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Structure-based design of flavone-based inhibitors of wild-type and T315I mutant of ABL

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ABSTRACT

The existence of drug resistance caused by mutations in the break-point cluster region-Abelson (BCR-ABL) tyrosine kinase domain remains a clinical challenge due to limited treatment options for effective CML therapies. Here, we report a series of flavone-based common inhibitors equipotent for the wild type and the most drug-resistant T315I mutant of BCR-ABL. The original hit **1** was extensively modified through a structure-based drug design strategy, especially by varying the C7 acetamide appendage of the scaffold to exploit extended interactions with P-loop residues. Structural features relevant to the stabilization of the newly identified inhibitors in the ATP-binding site of ABL are discussed in detail.

Chronic myeloid leukemia (CML) is a hematological malignancy characterized by the presence of Philadelphia (Ph) chromosome resulted from the reciprocal translocation involving chromosme 9 and chromosome 22. The translocation leads to the fusion of the Abelson tyrosine kinase (ABL) gene on chromosome 9 with the Breakpoint Cluster Region (BCR) gene on chromome 22 and the generation of a constitutively active chimeric BCR-ABL kinase which induces malignant cellular formation.[1] This knowledge has led the way for the development of targeted molecular therapies for CML, and the FDA approval and clinical success of imatinib. Imatinib, a potent first generation BCR-ABL tyrosine kinase inhibitor confirmed the BCR-ABL kinase as a key therapeutic target for CML.[2] Despite the clear benefits of imatinib, drug resistance has been frequently reported especially in patients with the advanced phases of CML, and is mainly caused by point mutations in the BCR-ABL kinase domain that reduces the binding affinity of imatinib to the protein.[3] Currently, more than 100 mutations have been discovered in patients with imatinib-resistant CML.[3b]

The need to address the imatinib-resistance resulted in the design of several second-generation BCR-ABL kinase inhibitors. Nilotinib, bafetinib, dasatinib, and bosutinib are currently in clinical use and are effective against the majority of imatinib-resistant BCR-ABL clones excluding the T315I kinase mutation which accounts for about 20% of all clinically relevant CML mutations.[4] The T315I mutation, the substitution of the threonine residue at position 315 of the BCR-ABL by a bulkier hydrophobic isoleucine, is a particular concern in drug design because it causes steric hindrance interrupting the access of most of the inhibitors to the ATP-binding pocket, and also eliminates a key hydrogen bond required for tight binding of inhibitors to the active site of ABL.[5] In this regards, the successful identification and development of potent inhibitors against the ABL^{T315I} mutation would have great therapeutic implications in CML. Recently, the third generation inhibitor, ponatinib[6] that target T315I mutation has been approved by the FDA for the treatment of CML patients with resistance to prior therapy.

The availability of the 3D-structure of therapeutic targets has enhanced opportunities for the rapid identification of new biologically active compounds utilizing structure-based drug design. Recently, we have identified several novel classes of common inhibitors against the wild type and T315I mutant utilizing docking simulations between ABL kinase and its candidate inhibitors.[7]

The ABL inhibitors **1** and **2** found in the previous study are equipotent for the wild type and T315I mutant of BCR-ABL, which are categorized into the two scaffolds, **1** (phenyl-(9*H*-purin-6-yl) amine) and **2** (phenyl-(5-phenyl-phthalazin-1-yl) amine) as shown in Figure 1. Considering tolerance within the ABL active site and low molecular weight of 400, compound **1** is expected to serve as a good scaffold from which much more potent inhibitors can be derivatized. In addition, compound **1** is attractive because a chromone scaffold can be easily diversified by standard synthetic chemistry, allowing for rapid exploration of structure–activity

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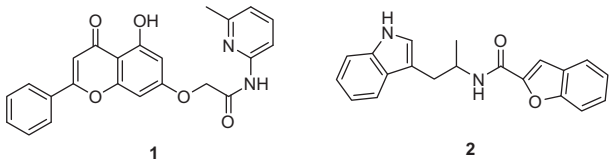


Figure 1. Chemical structures of inhibitor scaffolds under investigation.

