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Design, Synthesis, and Biological Evaluation of 3-(1*H*-1,2,3-Triazol-1-yl)benzamide Derivatives as Potent Pan Bcr-Abl Inhibitors Including the Threonine^{315}→Isoleucine^{315} Mutant

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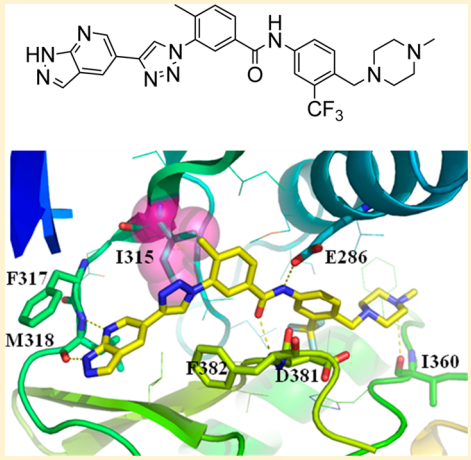
Supporting Information

ABSTRACT: A series of 3-(1*H*-1,2,3-triazol-1-yl)benzamide derivatives were designed and synthesized as new Bcr-Abl inhibitors by using combinational strategies of bioisosteric replacement, scaffold hopping, and conformational constraint. The compounds displayed significant inhibition against a broad spectrum of Bcr-Abl mutants including the gatekeeper T315I and p-loop mutations, which are associated with disease progression in CML. The most potent compounds **6q** and **6qo** strongly inhibited the kinase activities of Bcr-Abl^{WT} and Bcr-Abl^{T315I} with IC_{50} values of 0.60, 0.36 and 1.12, 0.98 nM, respectively. They also potently suppressed the proliferation of K562, KU812 human CML cells, and a panel of murine Ba/F3 cells ectopically expressing either Bcr-Abl^{WT} or any of a panel of other Bcr-Abl mutants that have been shown to contribute to clinical acquired resistance, including Bcr-Abl^{T315I}, with IC_{50} values in low nanomolar ranges. These compounds may serve as lead compounds for further development of new Bcr-Abl inhibitors capable of overcoming clinical acquired resistance against imatinib.



INTRODUCTION

Chronic myelogenous leukemia (CML) is a hematological malignancy representing about 20% of adult leukemia and characterized by the presence of the Philadelphia (Ph) chromosome. The chromosome is a truncated version of chromosome 22 resulting from a reciprocal translocation between chromosomes 9 and 22. The chimeric Bcr-Abl gene generated by the translocation encodes a fusion protein having constitutively active kinase activity.[1] This fusion protein Bcr-Abl kinase has become a well-validated target for development of small molecular inhibitors to treat CML.[2] The first-generation Bcr-Abl inhibitor imatinib (**1**, STI571) has achieved great clinical success and became the first-line drug for the treatment of CML.[3] However, emerging acquired resistance to drug **1** can be a major challenge,[4] with resistance rates of 3−4% reported in newly diagnosed chronic phase CML patients, up to 40−50% or over 80%, respectively, in accelerated or blastic phase disease.[5] The point mutations in the kinase domain of Bcr-Abl are a major mechanism to imatinib resistance, and more than 100 point mutations have been identified.[6]

To overcome the resistance against drug **1**, several classes of second-generation kinase inhibitors have been developed, among which nilotinib (**2**)[7] and dasatinib (**3**)[8] have been approved as the second-line drugs to treat adult patients in all phases of CML with resistance to drug **1** (Figure 1).[9] Bafetinib[10] and bosutinib[11] have also been developed into late-stage of clinical trials. Despite of the effectiveness of these second-generation compounds to inhibit most of the Bcr-Abl mutants, subsets of mutants remain resistant.[12] Particularly, none of the second-generation inhibitors inhibits Bcr-Abl^{T315I}, the "gatekeeper" mutation that accounts for approximately 15−20% of all clinical acquired mutations.[4,13] Bcr-Abl^{T315I} -induced drug resistance remains an unmet clinical challenge for CML treatment.[14]

Thr315 residue is critical for the binding of most Bcr-Abl inhibitors, regardless of whether they target the active or inactive conformation of the kinase, and in the T315I mutant loss of the side-chain hydroxyl group abrogates the possibility of a key hydrogen bond between the inhibitors and Thr315 residue.[6b] The bulky isoleucine side chain also hinders the inhibitors from accessing an important adjacent hydrophobic pocket.[7b,15] Several classes of inhibitors have been identified that potently suppress the function of Bcr-Abl^{T315I} mutant.[9b,16]

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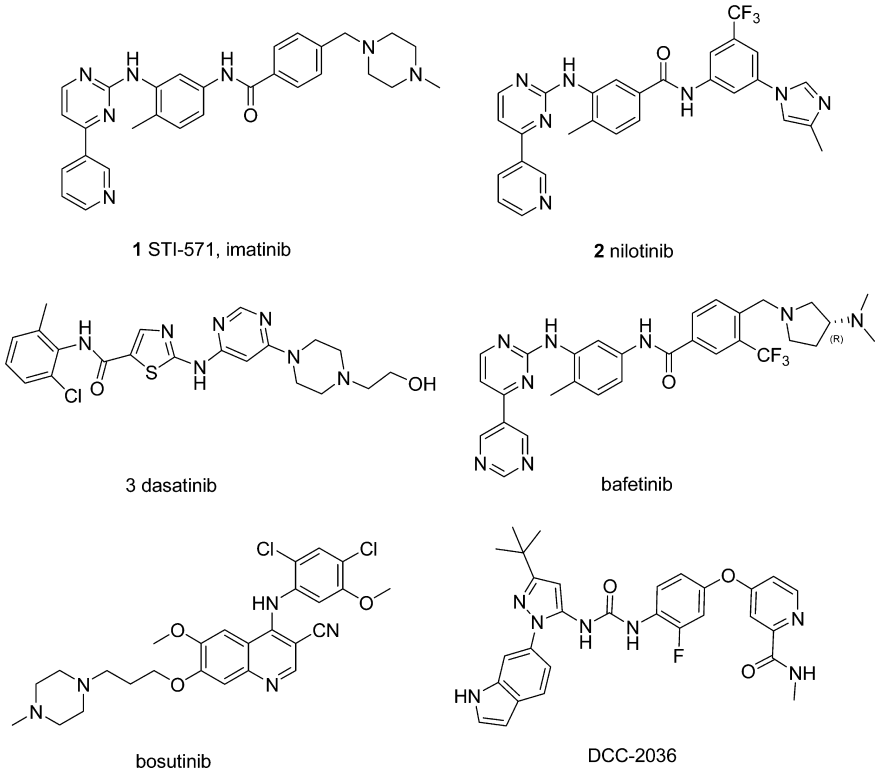

Figure 1. Chemical structures of FDA-approved Bcr-Abl inhibitors and newly developed molecules bafetinib, bosutinib, and DCC-2036.

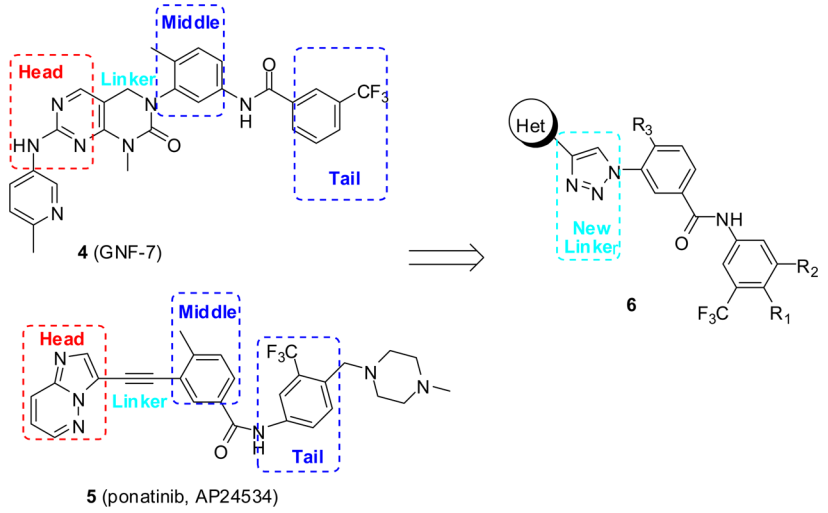

Figure 2. Design of 3-(1*H*-1,2,3-triazol-1-yl)benzamide derivatives as new Bcr-Abl inhibitors.

