A Computational Approach: Proposing Potential Inhibitors by Targeting SRC/BCR-ABL Kinase in Acute Lymphoblastic Leukemia through Drug Repurposing

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

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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 have acknowledged all main sources of help.

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Approval

The thesis titled “A Computational Approach: Proposing Potential Inhibitors by Targeting SRC/BCR-ABL Kinase in Acute Lymphoblastic Leukemia through Drug Repurposing” submitted by Nusrat Zahan Nisha (20346059), of Summer, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

Acute Lymphoblastic Leukemia (ALL) is a hematological malignancy characterized by the overproduction of immature white blood cells. Patients with Philadelphia chromosome-positive ALL (Ph+ ALL) face significant challenges, including drug resistance and disease relapse, primarily driven by the BCR-ABL fusion protein. This study investigates 500 FDA-approved drugs to identify potential candidates for repurposing. The goal was to find drugs that can inhibit the SRC/BCR-ABL protein. Molecular docking was carried out using dasatinib, a known inhibitor of the protein as a reference. The analysis found several candidates with superior binding affinities compared to dasatinib. Six drugs were selected based on their superimposition to the reference drug and interaction profiles. They are acarbose (anti-diabetic), amrubicin (anti-neoplastic), cetirizine (antihistamine), dabrafenib (anti-neoplastic), streptomycin (anti-mycobacterial), and teniposide (anti-neoplastic). These findings suggest promising alternatives for targeting the BCR-ABL fusion protein, necessitating further experimental validation through in vitro and in vivo studies to confirm their efficacy and safety.

Keywords: SRC/BCR-ABL; Acute Lymphoblastic Leukemia (ALL), Ph+ ALL, Molecular Docking

Dedication

***Dedicated to my late father*.**

Acknowledgement

First and foremost, I am deeply grateful to Almighty Allah (SWT) for granting me the strength, patience, and guidance throughout this journey. Without His countless blessings, this accomplishment would not have been possible.

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List of Acronyms

ALL	Acute Lymphoblastic Leukemia
WHO	World Health Organization
NOS	Not Otherwise Specified
ICD-O	The International Classification of Disease for Oncology
Ph+ ALL	Philadelphia chromosome-positive Acute Lymphoblastic Leukemia
GvHD	Graft vs Host Disease
SKF	SRC Kinase Family
BCR	Breakpoint Cluster Region
ABL	Abelson proto-oncogene
M-BCR	Major Breakpoint Cluster Region
CML	Chronic Myeloid Leukemia
m-BCR	Minor Breakpoint Cluster Region
minor-BCR	Minor Breakdown Cluster Region
CC	Coiled Coil
GEF	Guanine nucleotide Exchange Factor
Tyr177	tyrosine 177
GRB2	Growth Factor Receptor-Bound protein 2

FABD	F-actin binding domain
RAS	Rat sarcoma
GAB2	GRB2-Associated Binder 2
CRKL	CRK Like Proto-Oncogene, Adapter Protein
MAPK	Mitogen-activated protein kinases
PI3K	Phosphoinositide-3-kinase
mTOR	Mammalian target of rapamycin
JAK2	Janus Kinase 2
STAT5	Signal transducer and activator of transcription 5
TKIs	Tyrosine Kinase Inhibitors
FDA	Food and Drug Administration
GEO	Gene expression omnibus
ADME	Absorption-Distribution-Metabolism-Excretion
CADD	Computer-aided drug design
LBDD	Ligand based drug design
SBDD	Structure based drug design
QSAR	Quantitative structure activity relationship
EGFR	Epidermal growth factor receptor
PDB	Protein data bank

SDF	Structure data file
ADMET	Absorption-Distribution-Metabolism-Excretion-Toxicity
PPIs	Protein pump inhibitors
NSAIDs	Non-steroidal anti-inflammatory drugs
Caco-2	Cancer coli-2
QPlogBB	Brain/blood partition coefficient
PSA	Van der Waals surface area of polar nitrogen and oxygen atoms
CNS	Central nervous system
BBB	Blood brain barrier
LYS	Lysine
GLU	Glutamic ACID
ASP	Aspartic acid

Chapter 1

Introduction

1.1 Acute Lymphoblastic Leukemia

Acute Lymphoblastic Leukemia (ALL) is a type of cancer of lymphocytes where there are too many immature white blood cells in the blood and bone marrow. These immature lymphocytes, commonly referred to as lymphoblast lose their ability to carry out their primary function- fighting infection. ALL is considered the most common cancer in children which can occur both in children and adults; it has been reported that 80% of ALL occur in children at the age between the age of 1-14 years (Ekpa et al., 2023; Terwilliger & Abdul-Hay, 2017).

According to Ekpa et al (2023), in 2019 approximately 6000 cases of ALL were estimated, accompanied by 1500 deaths in the United States demonstrating 1.38 per 100,000 people diagnosed with ALL every year with the highest incidence observed in children between one to four years. The American Cancer Society estimated the new cases of ALL for both sexes in 2024 will rise by 6,550 whereas estimated deaths from ALL for both sexes in 2024 will be 1,330 ("2024-Cancer-Facts-and-Figures-Acs," 2024). Moreover, the overall survival rate of 5 years is 72%. But the survival rate is different for elderly patients (55 years) which is 20% indicating an inverse relation between age and survival (Aldoss et al., 2019). Complete remission, defined as the disappearance of all signs of cancer in response to treatment, was achieved in 76% of patients, increasing to 91% following second-line treatment. However, 40% of patients experienced relapse, with a median progression-free survival (PFS) of 12.2 months and a median overall survival (OS) of 18.3 months (Sas et al., 2019).

1.1.1 Types of Acute Lymphoblastic Leukemia

According to the World Health Organization (WHO), acute lymphoblastic leukemia is classified into three main types (Table 1) (Terwilliger & Abdul-Hay, 2017). The types are described below.

1) B-cell acute lymphoblastic leukemia, not otherwise specified (NOS), is the most common type of lymphoblastic leukemia. In this subtype, the cancer cells are moderate in size, contain limited cytoplasm, and develop from immature B-cell lymphocytes (Szymańska & Park, 2018).
2) B-cell acute lymphoblastic leukemia with recurrent genetic abnormalities is further subclassified into 11 categories according to the 5th edition of the WHO classification (Alaggio et al., 2022).

Table 1: Subtypes of B-cell lymphoblastic leukemia with recurrent genetic abnormalities (Alaggio et al., 2022; Lilljebjörn et al., 2016; Szymańska & Park, 2018; Tasian, 2023)

Name of Subclassifications	Occurrence
B-cell lymphoblastic leukemia with high hyperdiploidy	More common in children (approximately 25% of all B-ALLs) while occur in adult only 7%-8% of all B-ALL cases
B-cell lymphoblastic leukemia with hypodiploidy	About 1%-5% cases of all B-ALL cases in both children and adult
B-cell lymphoblastic leukemia with iamp21	Around 2% of all B-ALL cases in children

B-cell lymphoblastic leukemia with BCR::ABL1 fusion	t(9;22)(q34;q11.2), 25% common of all B-ALL cases in adult while 2%-4% in children
B-cell lymphoblastic leukemia with BCR::ABL1-like features	10%-25% of all B-ALL cases proportional with age
B-cell lymphoblastic leukemia with KMT2A rearrangement	t(v;11q23), mainly common in infants under 1 year old
B-cell lymphoblastic leukemia with ETV6::RUNX1 fusion	t(12;21)(p13;q22), mostly occur in children (25% cases of all B-ALL) but not in infants
B-cell lymphoblastic leukemia with ETV6::RUNX1-like features	Similar to ETV6::RUNX1 fusion but lacks its fusion gene, instead combined ETV6 and IKZH1 lesions present
B-cell lymphoblastic leukemia with TCF3::PBX1 fusion	t(1;19)(q23;p13.3), around 6% of B-ALL cases among children
B-cell lymphoblastic leukemia with IGH::IL3 fusion	t(5;14)(q31;q32), <1% of all ALL cases including both children and adult
B-cell lymphoblastic leukemia with TCF3::HLF fusion	T(17;19), <1% of all B-ALL cases

This study focuses on BCR-ABL1 fusion of B-ALL, also referred as Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia with translocation t(9;22)(q34;q11.2), which is one of the high-risk subtypes with 20-30% cure rate in children when treated with chemotherapy only, but gives promising result when patients are administered tyrosine kinase inhibitors in combination with chemotherapy (Schultz et al., 2010).

3) T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive subtype of acute lymphoblastic leukemia characterized by the proliferation of malignant immature T-cell lymphocytes, leading to blood cancer. This subtype accounts for approximately 15% of cases diagnosed in children and 25% in adults. Although combination treatments for T-ALL result in high overall survival rates, 20% of children and 40% of adult patients experience disease recurrence (Fattizzo et al., 2020; Szymańska & Park, 2018).

1.2 Available Treatment Options for ALL

For ALL, treatment outcomes differ in higher and lower-income countries based on the availability of modern treatments. In high-income countries, 8 to 9 of every 10 children diagnosed with ALL have been cured and experienced long-term survival (cure rate exceeds more than 80%). In comparison, at least 15% or more of the patients from low-income countries experience early death with the same treatment. This disparity may occur due to several factors including delayed diagnosis, inadequate hospital infrastructure, insufficient medical supplies, etc. (Rivera & Ribeiro, 2014). Treatment options available for Acute lymphoblastic Leukemia include the following:

i. The standard treatment option for ALL is chemotherapy following induction, consolidation and maintenance therapy. Here, induction is done to achieve complete remission of cancer in the body using vincristine, corticosteroids and anthracycline (Terwilliger & Abdul-Hay, 2017). Following induction therapy, consolidation therapy is given to ensure and achieve the strength and depth of previously achieved remission of cancer and lastly, maintenance therapy is given for extended periods for prolonged remission, thus increasing progression-free survival and overall survival (Joks et al., 2015). However, many patients, particularly lower-risk patients and children of ALL may die from the associated side effects of chemotherapy rather than the cancer cells. Severe acute side effects of chemotherapy can affect all organs of the body by causing mucositis (40% of the patients suffer from mucositis after using anti-metabolites or alkylating agents used in case of conditioning therapy before hematopoietic stem cell transplantation), central neurotoxicity (10 to 15% of the patients of ALL suffers from central neurotoxicity with symptoms such as steroid psychosis, seizures, posterior reversible encephalopathy syndrome-PRES, etc may lead to neurocognitive defects), peripheral neuropathy (caused by vincristine), bone toxicities (osteoporosis caused by leukemia and use of corticosteroids, prednisolone or dexamethasone, seen in 20% children newly diagnosed with ALL) and so on (Schmiegelow et al., 2017).
ii. Another treatment option for ALL is targeted therapy which targets the specific protein or cells causing ALL with specific genetic problems and it gives more effective results while reducing the amount of chemotherapy and associated side effects by using tyrosine kinase inhibitors such as imatinib, dasatinib, nilotinib, ponatinib (Lejman et al., 2021). Even though they give fewer side effects, the related side effects of targeted therapy cannot be ignored which include

pulmonary hypertension, thromboembolism, exanthema, colitis, hepatosis, cytokine -release syndrome, and many more (Kroschinsky et al., 2017).

