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Approaching the Active Conformation of 1,3-Diaminopyrimidine Based Covalent Inhibitors of Bruton's Tyrosine Kinase for Treatment of Rheumatoid Arthritis

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ABSTRACT

By applying conformational restrictions, we were able to discover highly potent 1,3-diaminopyrimidine based covalent inhibitors of BTK, such as **8a** (IC_{50} = 3.76 nM), and providing useful information of its active conformation. We are developing these novel small molecule covalent inhibitors of BTK toward oral agents for Rheumatoid arthritis.

Bruton's tyrosine kinase (BTK) is a cytoplasmic, non-receptor tyrosine kinase and belongs the Tec kinase family.[1] BTK is expressed in B cells, mast cells, monocytes/macrophages, neutrophils, dendritic cells, erythroid cells, platelets, hematopoietic stem cells, and multipotent progenitors.[2] It has been reported that BTK plays a critical role in B cell antigen receptor (BCR) activation, and that loss of function mutations in the BTK gene prevent the development of B cells, causing a depletion of B cells and circulating immunoglobulins.[3]

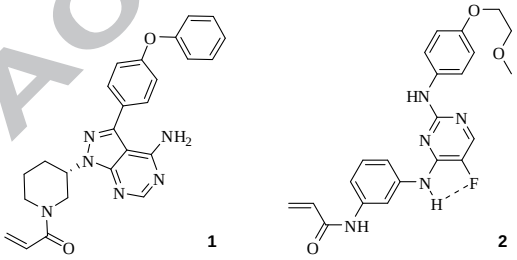

Figure 1. BTK inhibitor Ibrutinib (**1**) and Spebrutinib (**2**)

Because of these findings, BTK has been targeted for the treatment of autoimmune diseases, such as Rheumatoid arthritis (RA), and several research groups have recently reported small molecule BTK inhibitors which are efficacious in rodent models of collagen-induced arthritis (CIA) and systemic lupus erythematosus (SLE).[4] Several BTK inhibitors are currently under clinical development.[1] The most advanced BTK inhibitor, Ibrutinib (**1**, Figure 1) has been approved for the treatment of mantle cell lymphoma in 2013, and for chornic lymphocytic leukemia in 2014, and for Waldenström's macroglobulinemia, a form of non-Hodgkins lymphoma in January 2015.[5] Also in clinical development is Spebrutinib (**2**, Figure 1), and it is the first BTK inhibitor to be tested in patients with RA.[6] Both Ibrutinib and Spebrutinib are irreversible covalent inhibitors of BTK, and they represent the new trend of effective and safe covalent drugs in development.[7]

In search for novel small molecule BTK covalent inhibitor with improved selectivity and pharmacokinetic properties for the treatment of RA, we formulated our working hypothesis as follows: restriction of the free rotation of C-N single bonds between aromatic moieties of Spebrutinib would result in conformationally defined molecules that might have better alignment of its reactive group inside the BTK active site. Since the nitrogen atom at the 4-position of the pyrimidine is closer to the reactive site, we decided to explore this position first rather than the nitrogen atom at 2 position. Taken into consideration that this structural space has been heavily patented in recent years, we carried out an extensive search for novel chemotypies with better defined conformational space. In this letter, we report

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the discovery of novel covalent BTK inhibitors based on conformationally restricted 1,3-diaminopyrimidine.

Comparative conformational analysis revealed that to adopt the active conformation close to that of Ibrutinib, the benzene ring of benzene-1,3-diamine in Spebrutinib is most likely rotated away from the fluorine atom on the pyrimidine ring. It is conceivable that this particular conformation is stabilized by the intramolecular H-bond between the NH and electronegative fluorine atom (Figure 1, dotted line).[8] Based on this analysis, we decided, first, to replace the fluorine atom with sterically more demanding substituents, and second, to cyclize from this position onto the amino group (Figure 2). A quick SAR study showed that bulkier sulfur atom is a good replacement for fluorine.

