A Review on Bruton's Tyrosine Kinase Inhibitors to Outline the Scopes of Further Advancements in the Treatment of Cancer.

Submitted by,

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy

Brac University

2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

The thesis titled "A Review on Bruton's Tyrosine Kinase Inhibitors to Outline the Scopes of Further Advancements in The Treatment of Lymphocytic Leukemia" submitted by Muidul Hasan Khan (19346066), of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Dedication

Dedicated to my faculty members, family and friends.

Ethics Statement

This study did not involve any human participants, human specimens or tissue, vertebrate animals or cephalopods, vertebrate embryos or tissues and field research.

Abstract

In many B cell malignancies, Bruton's tyrosine kinase (BTK), a non-receptor kinase, plays a significant role in oncogenic signaling that is essential for the proliferation and survival of leukemic cells. BTK works as a transducer in B cell receptor and other cell surface receptors. Hence they can drive the proliferation of cancerous cells through the B cell receptor (BCR) signaling pathway. So, BTK inhibitors can play an important role in the management of such malignant tumors. This review is comprised of an updated compilation of drugs that bind to BTK and make them unable to play their role in the BCR signaling pathway. BTK inhibitors that are available in the market are, Ibrutinib, Acalbrutinib, Zanubrutinib, Tirabrutinib, Orelabrutinib and Pirtobrutinib. Most of these drugs are not highly selective to BTK and can cause resistance. So, to overcome the problems caused by the existing BTK inhibitors, new drugs are needed to be developed. Some promising drugs are currently under clinical trial which may overcome these problems. They are Spebrutinib, Evobrutinib, Vecabrutinib and Fenebrutinib.

Keywords: Bruton’s Tyrosine Kinase; BTK Inhibitors; B-cell Receptor Signaling Pathway; Chronic lymphocytic leukemia; B-cell Malignancy; BTKi Resistance.

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List of Acronyms

BTK	Bruton's Tyrosine Kinase
ITK	Interleukin-2-inducible T-cell kinase
WM	Waldenström Macroglobulinemia
BCR	B-cell Receptor
CLL	Chronic Lymphocytic Lymphomia
FCγR	Fragment Crystallizable Gamma Receptor
BMX	Bone Marrow Kinase on Chromosome X
pDC	PlasmaCytoid Dendritic Cells
TNF	Tissue Necrosis FActor
JAK3	Janus Kinase 3
EGFR	Epidermal Growth Factor Receptor
OI	Oppertunistic Infections
TLR	Toll-like Receptors
SLL	Small Lymphocytic Lymphoma
RA	Rehumatoid Arthritis
MS	Multiple Sclerosis
SLE	Systemic lupus erythematosus
MCL	Mantle Cell Lymphoma
PLCγ2	Phospholipase C Gamma 2
PKCβ	Protein Kinase C Beta
PH	Pleckstrin Homology
SRC	Steroid Receptor Coactivator
MRR	Major Response Rate
PFS	Progression-free Survival
OS	Overall Survival
CXCL13	Chemokine Ligand 13
MIP-1β	Macrophage Inflammatory Protein-1β
CTX-I	Carboxy-terminal Collagen Cross-linking Telopeptide

Chapter 1

Introduction

1.1 Overview

In 112 of the 183 countries for people under 70, the second most prevelant cause of demise is cancer (Martins *et al*, 2022). In 23 other countries, it is the 3rd or 4th most common cause of demise. (Martins *et al*, 2022). Protein Kinases play a major role in cancer (Das & Hong, 2020). Protein kinases are enzymes that facilitate the process of protein phosphorylation, leading to alterations in their activity or capacity to engage with other molecules. These modifications have significant implications for several cellular processes, such as growth, differentiation, survival, proliferation and transferring information (Das & Hong, 2020). Hence, when the activity of protein kinase is not properly regulated, it may act as an important part in the pathogenesis of several illnesses, including cardiovascular, autoimmune, neurological, and inflammatory disorders, alongside a diverse range of malignancies (Regot *et al*, 2014). Bruton's tyrosine kinase (BTK) has garnered increasing interest, as inhibitors targeting this kinase have exhibited notable anticancer efficacy in clinical studies. BTK is a signaling protein, residing in cytoplasm and it is a part of the Tyrosine protein kinase (Tec) family which is expressed in hepatocellular carcinoma (Tasso *et al*, 2021). In almost all hematopoietic cell, BTK is active unless they are T-lymphocytes and terminally differentiated plasma cells. (CarneroContentt i& Correale, 2020).

According to Pope (2023), BTK is linked to various diseases like, Waldenstrom macroglobulinemia, Chronic lymphocytic leukemia (CLL), Mantle cell lymphoma (MCL), Small lymphocytic lymphoma, Follicular lymphoma, Marginal zone lymphoma, Chronic graft-versus-host disease and other selective B cell malignancies.

1.2 Structure of BTK

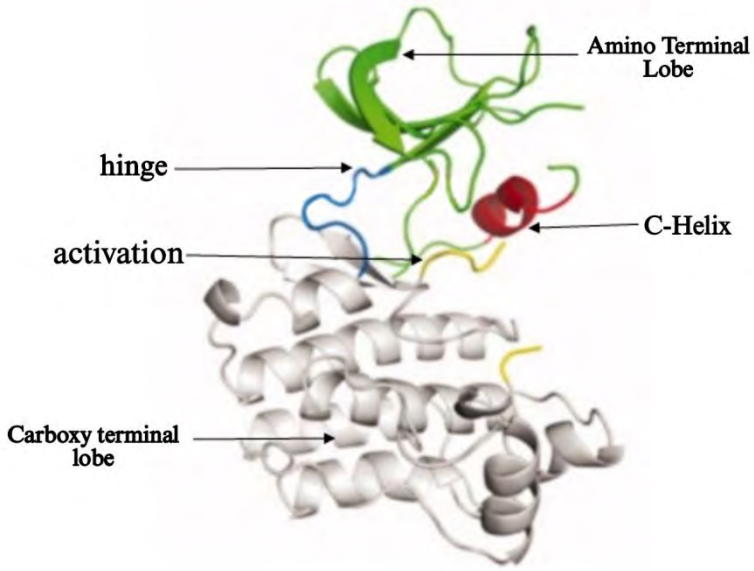

Figure 1.1: The protein structure of BTK . (Marcotte et al, 2010)