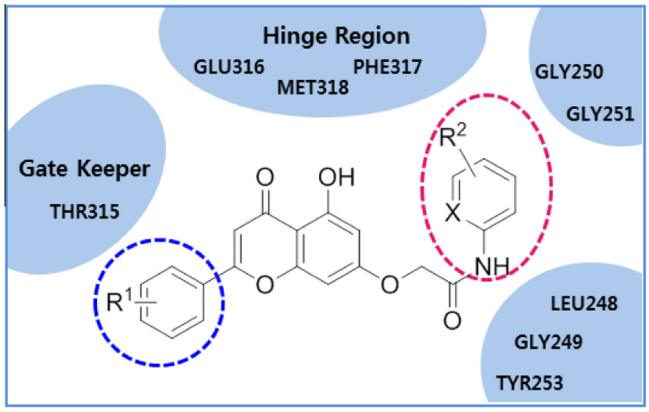


Figure 2. The simplified binding mode of flavones-based inhibitors with the ATP binding site of ABL and design strategy .

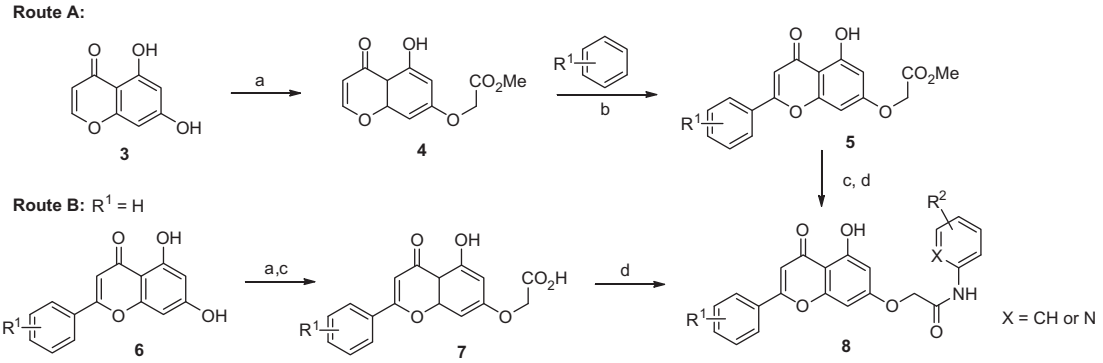
relationship (SAR). For these reasons, compound **1** was selected for further optimization through in-depth structural modification.

To develop more potent ABL inhibitors, we investigated the binding forces that are responsible for stabilization of compound **1** in the ATP-binding site of ABL kinase. In effort to enhance binding affinity, we planned to explore the space nearby the C2 and C7 positions of chromone moiety based on the calculated binding free energies of the derivatives with respect to the wild-type and T315I mutant ABL kinase. To identify the most suitable gate keeper region binder, our initial round of analogs was focused on the incorporation of substitution on the C2 phenyl group of chromone core while fixing the C7 acetamide moiety. The chromone scaffold can be easily installed with various aryl groups at the C2 position by direct arylation.[8] We next focused our design attempts on derivatizing the C7 acetamide appendage, to assess a glycine rich loop by installing variously substituted (hetero)arenes. Therefore, our design strategy prioritized the incorporation of suitable pharmacophores into chromone core at the C2 and C7 positions to generate new molecules with the expectation of combining high affinity for ABL kinase (Fig. 2).

The preparation of the target compounds is described in Scheme 1. To facilitate the exploration of an assortment of C2 phenyl group, the direct arylation of chromone was conducted prior to performing amide coupling. The synthesis commenced with treatment of the commercially available 5,7-dihydroxy-4*H*-chromen-4-one (**3**) with methyl 2-bromoacetate in DMF under basic conditions (Route A). We recently reported a straightforward and practical method for the oxidative cross-coupling of chromones and unactivated arenes via palladium-catalyzed twofold C–H functionalization.[8a] This general synthetic method offers the direct route for the C2-selective arylation of chromones. Utilizing the methodology, a variety of substituted phenyl groups could be installed to the C2-position to afford flavone derivatives **5**. To build amide group at C7 acetamide appendage, the resulting ester was converted into the desired products **8** using EDCI/DMAP conditions. Alternatively, commercially available chrysin (**6**) was treated with methyl 2-bromoacetate, followed by hydrolysis with lithium hydroxide to afford the corresponding carboxylic acid **7** (Route A). The various (hetero)anilines were then coupled with the carboxylic acid to furnish the target compounds **8**. The resulting compounds were tested over ABL and ABL^{T315I} to determine the inhibitory effects, and Table 1 lists the chemical structures and IC_{50} values of the representative inhibitors.[9] Among the flavone analogs prepared, six compounds were found to have a good potency against both the wild type and T315I mutant of ABL at the submicromolar level (Table 1).

