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Structure-based discovery of novel 4,5,6-trisubstituted pyrimidines as potent covalent Bruton's tyrosine kinase inhibitors

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ABSTRACT

A series of novel 4,5,6-trisubstituted pyrimidines were designed as potent covalent Bruton's tyrosine kinase (BTK) inhibitors based on the structure of ibrutinib by using a ring-opening strategy. Among these derivatives, compound **I_1** exhibited the most potent inhibitory activity with an IC_{50} value of 0.07 μM. The preliminary structure–activity relationship was discussed and the primary amino group at the C-4 position of pyrimidine was crucial for maintaining BTK activity. Furthermore, molecular dynamics simulations and binding free energy calculations were performed for three inhibitor-BTK complexes to determine the probable binding model, which provided a comprehensive guide for further structural modification and optimization.

Bruton's tyrosine kinase (BTK), a member of the Tec family of non-receptor tyrosine kinases, plays a vital role in the B-cell signaling pathway linking cell surface B-cell receptor (BCR) stimulation to downstream intracellular responses. The expression and activity of BTK are critical to several key steps in the life cycle of B-lineage cells including proliferation, development, differentiation, survival, and apoptosis.[1,2] Ample evidence has indicated that the dysregulation of BTK is closely associated with the pathogenesis and development of various B-cell malignancies and autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosis, multiple sclerosis, B-cell lymphomas and leukemias, such as mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL).[3,4] Therefore, BTK has been considered as a potential therapeutic target for treating these diseases.

In recent years, many BTK inhibitors have been reported, which can be divided into two major classes, reversible and covalent irreversible ones, and some of them have been investigated in clinical trials.[5] Among these, covalent BTK inhibitors are a unique class of drugs, which form a covalent bond with a noncatalytic cysteine (Cys481) located at the rim of the ATP-binding pocket of BTK. This type of irreversible inhibitors has distinct characteristics compared with traditional reversible one, such as rapid onset of inhibition, greater potency, and longer duration of drug action.[6] Ibrutinib is a first-in-class irreversible inhibitor with subnanomolar against BTK (IC_{50} = 0.5 nM),[7] and has been approved for the treatment of MCL,[8] CLL,[9] and Waldenström's macroglobulinemia (WM).[10] Subsequently, other fused pyrimidine-based covalent inhibitors of BTK, such as ONO-4059,[11] TM-71224,[12] and acalabrutinib,[13] were also quickly advanced into clinical trials for the treatment of B-cell malignancies and autoimmune disorders (Fig. 1). Inspired by the pioneering work on these fused pyrimidines, we initiated docking simulations to investigate the interactions between ibrutinib and BTK, and found that the pyrazole moiety of ibrutinib didn't appear to make any visible interactions with the ATP-binding pocket directly, which provided new direction for development of novel covalent BTK inhibitors by the structural modification of this skeleton.

The protein structure, cocrystallized with an ibrutinib analogue **B43**,[14] was firstly carefully analyzed (PDB code: 3GEN). Besides, we performed docking simulations using Glide[15] in Schrodinger Suite with the default setting to investigate the interactions between ibrutinib and BTK. As predicted, docking simulations suggested that ibrutinib interacted with the active site in a fashion similar to compound **B43** (Fig. 2). Briefly, the primary amine NH_2 formed two hydrogen bonds with the gatekeeper Thr474 hydroxyl and

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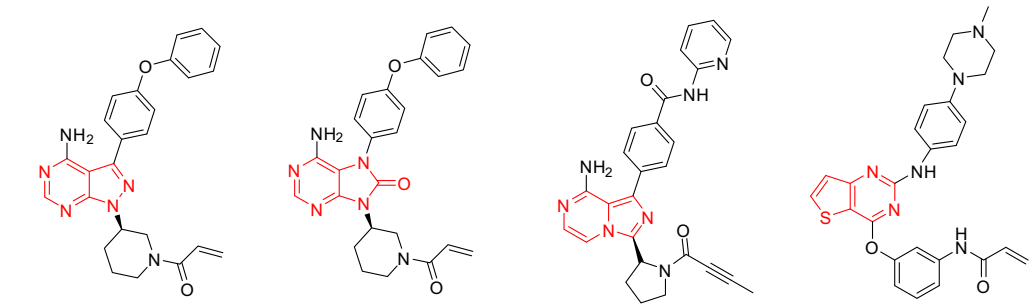

Ibrutinib
Celera /Pharmacyclics /Janssen
IC_{50}: 0.5 nM, Launched

ONO-4059
Ono /Gilead
IC_{50}: 23.9 nM, Phase II

Acalabrutinib
Acerta Pharma
IC_{50}: 3.1 nM, Phase III

HM-71224
Hanmi /Lilly
IC_{50}: 1 nM, Phase I

Figure 1. Representative fused pyrimidine-based covalent BTK inhibitors.