BTK, as a protein, basically has five parts and they are amino terminal indicated with green in the figure, the hinge region indicated with blue in the figure (ligand binding domains are present here which facilitates antigen binding), C-Helix indicated with red in the figure (a unique and dynamic regulatory element in protein kinase), activation zone indicated with yellow in the figure and the carboxy terminal lobe indicated with gray in the figure (Marcotte *et al*, 2010).

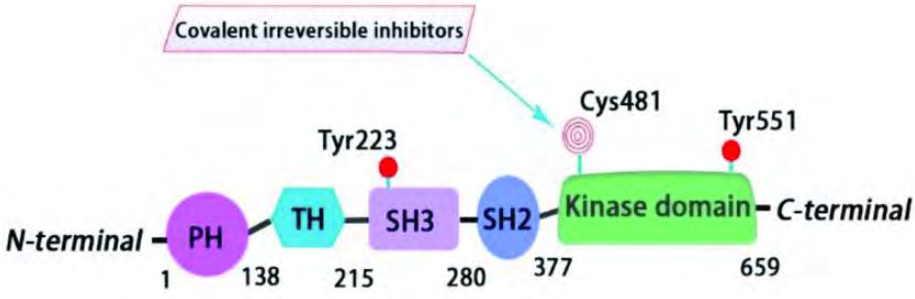

Figure 1.2: BTK structure diagram (Zhang et al, 2021).

The composition of BTK consists of four distinct domains: the TEC homology (TH) domain enriched with proline, the pleckstrin homology (PH) domain, the Steroid Receptor Coactivator (SRC) homology (SH) domains (also known as SH3 and SH2), and the catalytic domain. These domains collectively span a total of 659 amino acids (Zhang et al., 2021). The autophosphorylation site Tyr223 is located in the SH2 and SH3 domains, but the catalytic domain has two phosphorylation sites, Tyr551 and Cys481, which are targeted by irreversible inhibitors. (Tasso *et al*, 2021).

1.3 Function of BTK and its Role in Malignancy

The BTK plays an important role, when it comes to the formation of B-cells (Feng *et al*, 2019). BTK predominantly localizes within B lymphocytes, where it assumes a critical function in the processes of maturation, differentiation, and survival, alongside its involvement in cellular signaling (Ringheim, Wampole & Oberoi, 2021). The proper regulation of BTK in B cell receptor signaling pathway ensures the stability of immune system (McDonald *et al*, 2021). The BCR pathway according to Wiestner (2013) is described in the following paragraph.

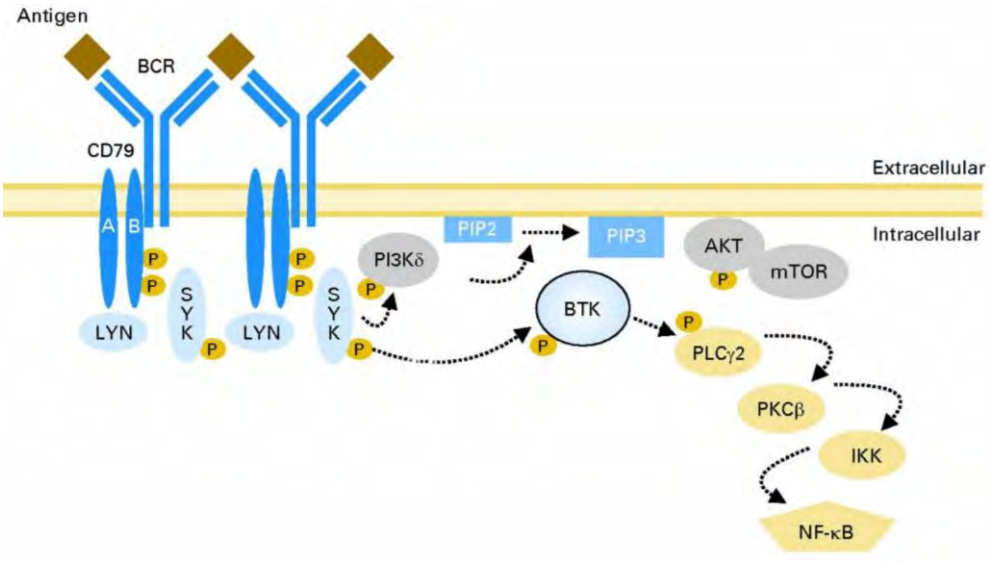

Figure 1.3: BCR pathway (Wiestner 2013).

Upon binding to the B-cell receptors, antigens cause the aggregation of the receptors with their co-receptors CD79A and CD79B. The co-receptors undergo phosphorylation by tyrosine kinases, specifically SYK and LYN, in order to enhance the original signal. SYK activates phosphoinositide 3-kinase (PI3K), which subsequently converts phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-triphosphate (PIP3). The intracellular enzymes Bruton's tyrosine kinase (BTK) and AKT utilize PIP3 as a specific location for binding. BTK phosphorylates and stimulates phospholipase C gamma 2 (PLCγ2), leading to the production of a cascade of second messengers that subsequently activate protein kinase C beta (PKCβ). PKCβ stimulates the activation of nuclear factor κB (NF-κB) transcription factors, which regulate the synthesis of several survival factors, by the phosphorylation of IκB kinase (IKK).