iii. Immunotherapy, another alternative treatment option, where medication strengthens the body's immune system so that it can recognize and destroy cancer cells in the body, it includes monoclonal antibodies (blinatumomab and Inotuzumab ozogamicin) and CAR T-cell therapy (brexucabtagene autoleucel and tisagenlecleucel). According to the American Cancer Society, monoclonal antibodies are not only associated with nervous system problems but also severe infusion reactions as well as veno-occlusive disease. In addition, CAR T-cell therapy can cause life-threatening cytokine release syndrome, nervous system problems, and low blood counts (Treating Acute Lymphocytic Leukemia (ALL), 2024).

iv. In stem cell transplant, after killing the cancer cells using a high dose of chemotherapy, blood-forming stem cells are transplanted in the patient (Treating Acute Lymphocytic Leukemia (ALL), 2018). Despite having 40%-45% complete remission rate, it gives an overall survival rate of less than 10% (Ribera, 2011). It also has treatment-related mortality rate of 10%-30% due to its toxicity involving infection and graft vs host disease (GvHD) (Calvo et al., 2022).

Despite the treatment options mentioned, there is a need to find better and affordable treatment options due to the available life-threatening side effects. Even though current treatments give 5-year overall survival rate exceeding 90%, the necessity of advanced strategies with manageable side effects of therapy cannot be overlooked.

1.3 SRC/BCR-ABL Kinase

SRC/BCR-ABL tyrosine kinase is involved in the development of ALL, specifically Ph^+ ALL where the proteins are overexpressed causing the production of too many white blood cells.

1.3.1 SRC Kinase

Src proto-oncogene is a non-receptor protein-tyrosine kinase (SKFs) that plays a role in signaling pathways involving cell growth, cell cycle progression, cytoskeletal rearrangement, transcription, nervous system function, division, migration, and cell survival. SKFs have a total of 11 enzymes and can be divided into three groups: group 1 includes Src, Fyn, Yes, and Fgr, group 2 includes Blk, Hck, Lck, and Lyn and lastly group 3 includes Frk, Srm, and Brk. Additionally, SKFs contain four Src domains- a unique domain (with an N-terminal 14 carbon myristoyl group), an SH3 domain, an SH2 domain and an SH1 domain (Roskoski, 2015).

1.3.2 ABL1 Kinase Domain

This structure is known as a bi-lobal structure as it consists of two lobe- N (myristoylated NH_2 terminal) and C lobe (COOH terminal). The N-lobe comprises αC helix and P-loop or phosphate binding loop which helps in ATP binding and initial recognition of substrate, while the C-lobe act as a peptide substrate binding site. Between the two lobes, the activation site is present, containing both an ATP-binding pocket and an activation loop or A-loop. When A-loop acts as a platform for substrate interaction due to its open confirmation, the ABL1 kinase domain switches to active confirmation which remains stable through phosphorylation of tyrosine residue Y393. The active state has a characteristic which is the "DGF-in" motif (comprises of three tyrosine residues- D381, F382, and G383 which are involved in pointing the ATP-binding site, coordinating with Mg^{2+} and positioning within the hydrophobic pocket) of the A-loop. During the inactive state, folding of the A-loop leads to the "DFG-out" motif feature. As a result, Y393 residue becomes unphosphorylated. Alongside, significant rotation occurs at DFG motif resulting in F382 occupying the ATP-binding site and interacting with other residues instead of positioning within

hydrophobic pocket. This helps stabilize the inactive confirmation and leads to the creation of a specificity pocket behind the T315 residue which enhances the binding specificity of several tyrosine kinase inhibitors (TKI) (Wu et al., 2024).

During the inactive state, together SH2 and SH3 domains act as a clamp in which, SH2 domain binds to C-lobe strongly, restraining its movement and the SH3 domain attaches with N-lobe, thus inhibiting ABL1 kinase activation. SH2 and SH3 domain creates a rigid structure through many intermolecular interactions which further gives an inhibitory effect on ABL1. However, phosphorylation of Tyr-245 in the SH2-kinase linker region may weaken the binding of SH3 domain, hence activation can occur. On the other hand, the SH2 domain may dissociate from the C-lobe if it binds to the phosphorylated tyrosine of the ABL1 substrate which may also result in the activation of the ABL1 domain. Both cases specifically result in the removal of the rigid structure of SH2-SH3 as well as the clamp. Due to the removal of rigid structure and clamp, SH2 and SH3 domain no longer gives an inhibitory effect on ABL1 kinase, allowing it to disrupt the autoinhibition and helps ABL1 to adopt elongated confirmation. Lastly, it initiates phosphorylation and downstream signaling pathways as shown in Figure 2 (Wu et al., 2024).

1.4 SRC/BCR-ABL1 Kinase in Acute Lymphoblastic Leukemia Development

BCR (breakpoint cluster region)-ABL1 (Abelson proto-oncogene) oncoprotein is a constitutively active tyrosine kinase resulting from the fusion of BCR and ABL gene which forms on chromosome 22 (Al Hamad, 2021). The fusion is formed by the fusion of the entire coding region of the ABL1 gene (Abelson murine leukemia virus homolog, exons 2-11, chromosome 9) to the BCR (breakpoint cluster region) gene on chromosome 22 (Bernt & Hunger, 2014). Breakpoint cluster of the BCR gene can occur at exons 13 or 14, known as Major Breakpoint Cluster Region

or M-BCR, and a combination of the breakpoints of BCR and ABL1 exon 2 (a2) results fusion translocation gene referred to as e13a2 (b2a2) or e14a2 (b3a2) as well as it generates a protein called $p^{210BCR-ABL1}$ with molecular weight of 210 kDa. Protein p210 is more commonly found in chronic myeloid leukemia (CML) patients and almost one-third of Ph+ ALL patients. But if the breaks of BCR gene occur in exon 1, also known as minor Breakpoint Cluster Region, m-BCR, combines with the same portion of ABL1, then it results in the generation of e1a2 gene alongside a protein called $p^{190BCR-ABL1}$ with a molecular weight of 190 kDa. p190 is found in two-thirds of Ph+ ALL patients and a few cases of CML. Another breakpoint cluster region known as the minor Breakdown Cluster Region (minor-BCR), between exon 19 BCR and ABL1 exon 2 results in a fusion protein called p230BCRABL1, present in chronic neutrophilic leukemia (Bernt & Hunger, 2014; Braun et al., 2020).

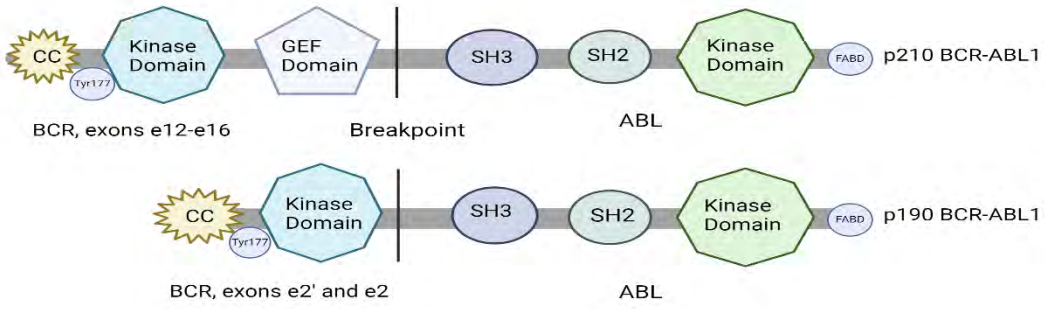


Figure 1: Two isoforms (p210 and p190) of fusion protein BCR-ABL1 with their domain arrangement (made using BioRender)

In Figure 1, CC represents coiled-coil domain which can facilitate dimerization and autophosphorylation, Tyr177 (tyrosine 177 serves as a docking site for GRB2 when phosphorylated), GEF domain or guanine nucleotide exchange factors for G-protein signaling, SH3 and SH2 domain binds to phosphorylated tyrosine residue and proline-rich peptide accordingly and lastly FABD as F-actin binding domain (Bernt & Hunger, 2014; Reckel et al., 2017).

Due to the fusion of BCR and ABL kinase, the constitutively active tyrosine kinase BCR-ABL1 gives rise to downstream signaling pathways leading to many oncogenic consequences. When Tyr177 becomes phosphorylated, it functions as the docking site for GRB2, which then initiates the RAS signaling pathway as shown in Figure 2. Besides, dimerization and autophosphorylation of coiled coil domain contribute to the phosphorylation of other substrates including GAB2, CRKL and CBL which induce RAS, MAPK, PI3K, AKT, and mTOR pathways (Braun et al., 2020). Among them, the PI3K-AKT-mTOR downstream pathway led to protein synthesis while RAS and MAPK pathway induced from GAB2 eventually leads to the growth and proliferation of cancer cells. On the other hand, BCR-ABL1 fusion protein also interacts with SRC family kinases and activates three of the SRC family kinases which are Lyn, Hck, and Fgr (Bernt & Hunger, 2014). They are also responsible for activating the downstream signaling pathway JAK2-STAT5 which causes increased transcription and cell survival, eventually helping in cell growth.