PPY-A is a pyrrolopyridine compound binding to the active conformation of Bcr-Abl and effectively inhibited T315I mutant with an IC_{50} value of 20 nM.[17] c-Src/Abl dual inhibitor TG100598 displayed an IC_{50} value of 3.4 nM in an enzymatic assay against Bcr-Abl^{T315I}.[18] Non-ATP-competitive or allosteric inhibitors (ON012380[19] or GNF-2[20]) were also identified to potently inhibit Bcr-Abl^{T315I}. Several ATP-competitive aurora kinase inhibitors such as MK-0457 (Tozasertib, VX680),[21] PHA-739358,[22] AT9283,[23] and XL-228[24] have been found to be active against Bcr-Abl^{T315I}. Some of the aurora inhibitors have been advanced into clinical investigation. However, these molecules have to be formulated for intravenous administration, and clinical development for MK-0457 has been discontinued due to cardiac toxicity including QTc prolongation.[25]

Most recently, some "third-generation" Bcr-Abl inhibitors were disclosed to inhibit almost the full range of Bcr-Abl kinase domain mutations as well as the native kinase.[26] A novel type II Bcr-Abl inhibitor GNF-7 (**4**) was reported to strongly inhibit Bcr-Abl^{T315I} and other mutants with low nanomolar IC_{50} values. It also showed promising in vivo efficacy without appreciable toxicity.[27] Ponatinib (**5**, AP24534)[28] and DCC-2036[29] have been advanced for clinical investigation. The overall clinical benefits of these inhibitors are eagerly awaited. Herein, we report the design, synthesis, and biological evaluation of 3-(1*H*-1,2,3-triazol-1-yl)benzamide derivatives as new pan Bcr-Abl inhibitors that potently inhibit native Bcr-Abl kinase and a

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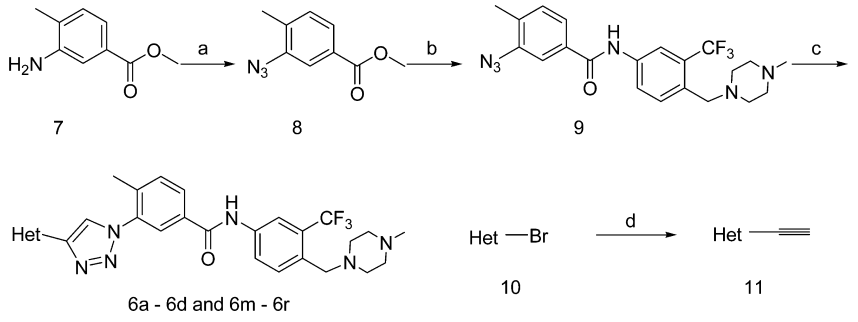
Scheme 1. Synthesis of Compounds 6a−6d and 6m−6r[a]



[a]Reagents and conditions: (a) 6 N HCl, $NaNO_2$, NaN_3, 0 °C to rt, 99%; (b) 4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline, ^tBuOK, THF, −20 °C to rt, 61%; (c) **11**, $CuSO_4$, sodium ascorbate, ^tBuOH/water (1:1), 90 °C, 22−69%; (d) (i) trimethylsilylacetylene, 10 mol % of $Pd(PPh_3)_2Cl_2$, 10 mol % of CuI, Et_3N, acetonitrile, reflux; (ii) K_2CO_3, methanol, rt, 68−80% (two steps).

wide-range of Bcr-Abl kinase domain mutants, including the gatekeeper Bcr-Abl^{T315I}.

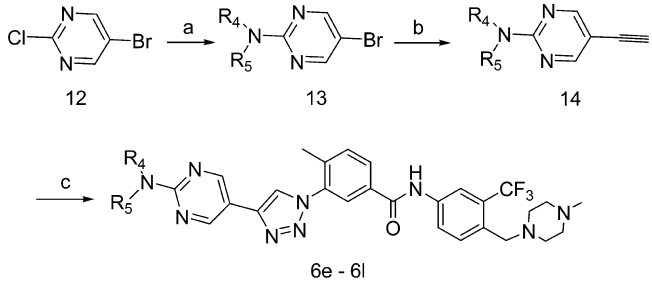
Molecular Design. Several cocrystal structures of Bcr-Abl with different type II third-generation inhibitors have been reported.[27,30] Structural analysis of chemical features of the inhibitors (i.e., **4** and **5**) and their interactions with Bcr-Abl kinase revealed that most of the compounds possessed some key structural elements which formed crucial interactions with the protein: (1) a heterocyclic moiety (so-called "head region") binding to the adenine pocket and forming a hydrogen bond network with residues in the hinge region; (2) a methylphenyl group ("middle part") occupying the hydrophobic pocket behind the gatekeeper residue; (3) an additional hydrophobic group ("tail region") binding the pocket induced by the DFG-out conformation; (4) a suitable linker between the head region and the middle part to avoid the steric clash with the Ile315 residue of Bcr-Abl. On the basis of this observation, a series of 3-(1*H*-1,2,3-triazol-1-yl)benzamide derivatives were designed as Bcr-Abl inhibitors in which a 1,2,3-triazol moiety was utilized as the linker between the head region and the middle part (Figure 2).

Chemistry. The synthesis of compounds **6a−6d** and **6m−6r** with alternative hinge-binding templates is illustrated in Scheme 1. Briefly, methyl 3-azido-4-methylbenzoate (**8**) was prepared by diazotization of methyl 3-amino-4-methylbenzoate (**7**) and coupled with 4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline to produce the benzamide (**9**). The coupling of **9** with **11**, which was prepared through a classical Sonogashira reaction,[31] following deprotection afforded final products **6a−6d** and **6m−6r** in moderate yields. It is noteworthy that the coupling reaction worked well for substrates with unprotected pyrazole, pyrrole, or amine functionalities, although a slight decrease of isolated yields was observed.

For the compounds containing 2-amino pyrimidinyl moieties (**6e−6l**, Scheme 2), *N*-substituted 5-bromo-2-amines **13** were first prepared by S_NAr displacement of 5-bromo-2-chloropyrimidine (**12**) with different amines. The final compounds could be obtained through procedures similar to that in Scheme 1.

The synthesis of **6qa−6qi** is outlined in Scheme 3. 4-Methyl-3-(trifluoromethyl)aniline (**15**) was protected with a mono-Boc group to provide intermediate **16**. Compound **17** was obtained by NBS bromination of **16**, followed by amine substitution and deprotection to afford intermediates **18**. Compounds **6qa−6qi** were readily synthesized using procedures similar to that in Scheme 1.

Scheme 2. Synthesis of Compounds 6e−6i[a]



[a]Reagents and conditions: (a) amines, ethanol, 80 °C, 75−98%; (b) (i) trimethylsilylacetylene, 10 mol % of $Pd(PPh_3)_2Cl_2$, 10 mol % of CuI, Et_3N, acetonitrile, reflux; (ii) K_2CO_3, methanol, rt, 64−90% (two steps); (c) **9**, $CuSO_4$, sodium ascorbate, ^tBuOH/water (1:1), 90 °C, 14−62%.

Compounds **6qj−6ql** were synthesized from 3-bromo-5-(trifluoromethyl)benzenamine (**20**). Compounds **21** were prepared by applying our previously developed protocol.[32] The inhibitors with different triazoles or imidazoles (**6qj−6ql**) could be synthesized similarly from the procedures outlined in Scheme 4.

Compounds **6qm−6qs** with different R_3 substituents were prepared by using commercially available or self-prepared 3-amino-4-substituted benzoates (**23**) as the starting materials, following procedures similar to that of other substrates (Scheme 5).

RESULTS AND DISCUSSION

Given the strong potency of inhibitor **4** against Bcr-Abl^{WT} and the clinical resistance relevant mutants, we initially selected the imidazo[1,2-*b*]pyridazine moiety, which was one of the most important pharmacophores of **4**, as the potential head template to interact with the hinge region of the kinase. The 4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)benzenamine tail group was utilized because of its physicochemical properties to keep ideal hydrophilic−lipophilic balance of the molecules. This group was also widely found in the chemical structures of many other kinase inhibitors.[15a,33]

A computational docking study suggested that **6a** could bind to the ATP binding site of the nonphosphorylated (DFG-out) form of Bcr-Abl^{WT} and different Bcr-Abl mutants, including Bcr-Abl^{T315I}, with similar binding modes to that of inhibitor **4** (Figure 3A,B). The imidazo[1,2-*b*]pyridazine moiety in **6a** formed an essential hydrogen bond with the NH of Met318 in

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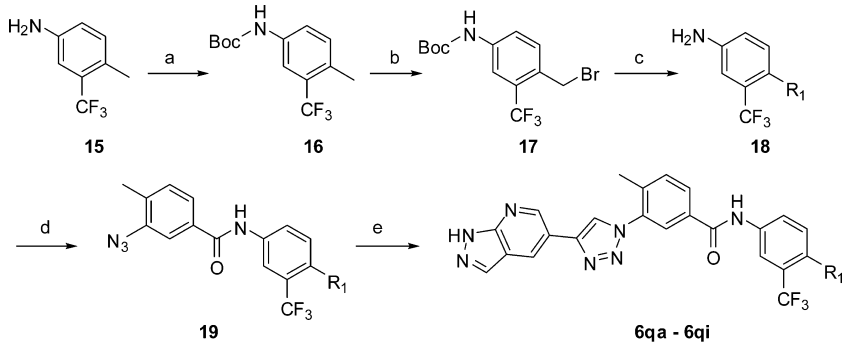
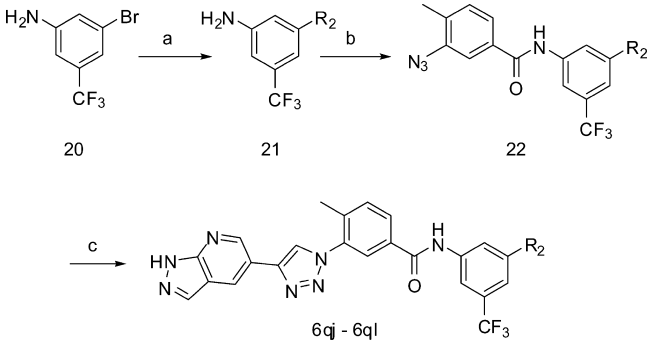
Scheme 3. Synthesis of 6qa−6qi Containing Different Hydrophilic Groups[a]



[a]Reagents and conditions: (a) di-*tert*-butyl dicarbonate, Na_2CO_3, dioxane/water (1:1), rt, 99%; (b) NBS, AIBN, CCl_4, reflux, 98%; (c) (i) hydrophilic amine, Et_3N, THF, 50 °C; (ii) TFA, DCM, rt, 45−88%; (d) **8**, ^{t}BuOK, THF, −20 °C to rt, 32−86%; (e) **11q**, $CuSO_4$, sodium ascorbate, ^{t}BuOH/water (1:1), 90 °C, 14−43%.

