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Broad spectrum alkynyl inhibitors of T315I Bcr-Abl

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ABSTRACT

A series of alkyne-containing type II inhibitors with potent inhibitory activity of T315I Bcr-Abl has been identified. The most active compound **4** exhibits an EC_{50} of less than 1 nM against wild-type Bcr-Abl and an EC_{50} of 10 nM against T315I mutant but is broadly active against a number of other kinases.

The success of Bcr-Abl inhibitor imatinib (Gleevec®, Novartis Pharma AG) for the treatment of Chronic Myelogenous Leukemia (CML) has provided the paradigm for targeting dominant oncogenes with small molecules.[1,2] Imatinib resistance is rare in chronic phase patients, however for patients with blast crisis phase CML or Philadelphia chromosome-positive CML, resistance is common after an initial response in the first year.[3,4] To address these relapses, two more potent ATP-site directed agents: nilotinib (AMN107)[5] and dasatinib (BMS-354825)[6] have been approved as second-line therapies. Although both compounds inhibit most of the protein mutants that induce resistance to imatinib, neither compound is capable of inhibiting the so-called 'gatekeeper' T315I mutant.[7] Because of the clinical importance of this mutation, there has been intense interest in the synthesis of novel inhibitors that are able to circumvent this mutation.

Recently, several compounds from the Type-II class[8] that recognize the 'DFG-out' conformation have been reported to inhibit T315I. These include cyclic urea compound **14**,[9] BGG463,[10] AP24163,[11] DSA series compounds,[12] HG-7-85-01[13] and AP24534.[14] A co-crystal structure of T315I with AP24534, an imidazo[1,2*b*]-pyridazine-based multitargeted inhibitor demonstrates how this compound can circumvent a larger residue at the gatekeeper site.[14]

In our efforts to identify new molecular scaffolds that could target T315I mutant of Bcr-Abl, we recently reported the discovery of HG-7-85-01, a small molecule type II inhibitor that inhibits the proliferation of cells expressing the major imatinib-resistant gatekeeper mutants, BCR-ABL-T315I, Kit-T670I, PDGFRα-T674M/I, as well as Src-T341M/I.[13] HG-7-85-01 was designed as a hybrid between the type I inhibitor dasatinib and the type II inhibitor, nilotinib. Specifically, a superposition of the Abl-bound conformation of dasatinib (PDB code: 2GQG)[15] and nilotinib (PDB code: 3CS9)[5] guided the choice of how to connect the aminothiazole hinge-interacting motif of dasatinib with the *N*-(3-(trifluoromethyl)phenyl)-benzamide substructure of nilotinib, which is known to be responsible for inducing the 'DFG-out' flip that is characteristic of type II kinase inhibitors. Our results demonstrate that it is possible to design a type II inhibitor that can circumvent

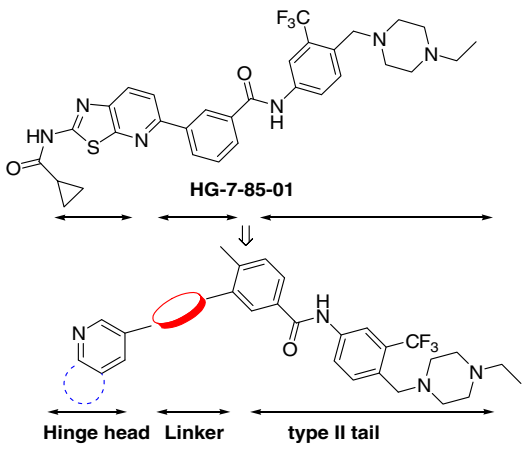

Figure 1. Scaffold design strategy.