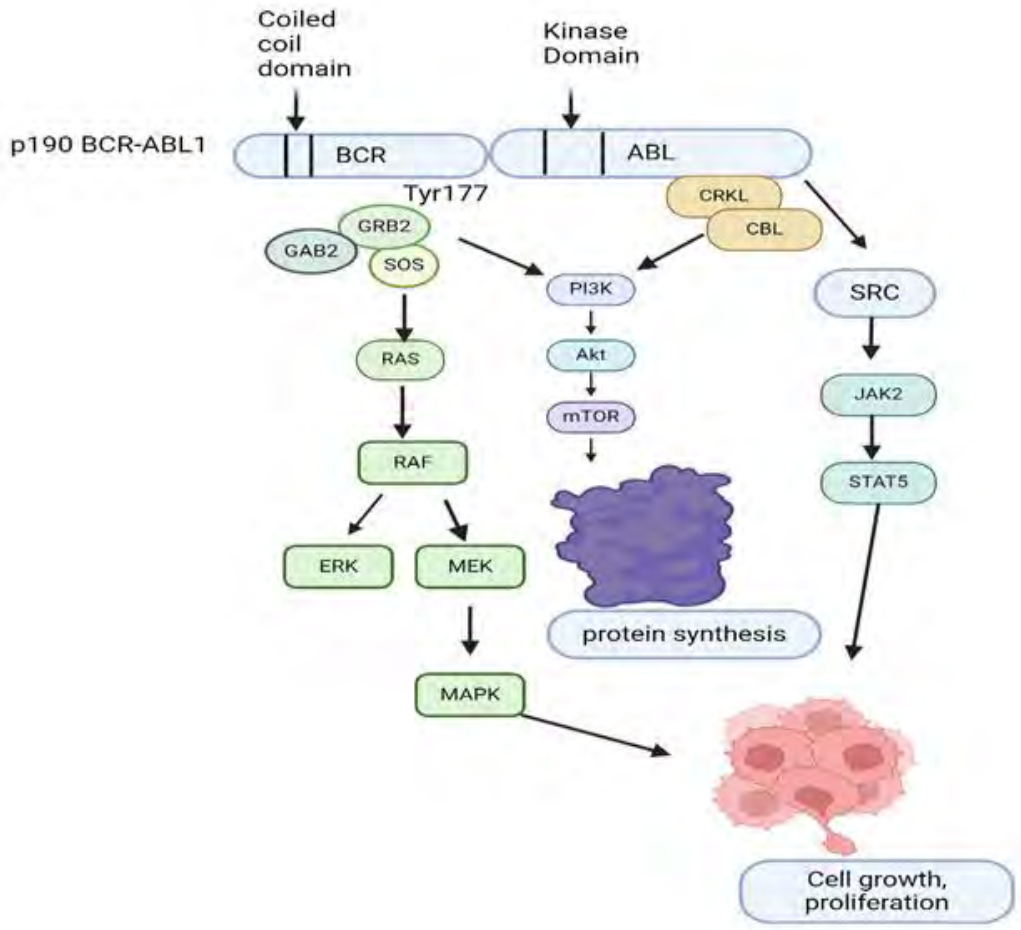


Figure 2: BCR-ABL1 induced downstream signaling pathway in Ph$^+$ ALL (made using BioRender)

1.5 Tyrosine Kinase Inhibitors (TKIs) and their Mechanisms of Resistance

The identification of BCR-ABL1 kinase as a key driver in the development of both chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) has provided a strong rationale for targeting this protein in cancer treatment development. Imatinib, first generation TKI was approved by FDA in 2001; it was found to be

more effective against CML and less effective against Ph^+ ALL. Imatinib exerts its action by inhibiting BCR-ABL1 kinase so that it cannot induce the downstream signaling pathways.

Despite that, 5-year follow-up results still demonstrated that 17% of patients developed resistance to imatinib and could not inhibit the targeted BCR-ABL1 kinase. Mechanism of resistance can be both BCR-ABL1 kinase-dependent where patients relapse after achieving remission and BCR-ABL1 kinase-independent where patients fail to achieve any response (Braun et al., 2020).

Kinase domain mutation is one of the common dependent resistance mechanisms involving G250E, Y253F/H, E255K/V, M351T, and F359V/C mutation in the kinase domain of ABL1 kinase (Poudel et al., 2022). This may occur due to the third intermediate confirmation of ABL1 kinase domain which is also called 'Src-like inactive ABL1'. In this confirmation, a compacted form of the A-loop of ABL1 kinase blocks the access of the substrate binding site causing the kinase domain to become inactivate. However, unlike typical inactive ABL1 which features a 'DGF-out' motif, Src-like inactivate ABL1 features a 'DGF-in' motif. Moreover, as the αC helix swings away from the active site, it results in a larger ATP binding site which facilitates DFG motif flipping allowing a transition between active/inactive confirmation switches. Due to this structural change, kinase domain mutation occurs which facilitates ABL1 kinase activity enhancing its ability to escape TKI targeting (Wu et al., 2024).

Apart from them, T3151 mutation also occurs, known as gatekeeper mutation in which the breaking of hydrogen bonding leads to mutation as the drug cannot bind any longer (Jędraszek et al., 2022). Second-generation TKIs- dasatinib and nilotinib were developed due to this mechanism of resistance. Dasatinib acts as a dual inhibitor by inhibiting both BCR-ABL1 kinase and SRC kinase, so it is more potent for Ph^+ ALL patients. Even though dasatinib and nilotinib are free from

imatinib-resistant mutations, they are resistant to T3151 mutation. Third-generation TKI, ponatinib, and recent FDA-approved asciminib can be used to treat patient-developed T3151 mutation.

Approximately 50% of patients with resistance are due to kinase domain mutations, indicating that the other half of people with poor response to treatment or recurrence of disease are due to BCR-ABL1 kinase-independent resistance. Independent resistance may involve leukemic cells creating alternate de novo parallel bypass pathways such as alternative activation of MAPK pathway, JAK/STAT pathway, PI3K/AKT pathway *etc* (Poudel et al., 2022).

The emergence of resistance mechanisms necessitates the need for even more effective therapies, expanding the available treatment options for Ph^+ ALL.

1.6. Computational Biology in Drug Repurposing

Drug repurposing involves utilizing a strategy of using an existing drug or drug candidate for a medical condition or indication for which it was not originally intended to use (Kulkarni et al., 2023). According to some reports, around 30% of repurposing attempts are successful in achieving approval for marketing in contrast around 10% of success rate for new drug application. Additionally, new drug discovery and development may take 10 to 17 years to be finally approved while repurposing drugs take 3-12 years alongside reducing half of the cost (Krishnamurthy et al., 2022).

Through drug repurposing, established safety profiles of approved drugs in humans are developed and tested for efficacy against specific diseases other than the one for which they were originally developed. With this approach drug development process is accelerated into preclinical and

clinical trial bypassing many steps, thereby minimizing the risk of failure and lowering cost (Kulkarni et al., 2023).

The drug repurposing approach involves connecting networks among drugs, genes, and diseases with the help of large sets of gene expression data, for instance, an existing drug was repurposed with a potential application in which drug-disease pair recognized by comparing drug-gene expression patterns with those associated with disease in the Gene Expression Omnibus (GEO). Moreover, investigation of biological pathways helps understand drug targets to identify diseases that a drug could effectively treat which is not currently indicated for that disease (Pan et al., 2014).

Drug repurposing incorporates several computational biology techniques in developing drugs. Here, computational biology consists of computer science, math, and statistics with life science. It helps to study and analyze biological systems of molecules, cells, tissue, organs, and even the entire organism by using computer-based *in silico* methods. This technique makes it easy for scientists to understand any complicated biological process, enhancing drug discovery and development as well as the whole drug repurposing process (Narang, 2023).

A new drug discovery usually takes 6-12 years costing billions of dollars with a success rate in clinical testing of less than 15% and 50% of the compounds fail due to poor ADME properties (Zhang et al., 2022). In contrast, the computational method allows rapid screening of a large compound library and identifies potential binders with simulation and visualization techniques, for example- this method was used in screening inhibitors for tyrosine phosphate-1B, linked to diabetes where among 365 virtually screened compounds, 127 compounds were blocked the enzyme, showing a success rate of 35% (Sliwoski et al., 2014). One of the most common techniques is computer-aided drug design (CADD) among computational biology techniques

which uses methods like molecular docking, virtual screening, and pharmacophore modeling to identify drug candidates. The drug discovery process is expedited through ligand-based (LBDD) and structure-based drug design (SBDD). For LBDD, known ligands with biological activity are chosen and their characteristics are analyzed to create a quantitative structure-activity relationship (QSAR) model. Using this model, virtual screening is conducted on a compound library to find new leads which are further optimized for drug development (Zhang et al., 2022).

Metformin, a well-known antidiabetic agent has been repurposed as an oncology drug, targeting not only breast cancer but also colorectal, endometrial, prostate, and non-small cell lung cancer with EGFR. It has already reached the phase 3 clinical trial against all five-cancer types both as monotherapy or combination therapy (Hijazi et al., 2023).

Thus, drug discovery can be accelerated using computational biology techniques as well as this technique reduces cost and failure rates in drug discovery.

1.7 Molecular Docking

The computational technique of analyzing the conformation and orientation of molecules into the binding site of a macromolecular target or a specified protein is called molecular docking (Torres et al., 2019). It helps to predict the binding affinity of ligands to receptor proteins by predicting the strength of binding interaction between them. Alongside the strength of binding interaction, it also reveals the biochemical process involving the interaction. Binding affinity assesses the ligands optimal binding of the drug to the receptor protein (Agu et al., 2023). Ligands with high binding affinity are considered potential drug candidates as high binding affinity means stronger binding interaction or stronger impact of ligand on the physiological function of the receptor protein (Seo

et al., 2021). Some of the commonly used docking software developed includes AutoDock Vina, DockThor, GOLD, FlexX, and Molegro Virtual Docker (Torres et al., 2019).

The process primarily consists of two steps: first, ligand conformations are tested for compatibility with the protein's active site, and then the binding affinity of the drug to the active site of the protein is found.

This method has been widely used in hit identification, lead optimization, ADMET prediction, structure elucidation and repurposing (Agu et al., 2023).

1.8 Rationale of the Study

The BCR-ABL fusion protein is a key driver in the pathogenesis of Acute Lymphoblastic Leukemia (ALL), especially in the Ph-positive (Philadelphia chromosome-positive) subtype. ALL is a type of leukemia that primarily affects children but can also occur in adults. It is characterized by the overproduction of immature white blood cells (lymphoblasts), which accumulate in the bone marrow and blood. ALL affects 1.8 per 100,000 people every year among which 25% are children (Ekpa et al., 2023; Kakaje et al., 2020). While survival rates for ALL have improved significantly due to advances in treatment, the disease remains challenging due to its heterogeneity and the potential for relapse. For instance, Ph-positive ALL, driven by the BCR-ABL fusion protein, is associated with a poorer prognosis and more difficult treatment outcomes. Despite the availability of some effective treatments like tyrosine kinase inhibitors (TKIs) for Ph-positive ALL, many patients experience resistance to these therapies or have inadequate responses. New drugs are needed to overcome resistance mechanisms and improve outcomes for patients with refractory or relapsed disease.

