Abstract

of

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“Design, Synthesis and Biological Evaluation of Novel Bruton's Tyrosine Kinase (BTK) Inhibitors”

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Abstract

Bruton's tyrosine kinase (BTK) is a cytoplasmic tyrosine kinase of the TEC family. It was named after pediatrician Ogden Bruton, who discovered XLA (X-linked agammaglobulinemia), a disease characterized by the absence of mature B cells. BTK is expressed in the majority of hemopoietic cells, primarily B cells and myeloid cells, but not in T cells or plasma cells. BTK has five domains, such as An N-terminal pleckstrin homology (PH) domain, a Tec homology domain (TH), SRC homology 3 (SH3) and SRC homology 2 (SH2) domains, and a C-terminal kinase domain.

B cell maturation, differentiation, and proliferation were mainly regulated by the B cell receptor (BCR), where the role of the BTK enzyme is very crucial. In the BCR pathway, BTK is activated by the upstream Src-family kinases, such as Blk, Lyn, and Fyn. In turn, BTK phosphorylates and activates phospholipase Cgamma2 (PLCγ2), leading to Ca^{+2} mobilisation and activation of NF-kB and MAP kinase pathways, which are essential for B cell survival. Immune cells like mast cells, basophils, monocytes, and macrophages play important roles in inflammatory and allergic responses. Constitutive BTK activation under autoimmune conditions leads to activation of the Fc receptors of IgG and IgE (FcγR, FcεR), in macrophages and mast cells. BTK is also important for the signalling of chemokine receptors and toll-like receptors (TLRs).

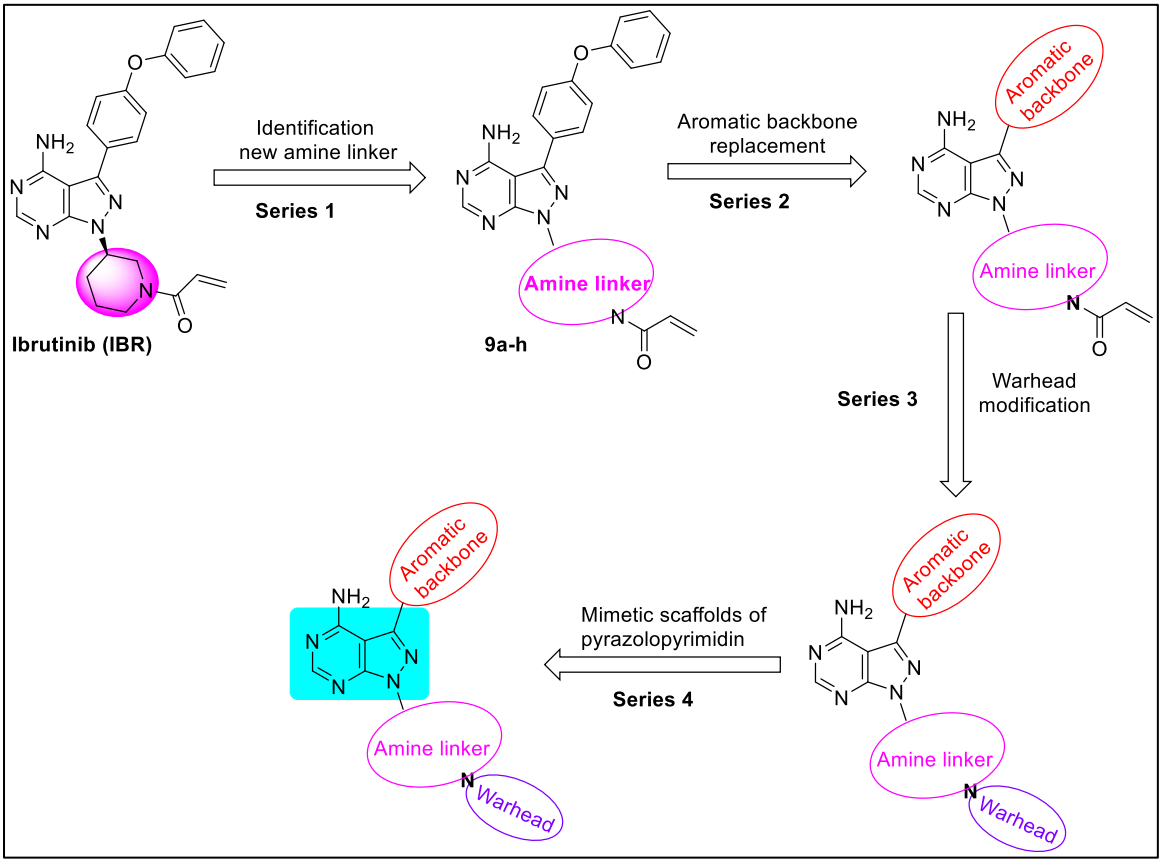
BTK is crucial in the development of inflammatory diseases, mainly autoimmune diseases. Loss of self-tolerance, atypical B cell activation, and the eventual formation of autoreactive antibodies are characteristics of autoimmune diseases. Animal studies demonstrate that BTK is essential for determining the threshold for B cell activation and

for the BCR signalling-mediated counter selection of autoreactive B cells. Mutant mice overexpressing BTK rapidly captured autoimmune disorders like systemic lupus erythematosus (SLE), which affect many organs. BTK is vital for innate immune cells to release inflammatory cytokines. Over activation of BTK could end up resulting in chronic inflammation or an acute hyper inflammatory condition, making BTK inhibition a potential target for treatment for autoimmune disorders.