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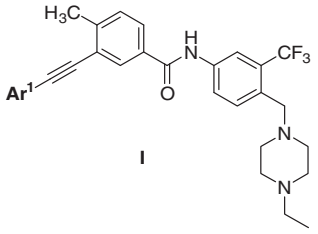
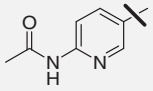
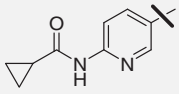
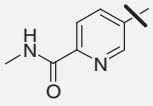
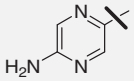
Table 1
SAR of mono-heterocyclic head alkynyl analogs

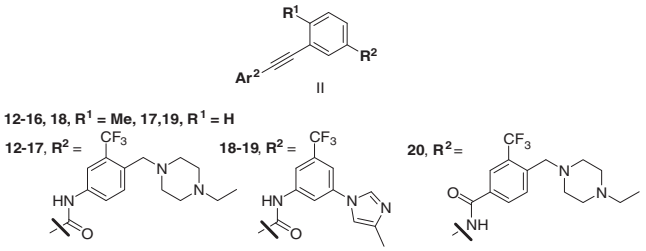


I

Compds	Ar^1	Cellular antiproliferative activity (EC_{50}, μM)		
		Bcr-Abl[a] (wt)	Bcr-Abl[b] (T315I)	BaF3[c]
3		<0.001	0.092	1.59
4		<0.001	0.010	2.05
5		<0.001	0.013	1.80
6		0.041	2.58	3.98
7		<0.001	0.99	2.19
8		0.006	3.03	2.49
9		<0.001	0.165	0.86

[a] Cellular antiproliferative activity (EC_{50}, μM) on wtBcr-Abl-Ba/F3.
[b] Cellular antiproliferative activity (EC_{50}, μM) on mutant Bcr-Abl-T315I-Ba/F3.
[c] Cytotoxicity (EC_{50}, μM) on wt-Ba/F_3.

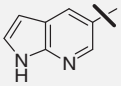
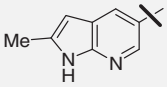
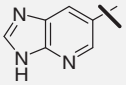
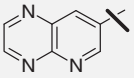
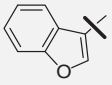
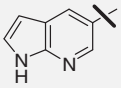
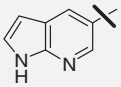
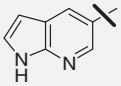
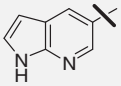
Table 2
SAR of bi-heterocyclic head alkynyl analogs



II

12-16, 18, R^1 = Me, 17,19, R^1 = H

12-17, R^2 = 18-19, R^2 = 20, R^2 =

Compds	Ar^1	Cellular antiproliferative activity (EC_{50}, μM)		
		Bcr-Abl[a] (wt)	Bcr-Abl[b] (T315I)	BaF3[c]
12		<0.001	0.080	1.00
13		0.026	0.56	1.00
14		<0.001	0.014	0.060
15		0.037	5.52	3.32
16		0.24	8.37	6.76
17		0.011	0.22	>10.0
18		0.037	0.47	>10.0
19		0.015	0.30	2.83
20		0.070	2.63	7.86

[a] Cellular antiproliferative activity (EC_{50}, μM) on wtBcr-Abl-Ba/F3.
[b] Cellular antiproliferative activity (EC_{50}, μM) on mutant Bcr-Abl-T315I-Ba/F3.
[c] Cytotoxicity (EC_{50}, μM) on wt-Ba/F_3.

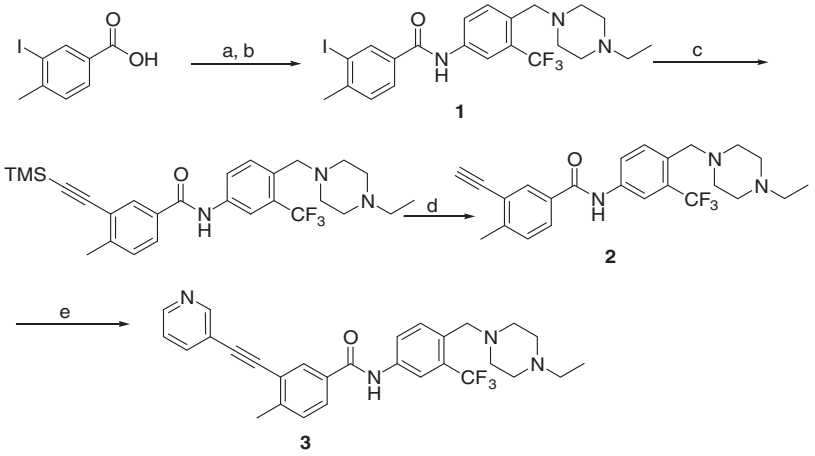
the T315I Bcr-Abl 'gatekeeper' mutation by bridging the ATP and allosteric binding sites using a linker segment that can accommodate a larger gatekeeper residue. Here we report on our efforts in applying this strategy towards the synthesis of type II inhibitors using an alkyne as a linear linkage segment that can traverse a larger gatekeeper residue. A number of compounds from this series exhibit highly potent activities against both the wild-type and T315I mutant of Bcr-Abl.