Scheme 4. Synthesis of Inhibitors 6qj−6ql with Different Triazoles or Imidazoles[a]

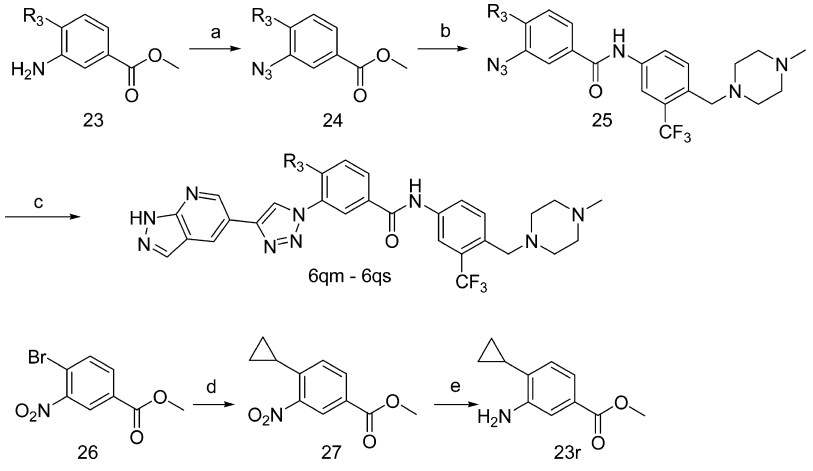


[a]Reagents and conditions: (a) triazoles or imidazoles, 1-(5,6,7,8-tetrahydroquinolin-8-yl)ethanone, CuI, Cs_2CO_3, DMF, Ar, 130 °C, 50−71%; (b) 6 N HCl, $NaNO_2$, NaN_3, 0 °C to rt, 79−95%; (c) **11q**, $CuSO_4$, sodium ascorbate, ^{t}BuOH/water (1:1), 90 °C, 8−68%.

the hinge region of Bcr-Abl. The tail part of the molecule also participated in hydrogen bonds and hydrophobic interactions with the DFG motif, among which the amide formed two hydrogen bonds with Glu286 and Asp381 and the trifluoromethylphenyl group bound deeply into the hydrophobic pocket. The 1,2,3-triazol linker could form favorable van der Waals interactions with the gatekeeper Ile315 of Bcr-Abl^{T315I} without causing steric clash.

Compound **6a** inhibited Bcr-Abl^{WT} and the Bcr-Abl^{T315I} mutant as assessed using a well-established FRET-based Z′-Lyte assay.[34] Compound **4** was used as the positive control to validate the screening conditions and for direct comparison. As shown in Table 1, compound **4** potently inhibited the enzymatic activity of Bcr-Abl^{WT} and Bcr-Abl^{T315I} mutant with mean IC_{50} values of 1.25 and 0.97 nM, respectively, which is comparable to the reported data.[28] The IC_{50} value of compound **6a** was 2.67 nM against Bcr-Abl^{WT} under the same screening conditions. The kinase inhibition of **6a** was further validated by investigating its suppression on the growth of murine Ba/F3 cells ectopically expressing Bcr-Abl^{WT}. Further evaluation indicated that it strongly inhibited the proliferation of Bcr-Abl^{WT} -positive K562 and KU812 CML cells. It also inhibited the growth of our imatinib-resistant K562R cells, shown to carry a Q252H mutation in Bcr-Abl, with a mean IC_{50} value of 1.4 nM. However, its inhibitory activity against Bcr-Abl^{T315I} mutant was significantly lower than that of wild-type

Scheme 5. Synthesis of Compounds 6qm−6qs with Different R_3 Substituents[a]



[a]Reagents and conditions: (a) 6 N HCl, $NaNO_2$, NaN_3, 0 °C to rt, 47−98%; (b) methyl 3-azido-4-methylbenzoate, ^{t}BuOK, THF, −20 °C to rt, 10−52%; (c) **11q**, $CuSO_4$, sodium ascorbate, ^{t}BuOH/water (1:1), 90 °C, 13−18%; (d) cyclopropylboronic acid, Pd(dppf)Cl_2-DCM, K_3PO_4, dioxane, Ar, 90 °C; (e) (i) TFA, NH_4Cl, THF/water (1:1); (ii) Fe powder, refluxing, 44% (two steps of d and e).

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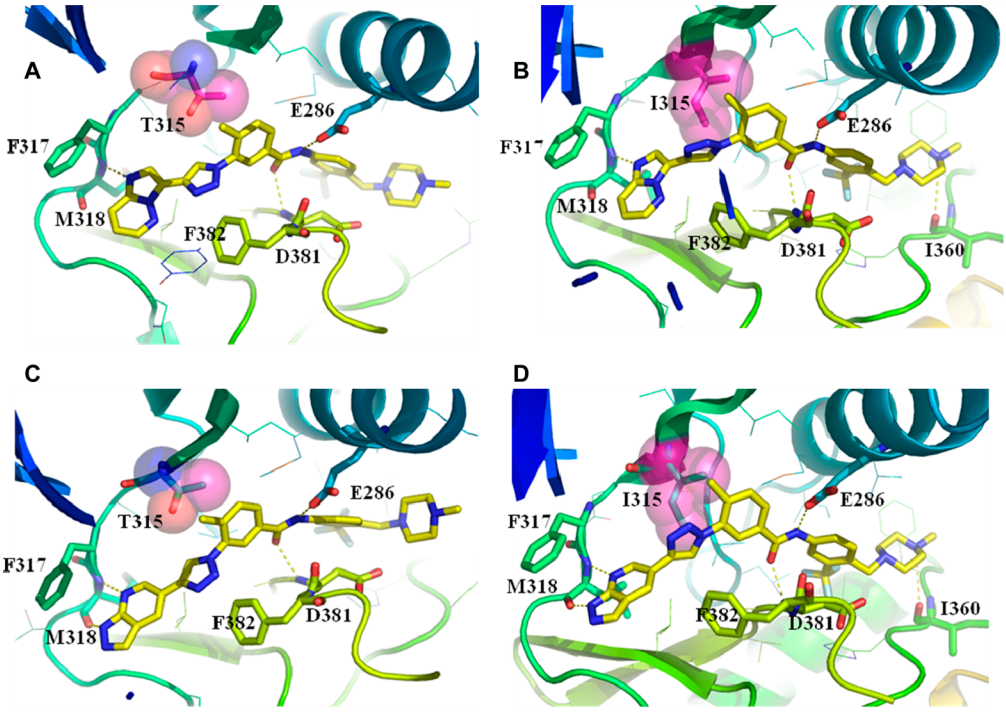

Figure 3. Binding models of inhibitor **6a** and **6q** to Bcr-Abl kinase. Hydrogen bonds are indicated by yellow hatched lines to key amino acids. (A) Binding model of **6a** with Bcr-Abl^{WT} (PDB: 3cs9). (B) Binding model of **6a** with Bcr-Abl^{T315I} (PDB: 3ik3). (C) Binding model of **6q** with Bcr-Abl^{WT} (PDB: 3cs9). (D) Binding model of **6q** with Bcr-Abl^{T315I} (PDB: 3ik3).

kinase (mean IC_{50} 92 nM). It also poorly inhibited the growth of murine Ba/F3 cells stably expressing Bcr-Abl^{T315I} (IC_{50} >10 μM), which might be due to poor solubility.

Other heterocyclic moieties such as 3-imidazo[1,2-*a*]-pyrimidinyl (**6b**), 3-pyrazolo[1,5-*a*]pyrimidinyl (**6c**), or 3-l*H*-imidazo[1,2-*a*]pyridinyl (**6d**) were introduced to maintain the potential hydrogen bond with the hinge region of Bcr-Abl. Compounds **6b** and **6c** displayed activities similar to that of **6a** in biochemical and cellular assays. Interestingly, **6d** was 5−6 times more potent than **6a** in the enzymatic inhibition assay. The superiority of **6d** became more significant in cell proliferation inhibition assays.

Our docking study has suggested that the imidazo[1,2-*b*]pyridazine moiety in **6a** could form a hydrogen bond with the NH of M318 in the hinge region of Bcr-Abl (Figure 3A). We hypothesized that the bioactivity might be improved by introducing an additional hydrogen-donating moiety into the molecule to provide another hydrogen bond with the hinge region. A 2-*N*-substituted amino pyrimidinyl motif has previously been successfully used as a hinge-binding template to catch two pairs of hydrogen bonds in the design of receptor tyrosine kinase inhibitors.[35] Therefore, a series of 3-(4-(2-(substituted amino)pyrimidin-5-yl)-1*H*-1,2,3-triazol-1-yl)-*N*-phenylbenzamides were designed and synthesized as new Bcr-Abl inhibitors. Computational studies suggested that compound **6f** could form two hydrogen bonds with the NH and the C═O motif of M318 in the hinge region of Bcr-Abl (Supporting Information). Biological evaluation revealed that compound **6f** strongly inhibited the activities of Bcr-Abl^{WT} and Bcr-Abl^{T315I} with IC_{50} values of 0.78 and 10.6 nM, respectively, which was about 3−8 times more potent than **6a**. This compound was also significantly more active than **6a** suppressing Bcr-Abl-dependent cell proliferation. Further structure−activity relationship analysis demonstrated that the substituted groups in the 2-amino moiety had a significant impact on the bioactivities of compounds. With increasing size of the substituted groups, the potencies decreased markedly. This might be due to the fact that the substituted groups could form steric clash to affect their potential hydrogen bond interaction with the hinge of Bcr-Abl. For instance, *N*-methyl analogue **6f** displayed activities similar to the *N*-unsubstituted inhibitor **6e**, but the *N*-benzyl **6k** showed a 46-fold loss in potency. Not surprisingly, the *N*,*N*-disubstituted compound **6l** was almost totally inactive in both enzymatic and cellular assays.