Drug repurposing, using molecular docking allows for the virtual screening of numerous compounds from databases, which can identify potential inhibitors of BCR-ABL without the need for immediate laboratory synthesis and testing. These in silico studies can rapidly assess a large number of compounds from databases like PubChem without the need for extensive laboratory work. This accelerates the drug discovery process and reduces associated costs. It provides preliminary data on how well various compounds might bind to the BCR-ABL protein, helping to prioritize which compounds should be tested further in experimental assays. It can reveal new potential drug candidates that might be more effective or have fewer side effects than current treatments. As such, different classes of FDA-approved drugs were investigated in this study to propose potential candidates that could possibly inhibit SRC/BCR-ABL1 kinase with better pharmacokinetic properties.

Molecular docking predicts the binding affinity and interaction modes of drugs with the BCR-ABL protein. This can reveal which drugs are most likely to inhibit the protein's activity. Understanding how drugs interact with BCR-ABL at the molecular level helps in elucidating their mechanism of action and in designing more potent and selective inhibitors. Furthermore, the study may help in overcoming drug resistance in ALL.

Chapter 2

Methodology

The study employed an *in silico* approach to investigate potential drug compounds for treating Acute Lymphoblastic Leukemia. This involved using computational biology techniques and molecular docking to analyze the binding affinities of FDA-approved drugs and their protein-ligand interactions. The study commenced with a comprehensive literature review to elucidate the characteristics of Acute Lymphoblastic Leukemia (ALL), including its classification, available treatments, targeted proteins, tyrosine kinase inhibitors (TKIs), and associated drug resistance mechanisms. Subsequently, three-dimensional structure of the target protein (SRC/BCR-ABL) and FDA approved ligands were retrieved from databases for molecular docking analysis to assess protein-ligand binding affinity. Finally, a detailed examination of protein-ligand interactions was undertaken to identify the precise binding pocket within the protein.

2.1 Software, Online Tools and Databases Used for the Study

Different software and online tools were used to obtain the three-dimensional structures of proteins and ligands involved in the study. The software and online tools were utilized to assess binding affinity and analyze the protein-ligand interactions to propose potential candidates against ALL. The protein, SRC/BCR-ABL, was retrieved from the RCSB Protein Data Bank (PDB). A compound library was constructed using the online databases PubChem and DrugBank.

Table 2 contains the list of major software and online tools used in the study with their corresponding version of each.

Table 2: Software and online tools used in the study.

SL No.	Software and Online tools	Version
1.	Open Babel GUI	2.4.1
2.	PyMOL	1.7.4.5
3.	AutoDock Tools	1.5.6
4.	AutoDock Vina	1.1.2
5.	Discovery Studio	16.1.0.15350
6.	Maestro, Schrodinger	11.0

To carry out the in silico experiments efficiently, six software programs and online tools were utilized. The software and online tools provide a wide range of functionality.

2.1.1 Open Babel GUI

This software specifically helps to overcome the challenges of converting chemical structures from one format to another in computational modeling which is why it is used in this study to convert the SDF format of ligands into PDB format. It acts as an open-source chemical toolbox involving over 110 formats available to interchange (O'Boyle et al., 2011).

2.1.2 PyMOL

PyMOL is a molecular graphics tool, extensively used in visualizing three-dimensional structures of compounds including protein, nucleic acids, small molecules, electron densities, and surfaces (Yuan et al., 2017). It is one of the major tools that is extensively used in this study to visualize the protein structure and to check the superimposition.

2.1.3 AutoDock Tools

A tool used in docking experiments to prepare molecule files and ligands before performing docking through protonation, charge calculation, and specifying bonds of proteins and ligands (Morris et al., 2009). The purpose of using this tool in this study is to prepare the ligands as well as protein prior to docking.

2.1.4 AutoDock Vina

AutoDock Vina is used for molecular docking and virtual screening with enhanced binding mode prediction and calculation of grid maps (Trott & Olson, 2010). Binding affinity which is the most important part of this study is determined by this software. It is considered the first choice in performing docking due to its rapid docking speed along with high accuracy (Tang et al., 2022).

2.1.5 Discovery Studio

Discovery Studio, a comprehensive software package was used to determine the amino acid complexes, distance, category and types of bonds between the ligands and the protein.

2.2 Preparation of Protein

The protein was selected based on certain criteria including its association with ALL, expression system, resolution, completeness, druggability, and validation report among others. As such, SRC/BRC-ABL (PDB ID: 3QRJ) was selected. The protein undergoes mutation at position 93. The 3D structure of the protein was downloaded in PDB format from RCSB-PDB (Protein Data Bank). The protein was curated using Maestro, and Schrodinger by removing co-crystallised ligand, and water molecules present in the structure, adding missing atoms, and refining and optimizing the structure. Binding pocket where the ligands will accommodate and interact with the protein are identified using PyMOL. As there were two chains (A, B) with similar amino acid

sequences, chain A was deleted from the protein using PyMOL. The curated protein file was prepared as the final protein for performing molecular docking through AutoDock Vina. With that purpose, the curated protein file was opened in AutoDock Tool and the polarity of the protein was changed by adding polar only hydrogen. In addition, amino acids of the protein were selected to cover them precisely by putting coordinates specifying the area of amino acids in grid box of AutoDock Tools. Finally, the protein was prepared and saved as 'protein.pdbqt' for performing docking using AutoDock Vina.

2.3 Preparation of Ligand

Different classes of drugs, including Protein Pump Inhibitors (PPIs), Antidepressants, Antiparkinson, Bronchodilators, Antidote, Anti-thyroid, Natural Compounds, local anesthetics, Anti neoplastic, Statins, Diuretics, Antidiabetic, NSAIDs, Antihypertensive, Anti-fungal, Anti histamines, Analgesic, Anti-viral, Anti mycobacterial, Anti emetics, Anti-convulsant, Antibiotics and Alpha blockers were chosen for this study.

3D structures of approximately 500 ligand molecules were downloaded as SDF files (https://pubchem.ncbi.nlm.nih.gov/) and PDB files (https://go.drugbank.com/). The SDF format ligands were converted to PDB format using Open Babel GUI. All the structures were converted to PDBQT format using AutoDock Tools and assigned charges.

All active bonds of every ligand were made non-rotatable through 'torsion tree' option of AutoDock Tool. Again 'set number of torsions' from 'torsion tree' option was selected to check whether active bonds of ligand became non-rotatable or not. After confirming the file was saved as 'ligand.pdbqt' and it was ready for further study.

2.4 Molecular Docking

Molecular docking was carried out using AutoDock Vina. The potential binding site was identified and defined using PyMOL. Based on the identification, a grid box was generated as per calculation around the binding site by setting the center and size parameters in the AutoDock Vina configuration file. The grid file and config.txt file based on the values obtained from the grid.txt file were saved. Protein.pdbqt and ligand.pdbqt files were placed in a folder. The folder then had 2 PDBQT files of the protein and ligand, the config.txt file and the grid.txt file. To initiate the docking, the Windows command was opened by typing Start Menu. This action opened a command prompt. The command used for docking is given below:

"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor protein.pdbqt --ligand ligand.pdbqt --config config.txt --log log.txt --out output.pdbqt.

The binding affinity values were compared to the reference drug. Only the drugs that gave better binding affinity than the reference drug were selected for further parts of the study.

2.5 Superimposition

The superimposition of a newly identified ligand with a reference drug after molecular docking is a crucial step. By overlaying the two molecules in PyMOL, structural similarities and differences can be identified. Superimposition allows for a direct comparison of the binding modes of the new ligand and the reference drug within the target protein's binding site. This analysis can reveal if the new ligand adopts a similar or different binding pose, which can impact its activity and selectivity. This step provides insights into how a drug interacts with the binding pocket. The specific interactions were analyzed in the next step.

2.6 Protein-Ligand Interaction

Discovery Studio (v16.1.0.15350) facilitates the visualization and quantification of interactions between small molecules and macromolecules, aiding in the identification of potential drug candidates and understanding their mechanisms of action. The output,pdbqt files obtained from docking results were needed along with the curated protein file. Firstly, the interaction was checked for the reference drug using Discovery Studio (v16.1.0.15350) where both output.pdbqt of reference drug and curated protein file were opened in two different windows. After that, the reference drug was selected as a ligand group and the structure of the reference drug was copied and pasted on the curated protein window, forming a drug-protein complex. Following that, hydrogen was added to the complex, and ligand interaction was chosen from non-bond interaction. Subsequently, the complex was labeled with amino acids. Thus, the complex showed binding sites of the reference drug. Using the "non-bond" option, the amino acid sequence, distance, category, and type of bond were analyzed. Following this process, the interactions of all ligands—selected based on strong binding affinity values—were examined to assess the overlap between the amino acids of the ligands and those of the reference drug. The ligand which did not overlap with any amino acid were discarded. The ligands with the most amino acid in common with those of the reference drug were selected.

Chapter 3

Result

3.1 Structure of the Protein

The structure of the selected protein, SRC/BCR-ABL was taken from RCSB Protein Data Bank (PDB ID:3QRJ) in PDB format. Then the protein was opened in PyMOL for further visualization.

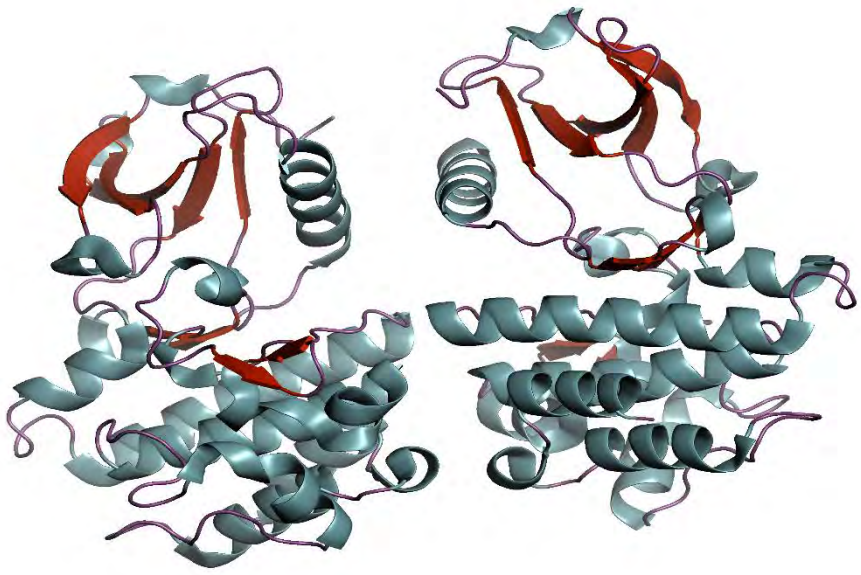


Figure 3: 3-Dimensional structure of protein SRC/BCR-ABL Kinase obtained from RCSB PDB (The crystal structure of human abl1 kinase domain T315I mutant in complex with DCC-2036)

3.2 Selection of Reference Drug

Dasatinib was chosen as the reference drug for this study. Dasatinib, a 2nd generation tyrosine kinase inhibitor, is also known as a dual inhibitor as it inhibits both SRC and BCR-ABL1 kinase. It is mainly used in the treatment of Philadelphia chromosome-positive (Ph+) acute lymphoblastic

leukemia and Ph+ chronic myeloid leukemia. It was approved by the FDA in 2006. 1st generation tyrosine kinase inhibitor, known as imatinib showed resistance in patients which led to the development of dasatinib. It overcame the imatinib resistance successfully as well as it can overcome resistance including ABL1 kinase domain mutations (Shen et al., 2020). At the same time, it inhibits both active and inactive forms of ABL kinase domain, resulting in overcoming resistance against ABL1 kinase domain mutation.