Molecular modeling suggested that the triple-bond linkage should be used to connect the toluene moiety of imatinib/nilotinib with a variety of heterocycles that would be capable of forming hydrogen bonding interactions with the kinase hinge region (Fig. 1). This scaffold is exemplified by structures I and II (Tables 1 and 2). Concise synthetic routes were developed to prepare I and II (Schemes 1 and 2). A Sonogashira coupling[16] was used as the key reaction in both synthetic routes. Scheme 1 depicts the synthesis of compound **3**, starting with the amide condensation of freshly prepared 3-iodo-4-methylbenzoyl chloride with 4-((4-ethyl-piperazin-1-yl)methyl)-3-(trifluoromethyl)-benzenamine to afford the iodo-intermediate **1**. Alkyne intermediate **2** was obtained using a Sonogashira coupling of intermediate **1** with ethynyltrimethylsilane followed by deprotection of the TMS group. The final product **3** was obtained using another Sonogashira coupling of **2** with 3-iodopyridine. Compounds **4**–**9** were synthesized analogously using different heteroaromatic iodides or bromides in the final coupling step.

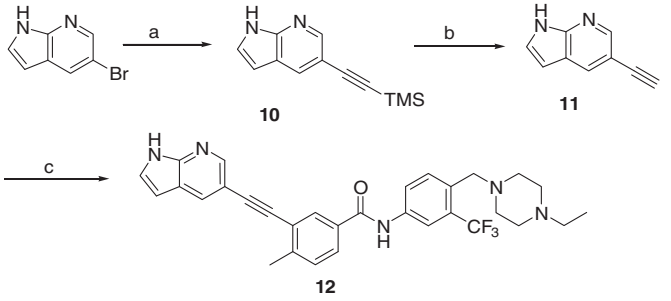
Synthesis of **12** was accomplished by introduction of an ethynyl group to 5-bromo-1*H*-pyrrolo[2,3-*b*]pyridine followed by coupling with the iodo-intermediate **1** (Scheme 2). Compounds **13–20** were obtained following this synthetic route.

To assess the cellular activity of the compounds, we tested them against parental, wild-type and T315I Bcr-Abl transformed Ba/F3 cells. Wild-type Ba/F3 cells proliferate only in the presence of interleukin-3 (IL-3) while Ba/F3 cells transformed with oncogenic kinases such as Bcr-Abl become capable of growing in the absence of IL-3 and provides a robust and commonly used assay for selective kinase inhibition.[17]

The first compound **3** we synthesized exhibited an EC_{50} of less than 1 nM on wild-type Bcr-Abl and an EC_{50} of 92 nM on T315I. The EC_{50} against parental Ba/F3 cells was 1.59 μM, demonstrating that the antiproliferative activity was derived from on-target inhibition of Bcr-Abl. Encouraged by this result, we



Scheme 1. Synthetic route of **3**. Reagents and conditions: (a) $SOCl_2$, reflux, 1 h; (b) 4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)-benzenamine, DIEA, CH_2Cl_2, 0 °C to rt, 56% over two steps; (c) ethynyltrimethylsilane, $Pd(PPh_3)_4$, CuI, DIEA, DMF, rt, 62%; (d) TBAF, THF, rt, 72%; (e) 3-iodopyridine, $Pd(PPh_3)_4$, CuI, DIEA, DMF, 50 °C, 72%.