Furthermore, BTK plays an important part in the liberation of histamine, degranulation, and the production of pro-inflammatory cytokines (Hata et al, 1998). Additionally, BTK plays a pivotal role in relaying signals derived from different receptors, including Toll-like receptors (TLRs) located in B-cells, Fragment crystallizable gamma receptor (FCγR) or Toll-like receptors (TLRs) found in macrophages or plasmacytoid dendritic cells (pDCs), as well as mast cells and basophils (Liu et al, 2021). Moreover, the function of BTK is pivotal in the synthesis of proinflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β). BTK also has a notable impact on degranulation and the subsequent liberation of histamine. The citation is from Tasso et al. (2021). Recent investigations have revealed that BTK plays a vital function in regulating the NLRP3 inflammasome. This control is achieved by altering the subcellular location and facilitating the assembly of the inflammasome. (Bittner *et al*, 2021).

The functions of BTK from the paragraphs above, can be summarized using the following diagram,

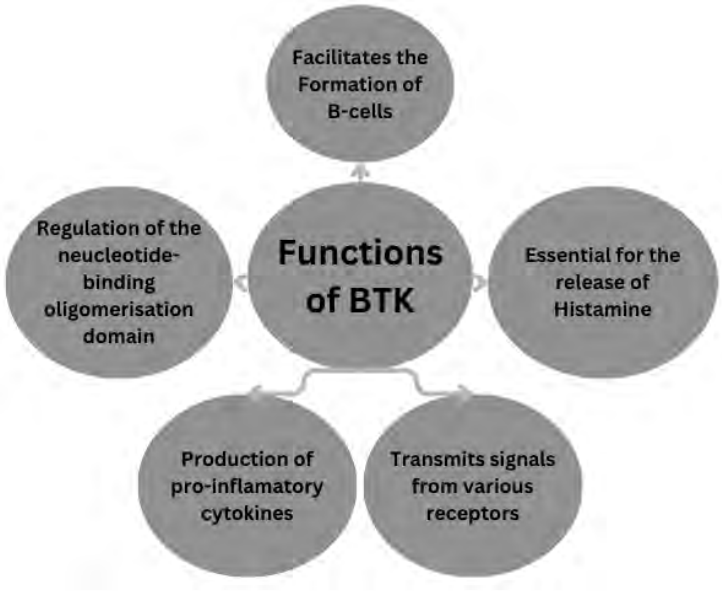

Figure 1.4: Functions of BTK .

In different B-cell malignancies, BTK has been discovered to control cell migration, survival, and proliferation This group of kinases can drive the proliferation of cancerous cells through the BCR (B cell receptor) signaling pathway (Burger, 2019). Basically due to genetic mutations, hyper activation of BTK can lead to uncontrolled proliferation of B-cells and lead to malignancy (Desikan *et al*, 2022). It seems that additional B-cell tyrosine kinases (BTKs) are mainly found in tissues and cancers that are not related to B-cells. The gene p65BTK exhibits increased expression levels in colon, lung, ovarian, esophageal, breast, gastric and bladder cancer (Rozkiewicz *et al*, 2023).

1.4 Rationale

The rationale of this project is to summarize the information available on BTK inhibitors to portray their importance in the treatment of cancer and to provide the background information to ease and find the scopes of further research on BTK inhibitors. BTK inhibitors possess the capacity to assume a crucial function in the management of cancer.. These drugs can be used to reduce the application of Chemo therapy. Currently approved BTK inhibitors, ibrutinib, acalabrutinib, zanubrutinib, pirtobrutinib, tirabrutinib and orelabrutinib, have several limitations and may cause unwanted effects. So, new drugs must be developed to overcome the short comings the approved drugs have. Currently spebrutinib, evobrutinib, vecabrutinib and fenebrutinib are under clinical trials while other compounds are being developed.

1.5 Objective

The objective of the review is to compile available data on Bruton's Tyrosine Kinase Inhibitors to find out the advantages of using them, the limitations they have and the improvements that can be brought in the future. This article contains information about the indication and activity(IC_{50}) on BTK, ITK and TEC of BTK inhibitors that are in the market as well as that are under clinical or pre-clinical trial. So, this paper can be used as an updated review of this class of drugs and can be used to facilitate further studies on the treatment of cancer.

Chapter 2

Methodology

An overview of BTK inhibitors is provided in the review. Peer-reviewed research publications from PubMed, Elsevier, ScienceDirect, Nature, Springer, Lancet, Taylor & Francis were used to compile all the data for this review report. To give readers a better knowledge of BTK inhibitors and their importance in the treatment of cancer, information was correctly gathered and referenced.

Chapter 3

BTK inhibitors

3.1 Overview of BTK Inhibitors

BTK inhibitors are part of a class of drug that usually prefers to bind to BTK either covalently or by any other weaker bond to hinder their phosphorilation in the BCR and carry out their actions (Rozkiewicz *et al*, 2023). Burger (2019) states that, in the past few years BTK inhibitors have been working as a great alternative to chemotherapy in case of mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL). So, by using BTKi several cancers that are linked with BTK can be treated.

Apart from cancer BTK inhibitors could be a viable therapeutic approach for managing immunoglobulin E (IgE)-related conditions such as allergies, asthma, and dermatitis. (Liu *et al*, 2021). Furthermore, BTKi canbe used in the treatment of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), and Sjögren's syndrome (Rozkiewicz *et al*, 2023).

Some examples of BTKi are, Ibrutinib, Acalabrutinib, Evobrutinib, Zanubrutinib, Tirabrutinib, Pirtobrutinib *etc*.