Dasatinib is an established inhibitor of the BCR-ABL protein, widely used in the treatment of Philadelphia chromosome-positive leukemias. Its well-characterized binding affinity and mechanism of action make it an ideal reference compound for molecular docking studies. By comparing other potential inhibitors to dasatinib, researchers can evaluate their effectiveness in disrupting BCR-ABL activity and identify promising candidates for further investigation (Fauziya et al., 2023).

The binding affinity of reference drug was -8.1 Kcal/mol. A more negative values indicates better binding affinity. The ligands that gave better binding affinities than the reference drug were selected for further study. A total of 283 drugs gave higher binding affinity values than the reference drug, dasatinib.

3.3 Results of Molecular Docking

After performing docking of approximately 500 ligands, 283 ligands were selected for further studies as they demonstrated better binding affinity values than the reference drug (-8.1 Kcal/mol). Table 3 demonstrates the binding affinities of the selected drugs.

Table 3: Docking Result of reference drug and selected ligands

Classification	Name of Drug	Binding Affinity (Kcal/mol)
Reference Drug	Dasatinib	-8.1
Local Anesthetics	Eletriptan	-9.1
	Ergotamine	-11.4
	Lasmiditan	-9.2
	Methysergide maleate	-8.6
	Bupivacine	-8.6
PPIs	Pantoprazole	-8.6
	Rabeprazole	-8.8
	Lansoprazole	-9.1
	Dexlansoprazole	-8.5
Antiparkinson	Apomorphine	-8.7
	Benzotropine	-8.8
	Biperiden	-8.4
	Bromocriptine	-11.1
	Levodopa	-8.2
Bronchodilators	Formoterol	-9
	Glycopyrronium bromide	-8.5
	Aclidinium	-9.1
	Tiotropium	-8.2
	Ipatropium	-9.3
	Aripiprazole	-9.6
	Asenapine	-8.4
	Brexpiprazole	-9.1
	Clozapine	-8.8

	Lurasidone	-13
	Iloperidone	-10.7
	Paliperidone	-8.4
	Quetiapine	-8.5
	Ziprasidone	-9.4
Antidote	Atropine	-8.6
	Flumazenil	-8.6
	Naloxone	-9.4
Natural Compounds	Sophoronol A	-9.4
	Calanquinone A	-8.5
	Cannabisin G	-9
	Acronine	-8.7
	Cudraphenone A	-9.6
	Oxocularine	-8.2
	Pumilin	-10.3
	Rosmanol	-9.8
	Curcumin	-10.1
	Oxyberberin	-9
	Bexarotene	-9.3
	Etoposide	-9.2
	Methotrexate	-9.2
	Trastuzumab Deruxtecan	-9.9
	Ponatinib	-10
	Asciminib	-9.9
	Mitomycin	-8.5

Anti-Neoplastic	Bexarotene	-9.3
	Sorafenib	-10.2
	Teniposide	-10
	Epirubicin	-9.7
	Alitretinoin	-8.4
	Erlotinib	-8.9
	Imatinib	-8.8
	Pemetrexed	-9.6
	Daunorubicin	-9.3
	Irinotecan	-9.4
	Etoposide	-14.6
	Doxorubicin	-9.4
	Topotecan	-10
	Capecitabine	-9.2
	Procarbazine	-8.3
	Estramustine	-9.4
	Mitoxantrone	-9.1
	Sunitinib	-8.3
	Omacetaxine mepesuccinate	-8.8
	Belinostat	-8.3
	Romidepsin	-8.2
	Pixantrone	-8.7
	Amrubicin	-12
	Lonidamine	-8.7
	Panobinostat	-8.3
	Trametinib	-8.6

	Dabrafenib	-9.8
	Idelalisib	-11.1
	Ceritinib	-10.4
	Olaparib	-10.3
	Lenvatinib	-8.6
	Nintedanib	-8.5
	Pirarubicin	-10.3
	Zorubicin	-8.6
	Megestrol acetate	-9.4
	Nilutamide	-8.5
	Fulvestrant	-10.6
	Exemestane	-9.3
	Bicalutamide	-10.1
	Bosutinib	-8.2
	Nilotinib	-9
	Vandetanib	-8.3
	Pazopanib	-8.4
	Vismodegib	-10
	Crizotinib	-8.8
	Vemurafenib	-9.9
	Regorafenib	-12.1
	Afatinib	-10
	Ibrutinib	-8.7
	Abiraterone	-9.5
	Midostaurin	-11.3
	Toremifene	-8.3

	Prednisolone	-9.6
	Testolactone	-8.9
	Methylprednisolone	-9.8
	Camptothecin	-9.8
	Halofuginone	-8.7
	Amonafide	-8.7
	((E)-9,10-Didehydroepothilone D)	-8.5
	Crocetin	-9.4
	Rubitecan	-10
	Seliciclib	-9.1
	Maxacalcitol	-10
	Annamycin	-10.8
	Semaxanib	-8.6
	Porfiromycin	-8.3
	Rabusertib	-10.8
	Niraparib	-8.8
	Ixabepilone	-8.8
	Sonidegib	-11.1
	Enzalutamide	-9.5
	Ribociclib	-9.3
	Dacomitinib	-10.7
	Binimetinib	-8.4
	Abemaciclib	-8.6
	Lorlatinib	-8.5
	Apalutamide	-9.2
	Larotrectinib	-9.1

	Entrectinib	-10.2
	Zanubrutinib	-12.1
	Pemigatinib	-9
	Darolutamide	-8.5
	Ripretinib	-10.4
	Capmatinib	-8.9
	Acalabrutinib	-11.3
	Vandetanib	-8.3
	copanlisib	-9.2
	Trimetrexate	-8.8
	Duvelisib	-10
	Momelotinib	-10.3
	Pralatrexate	-8.5
	Capivasertib	-8.7
Statins	Cerivastatin	-9.7
	Ezetimibe	-10.2
	Fluvastatin	-10.1
	Lovastatin	-10.7
	Mevastatin	-9.8
	Pitavastatin	-9.5
	Pravastatin	-8.6
	Rosuvastatin	-11.1
	Simvastatin	-10.8
	Chlorthalidone	-8.7
	Furosemide	-8.2
	Indapamide	-9

Diuretics	Metolazone	-8.9
	Bendroflumethiazide	-10.3
	Eplerenone	-9
	Spironolactone	-9.5
	Triamterene	-8.5
	Xipamide	-9.3
Antidiabetics	Acetohexamide	-9.1
	Dapagliflozin	-9.7
	Empagliflozin	-10.5
	Bexagliflozin	-10.5
	Ertugliflozin	-9.9
	Gemigliptin	-9.9
	Gliclazide	-9
	Glimepiride	-8.5
	Mitiglinde	-9
	Netoglitazone	-8.4
	Linagliptin	-8.7
	Saxagliptin	-9.5
	Sitagliptin	-9.8
	Vildagliptin	-9
	Pioglitazone	-8.4
	Repaglinide	-9.9
	Rosiglitazone	-8.4
	Tolzamide	-8.9
	Acarbose	-9.2
	Cangliflozine	-9.9

	Glipizide	-8.7
	Saroglitazar	-9.2
NSAIDs	Aclofenac	-8.8
	Celecoxib	-9.3
	Cimicoxib	-8.4
	Etoricoxib	-8.7
	Fenoprofen	-9
	Indomethacin	-8.8
	Indoprofen	-8.8
	Lumiracoxib	-8.2
	Naproxen	-8.4
	Phenylbutazone	-8.2
	Rofecoxib	-9.2
	Valdecoxib	-8.7
Antihypertensive – ACE Inhibitors	Benazepril	-8.7
	Lisinopril	-10.6
	Naftopidil	-9.8
	Perindopril	-8.6
	Quinapril	-10.1
	Ramipril	-9.1
	Trandolapril	-9.3
	Urapidil	-8.8
	Cilazapril	-9
	Enalapril	-9.4
Antihypertensive-ACE2 Receptor Antagonist	Azilsartan	-10.5
	Candesartan	-10.8