Scheme 2. Synthetic route of **12**. Reagents and conditions: (a) ethynyltrimethylsilane, $Pd(PPh_3)_4$, CuI, DIEA, DMF, 50 °C, 55%; (b) TBAF, THF, rt, 77%; (c) 1, $Pd(PPh_3)_4$, CuI, DIEA, DMF, rt, 82%.

next prepared a small set of compounds to investigate the SAR and validate our design strategy (Table 1). Introduction of a substituted amino-group to the pyridine 6-position (**4** and **5**) resulted in an approximate eight-fold improvement relative to **3** against T315I mutant Bcr-Abl. This might be attributed to the introduction of an additional hydrogen bond to the kinase hinge from the amino-group. Incorporation of a carbonyl group, as is present in Sorafenib at the 2-position, afforded compound **6** which was less potent against both wild-type and T315I Bcr-Abl relative to **3.** Replacing the pyridine head with pyrimidine and pyrazine resulted in approximately equipotent compounds **7** and **8** against wild-type but decreased potency on T315I mutant. The identification of highly potent compounds **3–5** clearly validates our design strategy. The results also demonstrate that T315I Bcr-Abl is less potently inhibited relative to wild-type by this inhibitor series.

We next investigated the effects of using 6-5 and 6-6 fused heterocyclic rings such as 7-aza-indole, imidazopyridine, pyridopyrazine, and benzofuran as hinge-interacting motifs (Table 2). Most of the resulting compounds exhibited EC_{50} values below 100 nM against wild-type Bcr-Abl, but only compounds **12** and **14** exhibited EC_{50} values below 100 nM against T315I. A comparison of potencies of compounds **12** and **17** demonstrate that the presence of the toluene methyl group is an important structural element for achieving potent inhibition against both wild-type and T315I Bcr-abl. An analysis of the Abl-bound conformations of imatinib, nilotinib and AP24534 suggests that the methyl group favors the twisted conformation required for high affinity binding.[5,14,18] The orientation of the amide found in nilotinib (**12**) is favored over the reverse amide orientation found in imatinib (**20**).

The selectivity of this scaffold was assessed using KINOME*scan*™ (Ambit Biosciences, San Diego, CA),[19] a high-throughput method for screening kinase inhibitors against a panel of 442 kinases. Compounds **3** and **12**, were screened at a concentration of 10 μM. This analysis revealed that the compounds possessed an extremely broad selectivity profile with compounds **3** and **12** inhibiting 44% and 46% of kinases on the panel respectively, with a score of less than 10% of the DMSO control. The kinase hits for **12** with ambit score less 0.1% of the DMSO control were highlighted in a spot tree[20] (Fig. 2, please see Supplementary data for full screening data of **12** and **3**). The potently targeted kinases were mainly from the TK, TKL, STE, and CMGC groups. The selectivity scores[19] for **3** (S_{10} = 0.427) and **12** (S_{10} = 0.449) indicate that these compounds are considerably less specific relative to compounds such as HG-7-85-01 (S_{10} = 0.056).[13] As compounds **3** and **12** are highly rigid structures they are most likely only to bind with high affinity to the 'DFG-out' conformation as confirmed by the recent co-structure of AP24534 with T315I Bcr-Abl.[14] These kinase profiling results demonstrate that a very large number of kinases can be potently targeted in this conformation and inhibitors **3** and **12** represent starting points for the design of multitargeted inhibitors with the potential to target diverse combinations of kinase targets.

In summary, we have used a structure-based design approach to design a new type II scaffold using an alkyne as a linker segment between a heterocyclic hinge-interacting motif and a trifluoromethylphenylamide motif that binds to the pocket created by the 'DFG-out' conformation. The compounds exhibit very potent cellular activity against both the wild-type and T315I Bcr-Abl. Despite being extremely promiscuous kinase inhibitors, compounds such as **3** and **12** are not general cytotoxic agents and exhibit up to 1000-fold selectivity for Bcr-Abl dependent cellular growth. Further medicinal chemistry efforts are in progress to develop analogs from this compound series whose multitargeted inhibition profile is tailored for optimal activity against particular cancer genotypes.