3.2 Classifications of BTK Inhibitors

BTK inhibitors can be divided into two categories, they are- Irreversible BTKi and Reversible BTKi. (Tullemans *et al*, 2021)

The irreversible BTKi is a group of drugs that bind to the particular protein by using a covalent bond. The aromatic core of this drug which has the terminal group attached to it mainly forms the covalent bond with Cys481 (Tasso *et al*, 2021). Some examples include, Ibrutinib, acaclabrutinib, zanubrutinib *etc.*

According to a research the four main parts of the pharmacophore model of irreversible BTK inhbitors are, (i) a large hydrophobic group, (ii) an aromatic heterocyclic nucleus, (iii) a linker, and (iv) a warhead terminal group. (Tasso *et al*, 2021).

The reversible group includes, imidazo-pyrazines, imidazo-pyrimidines, imidazo-quinoxalines, Vecabrutinib, Fenebrutinib *etc.* These do not bind with BTK by covalent bonds and thus reversible. They form weak hydrogen bonds or hydrophobic interactions to be attached to to a specificpart of the SH3 domain (Tasso *et al*, 2021)

3.3 Available BTK Inhibitors in the Market

Food and Drug Administration (FDA), an American regulatory organization had approved acalabrutinib (2017), ibrutinib (2013), and zanubrutinib (2020) up until 2023 (Rozkiewicz *et al*, 2023). Recently, on January 27, 2023, FDA approved Pirtobrutinib (Brook, 2023). There are two other BTK inhibitors named Tirabrutinib and Orelabrutinib. Orelabrutinib has got approval from the China Food and Drug Administration and Tirabrutinib has got approval from Japan Pharmaceuticals and Medical Devices Agency in 2020 (Rozkiewicz *et al*, 2023).

Table 1: Summary of BTK Inhibitors Available in Market . (Zain et al, 2021); (Rezaei et al, 2022);(Tasso et al, 2021); (Liu et al, 2021); (Estupiñán et al, 2021); (Carles et al, 2018); (Rosoki, 2019);

Name	Indication	Activity (IC_{50})	Year Approved
Ibrutinib Synonyms CRA-032765 PC-32765 PCI-32765 PCI-32765-00	Chronic lymphocytic leukemia, Mantle cell lymphoma, Marginal zone lymphoma, Graft-versus-host disease, Small lymphocytic lymphoma, Waldenström's macroglobulinemia.	BTK IC_{50} = 0.47 nM ITK IC_{50} =55 nM TEC IC_{50} = 3.2 nM	2013

Acalabrutinib Synonym ACP-196	Chronic lymphocytic leukemia, Mantle cell lymphoma, Small lymphocytic lymphoma	BTK IC_{50} = 2.5 nM ITK IC_{50} > 20,000 nM TEC IC_{50} = 37 nM	2017
Zanubrutinib Synonym BGB-3111	Non-Hodgkin lymphoma, Chronic lymphocytic leukemia, Mantle cell lymphoma	BTK IC_{50} = 0.3 nM ITK IC_{50} = 56 nM TEC IC_{50} = 2 nM	2020
Tirabrutinib Synonym ONO-4059	Waldenström's macroglobulinemi, Chronic lymphocytic leukemia, CNS lymphoma	BTK IC_{50} = 6.8 nM ITK IC_{50} > 20,000 nM TEC IC_{50} = 48 nM	2020
Orelabrutinib Synonym ICP-022	Chronic lymphocytic leukemia, Mantle cell lymphoma, Small lymphocytic lymphoma	BTK IC_{50} = 1.6 nM	2020
Pirtobrutinib Synonym LOXO-305	Chronic lymphocytic leukemia, Mantle cell lymphoma	BTK IC_{50} = 3.15 nM ITK IC_{50} > 5000 nM TEC IC_{50} = 1234 nM	2023

Ibrutinib:

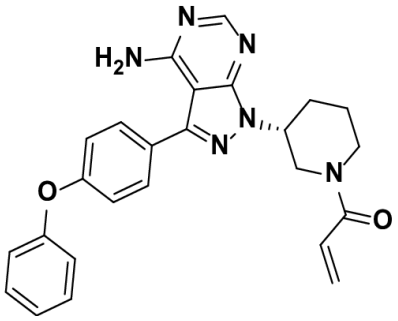

Figure 3.1: Ibrutinib structure. (Mehta et al, 2020)

Ibrutinib, a BTK inhibitor, which is a member of acrylamide class (National Center for Biotechnology Information, 2023) received its initial authorization from Food and Drug Administration (FDA) in the year 2013 (Messex *et al*, 2021). It is a first generation drug which is irreversible in nature and it binds covalently with Cys-481 (Tasso *et al*, 2021). After binding with BTK it hinders the BCR signaling path way, directly down regulating the BCR signaling pathway (Saleh *et al*, 2017). Along with BTK other kinases that are related to TEC family gets affected by this drug (McCa & Gribben, 2022). As an example, ibrutinib specifically attaches to interleukin-2-inducible T-cell kinase (ITK), which is a type of TEC family kinase. This interaction alters the movement of cells towards chemical signals, the exchange of information between cells, and the attachment of cells to one other inside the localized environment of the tumor (McCa & Gribben, 2022). ITK is associated with T-cells and plays a crucial role in the growth and development of T-cells. (Xia *et al*, 2020).

Acalabrutinib:

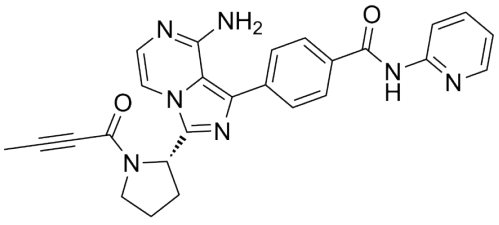

Figure 3.2: Acalabrutinib structure. (Bryd et al, 2021)

Acalabrutinib, classified as a second-generation BTK inhibitor belonging to the imidazopyrazines class (National Center for Biotechnology Information, 2023), was granted FDA approval in 2017. The approved uses for this medication are relapsed/refractory mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL). (Estupiñán *et al*, 2021). In contrast to ibrutinib, only the kinases BTK, BMX, and ErbB4 exhibit inhibition at levels that are considered clinically meaningful. Hence, it can be inferred that acalabrutinib exhibits a notable level of selectivity, thereby potentially reducing the occurrence of the intended adverse effects. (Liu *et al*, 2021). In contrast to ibrutinib, acalabrutinib has more advantageous pharmacological characteristics, including a rapid rate of oral absorption, a shorter duration of action, and a reduced incidence of adverse effects (Byrd *et al*, 2016) End of text. This medication has been extensively utilized in clinical studies for the management of myelofibrosis, B-cell malignancies, multiple myeloma, Hodgkin lymphoma, and ovarian cancer. (Liu *et al*, 2021).