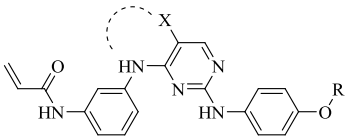
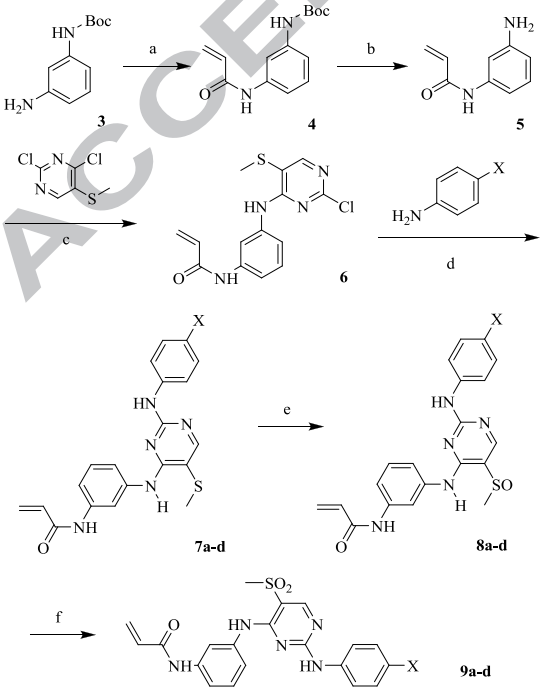


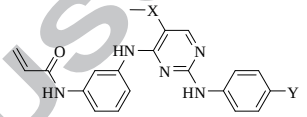
Figure 2. Conformationally restricted BTK inhibitor designs

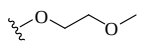
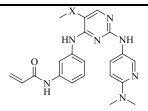
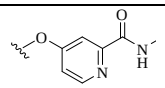
The synthesis of 1,3-diaminopyrimidine-4-sulfur substituted analogs (**7**, **8**, and **9**) is outlined in **Scheme 1**. The starting mono-Boc-protected 1,3-diaminobenzene was reacted with acryloyl chlodride under the conditions of potassium carbonate in THF. Product **4** was deprotected under acidic conditions to yield mono-acryloylated 1,3-diaminobenzene **5**. Aniline **5** was then reacted with 1,3-dichloro-4-(methylthio)pyrimidine in ethanol and anhydrous sodium acetate, and resulting pyrimidine 2-chloride **6** was treated with various para-substituted anilines in *n*-butanol with catalytic amounts of glacier acetic acid under mild heating to yield sulfide product **7a-d**. Oxidation of the sulfide **7a-d** to the corresponding sulfoxides (**8a-d**) was accomplished by treating the sulfides with hydrogen peroxide in acetic acid with mild heating, and enantiomerically enriched sulfoxide **8a-d** were obtained by asymmetric oxidation with either (*D*)-(-)-DET or (*L*)-(+)-DET ligand in the presence of titanium tetraisopropoxide and oxidizing agent TBHP. Selected sulfoxides were further oxidized to the corresponding sulfones with mCPBA.[9]



Scheme 1. Reaction keys: a) Acryloyl chloride, K_2CO_3, THF; b) HCl, DCM; c) NaOAc, EtOH; d) HOAc, n-BuOH, 110˚C; e) H_2O_2, HOAc; f) m-CPBA, DCM.

Table 1 shows the activities of some representative methanethioethers (**7**), methanesulfoxides (**8**), and methanesulfones (**9**). It is clear that the sulfones have reduced activities as compared to corresponding thioethers and sulfoxides, whereas the thioethers and the corresponding sulfoxides are similar in enzyme inhibitory activities. Further evaluation in B-Cell proliferation assay, we found that the thioethers (**7**) have bigger right shifts as compared to its sulfoxide analogs (**8**), probably due to the differences in unspecific reactivity. The sulfoxides showing in the table are racemates. Using chiral HPLC or chiral synthesis described earlier, we were able to resolve some of these racemic mixtures and obtain enantiomers. However, we found no noticeable differences between enantiomers with regard to BTK inhibition and B cell proliferation inhibition (data not shown).



Compd	-X-	-Y	BTK Inhibition IC_{50} (nM)[a]	B Cell Proliferation IC_{50} (nM)[a]
2[b]	F		4.61	3.28
7a	S		6.60	30.6
8a	SO		3.76	5.85
9a	SO2		31.2	N/A[c]
7b	S		1.08	64.0
8b	SO		0.903	3.55
8c	SO		1.48	14.9
8d	SO		5.65	19.4