Inspired by the contribution of the two hydrogen bonds between the 2-*N*-methyl amino pyrimidinyl template and the hinge of Bcr-Abl to the kinase inhibition, a series of compounds containing either a pyrrolo[2,3-*b*]pyridinyl or 1*H*-pyrazolo[3,4-*b*]pyridinyl motif were designed by utilizing a conformation-constraining strategy. As shown in Table 1, **6n**, **6q**, and **6r** displayed similar potencies against Bcr-Abl^{WT} to those of **6e** and **6f**. However, their inhibitory activities against Bcr-Abl^{T315I} were significantly increased by factors of 20, 22, and 10, respectively, compared to that of **6f**. The cell growth inhibitory activities of the compounds were also obviously improved. The importance of the hydrogen bond network was further demonstrated by selective removal of the hydrogen bond donor or receptor moieties. Compound **6m** lacking a hydrogen bond donor group showed mean IC_{50} values against Bcr-Abl^{WT} and Bcr-Abl^{T315I} of 22.6 and 206 nM, respectively, which was 38 and 200 times less potent than **6q**. On the other hand, the removal of a hydrogen bond acceptor (**6o** and **6p**) also caused dramatic potency loss compared to **6n** and **6q**.

We have successfully designed and synthesized **6n**, **6q**, and **6r** as new highly potent inhibitors by applying combinational strategies of bioisosteric replacement, scaffold hopping, and conformational constraint. The compounds potently inhibited

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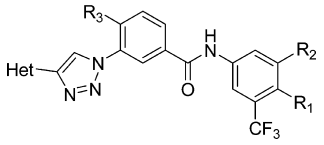
Table 1. Bioactivities of New Bcr-Abl Inhibitors[a]



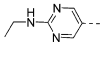
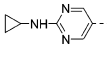
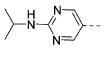
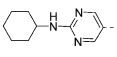
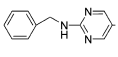
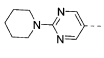
Cpds					abl kinase assay		Ba/F3 cell lines		human leukemia cell lines		
	Het	R_1	R_2	R_3	WT	T315I	WT[b]	T315I	K562	K562R[c]	KU812
6a			H	Me	2.67	92.2	27.4	>10000	35.8	1.41	14.6
6b			H	Me	3.44	95.7	47.9	8836	3.87	16.2	11.1
6c			H	Me	2.25	122	7.31	1444	2.45	17.1	1.51
6d			H	Me	0.52	14.4	4.54	178	0.63	1.85	0.16
6e			H	Me	0.78	10.6	3.35	494	0.85	17.1	0.20
6f			H	Me	0.54	25.2	2.74	299	0.67	11.0	0.16
6g			H	Me	1.18	29.6	3.04	405	1.26	20.2	0.20
6h			H	Me	1.01	89.4	3.01	579	1.24	19.2	0.22
6i			H	Me	5.96	341	32.8	799	7.26	81.2	1.44
6j			H	Me	12.8	342	28.3	3630	7.04	72.8	1.57
6k			H	Me	19.1	486	28.2	3499	8.82	181	1.89
6l			H	Me	1629	2130	1243	3588	798	893	158
6m			H	Me	22.6	206	316	533	40.9	76.8	11.2
6n			H	Me	0.69	1.22	3.16	66.9	0.42	3.89	0.14
6o			H	Me	302	334	435	999	70.4	800	19.6
6p			H	Me	17.8	250	56.2	754	12.4	109	12.2
6q			H	Me	0.60	1.12	2.92	63.2	0.40	5.46	0.11
6r			H	Me	0.52	2.18	2.03	79.6	0.39	4.74	0.15
6qa			H	Me	0.72	31.5	2.47	939	0.66	12.1	0.19
6qb			H	Me	0.52	5.09	3.01	91.7	0.32	2.24	0.68

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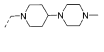
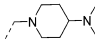
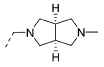
Table 1. continued

6qc			H	Me	0.45	14.2	3.43	544	0.31	3.15	0.24
6qd			H	Me	2.56	272	5.39	3514	3.22	31.1	0.47
6qe			H	Me	1.42	32.8	4.46	1557	3.41	41.1	0.46
6qf			H	Me	0.45	0.94	4.02	294	0.80	8.45	0.38
6qg			H	Me	0.52	18.0	23.8	873	1.25	4.32	1.48
6qh			H	Me	0.33	11.4	2.76	527	0.67	6.56	0.43
6qi			H	Me	0.41	18.7	3.66	473	0.25	3.55	0.40
6qj		H		Me	1.66	12.7	4.72	656	3.37	25.7	0.73
6qk		H		Me	1.03	10.6	4.54	652	3.50	21.9	0.46
6ql		H		Me	1.94	7.93	3.68	620	2.90	34.2	0.40
6qm			H	H	1.01	38.9	3.98	728	0.67	10.8	1.34
6qn			H	F	0.42	9.83	3.90	130	0.24	2.28	0.10
6qo			H	Cl	0.36	0.98	4.14	52.8	0.43	3.91	0.08
6qp			H	Br	0.70	1.94	3.28	46.4	0.23	1.49	0.10
6qq			H	Et	0.98	38.0	4.04	282	0.85	8.96	0.39
6qr			H		15.0	659	32.9	1156	5.69	37.5	4.05
6qs			H		66.0	130	420	2062	74	219	14.2
4					1.25	0.97	3.15	6.18	0.44	2.6	0.08

[a]The data are means of data from at least three independent experiments (IC_{50}, nM). [b]The data are means of results from two independent experiments. [c]The K562R is a self-established cell line which carries a Q252H Bcr-Abl mutation.

the enzymatic functions of Bcr-Abl^{WT} and Bcr-Abl^{T315I} with IC_{50} values in low nanomolar ranges. They also strongly suppressed the proliferation of K562, K562R, and KU812 human CML cells and murine Ba/F3 cells ectopically expressing Bcr-Abl^{WT} and Bcr-Abl^{T315I}. Taking **6q** as an example, the structure–activity relationships were further explored by investigating the influence of different R_1, R_2, and R_3 groups.

Our computational investigation suggested that **6q** could tightly bind to the ATP binding site of the nonphosphorylated (DFG-out) forms of Bcr-Abl^{WT} and different Bcr-Abl mutants, including Bcr-Abl^{T315I} (Figure 3B). The 1*H*-pyrazolo[3,4-*b*]pyridinyl moiety formed two hydrogen bonds with the hinge region of Bcr-Abl. It was also demonstrated that the trifluoromethylphenyl group in **6q** bound deeply into a hydrophobic pocket formed by DFG-out conformation. It was further suggested that the methylpiperazine moiety pointed toward the solvent-accessible domain of an allosteric binding pocket, and a positively charged terminal anime group might form favorable hydrogen bond(s) with the carbonyl oxygen atoms of the residues. The contribution of the terminal anime group has been clearly demonstrated in cocrystal structures of Bcr-Abl with inhibitor **1**, **4**, or others.[15a,30,36]

Therefore, we further investigated the potential influence of the tail region by introducing different hydrophilic groups in the R_1 or R_2 position. Consistent with our docking analysis, the 1-(4-methyl)piperazinyl group in compound **6q** was important for its strong inhibition against Bcr-Abl kinases. Although the

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compounds with 1-pyrrolidinyl (**6qa**), 1-piperidinyl (**6qd**), or *N*-morpholino (**6qe**) moieties displayed similar suppressive potencies against Bcr-Abl^{WT} to that of **6q**, their potencies against Bcr-Abl^{T315I} decreased approximately 30−250-fold. For instance, the IC_{50} values of compound **6q** against Bcr-Abl^{WT} and Bcr-Abl^{T315I} were 0.60 and 1.12 nM, while the corresponding values for compound **6qd** were decreased to 2.56 and 272 nM, respectively. Further investigation also revealed that the potency loss of compound **6qd** was able to be partially restored by introducing a 4-dimethylamino or 4-(1-(4-methyl)piperazin)yl group at the original piperidinyl moiety (**6qg** and **6qh**). Compounds **6qb** and **6qc** with a terminal (*S*)- or (*R*)-dimethylamino group as well as **6qi** were also more potent than the parent compound **6qa**. When the 1-(4-methyl)piperazinyl group was replaced with the 1-(4-methyl)-1,4-diazepanyl group, the resulting compound **6qf** was almost equally potent to compound **6q**. It was noteworthy that compounds displayed comparable potencies when the 3-methyl-1*H*-imidazol (**6qj**), 1*H*-1,2,4-triazol (**6qk**), or 3-methyl-1*H*-1,2,4-triazol (**6ql**) group was introduced at the R_2 position.

Several studies have suggested that the "flag methyl" group at the R_3 position was important for drug **1** and other inhibitors to maintain a favorable conformation for binding with Bcr-Abl kinase and imparting kinase selectivity.[33,37] A brief investigation was also conducted to evaluate its impact on inhibitory activities of new inhibitors, and the results are summarized in Table 1. It was shown that the methyl group in **6q** could be replaced with Cl (**6qo**) or Br (**6qp**) atoms without obvious influence on the kinase inhibition and cell proliferation inhibition. When the methyl group was replaced by H (**6qm**), F (**6qn**), or an ethyl group (**6qq**), the resulting inhibitors displayed inhibition against Bcr-Abl^{WT} and antiproliferation on K562, K562R, KU812, and Ba/F3 cells expressing Bcr-Abl^{WT} similar to that of **6q**. However, their activities on Bcr-Abl^{T315I} kinase and Ba/F3 cells expressing Bcr-Abl^{T315I} were dramatically decreased. The results also clearly revealed that large hydrophobic groups were detrimental to the activities both on kinase and cellular levels. For instance, replacement of the methyl group at the R_3 position with a cyclopropyl (**6qr**) or isopropyl (**6qs**) group caused 20−500-fold potency loss in Bcr-Abl^{WT} and Bcr-Abl^{T315I} inhibition.