Zanubrutinib:

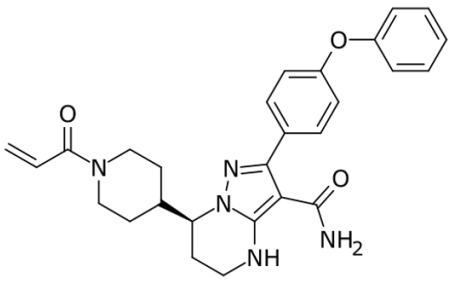

Figure 3.3: Acalabrutinib structure. (Brown et al, 2021)

Zanubrutinib, a novel compound (National Center for Biotechnology Information, 2023), is a 2nd-generation BTK inhibitor (Estupiñán *et al*, 2021). A predilection exists for BTK in comparison to TEC, and the aforementioned compound does not impede ITK functionality (Zain & Vihinen, 2021). Consequently, zanubrutinib exhibits a considerably reduced incidence of adverse effects compared to ibrutinib, encompassing atrial fibrillation, hypertension, and bleeding (Rozkiewicz *et al*, 2023). In a recent multicenter phase I research, zanubrutinib was delivered as monotherapy at doses of 160 mg two times or 320 mg once a day. The study found that zanubrutinib exhibited favorable tolerability and demonstrated clinically significant antitumor activity (Song *et al* 2022). Consequently, zanubrutinib exhibits a superior safety profile in individuals diagnosed with B-cell malignancies when compared to ibrutinib. Furthermore, it was observed that zanubrutinib exhibited a significantly extended duration of survival without any progression compared to ibrutinib in individuals diagnosed with SLL or relapsed or refractory CLL. Additionally, zanubrutinib was associated with a reduced incidence of adverse cardiac events (Brown *et al*, 2023). Moreover, an analysis of the ASPEN trial data comparing the economic implications of zanubrutinib and ibrutinib for the treatment of Waldenström macroglobulinemia (WM) in the USA demonstrated that this drug is a superior option in terms of cost-effectiveness when compared to ibrutinib (Muñoz *et al*, 2022)

Orelabrutinib:

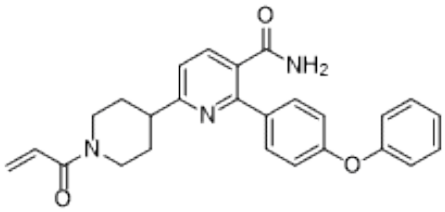

Figure 3.4: Orelabrutinib structure. (Dhillon, 2021)

In 2020, the China Food and Drug Administration authorized the use of Orelabrutinib, a new compound (Dhillon, 2021), for treating adults with mantle cell lymphoma who have received at least one prior treatment, and also adults with chronic lymphocytic leukemia or small lymphocytic lymphoma (CLL or SLL). (Dhillon, 2021). This binds with BTK irreversibly with covalent bonds and exerts a high level of inhibition (>90%) (Gu *et al*, 2022). At now, clinical trials are underway to investigate the potential applications of this treatment in the context of lymphoid malignancies and autoimmune illnesses (Robak *et al*, 2022)

Tirabrutinib:

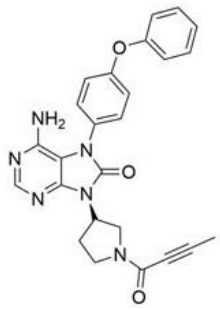

Figure 3.5: Tirabrutinib structure. (Lin & Andreotti, 2023).

In 2020, the Japan Pharmaceuticals and Medical Devices Agency granted approval for tirabrutinib to be used to treat recurrent or refractory primary central nervous system

lymphoma (PCNSL), as well as later for Waldenström macroglobulinemia (WM) and lymphoplasmacytic lymphoma. (Dhillon,2020). Based on the table it can be seen that ibrutinib Tirabrutinib is less selective than tirabrutinib. Tirabrutinib was delivered as a monotherapy to individuals with previously untreated or relapsed/refractory Waldenström macroglobulinemia (WM) in a phase II research trial called ONO-4059-05. The patients were administered a daily dose of 480 mg while in a fasting state. The study revealed that Tirabrutinib exhibited favorable efficacy and a well-tolerated safety profile. At 24 months, the progression-free survival (PFS) rate was 85% and the major response rate (MRR) was 78%. These results are either greater or comparable to those shown in earlier trials with BTK inhibitors, such as ibrutinib (MRR 78%, 18-month PFS 84%) and zanubrutinib (MRR 77%, 18-month PFS 85%). (Sekiguchi *et al*, 2022) (Tam *et al* , 2020)

Pirtobrutinib:

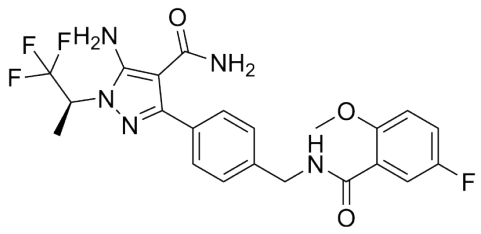

Figure 3.6: Pirtobrutinib structure (Aslanet al, 2022)