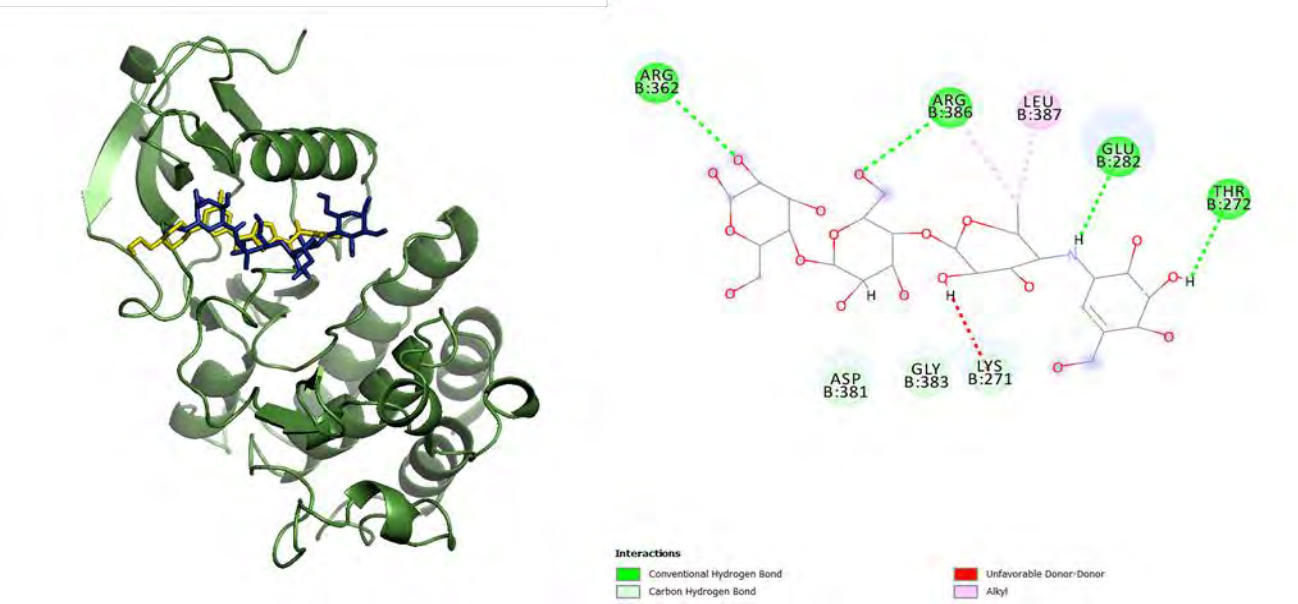
	Irbesartan	-9.9
	Losartan	-9.4
	Olmesartan	-11.5
	Valsartan	-10.6
	Eposartan mesylate	-9.4
	Fimasartan	-9.7
	Forasartan	-9.4
	Pratosartan	-11.4
	Tasosartan	-10.5
	Telmisartan	-10.1
Antihypertensive-Beta Blockers	Acebutolol	-8.2
	Labetolol	-9.5
	Penbutaolol	-8.3
	Talinolol	-8.9
Antihypertensive-Calcium Channel Blockers	Clevidipine	-8.2
	Diltiazem Hydrochloride	-8.2
	Nicardipine	-8.7
	Verapamil Hydrochloride	-8.7
Alpha Blockers	Alfuzosin	-8.8
	Terazosin	-8.3
	Doxazosin	-10
Anti-Fungal	Itraconazole	-10.3
	Terbinafine	-8.3
	Terbinafine Hydrochloride	-8.3
	Voriconazole	-9
	Terconazole	-9.8

	Butenafine	-9.2
	Fenticonazole	-8.9
	Miconazole	-8.5
	Amorolfine	-8.6
Anti-Histamines	Cetirizine	-8.9
	Desloratidine	-8.2
	Hydroxyzine	-9.1
	Levocabastine	-9
	Levocetrizine	-9
	Loratidine	-8.6
	Cinnarazine	-9.3
	Mizolastine	-9.1
Analgesics	Bendazac	-8.9
	Clonixin	-8.4
	Donepezil	-8.9
	Slidenafil	-9.1
Anti-Viral	Abacavir	-8.6
	Nelfinavir	-10.3
	Netivudine	-8.2
	Famciclovir	-8.4
	Tenofovir alafenamide	-8.5
	Rilpivirine	-8.9
Anti-Mycobacterial	Streptomycin	-8.9
Anti-Emetics	Dexamethasone	-9.3
	Dolasetron	-8.8
	Aprepitant	-8.8

	Granisetron	-8.7
	Ondasetron	-8.4
	Palonosetron	-8.8
	Rolipitant	-11.1
Antidepressants	Citalopram	-8.4
	Escitalopram	-8.4
	Fluoxetine	-9.4
	Fluvoxamine	-9
	Vilazodone	-9.9
	Donepezil	-8.2
	Galantamine	-8.3
	Amoxapine	-8.3
	Protyrptiline	-8.3
	Maprotiline	-9
	Duloxetine	-8.3
Anti Convulsants	Carbamazepine	-9.2
	Oxcarbazepine	-9.3
Antibiotics	benzylpenicillin	-8.6
	Ciprofloxacin	-8.4
	Ampicillin	-8.5
	Doxycyclin	-10.4
	Nafcillin	-9.1
	Penicillin V	-8.7
	Oxacillin	-8.7
	Nemonoxacin	-9.3
	Carindacillin	-10.1

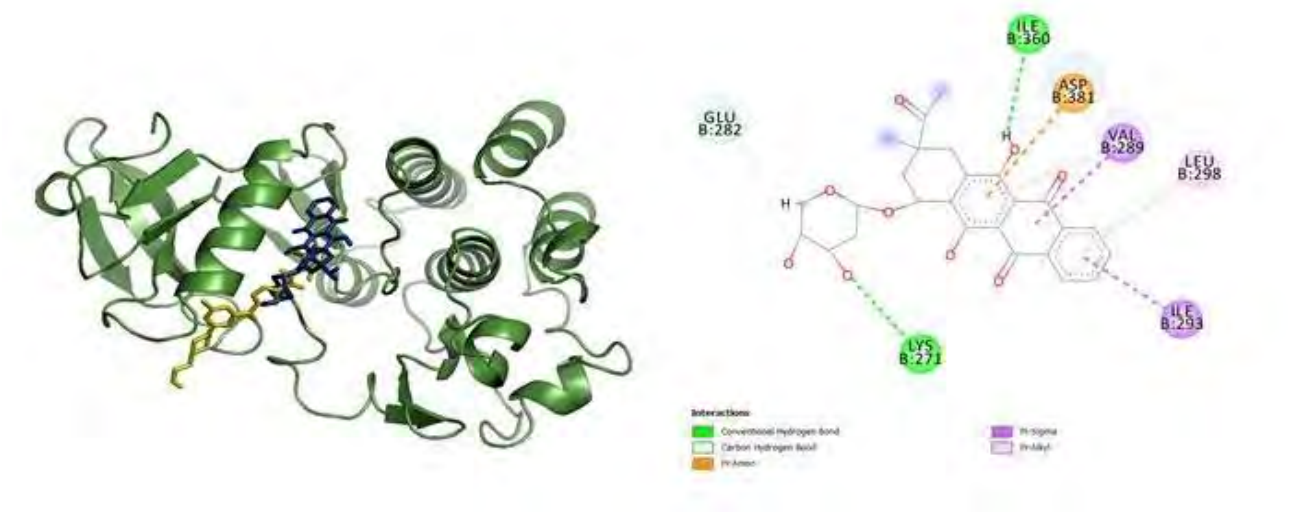
3.4 Superimposition

All the selected ligands after docking, were superimposed on the reference drug to find out if the reference drug and selected ligand overlap on each other within the target protein's binding site. The ligands that did not superimpose on the reference drug were discarded, resulting in a narrowed list of potential six ligands selected for further screening using Discovery Studio. Figures 4-9 show the potential ligands that effectively superimposed on the reference drug.



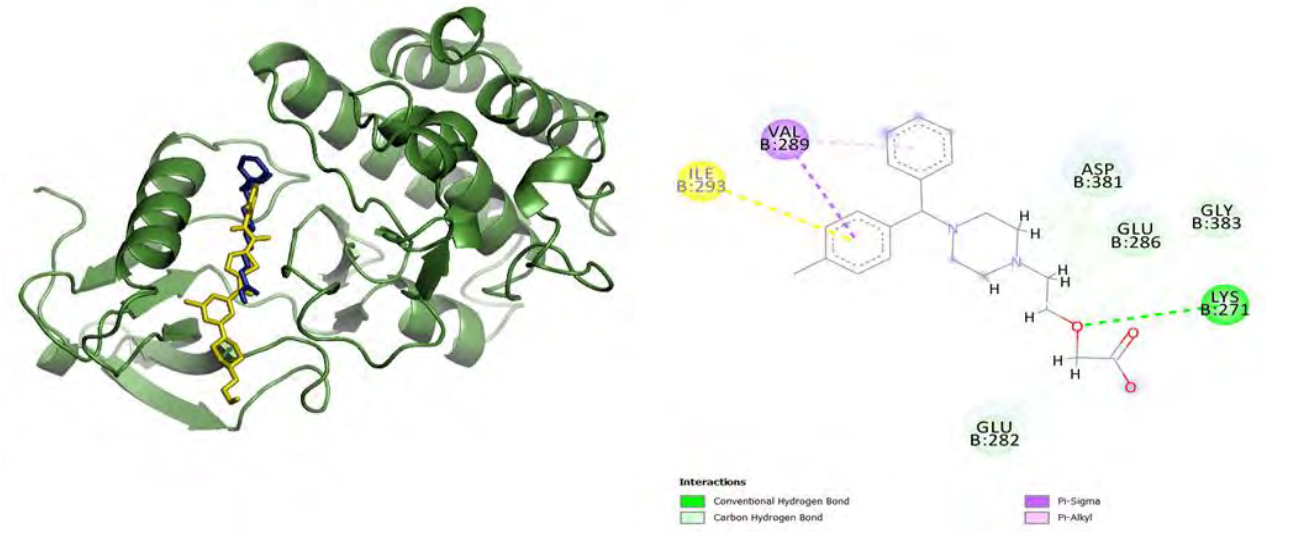
(a) **(b)**

Figure 4: (a) Superimposition of reference drug (Dasatinib, yellow) and ligand (Acarbose, blue) in the same binding pocket. (b) 2D diagram representing interaction of targeted protein (SRC/BCR-ABL) and Acarbose.



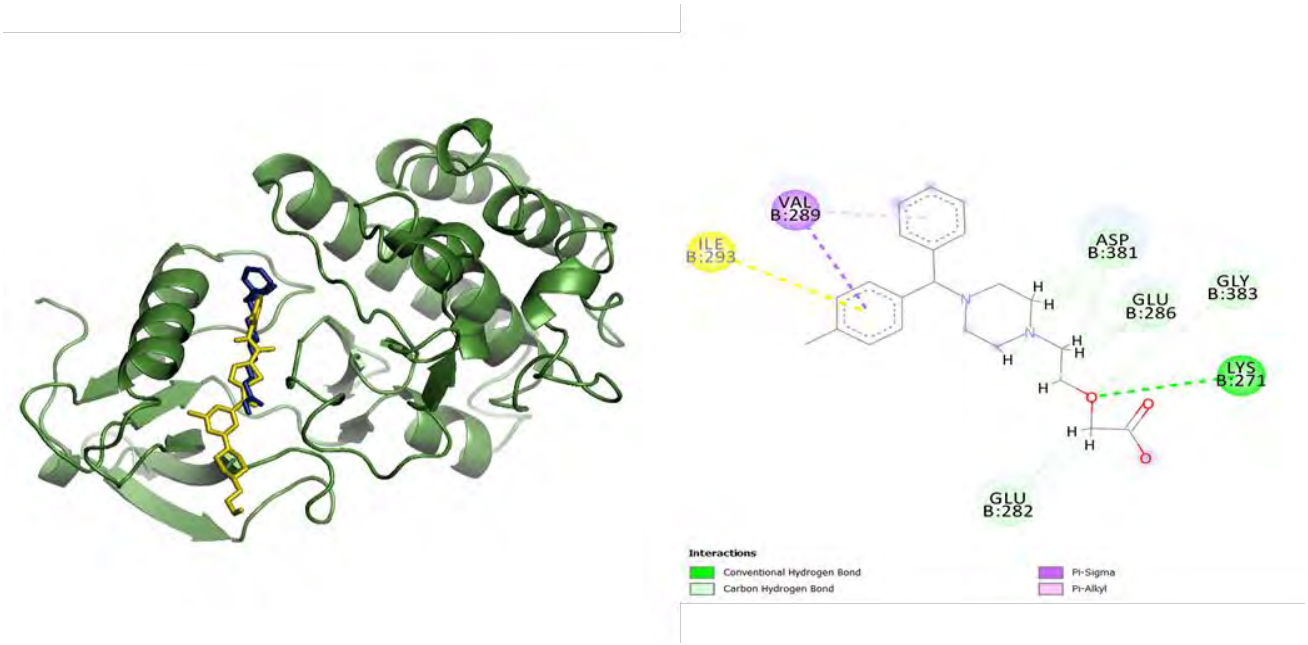
(a) **(b)**

Figure 5: (a) Superimposition of reference drug (Dasatinib, yellow) and ligand (Amrubicin, blue) in the same binding pocket. (b) 2D diagram representing interaction of targeted protein (SRC/BCR-ABL) and Amrubicin.