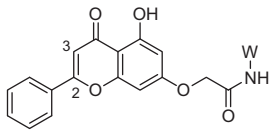
We first studied the effect of the substitution in the C2 phenyl group on the inhibitory activity. The substituent of the benzene ring was varied to include methyl, dimethyl, nitro or chloro groups. Unlike our expectation, the substitution on the C2 aryl ring were deleterious, exhibiting about 10-fold reduction in potency (IC_{50} >20 μM) when compared with hit compound **1**. Only fluoride group in the phenyl group is tolerated (**9**, ABL IC_{50} = 4.31 μM; ABL^{T315I} IC_{50} = 1.77 μM), which indicated that unsubstituted benzene at the C2 position could form favorable van der Waals interactions with the gatekeeping region without causing steric clash. Interestingly, removal of the 5-hydroxyl group resulted in a loss of activity (ABL IC_{50} = 15.2 μM), thus revealing the indispensible nature of the 5-hydroxyl group in maintaining activity in this series.

On the basis of these findings, C2-benzene moiety and C5-hydroxyl group were fixed to embark upon in-depth structural modification in order to optimize activity. We planned to exploit the extended interactions with P-loop residues by preparing related series of derivatives. Based on the docking study, it appeared that the substitution pattern around the pyridyl ring of the C7-acetamide appendage was important to its function. Removal of methyl group in the pyridyl ring decreased the inhibitory activity, implying the importance of the methyl group at this region for ABL inhi-



Scheme 1. Reagents and conditions: (a) methyl 2-bromoacetate, K_2CO_3, DMF, rt, 3 h, 92–95%; (b) $Pd(TFA)_2$, AgOAc, CsOPiv, PivOH, 100 °C, 30–60%; (c) LiOH·H_2O, THF/H_2O (1:1), rt, 2 h, 95–99%; (d) amine, EDCI, DMAP, CH_2Cl_2, rt, overnight, 55–70%. See Supplementary data for detail.

Table 1
Array of various (hetero)cycles and inhibition data against wild-type and T315I mutant of BCR-ABL



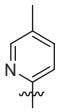
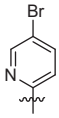
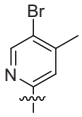
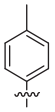
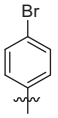
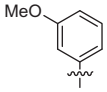
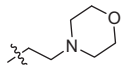
Compd	W	IC_{50} (μM)	
		ABL1	ABL(T315I)
1		3.30	2.71
9[a]		4.31	1.77
10		0.74	0.88
11		0.26	0.64
12		0.12	0.17
13		1.23	0.92
14		1.53	1.18
15		11.2	–
16		2.17	2.26
17		1.88	4.08
18		0.31	0.56
19		0.29	0.49
20		1.04	1.20
21		17.6	–
22		0.82	0.67

Table 1 (*continued*)

Compd	W	IC_{50} (μM)	
		ABL1	ABL(T315I)
23		2.27	3.16
24		4.31	2.77

[a] The benzene ring at the C2 position is replaced with 2,5-diflouorobenzene.

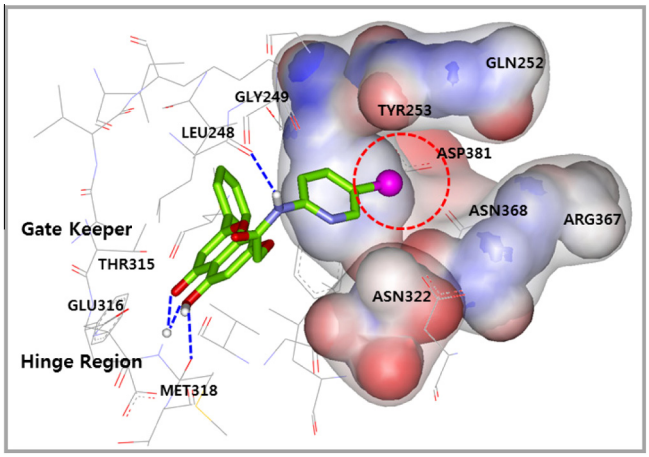


Figure 3. Predicted binding mode of **12** in the ATP-binding sites of ABL. Carbon atoms of the ligand are indicated in green. Each dotted blue line indicates a hydrogen bond.