Pirtobrutinib, also known as LOXO-305, is an inhibitor with high selectivity that may reversibly bind to both wild-type BTK and BTK with kinase domain mutations through non-covalent interactions (Lewis & Cheah, 2021). Moreover, pirtobrutinib exhibits a significantly higher peak level, approximately 90 times greater, and a longer retention in human plasma, approximately 2.5 times longer, compared to ibrutinib. As a result, pirtobrutinib possesses a more favorable pharmacokinetic profile. (Aslan *et al*, 2022). It is presumed when this drug is

given in initial BTK inhibitor therapy in relapsed mantle cell lymphoma (MCL), it will exhibit superior efficacy compared to the covalent BTK inhibitors (acalabrutinib, ibrutinib or zanubrutinib). (Eyre *et al*, 2022)

3.4 BTK Inhibitors in Clinical Phase

Mutations in BTK that particularly impact Cys481 and the gatekeeper residue Thr474 have restricted the efficacy of irreversible BTK inhibitors. Researchers working in this specific setting have created reversible inhibitors that don't interact with Cys481. (Gomez *et al*, 2023) Presently, the majority of these entities are undergoing clinical or preclinical examination.

Table 2: Summary of BTK Inhibitors in Clinical Phase. (*Zhang et al, 2021); (Liu et al, 2021); (Estupiñán et al, 2021)*

Name	Type of Inhibitor	Indication	Activity (IC50)
Spebrutinib Synonyms CC- 292 AVL- 292	Irreversible	Chronic lymphocytic leukemia, Non-Hodgkin lymphoma	BTK IC_{50} = 9.2 nM ITK IC_{50} = 1050 nM TEC IC_{50} = 8.4 nM
Evobrutinib Synonyms M2951 MSC-2364447C	Irreversible	Rheumatoid arthritis, Multiple sclerosis	BTK IC_{50} = 8.9 nM
Vecabrutinib Synonym SNS-062	Reversible	Chronic lymphocytic leukemia, Small lymphocytic lymphoma	BTK IC_{50} = 1.9 nM
Fenebrutinib Synonym GDC-0853	Reversible	Rheumatoid arthritis, Systemic lupus erythematosus, Chronic spontaneous urticaria	BTK IC_{50} = 2.3 nM ITK IC_{50} = 1000 nM TEC IC_{50} = 1000 nM

Spebrutinib:

Spebrutinib, a member of anilides class (National Center for Biotechnology Information, 2023), is an orally accessible compound that has great selectivity and efficacy as a drug that covalently bindes to BTK and inhibits its activity. (Ribrag *et al*, 2022).

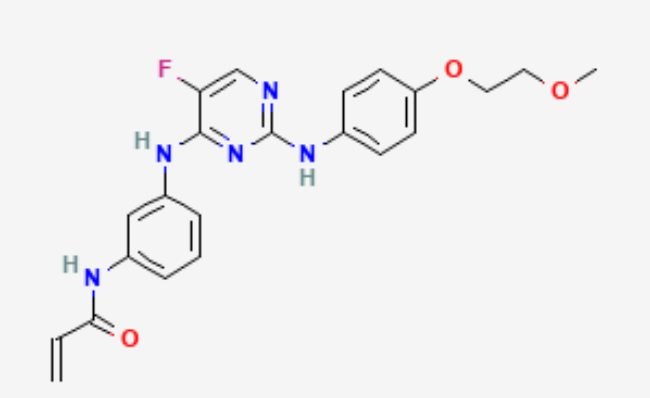

Figure 3.7: Spebrutinib structure. (National Center for Biotechnology Information, 2023)

Phase I studies enrolled individuals with relapsed or refractory CLL, Waldenström macroglobulinemia or B-cell non-Hodgkin lymphoma. The findings indicated that CC-292, when administered orally as a daily monotherapy, was well-tolerated at doses of up to 1000 mg one time or 500 mg two times daily. Patients who were administered CC-292 twice daily had BTK receptor occupancy exceeding 90% at both the 4 and 24-hour intervals. In contrast to ibrutinib and acalabrutinib, venetoclax exhibited comparatively reduced clinical efficacy, particularly in terms of response durability (Brown *et al*, 2016).

The safety and effectiveness of spebrutinib in treating patients with active rheumatoid arthritis (RA) were assessed in a separate phase II multicenter clinical investigation. In vitro, it was observed that spebrutinib exhibited a greater inhibitory effect on B-cell proliferation compared to T-cell proliferation. Spebrutinib further showed that it might inhibit osteoclastogenesis and the synthesis and release of myeloid and lymphoid cytokines. The study revealed that those who received spebrutinib saw elevated levels of overall CD19+ and

mature naive CD27−CD38−IgD+ B-cells, while observing a decline in transitional CD27−CD38+ B-cells. The median occupancy of the BTK receptor in peripheral blood was found to be 83%. Following the administration of spebrutinib, there was a significant reduction observed in the serum concentrations of chemokine ligand 13 (CXCL13), macrophage inflammatory protein-1β (MIP-1β), and carboxy-terminal collagen cross-linking telopeptide (CTX-I), which serves as a biomarker for bone resorption (Schafer *et al*, 2020)

Evobrutinib:

Evobrutinib, a member of the class of diphenylethers (National Center for Biotechnology Information, 2023), a new and highly selective inhibitor of the central nervous system-penetrating BTK, exhibits potent suppression of signaling pathways mediated by BCR and Fragment Crystallizable receptor. Evobrutinib's property makes it a promising therapeutic agent for treating autoimmune diseases, for instance, rheumatoid arthritis (RA), multiple sclerosis (MS), and related ailments. (Rozkiewicz *et al*, 2023)