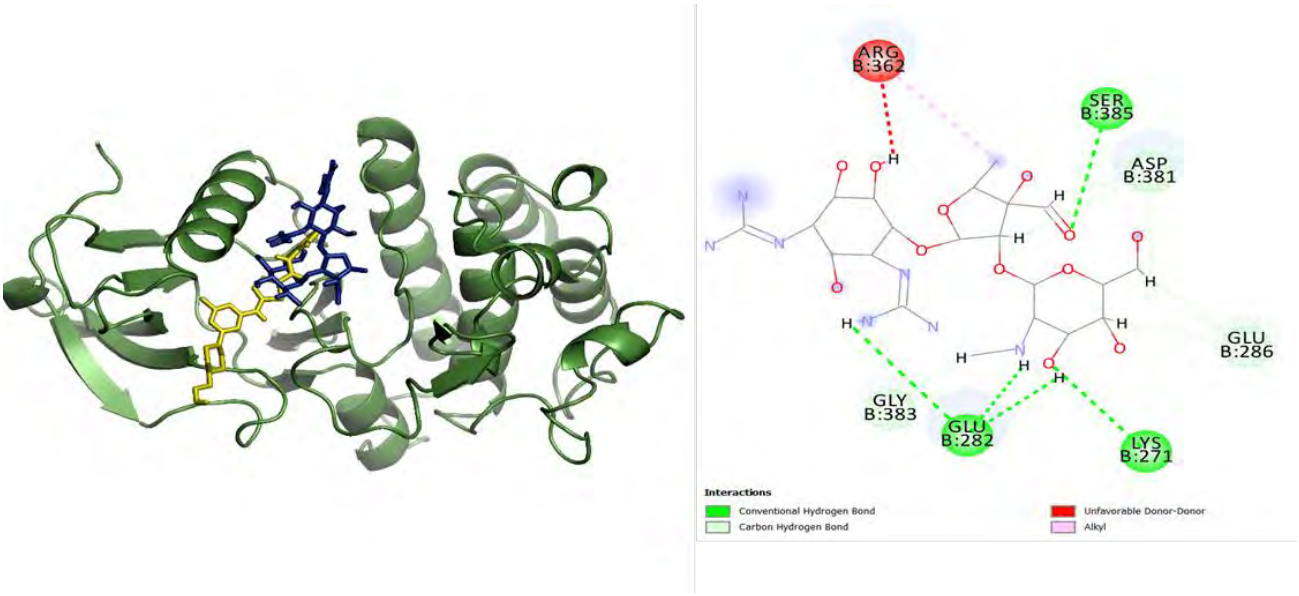
(a) **(b)**

Figure 6: (a) Superimposition of reference drug (Dasatinib, yellow) and ligand (Cetirizine, blue) in the same binding pocket. (b) 2D diagram representing interaction of targeted protein (SRC/BCR-ABL) and cetirizine.



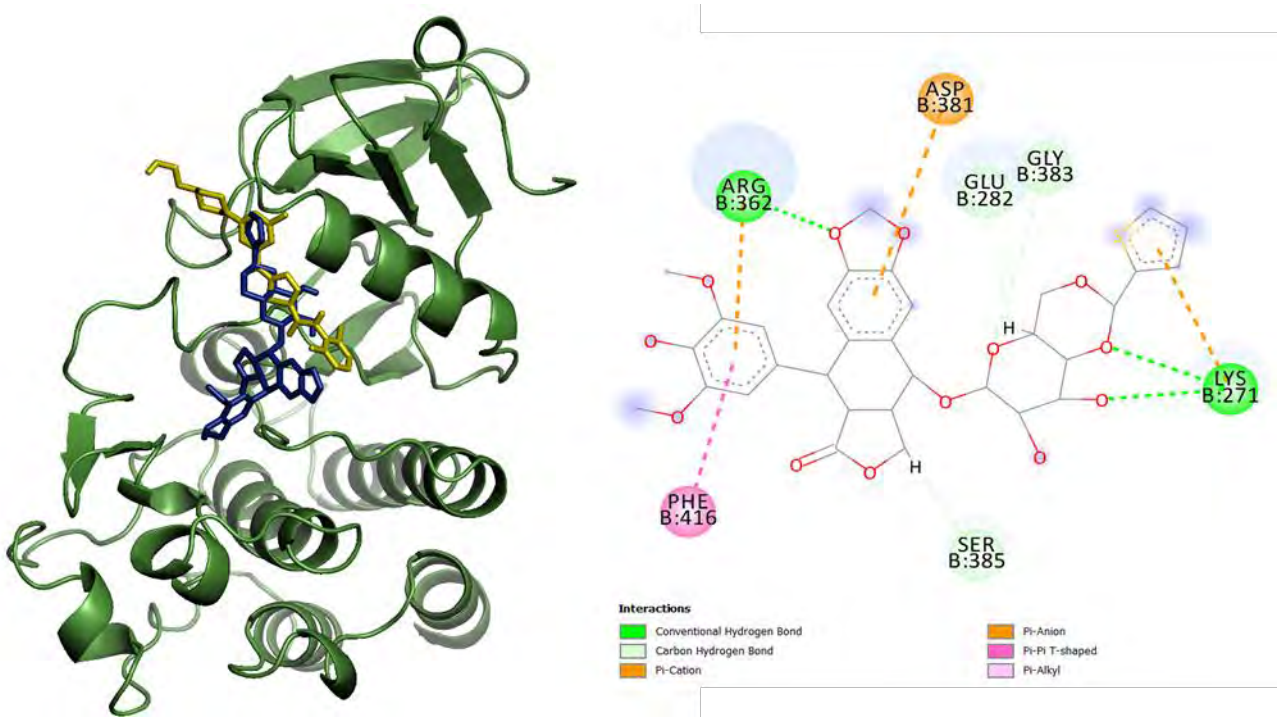
(a) **(b)**

Figure 7: (a) Superimposition of reference drug (Dasatinib, yellow) and ligand (Dabrafenib, blue) in the same binding pocket. (b) 2D diagram representing interaction of targeted protein (SRC/BCR-ABL) and dabrafenib.



(a) **(b)**

Figure 8: (a) Superimposition of reference drug (Dasatinib, yellow) and ligand (Streptomycin, blue) in the same binding pocket. (b) 2D diagram representing interaction of targeted protein (SRC/BCR-ABL) and streptomycin.



(a) **(b)**

Figure 9: (a) Superimposition of reference drug (Dasatinib, yellow) and ligand (Teniposide, blue) in the same binding pocket. (b) 2D diagram representing interaction of targeted protein (SRC/BCR-ABL) and teniposide.

3.5 Interaction Visualization of Protein and Ligand

Ligands that superimposed effectively with the reference drug were chosen for further study. Total 222 ligands were chosen for visualizing interaction. Through Discovery Studio, the interaction between protein and ligand was visualized as well as the amino acid sequences, distances, categories, and types of bonds were determined by observing the non-bonding interactions between them. Depending on the common amino acid sequence with the reference drug, ligands were identified for further evaluation. The drugs that had 2-3 common amino acids with dasatanib are listed in Table 4 below.

Table 4: Protein-ligand interaction demonstrating common amino acid sequence

Classification	**Name of Drug**	**Amino Acid**
Reference Drug	Dasatinib	LYS271, GLU282, ASP381
PPIs	Lansoprazole	LYS271, GLU282
	Dexlansoprazole	ASP381, GLU282
Bronchodilators	Brexpiprazole	ASP381, GLU282
	Clozapine	ASP381, GLU282
	Quetiapine	ASP381, GLU282
Natural Compounds	Cannabisin G	LYS271, ASP381
	Oxyberberin	ASP381, GLU282
Anti Neoplastics	Bexarotene	ASP381, GLU282
	Methotrexate	ASP381, GLU282
	Trastuzumab Deruxtecan	ASP381, GLU282
	Ponatinib	LYS271, ASP381
	Bexarotene	ASP381, GLU282
	Teniposide	LYS271, GLU282, ASP381
	Epirubicin	ASP381, GLU282
	Imatinib	ASP381, GLU282
	Daunorubicin	ASP381, GLU282
	Irinotecan	LYS271, ASP381
	Doxorubicin	ASP381, GLU282

	Topotecan	ASP381, GLU282
	Mitoxantrone	ASP381, GLU282
	Romidepsin	ASP381, GLU282
	Amrubicin	LYS271, GLU282, ASP381
	Dabrafenib	LYS271, GLU282, ASP381
	Ceritinib	ASP381, GLU282
	Pirarubicin	LYS271, ASP381
	Pazopanib	ASP381, GLU282
	Vemurafenib	ASP381, GLU282
	Regorafenib	LYS271, ASP381
	Ibrutinib	ASP381, GLU282
	Midostaurin	ASP381, GLU282
	Seliciclib	ASP381, GLU282
	Enzalutamide	ASP381, GLU282
	Binimetinib	ASP381, GLU282
	Abemaciclib	ASP381, GLU282
	Lorlatinib	ASP381, GLU282
	Ripretinib	ASP381, GLU282
	Momelotinib	ASP381, GLU282
	Pralatrexate	ASP381, GLU282
Statins	Cerivastatin	ASP381, GLU282