BTK has been shown to play a key role in several autoimmune disorders like lupus, Rheumatoid arthritis (RA), multiple sclerosis (MS), and various types of B-cell malignancies (mantle cell lymphoma (MCL), chronic lymphocytic lymphoma (CLL), or small lymphocytic lymphoma (SLL)). In the last decade, intense efforts have been made to develop selective BTK inhibitors, especially against closely associated cysteine kinases such as EGFR, JAK3, BLK, BMX, and TEC, for the safe and effective treatment of autoimmune disorders. BTK inhibitors can be classified into two groups based on their chemical scaffold, mode of action, and binding mode. (1) Covalent or irreversible, and (2) non-covalent or reversible.

Ibrutinib (IBR) is the first generation orally administered irreversible BTK inhibitor, which covalently binds to Cys481 of the active adenosine triphosphate (ATP) binding domain of a BTK enzyme. IBR was approved by the FDA in 2013 to treat mantle cell lymphoma (MCL) and was subsequently approved for various indications, such as chronic lymphocytic leukemia (CLL), Waldenström's macroglobulinemia (WM), and marginal zone lymphoma (MZL).

Acalabrutinib and Zanubrutinib are the second-generation irreversible BTK inhibitors that have been approved by the FDA for the treatment of MCL. Other irreversible BTK inhibitors like Tirabrutinib (for BCL), Evobrutinib (for MS), Branebrutinib (for RA, Systemic lupus erythematosus (SLE), and Stevens-Johnson syndrome (SjS)), and Orelabrutinib (for B-cell malignancies and autoimmune diseases) are in clinical development. Reversible BTK inhibitors, Fenebrutinib (for RA and SLE) and Rilzabrutinib (for immune thrombocytopenia (ITP)), are also in clinical development.



As already pointed out, there are various BTK inhibitors on the market or in clinical trials, although they have adverse effects such toxicities, off-target activities, and poor pharmacokinetics. Therefore, safe, potent, and selective BTK inhibitors still need to be developed for the effective treatment of autoimmune diseases such B-cell malignancies

and RA. In the present investigation, to discover novel, potent, selective, and orally bioavailable BTK inhibitor, stepwise structural modifications were carried out in the IBR and 64 target compounds were designed and synthesised through four distinct series.

A novel **Series 1** of 3-(4-phenoxyphenyl)-pyrazolo[3,4-d]pyrimidin-4-aminescaffold-based BTK inhibitors (**24a-h**) were synthesized, wherein the linker amine (piperidine) of IBR was replaced with eight saturated bicyclic linker amines. The most efficacious of the set was discovered to be compound **24e** (whose in vitro potency was equivalent to IBR). Moreover, **24e** possessed a superior pharmacokinetic profile and was devoid of CYP and hERG liabilities. The significant potency of 24e was also validated by molecular modelling studies.

In **Series 2**, to optimize the aromatic backbone (phenoxy phenyl) of **24e**, a total of forty compounds (**14a–an**) were synthesized. In this series, benzamide and thioether analogues as aromatic backbones demonstrated excellent BTK inhibitory and anti-proliferative activity, particularly **32b**, **32d**, **32e**, **32u**, and **32v**. During subsequent biological evaluation, only **32b** was identified to have superior bioavailability and it was found to be devoid of CYP and hERG at 10 μM concentration. Molecular docking revealed that the N-2-pyridyl ring of **32b** exhibits the important π-π interaction and also additional bonding in the catalytic domain of BTK enzyme with Ser538 and Gln412, which was believed to be contributing to its potent BTK inhibitory activity.

To investigate the influence of the warhead on in vitro activities in **Series 3**, acrylamide was swapped with specific α, β-unsaturated amide to construct **32ao-av**. butynamide (**32ao**), as the warhead displayed excellent potency in the BTK enzyme and TMD8 cell

proliferation assays, with IC_{50} values of 1.2 nM and 0.9 nM, respectively. **32ao** was found to be free of CYP and hERG liabilities and had a better PK profile than IBR, although **32ao** has a slightly inferior PK profile compared to **32b**.

In the final set (**Series 4**) of compounds (**41**, **42**, **51**, **52**, **61**, **62**, **71**, and **72**), attempts were made to replace the Pyrazolo-pyrimidin-4-amine scaffold with its mimetic aromatic heterocycles, particularly Pyrrolo-pyrimidin, Oxo-purine, Imidazo-pyrazine, and Pyrazole scaffolds. However, none of them were found to be as efficacious as its Pyrazolo-pyrimidin-4-amine counterpart.

In a comparative biological assessment of pharmacokinetics and in vitro assays, **32b** was found to be superior and devoid of CYP and hERG. Thus, **32b** was designated for developmental studies.

The anti-tumor potential of **32b** was assessed in TMD-8 xenograft tumor-bearing mice for 20 days via oral administration. The findings revealed that **32b** suppressed tumor growth in a dose-dependent manner (10%, 50%, and 88%, respectively), and its growth-inhibitory effects became more prominent after 7 days and onwards.

32b was found to be extremely effective in alleviating arthritis, causing a 97% reduction in the clinical score in a collagen-induced arthritis (CIA) mice model. In histological evaluation, treatment with **32b** demonstrated a substantial reduction of mice paw swelling as compared to the control group. The histologic severity scores for the mice treated with **32b** at dosages of 0.125, 0.25, 0.5, and 1 mg/kg were 5, 3.75, 1, and 0.2, compared to a histologic severity score of 10.4 for the paws from the vehicle-treated control group.

32b has been found to be BTK selective and covalently binds to the BTK enzyme.

At doses up to 300 mg/kg (100x of the ED_{50}), **32b** exhibited an ideal preclinical safety profile, with no negative effects seen in rats.

Hence, the pre-clinical profile of **32b** indicates that the new class of BTK inhibitor could be a viable therapeutic option for the treatment of autoimmune diseases like cancer and RA.

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