bition. Therefore, we hypothesized that the overall activity might be improved by gaining tight hydrophobic interactions with the amino acid residues in the P-loop if a substituent in the pyridyl ring is properly oriented. To our delight, having a substituent at the 4 or 5 position of the pyridyl ring enhanced its potency about 5- to 10-fold increase in inhibitory activity against both ABL wild-type and T315I mutant (**10**, ABL IC_{50} = 0.74 μM, ABL^{T315I} IC_{50} = 0.88 μM: **11**, ABL IC_{50} = 0.26 μM, ABL^{T315I} IC_{50} = 0.64 μM). This significant enhancement of potency most likely stemmed from the strengthening of hydrophobic interactions between the inhibitor and amino acid residues (in red circle, Fig. 3). The successful replacement prompted further investigation on the substituent through synthesis of a series of related analogs. Intriguingly, the bromo group at the 4-position in the pyridyl ring of **12** was most effective in producing the inhibitory activity (ABL IC_{50} = 0.12 μM, ABL^{T315I} IC_{50} = 0.17 μM). Attempts to further increase potency through di-substitution were unremarkable, and the introduction of an additional methyl group into the 4-bromo-pyridyl ring resulted in reduction of inhibitory activity (**14**, ABL IC_{50} = 1.53 μM, ABL^{T315I} IC_{50} = 1.18 μM).

In the next design cycle, we turned our attention to the modification of the pyridyl ring since the amidic NH in the 2-pyridyl acetamide moiety is generally acidic and prone to form an intramolecular hydrogen bond. In order to determine any negative effect of the 2-pyridyl moiety in this series, the pyridyl ring was replaced with a phenyl ring. This modification was not critical, and phenyl derivatives **18** and **19** resulted in compounds which were nearly of equivalent enzyme potency with the corresponding pyridyl derivatives. In general, having a substituent at the *meta*- or

para position of the phenyl group enhanced its potency, whereas a substitution at the *ortho* position was detrimental to ABL inhibitory activity. For example, when the *p*-bromo group was moved to the *ortho*-position, it resulted in a great loss of activity (**21**: ABL IC_{50} = 17.6 μM). Of these, the *p*-methyl or *p*-bromo groups in the phenyl ring were found to be most effective. This trend was similar to the one found for the substituent of the pyridyl ring, demonstrating the importance of the orientation of substituent in this region in determining activity. In case of the methoxy group, *meta*-substituted derivative was tolerable in this region (**22**, ABL IC_{50} = 0.82 μM, ABL^{T315I} IC_{50} = 0.67 μM). The introduction of the morpholine moiety was intended to improve the water solubility of these derivatives and replacement with morpholinoethane resulted in a decreased potency (**24**, ABL IC_{50} = 4.31 μM, ABL^{T315I} IC_{50} = 2.77 μM).

To obtain some energetic and structural insight into the inhibitory mechanisms of the newly identified inhibitors, the binding modes in the ATP-binding sites of ABL kinase were investigated using the modified AutoDock scoring function.[10] The predicted binding mode of **12** with respect to ABL is presented in Figure 3. The overall structural features derived from the docking simulations indicated that the inhibitory activities of compound **12** were attributed to the multiple hydrogen bonds and hydrophobic interactions established simultaneously in the ATP-binding site of ABL. Consistent with the general structural features in the interactions between ABL kinase and its potent inhibitors, the backbone of Met318 in the hinge region appeared to play a significant role in stabilizing **12** in the ATP-binding site.[11] The central phenolic group and carbonyl group of **12** receives and donates a hydrogen bond from the backbone amidic nitrogen of Met318 and to the backbone aminocarbonyl oxygen of Glu316, respectively. In addition, acetamide of the C7 appendage donates a hydrogen bond to Leu248. These hydrogen bonds seem to play a role of anchoring **12** at the ATP-binding site, which indicates a key structural element for high affinity. Of particular significance was the observation that the presence of 4-bromo group on the pyridyl ring of **12** would allow for a favorable hydrophobic interaction with the pocket created by Tyr253, Gln252, Asn322, Asn368, and Arg367 at the P-loop (in red circle, Fig. 3). This hydrophobic interaction explains the enhanced activity of **12** and related analogs.

In conclusion, a new series of potent flavone-based inhibitors that were effective against ABL and ABL^{T315I} have been developed by the structure-based design. Through the systematic exploration of the functional groups in the C2 and C7 positions of hit compound **1**, we successfully developed the SAR profile for the series. Our results showed that benzene at the C2 position and 4-bromopyridyl acetamide at the C7 position of the chromone scaffold resulted in optimal activity in this series. These newly identified inhibitors may serve as lead compounds for further development of new therapeutic agents to treat drug-resistant cases of CML with T315I mutation.

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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.2013.05.095.

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