Fig. 4. Binding poses of compound **34** with c-Met. The proteins were displayed by grid ribbon. Compound **34** were displayed by multicolor sticks. H-bonding interactions between the **34** and c-Met were indicated with dashed lines in yellow.

EGFR although the potency was 51.3-, 193.2-, and 346.5-fold lower than that of c-Met. These data suggested that compound **34** is a promising multi-target kinase inhibitor.

For docking purposes, the three-dimensional structure of the c-Met (PDB code: 3LQ8) were obtained from RCSB Protein Data Bank. The docking simulation was conducted using SURFLEX-DOCK module of SYBYL 8.1 package version. The binding model was exemplified by the interaction of compound **34** with c-Met. As shown in Figure 4, the pyrrolo[2,3-b]pyridine fragment formed two hydrogen bonds with MET1160. In the 5-atom linker, two nitrogen atoms of 1,2,3-triazole and hydrogen atom on amide linkage formed three hydrogen bonds with ASP1222. In addition, the trifluoromethyl group formed two hydrogen bonds with LYS1110. Therefore, compound **34** formed seven hydrogen-bonding interactions with c-Met.

In summary, we designed and synthesized 28 pyrrolo[2,3-b] pyridine derivatives bearing 1,2,3-triazole moiety. c-Met kinase and 4 human cancer cell lines (HT29, A549, MCF-7, and PC-3) were used to evaluate the potency of the synthesized compounds. 4 of the derivatives showed high potency with IC_{50} values in single-digit nanomole range. Compound **34** (c-Met IC_{50} = 1.68 nM, a multi-target tyrosine kinase inhibitor) showed the strongest cytotoxic activities against HT29, A549, and MCF-7 cell lines (IC_{50} values: 0.029, 0.082, and 0.11 μM, respectively). Analysis of SARs indicated that EWGs (X = CF_3, R^1 = F, R^2 = 4-F) were required to decrease the higher electron density on the 5-atom linker to a proper degree to improve the inhibitory activity.

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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.2016.02.059.

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22. *In vitro kinase assay*: The kinase assays were performed by homogeneous time-resolved fluorescence (HTRF) assay as previously reported protocol. Briefly, 20 μg/mL poly (Glu, Tyr) 4:1 (Sigma) was preloaded as a substrate in 384-well plates. Then 50 μL of 10 mM ATP (Invitrogen) solution diluted in kinase reaction buffer (50 mM HEPES, pH 7.0, 1 M DTT, 1 M MgCl2, 1 M MnCl2, and 0.1% NaN3) was added to each well. Various concentrations of compounds diluted in 10 μL of 1% DMSO (v/v) were used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 39 μL of kinase reaction buffer solution. The incubation time for the reactions was 30 min at 25 °C, and the reactions were stopped by the addition of 5 μL of Streptavidin-XL665 and 5 μL Tk Antibody Cryptate working solution to all of wells. The plates were read using Envision (PerkinElmer) at 320 nm and 615 nm. The inhibition rate (%) was calculated using the following equation: % inhibition = 100 − [(Activity of enzyme with tested compounds − Min)/(Max − Min)] × 100 (Max: the observed enzyme activity measured in the presence of enzyme, substrates, and cofactors; Min: the observed enzyme activity in the presence of substrates, cofactors and in the absence of enzyme). IC_{50} values were calculated from the inhibition curves.
23. *Analytic data of potent inhibitor* ***34***: ^{1}H NMR (400 MHz, DMSO) δ 11.12 (s, 1H), 8.42 (d, J = 5.6 Hz, 1H), 8.35 (d, J = 4.1 Hz, 1H), 7.99 (d, J = 4.9 Hz, 1H), 7.96 (s, 1H), 7.93 (d, J = 4.9 Hz, 1H), 7.87 (d, J = 4.9 Hz, 1H), 7.72 (s, 1H), 7.70 (s, 1H), 7.67 (d, J = 2.2 Hz, 2H), 6.95 (d, J = 4.1 Hz, 1H), 6.83 (d, J = 5.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO) δ 163.86, 162.58, 161.40, 159.71, 159.16, 157.46, 140.45, 146.32, 141.03, 139.07, 138.32, 137.83, 136.64, 131.35, 128.04 (2C), 123.54, 116.72 (2C), 112.20, 108.88, 104.39, 101.79; MS m/z (ESI): 501.3 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{13}F_5N_6O_2$: C, 55.25; H, 2.63; N, 18.79. Found: C, 55.19; H, 2.58; N, 18.76.
24. *Cytotoxicity assay:* A standard MTT assay was used to measure cell growth. The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximate 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The compounds tested at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 mL DMSO of each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested in triplicate in each cell line. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.