As the two most potent compounds shown in Table 1, the effects of **6q** and **6qo** were further investigated on the activation of Bcr-Abl and its downstream signals in cells harboring different status of Bcr-Abl kinases. K562 is a human CML cell line with positive Bcr-Abl^{WT}, and Ba/F3 T315I cells are murine Ba/F3 cells ectopically expressing Bcr-Abl^{T315I}. As shown in Figure 4, **6q** and **6qo** dose-dependently inhibited the phosphorylation of Bcr-Abl in both K562 and Ba/F3 cells expressing Bcr-Abl^{T315I}. The phosphorylation level of the downstream signal proteins such as CrkL and STAT5 were also obviously decreased, while the total protein levels of Bcr-Abl, CrkL, and STAT5 and GAPDH remained unchanged. Further evaluation also suggested that **6q** and **6qo** could potently induced apoptosis and G0/G1 phase arrest in both K562 and Ba/F3 T315I cells (Supporting Information). These data further supported strong target inhibition of the designed compounds.

In addition to mutations in the gatekeeper region (T315I/A), a variety of other mutations in the p-loop region (e.g., G250E, Q252H, Y253, E255K/V) and hinge region (F317L/V) conferring imatinib resistance have also been identified.[6c] The

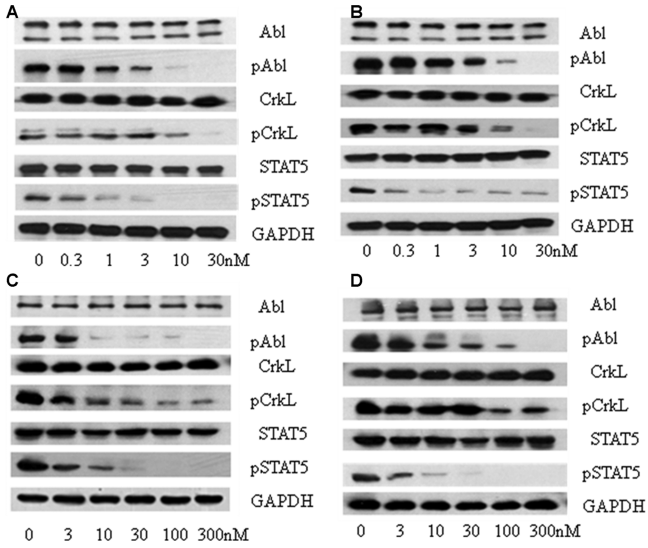

Figure 4. Compounds **6q** and **6qo** dose-dependently inhibit the phosphorylation of Abl, CrkL, and STAT5 in both K562 (A,B) and Ba/F3 cells transformed by Bcr-Abl^{T315I} (C,D). (A) **6q** suppresses the activation of Abl and downstream signals in K562 cells; (B) **6qo** suppresses the activation of Abl and downstream signals in K562 cells; (C) **6q** suppresses the activation of Abl and downstream signals in Ba/F3 T315I cells; (D) **6qo** suppresses the activation of Abl and downstream signals in Ba/F3 T315I cells. The data are representative of three independent experiments.

inhibitory activities of **6q** and **6qo** against a panel of other resistance relevant Bcr-Abl mutants were further investigated by evaluating their antiproliferation effects on Ba/F3 cells stably expressing different Bcr-Abl mutants (Table 2). The results clearly demonstrated that the compounds displayed strong activity against all of the mutant Bcr-Abl-dependent Ba/F3 cells, which might be due to the fact that the mutated residues did not form direct interaction with the compound in most of the mutants. It was noteworthy that the compounds were more than 30 times less potent on untransformed Ba/F3 cells than

Table 2. Inhibitory Activities of 6q and 6qo against 14 Types of Bcr-Abl Mutants as Well as Bcr-Abl^{WT}[a]

transformed Ba/F3 cells	1	2	3	6q	6qo
WT	1834	65.2	4.50	2.44	2.49
M351T	424	10.3	0.83	0.40	0.45
F486S	2547	21.0	1.71	1.43	4.17
Q252H	3763	150	6.55	5.49	7.55
Y253H	17083	314	1.29	1.61	2.97
E355G	362	8.38	0.58	0.37	0.30
M244V	129	1.54	0.12	0.12	0.61
T315I	3005	775	1714	13.9	11.9
H396P	110	6.34	0.31	0.28	0.30
E255K	2951	292	5.72	3.10	2.75
E255V	8615	473	1.91	2.96	3.41
F359V	960	159	1.94	1.44	2.31
H396R	2377	87.2	1.72	3.73	5.40
Y253F	1444	60.7	0.58	0.35	0.52
G250E	1863	38.6	0.51	1.67	1.97
parent	18520	40750	6589	416.4	372.3

[a]Antiproliferation assays of the compounds are evaluated by using cell counting kit (CCK-8). Data are the means of three experiments (IC_{50}, nM).

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those expressing Bcr-Abl kinases, supporting the selective inhibition on Bcr-Abl kinase.

In summary, we have successfully designed and synthesized a series of 3-(1*H*-1,2,3-triazol-1-yl)benzamide derivatives as new Bcr-Abl inhibitors which maintain significant inhibition against a broad spectrum of Bcr-Abl mutants including the most refractory gatekeeper T315I mutant associated with disease progression in CML. The most potent compounds **6q** and **6qo** strongly inhibited the kinase activities of Bcr-Abl^{WT} and Bcr-Abl^{T315I} with mean IC_{50} values of 0.60, 0.36 and 1.12, 0.98 nM, respectively. They also potently inhibited the proliferation of K562, KU812 human CML cells, and a panel of murine Ba/F3 cells ectopically expressing Bcr-Abl^{WT} and a broad spectrum of other Bcr-Abl mutants contributing to clinical TKI resistance, including Bcr-Abl^{T315I}. Western blot analysis on their effects on Bcr-Abl signal pathway further supported their strong kinase inhibition against Bcr-Abl and Bcr-Abl^{T315I}. These compounds may serve as new promising lead compounds for further development of new Bcr-Abl inhibitors overcoming the clinical acquired resistance against imatinib.

EXPERIMENTAL SECTION

Chemistry. Reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed using silica gel (300−400 mesh). All reactions were monitored by TLC, using silica gel plates with fluorescence F_{254} and UV light visualization. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz and Bruker AV-500 spectrometer at 125 MHz, respectively. Coupling constants (J) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to internal control (TMS). The low- or high-resolution of ESI-MS was recorded on an Agilent 1200 HPLC-MSD mass spectrometer or Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer, respectively. The purity of compounds was determined by reverse-phase high-performance liquid chromatography (HPLC) analysis confirming to be over 95% (>95%). HPLC instrument: Dionex Summit HPLC (column: Diamonsil C18, 5.0 μm, 4.6 × 250 mm (Dikma Technologies); detector: PDA-100 photodiode array; injector: ASI-100 autoinjector; pump: p-680A). Elution, MeOH in water; flow rate = 1.0 mL/min.

*Methyl 3-Azido-4-methylbenzoate (**8**).* Methyl 3-amino-4-methylbenzoate 7 (8.4 g, 0.05 mol) was dissolved in HCl (6.0 mol/L, 40.0 mL, 0.24 mol) at room temperature, cooled to 0 °C, treated with a solution of $NaNO_2$ (3.57 g in 8.4 mL of H_2O, 0.05 mol) and stirred for 10 min at −5 to ∼0 °C. Sodium azide (3.97 g in 12.6 mL of H_2O, 0.06 mol) was added slowly, and the mixture was stirred at room temperature for 2 h. The mixture was diluted with ethyl acetate, and the organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated to give the crude product as yellow oil which was pure enough for subsequent reaction (9.7 g, yield 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 1.2 Hz, 1H), 7.63 (dd, J = 7.6 Hz, 1.2 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 3.85 (s, 3H), 2.18 (s, 3H).

*3-Azido-4-methyl-N-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)benzamide (**9**).* To a mixture of **8** (9.6 g, 0.5 mol) and 4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)-aniline (13.7 g, 0.5 mol) in anhydrous THF (50.0 mL) was slowly added potassium *tert*-butoxide (16.8 g, 0.15 mol) in anhydrous THF (50.0 mL) at −20 °C. Then the reaction mixture was stirred for 1 h, then slowly warmed to room temperature and stirred for another 8 h. After evaporation of the solvent, the residue was disssolved in ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the desired compound as red solid (13.5 g, yield 61%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.77 (m, 3H), 7.73 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 1.4 Hz, 1H), 7.39 (dd, J = 7.8 Hz, 1.4 Hz, 1H), 7.21 (d, J = 8.3 Hz, 1H), 3.57 (s, 3H), 2.45 (br, 8H), 2.23 (s, 3H), 2.21 (s, 3H); MS (ESI) m/z 433 $[M + H]^+$.

*5-Ethynyl-1H-pyrazolo[3,4-b]pyridine (**11q**).* A mixture of 5-bromo-1*H*-pyrazolo[3,4-*b*]pyridine (10.0 g, 0.05 mol), Cu (I) iodide (0.5 g, 0.5 mmol), and $Pd(PPh_3)_2Cl_2$ (1.8 g, 0.5 mmol) were added to a 250 mL round-bottom flask with a reflux condenser. The flask was evacuated and backfilled with argon (3 cycles). Acetonitrile (50.0 mL), Et_3N (21.0 mL, 0.1 mol), and trimethylsilylacetylene (21.0 mL, 0.1 mol) were added by syringe at room temperature. After stirring at 100 °C for 20 h, the reaction mixture was filtered and concentrated. The residue was dissolved in methanol (50.0 mL) with addition of K_2CO_3 (14.0 g, 0.1 mol). The reaction mixture was stirred at room temperature for 0.5 h and then filtered and concentrated for further purification by flash column chromatography on silica gel to afford the desired product as gray solid (4.9 g, yield 68%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.27 (s, 1H), 8.69 (d, J = 1.6 Hz, 1H), 8.25 (d, J = 2.0 Hz, 1H), 8.11 (s, 1H), 3.17 (s, 1H); ^{1}H NMR (400 MHz, DMSO-*d*) δ 13.89 (br, 1H), 8.59 (d, J = 1.6 Hz, 1H), 8.41 (d, J = 1.6 Hz, 1H), 8.17 (s, 1H), 4.28 (s, 1H).