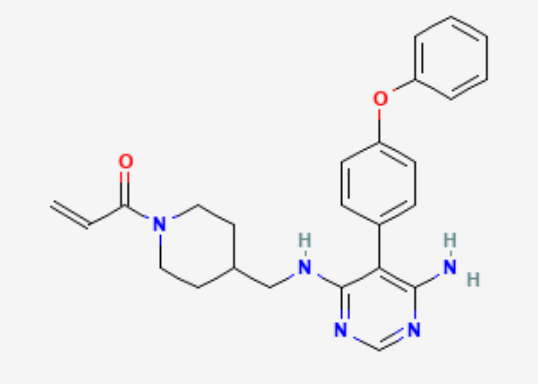

Figure 3.8: Evobrutinib structure. (National Center for Biotechnology Information, 2023)

Evobrutinib exhibits the ability to effectively inhibit its target for a significant duration following its elimination from the body by establishing a covalent connection with BTK, as indicated by previous studies. Moreover, evobrutinib exhibited a high degree of selectivity in an assay involving a comprehensive panel of 267 distinct kinases. Only two additional

kinases were found to selectively inhibit BTK to a degree more than 80% at a dose of 1 μM. In contrast, ibrutinib exhibited inhibition of 25 off-target kinases by more than 80% at the same concentration of 1 μM (Haselmayer *et al*, 2019). The results from Phase II trials demonstrated that evobrutinib exhibited favorable tolerability profiles among individuals diagnosed with MS, RA and SLE (Montalban *et al*, 2023); (Wallace *et al*, 2023).

Vecabrutinib:

A reversible, selective non-covalent BTK inhibitor is vecabrutinib. Preclinical research has effectively shown the efficacy against the C481 mutant and the wild-type BTK (Lewis & Cheah, 2021); (Aslan *et al*, 2021).

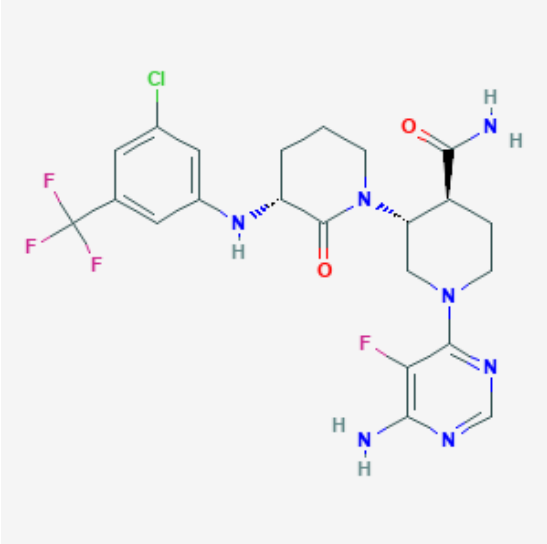

Figure 3.9: Vecabrutinib structure. (National Center for Biotechnology Information, 2023)

A recent phase Ib trial focused on dose escalation demonstrated that vecabrutinib exhibited favorable tolerability at a maximum dosage of 410 mg administered two times a day, which was the highest dose examined. However, the observed efficacy in patients resistant to BTK inhibitors, as evaluated at the explored levels of dose, was deemed insufficient to warrant the extension of this patient cohort in phase II trials (Allan *et al*, 2022)

Nevertheless, preclinical research shown that the coadministration of vecabrutinib and venetoclax resulted in a substantial augmentation of therapeutic efficacy, leading to a significant improvement in overall survival (Jebaraj *et al*, 2022).

Fenebrutinib:

Fenebrutinib is a selective, reversible BTKi. (Lewis & Cheah, 2021). The compound in question engages in hydrogen bonding interactions with residues K430, M477, and D539, rather than forming covalent connections with residue C481. Consequently, this compound exhibits potential utility in persons harboring the C481 mutation (Lewis & Cheah, 2021).

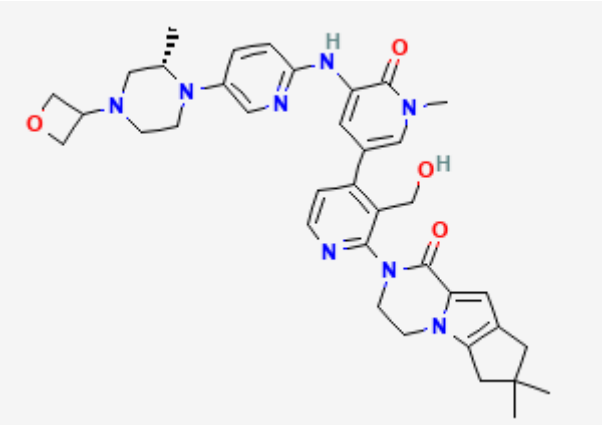

Figure 3.10: Fenebrutinib structure. (National Center for Biotechnology Information, 2023)

Only three out of the 286 examined kinases were found to be inhibited. The IC50 values obtained from the measurements demonstrated that the compound had a BTK selectivity that was more than 100-fold higher than that of BMX (153-fold), FGR (168-fold), and SRC (131-fold) (Qiu *et al*, 2021). Phase II clinical studies including individuals with RA, SLE, CSU, and B-cell malignancies have shown the effectiveness of fenebrutinib. (Isenberg *et al*, 2021).

