CHAPTER 3

3. Summery, Conclusion, and Way Forward

In our ongoing quest to discover a novel BTK inhibitor, 64 target compounds were designed and synthesised through four distinct series. In **Series 1**, we have selected eight saturated bicyclic amine analogues for bioisosteric replacement of piperidine in IBR. In **Series 2**, for aromatic backbone optimisation, three sets of aromatic backbone were selected. In the first set, benzamide and picolinamide-based aromatic backbones were selected, whereas in the second set, 4-phenyl ether, 4-phenyl thioether, and 4-phenyl alkyl derivatives were used as aromatic backbones. Phenyl-containing fused heterocycles were enlisted as the aromatic backbone for the third set.

To explore the impact of the warhead on BTK inhibitory activities, conventional warhead acrylamide was swapped with α, β-unsaturated amide in **Series 3**. In **Series 4**, Pyrazolo-pyrimidine, a hinge binder of BTK inhibitors, was replaced with mimetic heterocycles of Pyrazolo-pyrimidine, such as Pyrrolo-pyrimidin, Oxo-purine, Imidazo-pyrazine, and Pyrazole scaffolds.

Analytical data were used to characterise all of the target compounds, which were then evaluated for *in vitro* BTK inhibitory and anti-proliferative activities. Compounds having significant *in vitro* activity were studied for CYP and hERG inhibition, pharmacokinetic properties, and molecular docking. Out of the four series, the most potent molecule **(32b)** was ultimately selected for *in vivo* pharmacological evaluation, such as anti-tumour activity using the TMD8 xenograft model, anti-arthritic efficacy using the CIA mice model, kinase selectivity, an irreversible BTK inhibitory binding study, and acute toxicities studies.

A novel **Series 1** of 3-(4-phenoxyphenyl)-pyrazolo[3,4-d]pyrimidin-4-amine scaffold-based BTK inhibitors (**24a-h**) were synthesised, wherein the linker amine (piperidine) of Ibrutinib (IBR) was replaced with eight saturated bicyclic linker amines. The most efficacious of the set was discovered to be compound **24e (***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 optimise the aromatic backbone (phenoxy phenyl) of compound **24e**, a total of forty compounds (**32a–an**) were synthesised. 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 compound **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 tumour 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.

In summary, 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 rheumatoid arthritis.

Future plan for 32b:

Additional profiling studies will be carried out with **32b**, such as single and repeated dose PK studies in higher species (dogs or primates), including chronic toxicity studies in higher animal species. If **32b** fulfils pre-clinical candidate selection criteria, it will be subjected to IND enabling studies.

Overall project outcome:

Rational designing and synthesis of novel BTK inhibitors, SAR generation, *in vitro*, and *in vivo* profiling of lead compounds resulted in a novel BTK inhibitor **(32b)** in the present investigation. Overall, **32b** looks like a promising candidate in terms of efficacy and safety margin in acute animal studies. If our initial observations translate into chronic animal studies, **32b** may turn out to be a safe and efficacious BTK inhibitor for the treatment of various autoimmune disorders such as RA and cancer.