11a−11d and **11m−11r** were prepared by the method similar to that of **11q**.

*3-(4-(1H-Pyrazolo[3,4-b]pyridin-5-yl)-1H-1,2,3-triazol-1-yl)-4-methyl-N-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)-phenyl)benzamide (**6q**).* To a solution of 5-ethynyl-1*H*-pyrazolo[3,4-*b*]pyridine **11q** (1.5 g, 10 mmol) and 3-azido-4-methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)benzamide **9** (4.5 g, 10 mmol) in *tert*-butyl alcohol/water (1:1, 20.0 mL) were added $CuSO_4$ (0.17 g, 1 mmol) and sodium ascorbate (0.41 g, 2 mmol). Then the mixture was heated at 90 °C overnight. The reaction mixture was cooled to room temperature and extracted three times with ethyl acetate. Then the combined organic phases were washed with brine, dried over anhydrous sodium sulfate, concentrated, and purified by silica gel column chromatography to give desired product as white solid (1.31 g, yield 22%): ^{1}H NMR (400 MHz, DMSO-*d*) δ 13.81 (s, 1H), 10.60 (s, 1H), 9.15 (d, J = 9.6 Hz, 2H), 8.77 (s, 1H), 8.26 (s, 1H), 8.19 (d, J = 5.2 Hz, 2H), 8.14 (d, J = 6.4 Hz, 1H), 8.06 (d, J = 6.8 Hz, 1H), 7.72 (dd, J = 6.4 Hz, 2.8 Hz, 2H), 3.56 (s, 2H), 2.36 (br, 10H), 2.15 (s, 3H); ^{13}C NMR (125 MHz, DMSO-*d*) δ 164.04, 146.89, 144.77, 137.95, 137.15, 136.09, 133.65, 133.11, 132.26, 131.81, 131.24, 129.10, 127.38 (J = 30 Hz), 126.15, 125.36, 125.07, 123.53, 123.18, 123.01, 119.70, 117.27, 114.36, 57.40, 54.68, 52.66, 45.68, 17.70; HR MS (EI) calcd for $C_{29}H_{28}F_3N_9O$ 576.2442 $[M + H]^+$; found 576.2434, purity 96.4% (t_R 6.67 min).

Compounds **6a−6d** and **6m−6r** were prepared from compound **9** and the corresponding reactant **11** by a method similar to that of **6q**.

*5-Bromo-N-cyclohexylpyrimidin-2-amine (**13j**).* A solution of 5-bromo-2-chloropyrimidine **12** (1.93 g, 10.0 mmol) and cyclohexylamine (2.29 mL, 20 mmol) in ethanol (15.0 mL) was heated at 80 °C with stirring in a sealed tube overnight. The reaction mixture was cooled to room temperature. After evaporation of the solvent, the off-white solid was disssolved in ethyl acetate. Then the organic phase was washed with water, dried over anhydrous sodium sulfate, and concentrated to give desired product as off-white solid (2.4 g, yield 94%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 2H), 5.06 (d, J = 5.6 Hz, 1H), 3.78−3.69 (m, 1H), 2.03−1.99 (m, 2H), 1.77−1.71 (m, 2H), 1.66−1.62 (m, 1H), 1.46−1.35 (m, 2H), 1.27−1.16 (m, 3H).

*N-Cyclohexyl-5-ethynylpyrimidin-2-amine (**14j**).* A mixture of 5-bromo-*N*-cyclohexylpyrimidin-2-amine **13j** (1.28 g, 5.0 mmol), Cu(I) iodide (0.048 g, 0.25 mmol), and $Pd(PPh_3)_2Cl_2$ (0.18 g, 0.25 mmol) were added to a sealed tube. The tube was evacuated and backfilled with argon (3 cycles). Acetonitrile (15.0 mL), Et_3N (2.1 mL, 15.0 mmol), and trimethylsilylacetylene (2.1 mL, 15.0 mmol) were added by syringe at room temperature. After stirring at 100 °C for 24 h, the reaction mixture was filtered and concentrated. The residue was dissolved in methanol (20.0 mL) and treated with K_2CO_3 (1.4 g, 10.0 mmol). The reaction mixture was stirred at room temperature for 0.5 h and then filtered and concentrated for further purification by flash column chromatography on silica gel to afford the desired product as yellow solid (0.9 g, yield 89%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 2H), 5.24 (d, J = 6.4 Hz, 1H), 3.87−3.78 (m, 1H), 3.16 (s, 1H),

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2.04−2.00 (m, 2H), 1.77−1.72 (m, 2H), 1.66−1.61 (m, 1H), 1.47−1.36 (m, 2H), 1.27−1.18 (m, 3H).

Other *N*-substituted 5-ethynylpyrimidin-2-amines **14e−14l** were synthesized by using the procedure similar to that of **14j**.

*3-(4-(2-(Cyclohexylamino)pyrimidin-5-yl)-1H-1,2,3-triazol-1-yl)-4-methyl-N-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)-phenyl)benzamide (**6j**).* To a solution of *N*-cyclohexyl-5-ethynyl-pyrimidin-2-amine **14j** (0.20 g, 1.0 mmol) and 3-azido-4-methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-benzamide **9** (0.43 g, 1.0 mmol) in *tert*-butyl alcohol/water (1:1, 4.0 mL) were added $CuSO_4$ (16 mg, 0.1 mmol) and sodium ascorbate (40 mg, 0.2 mmol). Then the mixture was heated at 90 °C overnight. The reaction mixture was cooled to room temperature and extracted three times with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, concentrated, and purified by silica gel column chromatography to give desired product as white solid (0.31 g, yield 50%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 2H), 8.41 (s, 1H), 7.95−7.88 (m, 5H), 7.78 (d, J = 8.8 Hz, 1H), 7.51 (d, J = 7.6 Hz, 1H), 5.21 (d, J = 7.6 Hz, 1H), 3.88−3.86 (m, 1H), 3.63 (s, 2H), 2.50 (br, 8H), 2.35 (s, 3H), 2.30 (s, 3H), 2.07−2.04 (m, 2H), 1.75 (m, 2H), 1.67−1.64 (m, 1H), 1.48−1.39 (m, 2H), 1.29−1.24 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.30, 161.45, 155.66, 143.58, 137.99, 136.47, 136.37, 134.12, 133.53, 132.24, 131.40, 129.27 (J = 32.4 Hz), 128.57, 125.08, 124.71, 123.36, 122.90, 119.75, 117.69, 113.39, 57.77, 55.23, 53.14, 49.83, 46.05, 33.13, 25.68, 24.80, 18.22; HR MS (EI) calcd for $C_{33}H_{38}F_3N_9O$ 634.3224 $[M + H]^+$; found 634.3214, purity 99.5% (t_R 13.24 min).

Compounds **6e−6l** were prepared from compound **9** and the corresponding reactant **14** by a method similar to that of **6j**.

*tert-Butyl 4-Methyl-3-(trifluoromethyl)phenylcarbamate (**16**).* To 4-methyl-3-(trifluoromethyl)aniline **15** (25.0 g, 0.14 mol) in dioxane (50.0 mL) was added sodium carbonate (22.3 g, 0.21 mol) in water (50.0 mL) followed by di-*tert*-butyl dicarbonate (39.0 mL, 0.17 mol). After stirring at room temperature overnight, the reaction was diluted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give the desire product as red solid which was used without further purification (39.0 g, yield 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 2.0 Hz, 1H), 7.42 (d, J = 7.6 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.53 (br, 1H), 2.41 (d, J = 1.6 Hz, 3H), 1.52 (s, 9H).

*tert-Butyl 4-(Bromomethyl)-3-(trifluoromethyl)phenylcarbamate (**17**).* To a preheated solution of CCl_4 (50.0 mL) were added *tert*-butyl 4-methyl-3-(trifluoromethyl)phenylcarbamate **16** (18.3 g, 0.066 mol), NBS (23.6 g, 0.133 mol), and AIBN (1.1 g, 6.6 mmol) in one portion. After heating under reflux overnight, the mixture was filtered. The filtrate was washed with brine, dried over anhydrous Na_2SO_4, and concentrated to red oil which was used without further purification (23.0 g, yield 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 1.6 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 6.80 (br, 1H), 4.59 (s, 2H), 2.76 (s, 9H).

*4-((4-Methyl-1,4-diazepan-1-yl)methyl)-3-(trifluoromethyl)-aniline (**18f**).* *tert*-Butyl 4-(bromomethyl)-3-(trifluoromethyl)phenyl-carbamate **17** (1.8 g, 5.0 mmol) was added to anhydrous THF (10.0 mL) followed by *N*-methylhomopiperazine (1.5 mL, 12.0 mmol) and Et_3N (2.1 mL, 15.0 mmol). The stirred mixture was heated at 50 °C overnight. After evaporation of the solvent, the residue was diluted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $NaSO_4$, and concentrated for purification by silica gel column chromatography (DCM/CH_3OH = 20:1, 0.5% $NH_3{\cdot}H_2O$) to give desired product as yellow oil (1.75 g, yield 88%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 2.0 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 6.75 (s, 1H), 3.70 (s, 2H), 2.71−2.66 (m, 6H), 2.63−2.60 (m, 2H), 2.37 (s, 3H), 1.86−1.80 (m, 2H), 1.51 (s, 9H). Then the product (1.75 g, 4.5 mmol) was added to a solution of DCM (5.0 mL) and TFA (5.0 mL). After stirring for 1 h at room temperature, the solvent was evaporated off under reduced pressure. The residue was neutralized with saturated $NaHCO_3$ and extracted with ethyl acetate. The combined organic layers were dried and concentrated to yellow oil which was used directly in the next step (1.3 g, quantitative).