Chapter 4

Role of BTK Inhibitors for Advancement of Cancer Therapy

4.1 Benefits of BTK Inhibitors

BTK inhibitors basically work by hindering the BCR path way, hence stopping the proliferation of neo-plastic cells (Feng *et al*, 2019). BTK has played a pivotal role in leading the significant transition in therapeutic strategies for chronic lymphocytic leukemia (CLL), shifting the focus from chemoimmunotherapy to targeted therapy (Ahn & Brown, 2021). Randomized studies have been carried out to evaluate the effectiveness of ibrutinib with various chemoimmunotherapy techniques, revealing favorable outcomes in favor of ibrutinib (Gaballa & Pinilla-Ibarz, 2021). So, it can help reduce the adverse effects of chemo-therapy by reducing the usage of chemo-therapy. Combining BTKis and other classes of drugs can substantially increase the survival rate of patients (Timofeeva & Gandhi, 2021). The combined administration of everolimus, an inhibitor of the mammalian target of rapamycin (mTOR), and PLS-123, a BTK inhibitor, exhibits a synergistic effect in suppressing the migratory and invasive properties of mantle cell lymphoma (MCL) cells. Furthermore, this cotreatment demonstrates a significant reduction in tumor development by 84.8% in the Granta519 xenograft model (Li *et al*, 2018). The administration of BTK inhibitors (BTKis) significantly prolonged the overall survival of individuals diagnosed with cancer (O'Brien *et al*, 2016). The 5-year progression-free survival (PFS) and overall survival (OS) rates were recorded as 70% and 85%, respectively, when ibrutinib was administered as the initial treatment (Ahn *et al*, 2020).

4.2 Limitations of BTK Inhibitors

While covalent BTK inhibitors have demonstrated effective disease control in most of patients, it is noteworthy that BTK inhibition as a standalone treatment is not adequate for

completely eliminating chronic lymphocytic leukemia (CLL) or achieving significant improvements in patient outcomes (Burger *et al*, 2019).

BTK inhibitors (BTKis) elevate the likelihood of hemorrhaging by impeding the process of platelet aggregation and adhesion (Lipsky *et al*, 2015). Hypertension and atrial fibrillation (Afib) are widely recognized as the prevailing cardiovascular toxicities associated with BTKis. In a retrospective analysis encompassing a cohort of 562 patients who underwent treatment with ibrutinib, it was observed that a considerable proportion (78%) experienced the onset or exacerbation of hypertension. Notably, this occurrence manifested early in the treatment regimen, with approximately half (50%) of the events transpiring within the initial two months of treatment initiation. Furthermore, this hypertension-related phenomenon exhibited an association with significant cardiovascular events, including atrial fibrillation (Dickerson *et al*, 2019).

Further added, apart from its effects on BTK, ibrutinib also acts as an inhibitor for many intracellular kinases such as B lymphoid tyrosine kinase (BLK), bone marrow kinase on chromosome X (BMX), and Janus kinase 3 (JAK3) (Messex *et al*, 2021). The absence of selectivity leads to several unintended side effects, such as skin and dermatological issues, allergic responses, elevated body temperature, swelling of lymph nodes, excessive fluid retention, presence of albumin in urine, gastrointestinal disturbances, hemorrhaging, susceptibility to infections, migraines, and the occurrence of atrial fibrillation (Tasso *et al*, 2021). However, it should be noted that ibrutinib exhibits off-target effects by inhibiting JAK3, ITK, and EGFR. (Rozkiewicz *et al*, 2023).A retrospective research conducted by Rogers *et al*. revealed an incidence rate of opportunistic infections (OI) of 1.9 per 100 person-years in a cohort of more than 500 patients who had BTKis treatment (Rogers *et al*, 2019). The termination of BTKi in chronic lymphocytic leukemia (CLL) is frequently

attributed to disease progression. The long-term evaluation of patients who had treatment with ibrutinib monotherapy revealed a 5-year progression-free survival (PFS) rate ranging from 70% to 92% for individuals receiving ibrutinib as their initial treatment, whereas those with relapsed chronic lymphocytic leukemia (CLL) exhibited a PFS rate of 40% to 44% (O'Brien *et al*, 2018)

The majority of BTKIs that are currently on the approved list target Cys481. Cys481 mutations in B-cell malignancies can lead to resistance development because of BTKCys481 mutations. In such a situation, it might be pointless to switch to an alternative inhibitor when resistance arises. (Chen *et al*, 2018)

Chapter 5

Conclusion

In the last few years, studying Btk has become more important in the area of medical chemistry. A lot of information about a number of Btk inhibitors (BtkIs) has been published in science journals and patents. The way leukemias and lymphomas are treated has changed a lot since BTK inhibitors came out. More and more scientific and medical proof has shown that Bruton's tyrosine kinase (BTK) is important in more than just B-cell cancers. It is also important in solid tumors, autoimmune diseases, and inflammatory diseases. A few BTKIs are being tested right now, and early results show that they may be safe and help cure different types of cancer. Still, because the follow-up time in the study was so short, it's still not clear how well these treatments will work in the long run. At the moment, there are only six approved Bruton's tyrosine kinase (BtkIs) inhibitors that can be used to treat different kinds of leukemia and lymphomas. Still, most of the first genaration BtkIs are currently going through clinical studies to see if they can release drugs for longer periods of time to treat inflammatory diseases like rheumatoid arthritis (RA) and multiple sclerosis (MS).

Regrettably, the emergence of resistance to first genaration inhibitors, specifically ibrutinib, as well as the occurrence of off-target adverse effects, particularly skin and dermatological issues, have necessitated the exploration of selective second-generation BtkIs that pose a reduced risk of toxicity in comparison to irreversible drugs. The following examples of approaches that have been used to combat resistance issues include,

- (Proteolysis targeting chimeras) PROTAC molecules (Tasso *et al*, 2021)
- A multi-target strategy using substances that can simultaneously target BTK and FAK (Yang *et al*, 2020)

- The use of traditional BTK inhibitors in conjunction with other chemotherapeutic agents, antibodies, or immunotherapies is undertaken with the objective of impeding various intracellular signal pathways. (Tasso *et al*, 2021)
- The nanoformulations of BTKIs, such as gold nanoparticles, polymeric nanoparticles, and aqueous nanosuspensions, have demonstrated the potential to mitigate toxicity while enhancing absorption and bioavailability. (Brullo *et al*, 2021)

The pursuit of novel BTKIs continues to captivate the attention of both the academic community and the pharmaceutical business, as it remains a crucial focus in the realm of medicinal chemistry research, given its therapeutic significance.

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