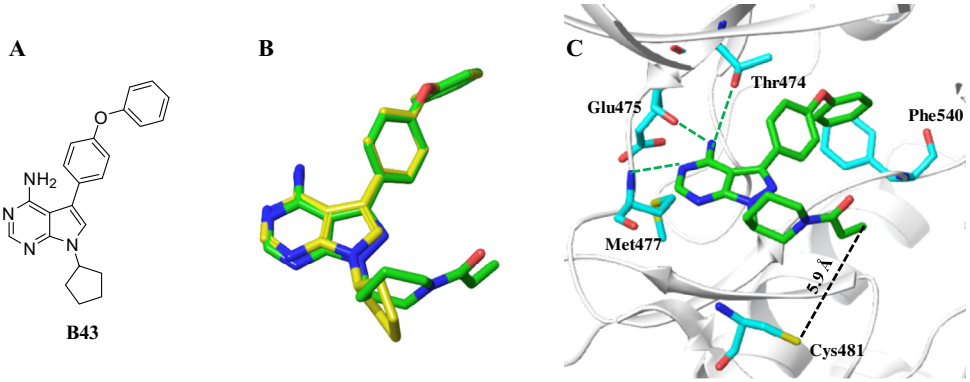

Figure 2. Docking mode of ibrutinib with BTK. (A) Chemical structure of **B43**. (B) Superposed docking poses of **B43** (yellow) and ibrutinib (green). (C) Docking pose of ibrutinib (green) with BTK. PDB ID: 3GEN.

the backbone carbonyl of Glu475, the N-3 nitrogen of the pyrimidine ring interacted with the backbone NH of Met477 at the hinge region, and the diphenyl ether moiety occupied the hydrophobic pocket behind the Thr474 gatekeeper residue and displayed an edge-to-face aromatic interaction with Phe540. The sulfhydryl group of Cys481 of BTK was about 6 Å away from the acrylamide moiety at the head region of ibrutinib, which bound covalently to a cysteine residue proximal to the ATP-binding pocket of the kinase catalytic domain by Michael addition reaction in vivo. Meanwhile, we speculated that the pyrazolyl group of ibrutinib might act the role of maintaining its bioactive conformation merely as it didn't seem to make any visible interactions with the ATP-binding site.

Based on the above analysis, we designed a series of novel pyrimidine-based BTK inhibitors by evolved the pyrazole ring of ibrutinib to 5-carbonyl and 6-amino using a ring-opening strategy (Fig. 3).[16,17] We hoped that the active conformation could be retained through the formation of a pseudo-ring which was formed by intramolecular hydrogen bonding between carbonyl and amino group. The intramolecular hydrogen bond plays a unique role in drug design and discovery, as it could control the conformation of the molecules.[18,19] Two main factors were considered in the whole process of compound designing: the binding mode of the designed compounds should be overlapped well to that of ibrutinib, and their distances between Michael receptor and the sulfhydryl group of BTK Cys481 had to be similar to each other (around 6 Å).

After opening the pyrazole ring, compound $\mathbf{I_1}$ was firstly designed and synthesized (Fig. 3). As can be seen from Figure 4A, docking simulations of the proposed compound $\mathbf{I_1}$ within the ATP binding site of BTK displayed almost the same binding mode to ibrutinib and the distance between Michael receptor and the sulfhydryl group of Cys481 of BTK was 5.5 Å. The preparation of compounds $\mathbf{I_{1-9}}$ was exemplified in Scheme 1. The commercially available pyrimidine-4,6-diol was subjected to Vilsmeier reaction with $POCl_3$ to give the aldehyde **1**, followed by oxidation with NaH_2PO_4 and $NaClO_2$ to obtain the corresponding carboxylic acid **2** in quantitative yields. Subsequent compound **2** was reacted with oxalyl chloride in anhydrous THF to give the corresponding acyl chloride which was used directly to assemble the key intermediate **3** by utilizing an intermolecular Friedel–Crafts acylation. The intermediates **3a–b** were then subjected to nucleophilic attack by (*R*)-1-Boc-3-aminopiperidine and substituted aliphatic amines to furnish compounds **5a–g**, respectively. After removing the Boc-protecting group of **5a–g**, the newly formed compounds were treated with acryloyl chloride to give the target compounds $\mathbf{I_{2-6}}$ and $\mathbf{I_{8-9}}$. Meanwhile, the intermediates **3a–b** were condensed successively with ammonia and (*R*)-1-Boc-3-aminopiperidine to generate compounds **8a–b**, followed by removal of the Boc-protecting group to give **9a–b** as intermediates. Finally, the desired products $\mathbf{I_1}$ and $\mathbf{I_7}$ were prepared via the reaction of **9a–b** and acryloyl chloride under an atmosphere of nitrogen.

Visual inspection of docking result also revealed that the para position of substituted piperidine ring of compound $\mathbf{I_1}$ was closer to Cys481 when compared to the meta position (6.5 Å and 6.0 Å, respectively) (Fig. 4B). We anticipated that the addition of Michael acceptor extending from the para position of this piperidine ring would yield substituents which also could directly interact with the sulfhydryl group of Cys481. Thus, compounds $\mathbf{II_{1-8}}$ were designed for this goal and the docking results indicated that the