*3-Azido-4-methyl-N-(4-((4-methyl-1,4-diazepan-1-yl)methyl)-3-(trifluoromethyl)phenyl)benzamide (**19f**).* To a solution of methyl 3-azido-4-methylbenzoate **8** (1.03 g, 5.4 mmol) and 4-((4-methyl-1,4-diazepan-1-yl)methyl)-3-(trifluoromethyl)aniline **18f** (1.3 g, 4.5 mmol) in anhydrous THF (5.0 mL) was added potassium *tert*-butoxide (1.5 g, 12.6 mmol) in anhydrous THF (15.0 mL) slowly at −20 °C. Then the reaction mixture was stirred for 1 h then slowly at room temperature and stirred overnight. After evaporation of the solvent, the residue was diluted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the desired compound as yellow oil (1.5 g, yield 74%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.95 (s, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.73 (d, J = 1.2 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.53 (dd, J = 8.0 Hz, 1.2 Hz, 1H), 7.26−7.25 (m, 1H), 3.76 (s, 2H), 3.16−3.14 (m, 2H), 3.03−3.01 (m, 2H), 2.81−2.78 (m, 2H), 2.62 (s, 3H), 2.06−2.00 (m, 4H).

*3-(4-(1H-Pyrazolo[3,4-b]pyridin-5-yl)-1H-1,2,3-triazol-1-yl)-4-methyl-N-(4-((4-methyl-1,4-diazepan-1-yl)methyl)-3-(trifluoromethyl)phenyl)benzamide (**6qf**).* To a solution of 5-ethynyl-1*H*-pyrazolo[3,4-*b*]pyridine **11q** (0.30 g, 2.1 mmol) and 3-azido-4-methyl-*N*-(4-((4-methyl-1,4-diazepan-1-yl)methyl)-3-(trifluoro-methyl)phenyl)benzamide **19f** (0.3 g, 0.7 mmol) in *tert*-butyl alcohol/water (1:1, 5.0 mL) were added $CuSO_4$ (11 mg, 0.07 mmol) and sodium ascorbate (28 mg, 0.14 mmol). Then the mixture was heated at 90 °C overnight. The reaction mixture was cooled to room temperature and was extracted three times with ethyl acetate. Then the combined organic phases were washed with brine, dried over anhydrous sodium sulfate, concentrated, and purified by silica gel column chromatography to give the desired product as yellow solid (0.24 g, yield 59%): ^{1}H NMR (400 MHz, DMSO-*d*) δ 13.84 (s, 1H), 10.60 (s, 1H), 9.16 (s, 1H), 9.14 (d, J = 1.2 Hz, 1H), 8.77 (d, J = 1.2 Hz, 1H), 8.26 (s, 1H), 8.20 (s, 1H), 8.17 (s, 1H), 8.14 (d, J = 6.4 Hz, 1H), 8.06 (d, J = 6.4 Hz, 1H), 7.76 (d, J = 6.8 Hz, 1H), 7.71 (d, J = 6.4 Hz, 1H), 3.69 (s, 2H), 2.64−2.60 (m, 4H), 2.58−2.55 (m, 2H), 2.52 (m, 2H), 2.35 (s, 3H), 2.24 (s, 3H), 1.72−1.70 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*) δ 163.94, 151.46, 146.78, 144.67, 137.70, 136.98, 136.00, 133.39, 133.09, 131.61, 130.96, 128.88, 127.14 (J = 31.0 Hz), 126.04, 125.55, 124.93, 123.45, 122.80, 119.62, 117.26, 114.24, 57.25, 57.15, 55.92, 54.19, 53.81, 46.14, 26.79, 17.46; MS (ESI) m/z 590 $[M + H]^+$; HR MS (EI) calcd for $C_{30}H_{30}F_3N_9O$ 590.2598 $[M + H]^+$; found 590.2590, purity 97.0% (t_R 18.19 min).

Compounds **6qa−6qi** were prepared from compound **11q** and the corresponding compound **19** by a method similar to that of **6qf**.

*3-(4-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)aniline (**21j**).* A mixture of CuI (190 mg, 1 mmol), 4-methyl-1*H*-imidazole (1.64g, 20 mmol), Cs_2CO_3 (3.25g, 10 mmol), 3-bromo-5-(trifluoromethyl)-aniline **20** (2.40g, 10 mmol), 1-(5,6,7,8-tetrahydroquinolin-8-yl)-ethanone (350 mg, 2 mmol), and DMF (30 mL) was added to a round-bottom flask. The flask was evacuated and backfilled with argon (this procedure was repeated three times), and then the mixture was heated at 130 °C for 24 h under argon. After cooling to room temperature, the solvent was removed under vacuum and the residue was purified by column chromatography on silica gel to afford the crude product. The crude product was recrystallized from toluene to afford **21j** as a white solid (1.7 g, 71%): ^{1}H NMR (400 MHz, DMSO-*d*) δ 8.06 (s, 1H), 7.35 (s, 1 H), 6.97 (s, 1 H), 6.93 (s, 1 H), 6.81 (s, 1 H), 5.87 (br, 2H), 2.15 (s, 3H); MS (ESI) m/z 242 $[M + H]^+$.

*3-Azido-4-methyl-N-(3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl)benzamide (**22j**).* To a solution of methyl 3-azido-4-methylbenzoate **8** (0.19 g, 1.0 mmol) and 3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)aniline **21j** (0.24 g, 1.0 mmol) in anhydrous THF (2.0 mL) was added potassium *tert*-butoxide (0.34 g, 3.0 mmol) in anhydrous THF (5.0 mL) slowly at −20 °C and stirred for 1 h. Then the reaction mixture was slowly warmed to room temperature and stirred for another 8 h. After evaporation of the solvent, the residue was diluted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated for purification by silica gel column chromatography to afford the desired compound as white solid (0.37 g, yield 92%): ^{1}H

NMR (400 MHz, DMSO-*d*) δ 10.71 (s, 1H), 8.28 (s, 1 H), 8.21 (s, 1 H), 8.12 (s, 1 H), 7.81 (s, 1 H), 7.75−7.74 (m, 2H), 7.49 (s, 1 H), 7.44 (d, J = 8.0 Hz, 1H), 2.24 (s, 3H), 2.18 (s, 3H); MS (ESI) m/z 401 $[M + H]^+$.

*3-(4-(1H-Pyrazolo[3,4-b]pyridin-5-yl)-1H-1,2,3-triazol-1-yl)-4-methyl-N-(3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-phenyl)benzamide (**6qj**).* 5-Ethynyl-1*H*-pyrazolo[3,4-*b*]pyridine **11q** (0.24 g, 1.7 mmol) and 3-azido-4-methyl-*N*-(3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl)benzamide **22j** (0.22 g, 0.55 mmol) was added to a solution of THF/water (1:1, 5.0 mL) followed by $CuSO_4$ (8.8 mg, 0.55 mmol) and sodium ascorbate (22 mg, 1.1 mmol). Then the mixture was heated at 90 °C overnight. The reaction mixture was cooled to room temperature and extracted three times with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, concentrated, and purified by silica gel column chromatography to give desired product as yellow solid (0.21 g, yield 70%): ^{1}H NMR (400 MHz, DMSO-*d*) δ 13.81 (s, 1H), 10.84 (s, 1H), 9.18 (s, 1H), 9.14 (s, 1H), 8.77 (s, 1H), 8.50 (s, 1H), 8.36 (s, 1H), 8.26 (s, 1H), 8.24 (s, 1H), 8.17 (s, 2H), 7.79 (s, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.60 (s, 1H), 2.37 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (125 MHz, DMSO-*d*) δ 164.82, 151.90, 147.32, 145.27, 141.52, 138.01, 137.76, 136.61, 135.30, 134.19, 133.15, 132.43, 131.32 (J = 27.5 Hz), 129.64, 126.63, 125.59, 123.96 (J = 271.2 Hz), 123.48, 120.13, 115.87, 115.29, 114.84, 112.76, 18.18, 9.02; HR MS (EI) calcd for $C_{27}H_{20}F_3N_9O$ 544.1816 $[M + H]^+$; found 544.1805, purity 95.0% (t_R 5.20 min).

Compounds **6qk** and **6ql** were prepared from compound **11q** and the corresponding compound **22** by a method similar to that of **6qj**.

*Methyl 4-Cyclopropyl-3-nitrobenzoate (**27**).* To a solution of methyl 4-bromo-3-nitrobenzoate **26** (1.0 g, 3.9 mmol) and cyclopropylboronic acid (0.34 g, 3.9 mmol) in dioxane (10.0 mL) were added Pd(dppf)Cl_2-DCM (64 mg, 0.078 mmol) and K_3PO_4 (2.08 g, 7.8 mmol). The reaction mixture was evacuated and backfilled with argon (3 cycles). After stirring at 90 °C overnight, the reaction mixture was filtered and concentrated. The residue was diluted with EtOAc and washed with brine. Then the organic layer was dried over anhydrous sodium sulfate and concentrated to afford the desired product which was used directly in the next step due to difficulty in purification by column chromatography.