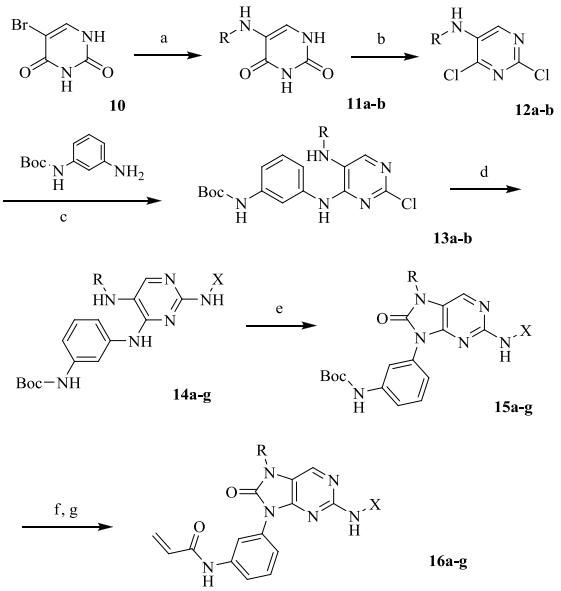
[a]Values reported are the mean of a minimum of two replicates with a standard deviation <25% of the mean, and the same for data in other tables. [b]Reported values, see ref. 10; [c]Not available, and same in other tables.

Table 1 BTK inhibition of sulfur containing analog **7**, **8** and **9** in Scheme 1

Although there was no noticeable improvement in BTK inhibition from that of the original lead Spebrutinib, we were quite encouraged to observe that, by and large, structural modification at that particular site can be tolerated. We then applied cyclization strategy outlined earlier to further restrict the accessible conformation of the lead compound.

The synthesis of the cyclized derivatives is outlined in **Scheme 2**. The starting 5-bromopyrimidine-2,4-dione was substituted with alkylamines under the conditions of potassium carbonate in THF. Product **11a-b** was treated with phosphoryl trichloride to yield 2,4-dichloropyrimidine **12a-b**, which was then reacted with mono protected1,3-benzendiamine in ethanol and with catalytic amounts of glacier acetic acid and heating to yield sulfide product **13a-b**. The resulting 2-chloropyrimidine was then substituted with various aromatic amines under

microwave heating. Cyclization was accomplished by heating the product (**14a-g**) with oxylylchloride. Final product **16a-g** were obtained after removal of Boc protecting group under acidic conditions, followed by acylation with acryloyl chloride to install the reactive Michael acceptor for covalent inhibition.[9]



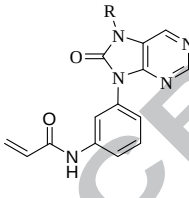
Scheme 2. Reaction keys: a) R-NH_2, K_2CO_3, THF; b) $POCl_3$; c) n-BuOH, Δ; d) X-NH_2, microwave, 110˚C; e) $(COCl)_3$; f) HCl; g) Acryloyl Chloride, DIEA, THF.

The synthesis of the cyclized derivatives is outlined in **Scheme 2**. The starting 5-bromopyrimidine-2,4-dione was substituted with alkylamines under the conditions of potassium carbonate in THF. Product **11a-b** was treated with phosphoryl trichloride to yield 2,4-dichloropyrimidine **12a-b**, which was then reacted with mono protected1,3-benzendiamine in ethanol and with catalytic amounts of glacier acetic acid and heating to yield sulfide product **13a-b**. The resulting 2-chloropyrimidine was then substituted with various aromatic amines under microwave heating. Cyclization was accomplished by heating the product (**14a-g**) with oxylylchloride. Final product **16a-g** were obtained after removal of Boc protecting group under acidic conditions, followed by acylation with acryloyl chloride to install the reactive Michael acceptor for covalent inhibition.[9]

Although some of these cyclic imidazolinone derivatives, such as **16b**, are rather potent BTK inhibitors (Table 2), they do not show improvement in terms of intrinsic activities when compared to the acyclic sulfur containing analogs (**7** and **8**).

These results are consistent with our proposed active conformation depicted in Figures 1 and 2. One could further argue that, although acyclic, our sulfoxide derivatives are also stabilized by the same intramolecular hydrogen bond in adopting the conformation necessary for covalent inhibition. We also believe that by fine tuning of the juxtaposition of reactive acrylamide and the binding domain of the molecule with various conformation restriction will eventually lead to more potent covalent inhibitors of BTK.

Optimization of irreversible kinase inhibitors is somewhat different from that of reversible inhibitors, because not all ADME parameters are of equal importance.[7] Since, by definition, covalent inhibitors are reactive in nature, it is imperative that we examine the non-specific reactivity of these new inhibitors toward plasma proteins.[10] So, for plasma stability, samples with initial concentration of 1μM was incubated in SD rat, beagle dog and human plasma samples for up to 4 h at 37 ℃. At the desired time points, an aliquot from the incubation was quenched by adding 10 volumes of 100% methanol supplemented with internal standard. Following the last collection, the samples were centrifuged at 12,000 rpm for 5 min and the supernatants were transferred to a new plate containing an equal volume of water for analysis by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS).[11] The results of representative compounds were summarized in Table 3.