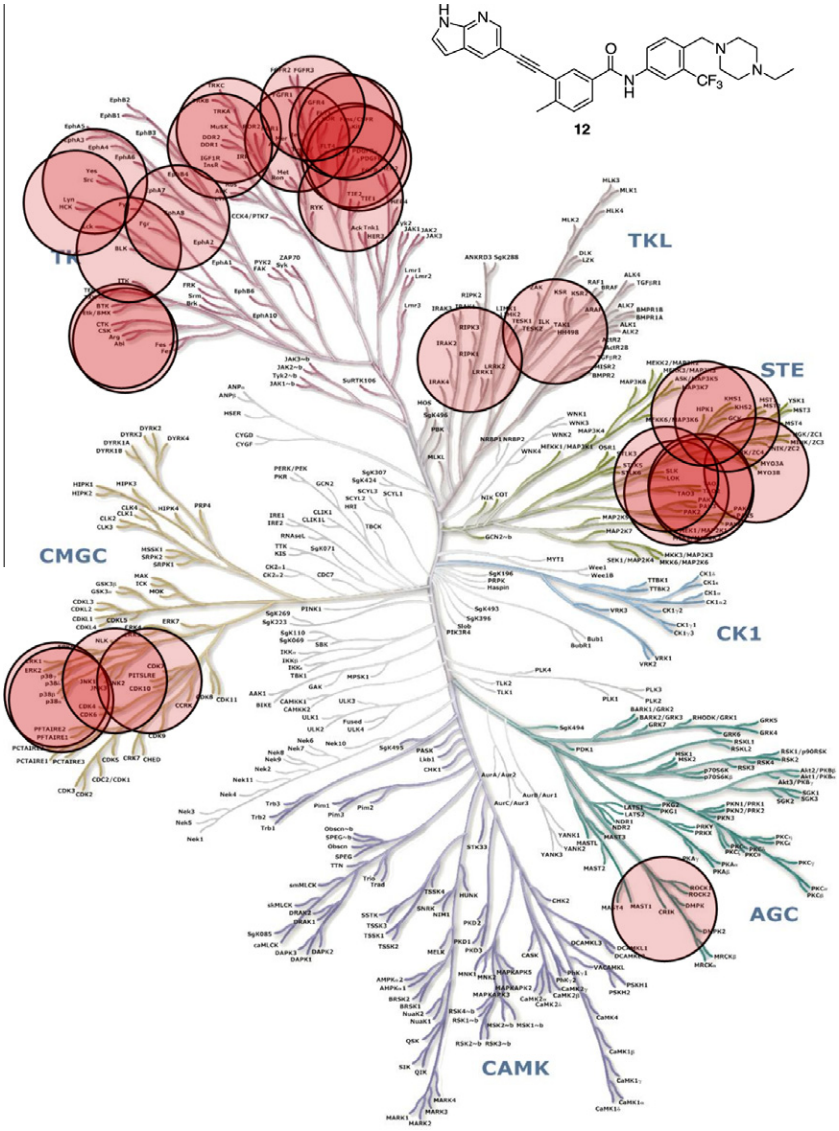


Figure 2. KINOME*scan*™ profiling of **12**. Compounds were screened at a concentration10 μM against 442 kinases and the most potently bound kinases are indicated by red circles and include: ABL, ARG, BLK, PITSLRE, CRIK, FMS/CSFR, DDR1, EphA8, FGFR4, HPK1, JNK2, KIT, LOK, LYN, GCK, MUSK, MYO3B, p38α, p38β, PDGFRα, PDGFRβ, RET, RIPK1, SRC, TAO1, TAO2, TAO3, and TIE2. The kinase dendrogram was adapted and reproduced with permission from Cell Signaling Technology, Inc.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.043.

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20. The image was generated by using the web-based TREE*spot*™ software (Ambit Biosciences).