*Methyl 3-Amino-4-cyclopropylbenzoate (**23r**).* To a solution of methyl 4-cyclopropyl-3-nitrobenzoate **27** (20 g, 91 mmol) in THF−water (1:1) (200 mL) were added CF_3COOH (4.38 g, 73 mmol) and NH_4Cl (0.48 g, 9.1 mmol). After heating under reflux, Fe powder (20.5 g, 364 mmol) was added in portion. The reaction was stirred for a further 3 h. The reaction mixture was filtered and concentrated. The residue was diluted with EtOAc and washed with brine. Then the organic layer was dried over anhydrous sodium sulfate, concentrated, and purified by column chromatography to afford the desired product as yellow solid (7.64 g, yield 44% of two steps): ^{1}H NMR (400 MHz, DMSO-*d*) δ 7.25 (d, J = 2.0 Hz, 1H), 7.06 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 5.27 (s, 2H), 3.78 (s, 3H), 1.75−1.71 (m, 1H), 0.92−0.88 (m, 2H), 0.56−0.52 (m, 2H).

*Methyl 3-Azido-4-cyclopropylbenzoate (**24r**).* This compound was synthesized through an analogous procedure to that used for the synthesis of **8** (yield 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 2.0 Hz, 1H), 7.70 (dd, J = 8.0 Hz, 1.6 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 3.92 (s, 3H), 2.16−2.11 (m, 1H), 1.06−1.02 (m, 2H), 0.75−0.71 (m, 2H).

*3-Azido-4-cyclopropyl-N-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)benzamide (**25r**).* This compound was synthesized through an analogous procedure to that used for the synthesis of **9** (yield 15%): m/z 459 $[M + H]^+$.

*3-(4-(1H-Pyrazolo[3,4-b]pyridin-5-yl)-1H-1,2,3-triazol-1-yl)-4-cyclopropyl-N-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)benzamide (**6qr**).* This compound was synthesized through an analogous procedure to that used for the synthesis of **6q** (yield 25%): ^{1}H NMR (400 MHz, DMSO-*d*) δ 9.16 (s, 1H), 9.13 (d, J = 1.6 Hz, 1H), 8.76 (d, J = 1.6 Hz, 1H), 8.25 (s, 1H), 8.18 (s, 2H), 8.14 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 3.56 (s, 2H), 2.38 (br, 4H), 2.33 (br, 4H), 2.15 (s, 3H), 1.82−1.75 (m, 1H), 1.01−0.99 (m, 2H), 0.85−0.84 (m, 2H); ^{13}C NMR (125 MHz, DMSO-*d*) δ 163.97, 151.82, 146.74, 144.84, 143.22, 138.10, 136.42, 133.50, 132.24, 132.17, 131.23, 129.60, 127.43 (J = 30.0 Hz), 126.09, 125.71, 125.29, 124.29 (J = 272.5 Hz), 123.56, 123.52, 119.53, 117.30, 114.38, 57.42, 55.99, 54.69, 52.66, 45.68, 18.51, 11.07, 9.74; HR MS (EI) calcd for $C_{31}H_{30}F_3N_9O$ 602.2598 $[M + H]^+$; found 602.2590, purity 95.5% (t_R 7.63 min).

Compounds **6qm**−**6qs** were prepared from compound **11q** and the corresponding compound **25** by a method similar to that of **6qr**.

Computational Study. The structures of Bcr-Abl^{WT} protein and Bcr-Abl^{T315I} protein were retrieved from the Protein Data Bank (PDB code: 3CS9.pdb and 3IK3.pdb). The proteins were processed using protein preparation wizard, which assigns bond orders and adds hydrogens and missing atoms. Inhibitors were built by in LigPrep (LigPrep, version 2.5, Schrödinger, LLC, New York, NY, 2011) module using OPLS-2005 force field. Molecular docking was performed in Glide module (Glide, version 5.7, Schrödinger, LLC, New York, NY, 2011) with standard precision scoring function.

FRET-Based Z′-Lyte Assay Detecting Peptide Substrate Phosphorylation. The effects of compounds on the kinase activity of Bcr-Abl and its mutants were assessed in 384-well plates using the FRET-based Z′-Lyte assay system according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Briefly, 10 μL per well reactions contained ATP concentration at 10 μM (for Bcr-Abl wild-type) or 5 μM (for T315I mutant), 2 μM Tyr2 peptide substrate in 50 mM HEPES (PH 7.5), 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA, 0.0247 μg/mL Bcr-Abl, and inhibitors as appropriate. The reaction was performed at room temperature for 2.0 h, and then 5 μL of development reagent was added for a further 2 h room temperature incubation followed by the addition of 5 μL of stop solution. Fluorescence signal ratio of 445 nm (coumarin)/520 nm (fluorescein) was examined on EnVision Multilabel Reader (Perkin-Elmer, Inc.). The data were analyzed using Graphpad Prism5 (Graphpad Software, Inc.). The data were the mean values of three experiments.

Cells and Reagents. The leukemia cell lines (K562, KU812) were purchased from ATCC and maintained as recommended by ATCC (Manassas, VA, USA). Imatinib, dasatinb, and nilotinib were purchased from Biocompounds Pharmaceutical Inc. (Shanghai, China). CCK-8 was purchased from Dojindo Molecular Technologies Inc. (Kumamoto, Japan). Dimethyl sulfoxide (DMSO) and Cremophor were purchased from Sigma-Aldrich (Dorset, USA). Antibodies against Abl, p-Abl, Crkl, p-Crkl, STAT5, p-STAT5 were all purchase from Cell Signaling Technology Inc. (Danvers, USA).

Cellular Antiproliferation Assay Using Cell Counting Kit (CCK-8). Cells in the logarithmic phase were plated in 96-well culture dishes (~3000 cells/well). Twenty-four hours later, cells were treated with the corresponding compounds or vehicle control at the indicated concentration for 72 h. CCK-8 was added into the 96-well plates (10 μL/well) and incubated with the cells for 3 h. OD450 and OD650 were determined by a microplate reader. Absorbance rate (A) for each well was calculated as OD450 − OD650. The cell viability rate for each well was calculated as $V\% = (A_s - A_c)/(A_b - A_c) \times 100\%$, and the data were further analyzed using Graphpad Prism5 (Graphpad Software, Inc.) (A_s, absorbance rate of the test compound well; A_c, absorbance rate of the well without either cell or test compound; A_b, absorbance rate of the well with cell and vehicle control).

Stably Transformed Ba/F3 Cells. The Ba/F3 cell lines stably expressing native Bcr-Abl or various Bcr-Abl mutants were established by following a procedure similar to that described by von Bubnoff.[38] Briefly, wild-type Bcr-Abl p210 was cloned into pCDNA3.1(+) (Invitrogen, Carlsbad, CA, USA). Point mutations were introduced to pCDNA3.1(+) Bcr-Abl using the QuickChange XL site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Ba/F3 cells were transfected with the constructs using transfected cells cloning kit (Stem Cell Technologies, Vancouver, Canada) by electroporation. Stable lines were selected in Amaxa cell line nucleofector kit V (Lonza, Cologne, Germany) with G418 (Merck, Whitehouse Station, USA) and withdrawal of interleukin-3 (IL-3, R&D). All of the Ba/F3 stable cell lines were verified by monitoring both DNA sequences through DNA sequencing and protein expression levels of the corresponding

Bcr-Abl mutants through Western blotting analysis. Their responses to the imatinib, nilotinib, and dasatinib were also hired for selecting the right clones. Parent Ba/F3 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and IL-3 (10 ng/mL), while all Bcr-Abl-transformed Ba/F3 stable cell lines were cultured in the similar medium except without IL-3. Imatininib-resistant K562 cells was self-established. Briefly, K562 cells were treated with graded imatinib (from 0.1 to 5 μM) during a 3 month period. Single clones were then selected and identified through DNA sequencing, and their responds toward imatinib, nilotinib, and dasatinb were also monitored as references.

Western Blot Analysis. The Western blot analysis was carried out by following the protocol described before.[39] Briefly, after the indicated treatment, cell lysates were collected dissolving cells in 1× SDS sample lysis buffer. After being sonicated and boiled, the supernatant of cell lysate was used for Western blot analysis. Cell lysates were loaded to 8−12% SDS-PAGE and separated by electrophoresis. Separated proteins were then electrically transferred to a PVDF film. After being blocked with 1× TBS containing 0.1% Tween-20 and 5% nonfat milk, and the film was incubated with corresponding primary antibody followed by HRP-conjugated secondary antibody. The protein lanes were visualized using ECL Western blotting detection kit (GE Healthcare, Piscataway, NJ, USA).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

More chemical information of the final compounds, the preliminary pharmacokinetics analysis on compounds **6q** and **6qo** and other biological data as well as computational docking results can be obtained in Supporting Information. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Bcr, breakpoint cluster region; Abl, abelson murine leukemia viral oncogene; T, Thr, threonine; I, Ile, isoleucine; CML, chronic myelogenous leukemia; WT, wild-type; DFG, Asp-Phe-Gly; Boc, *tert*-butoxycarbonyl; THF, tetrahydrofuran; rt, room temperature; NBS, *N*-bromosuccinimide; AIBN, azodiisobutyronitrile; TFA, trifluoroacetic acid; DCM, dichloromethane; DMF, dimethylformamide; E, Glu, glutamic acid; Q, Gln, glutamine; H, His, histidine; M, Met, methionine; A, Ala, alanine; G, Gly, glycine; Y, Try, tyrosine; K, Lys, lysine; V, Val, valine; F, Phe, phenylalanine; L, Leu, leucine; P, Pro, proline; S, Ser, serine; R, Arg, arginine; TKI, tyrosine-kinase inhibitor; ATP, adenosine triphosphate

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