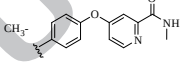
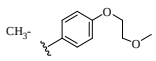
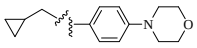
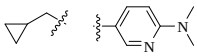
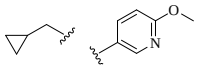
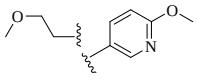
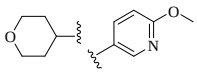
Compd	R-, -X	BTK Inhibition IC_{50} (nM)	B Cell Proliferation IC_{50} (nM)
16a		13.0	13.7
16b		4.11	6.84
16c		31.0	70.6
16d		30.6	818.4
16e		222	N/A
16f		265	N/A
16g		318	N/A

Table 2 BTK inhibition of imidazolinone derivative **16**.

Compd	Human	Beagle dog	SD rat
8a	86.7%	94.3%	90.7%
16b	42.2%	16.2%	27.5%
16c	40.8%	93.4%	80.5%

% remaining after 4 h incubation in plasma at 37˚C with initial concentration of 1μM.

Table 3. In vitro plasma stability of representative compounds.

Compound **8a** showed remarkable stability across different species. However, somewhat unexpected, cyclic imidazolinone analog **16b** has much reduced stability across the species, and **16c** has good stability in dog and SD rat plasma, by not in human plasma. These results indicated that for cyclic analogs (**16**), non-specific reactivity is moderate to high, and this could be due to the slightly increased reactivity of the acrylamide or the more easily accessible of its reactive group from conformational restriction.

We further studied the pharmacokinetic properties of these compounds, and results were summarized in Table 4. Not too surprisingly, these compounds showed moderate plasma clearance and very low oral bioavailability. Clearly these parameters need optimizing, however, we were delighted to observe that these compounds have short half-lives in rats. Since no excessive circulating levels are required to maintain efficacy with irreversible inhibitors, rapid clearance should lead to a lower propensity for off-target related adverse effects, provided that covalent inhibitors have fast on-rate (k_{on}) and slow off-rate (k_{off}).[12]

	Pharmacokinetic Parameter				
Compd	CL (L/h/kg)	$t_{1/2}$ (hr)	F_{oral} (%)	C_{max} (ng/mL)	AUC_{0-t} (ng*hr/mL)
8a	6.5±1.2	1.3±0.5	23±4.0	55±7.0	73±16
16a	1.8±0.1	2.4±2.3	9.0±1.1	107±112	100±91
16b	15.2±4.6	1.4±0.9	20±1.1	41±24	27±17

Data are presented as Mean±SD. Cl_p, plasma clearance after iv dosing; $t_{1/2}$, half life after po dosing; F_{oral}, oral bioavailability; C_{max}, peak plasma concentration after indicated oral dosing; AUC_{0-t}, area-under-curve after indicated oral dosing. Pharmacokinetic parameters were obtained following an IV (2 mg/kg) in 2%DMSO/15%Kolliphor HS15/5%Glucose /Water (pH=4.0) or P.O. (2 mg/kg) dose (amorphous free base) in 2%DMSO/15% Kolliphor HS15/5%Glucose /Water (pH=4.0).

Table 4. Pharmacokinetic Parameters of representative compounds in SD rats.

In summary, we have discovered novel covalent BTK inhibitors based on conformationally restricted 2-Amino-7,9-dihydro-8H-purin-8-one, which led toward better understanding of the reactive conformation of the covalent inhibitors. Some of our inhibitors are highly potent and have shown good stability in plasma, indicating little unspecific covalent labeling of plasma proteins. We are working to expand the SAR's to new cyclic systems, and new structure motifs for better overall profiles. We are advancing the BTK program by carrying out in vivo efficacy studies in rat CIA models, and eventually to preclinical candidate nomination.

Acknowledgments

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Graphical Abstract

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Approaching the Active Conformation of 1,3-Diaminopyrimidine Based Covalent Inhibitors of Bruton's Tyrosine Kinase for Treatment of Rheumatoid Arthritis

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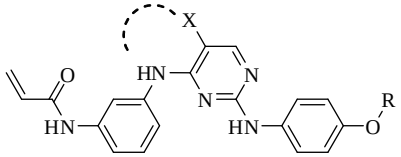
Zhenhua Huang*, Qian Zhang, Lianzhong Yan, Guizhen Zhong, Linqi Zhang, Xiaojuan Tan and Yanli Wang



8a, X = MeSO-, IC_{50} = 3.76 nM

16b, X = -N(Me)CO-, IC_{50} = 4.11 nM