	Ezetimibe	ASP381, GLU282
	Mevastatin	LYS271, ASP381
Anti-Diabetics	Bexagliflozin	ASP381, GLU282
	Linagliptin	ASP381, GLU282
	Acarbose	LYS271, GLU282, ASP381
	Cangliflozine	ASP381, GLU282
	Glipizide	ASP381, GLU282
Antihypertensive – ACE Inhibitors	Quinapril	ASP381, GLU282
	Ramipril	ASP381, GLU282
Antihypertensive-ACE2 Receptor Antagonists	Olmesartan	ASP381, GLU282
Antihypertensive-Calcium Channel Blockers	Verapamil Hydrochloride	ASP381, GLU282
Anti-Fungal	Itraconazole	ASP381, GLU282
	Fenticonazole	LYS271, ASP381
Anti-Histamines	Cetirizine	LYS271, ASP381, GLU282
	Hydroxyzine	ASP381, GLU282
	Mizolastine	LYS271, GLU282
Analgesics	Bendazac	ASP381, GLU282
	Slidenafil	ASP381, GLU282
Anti-Viral	Rilpivirine	ASP381, GLU282
Anti Mycobacterials	Streptomycin	LYS271, ASP381, GLU282

Anti-Emetics	Dexamethasone	ASP381, GLU282
	Aprepitant	LYS271, GLU282
Anti-Depressants	Vilazodone	ASP381, GLU282
	Donepezil	ASP381, GLU282
Antibiotics	Nafcillin	ASP381, GLU282
	Nemonoxacin	ASP381, GLU282

3.5.1 Non-Bond Interaction

Six ligands were selected from all the ligands as they had all three amino acids in common with that of the reference drug. Table 5-11 shows the non-bond interactions of six ligands with targeted protein alongside distance, category, and type of bond.

Table 5: Non-bond binding interaction of dasatinib and protein SRC/BCR-ABL

Name	Distance	Category	Type
B: LYS271:NZ -: UNL1: N	3.28046	Hydrogen Bond	Conventional Hydrogen Bond
B: LYS271:NZ -: UNL1: N	2.90217	Hydrogen Bond	Conventional Hydrogen Bond
B: LYS271:NZ -: UNL1	3.4375	Electrostatic	Pi-Cation
B: GLU282:OE2 -: UNL1	3.5134	Electrostatic	Pi-Anion
B: ASP381:OD2 -: UNL1	3.72394	Electrostatic	Pi-Anion

Table 5 demonstrates non-bond interaction between the targeted protein and the reference drug, dasatinib. Among the five complexes formed, two hydrogen bonds and three electrostatic

interactions are present. One electrostatic interaction was a pi-cation type and the other two electrostatic interaction were pi-anion type. The distance of the bond ranged between 2.90-3.72 Å.

Table 6: Non-bond binding interaction of acarbose and protein SRC/BCR-ABL

Name	Distance	Category	Type
: UNL1:H - B: GLU282:OE2	2.09387	Hydrogen Bond	Conventional Hydrogen Bond
B: LYS271: CE -: UNL1:O	3.72756	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H24 - B: ASP381:OD2	2.0229	Hydrogen Bond	Carbon Hydrogen Bond

Table 6 shows information involving non-bond interaction between targeted protein and an anti-diabetic drug named acarbose. Only three amino acid which are in common with reference drug are shown in the table. All of the complexes formed hydrogen bonds which is important in protein-ligand interaction as well as enhancing receptor-ligand interaction (Chen et al., 2016). The distance of the bonds ranged between 2.02-3.72 Å.

Table 7: Non-bond interaction of amrubicin and protein SRC/BCR-ABL

Name	Distance	Category	Type
B: LYS271:NZ -: UNL1:O	3.06973	Hydrogen Bond	Conventional Hydrogen Bond
: UNL1:H18 - B: GLU282:OE1	3.09218	Hydrogen Bond	Carbon Hydrogen Bond
B: ASP381:OD2 -: UNL1	4.15588	Electrostatic	Pi-Anion

In Table 7, non-bond interaction of targeted protein and an anti-neoplastic drug named amrubicin is shown. Among the eight complexes formed, three are shown in the table which are in common with reference drug. There are two hydrogen bonds and one electrostatic (charged-charged)

interaction present. The electrostatic interaction was pi-anion in type aromatic ring interact with an electron donor molecule. The distance of the bonds ranged between 3.06-4.15 Å.

Table 8: Non-bond interaction of cetirizine and protein SRC/BCR-ABL

Name	Distance	Category	Type
B: LYS271:NZ -: UNL1:O	3.26384	Hydrogen Bond	Conventional Hydrogen Bond
: UNL1:H9 - B:ASP381:O	2.36948	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H10 - B:ASP381:O	2.32987	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H12 - B: ASP381:OD2	2.66144	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H14 - B:ASP381:O	2.87313	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H15 - B:ASP381:O	3.07296	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H27 - B: GLU282:OE2	2.69276	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H28 - B: GLU282:OE2	2.76218	Hydrogen Bond	Carbon Hydrogen Bond

Table 8 includes non-bond interaction of targeted protein and an anti-histamine drug named cetirizine. Eight complexes were in common with the reference drug, which is shown in the table. All eight complexes formed hydrogen bonds with distance ranging between 2.32-3.26 Å. It represents a good protein-ligand interaction due to the presence of all hydrogen bonds.

Table 9: Non-bond interaction of dabrafenib and protein SRC/BCR-ABL

Name	Distance	Category	Type
B: LYS271:NZ -: UNL1: N	3.39589	Hydrogen Bond	Conventional Hydrogen Bond
B: GLU282:OE1 -: UNL1	4.54172	Electrostatic	Pi-Anion
B: ASP381:OD2 -: UNL1	4.12649	Electrostatic	Pi-Anion

Table 9 shows non-bond interaction of targeted protein and an anti-neoplastic drug named dabrafenib. Seven amino acid complexes were formed among which common three amino acid complexes are shown in the table. The three amino acids formed one hydrogen bond and two pi-anion-type electrostatic interactions. The distance of the bonds ranged from 3.39-4.54 Å.

Table 10: Non-bond interaction of streptomycin and protein SRC/BCR-ABL

Name	Distance	Category	Type
B: LYS271:NZ -: UNL1:O	3.37151	Hydrogen Bond	Conventional Hydrogen Bond
: UNL1:H - B: GLU282:OE2	2.61939	Hydrogen Bond	Conventional Hydrogen Bond
: UNL1:H - B: GLU282:OE2	1.90199	Hydrogen Bond	Conventional Hydrogen Bond
: UNL1:H - B: GLU282:OE1	2.52559	Hydrogen Bond	Conventional Hydrogen Bond
: UNL1:H17 - B: ASP381:OD2	2.31881	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H34 - B: ASP381:OD1	2.55576	Hydrogen Bond	Carbon Hydrogen Bond
: UNL1:H36 - B:ASP381:O	2.65722	Hydrogen Bond	Carbon Hydrogen Bond

Table 10 contains information regarding non-bond interaction of targeted protein and an anti-mycobacterial drug named streptomycin. Seven amino acid complexes were formed and all seven complexes formed hydrogen bonds with the distance ranging from 1.90-3.37 Å. As all the complexes gave hydrogen bonds, it represents a good ligand-bond interaction.

Table 11: Non-bond interaction of teniposide and protein SRC/BCR-ABL

Name	Distance	Category	Type
B: LYS271:NZ -: UNL1:O	2.74061	Hydrogen Bond	Conventional Hydrogen Bond
B: LYS271:NZ -: UNL1:O	2.55766	Hydrogen Bond	Conventional Hydrogen Bond

: UNL1:H14 - B: GLU282:OE2	2.49797	Hydrogen Bond	Carbon Hydrogen Bond
B: LYS271:NZ -: UNL1	4.36808	Electrostatic	Pi-Cation
B: ASP381:OD2 -: UNL1	4.97654	Electrostatic	Pi-Anion

In Table 11 non-bond interactions of targeted protein and an anti-neoplastic drug named teniposide is shown. A total of eleven amino acid complexes were formed among which five amino acid complexes were given in Table 10 as they are in common with reference drug. There were three hydrogen bond and two electrostatic interactions present. Between the two electrostatic interactions, one was pi-cation and the other one was pi-anion type. All the bonds ranged from 2.49-4.97 Å in distance.

Chapter 4

Discussion

Under normal physiological cellular condition, the SRC family of tyrosine kinase plays crucial role in cell survival, proliferation, differentiation, and migration. But overactivation of the SRC kinase may lead to the development of cancer onset as well as cancer progression (Bagnato et al., 2020). On the contrary, the Abelson or ABL kinase is important in regulating many cellular activities during development and cellular homeostasis including survival, growth and migration under healthy conditions of a human body. However, factors such as DNA damage, oxidative stress, growth signals, and chemokines influence the overexpression of ABL kinase which may give rise to inflammation, tissue damage response, tumor growth, metastasis, etc. (Luttman et al., 2021).

This study initially emphasizes the contribution of SRC/BCR-ABL in the developing and progression of Acute Lymphoblastic Leukemia (ALL). More specifically, it explores the expression of BCR-ABL1 fusion protein in Philadelphia chromosome-positive ALL. The BCR-ABL1 fusion protein is found to be associated with 20%-30% of patients diagnosed with Ph+ ALL. Among them, 50% of the patients are aged more than 50 years (Eghbali, 2024).

Even though several treatment options are available against ALL, life-threatening side effects cannot be overlooked of these treatments. Some of the potent kinase inhibitors are accessible which are commonly used in the case of Ph+ ALL to target the BCR-ABL1 fusion protein. However, according to previous reports, resistance mechanisms against some tyrosine kinase inhibitors and recurrence of the disease emerge the necessity of a more potent drug. For this reason, drug

repurposing has been chosen as a way of finding an existing drug that can potently inhibit the SRC/BCR-ABL protein.

In this study, dasatinib, a 2nd generation tyrosine inhibitor was chosen as reference drug as it inhibits both SRC and BCR-ABL protein. Around 500 drugs of 23 different class were downloaded from PubChem. Molecular docking was performed of all the drugs with the targeted protein including reference drug utilizing AutoDock Vina and AutoDock Tool. Drugs with higher binding affinity than the reference drug (-8.1 Kcal/mol) were chosen for further study. 283 drugs gave higher binding affinity (Table 1). Then superimposition was performed to analyze the binding pocket of the drugs using PyMOL. The drugs with the same binding pocket of reference drug were selected for subsequent study. Examples of some drugs with the same binding pocket of reference drug and binding interaction (2D diagram) of the drugs and targeted protein were shown from Figure 4 to Figure 9. So, the list of potential drugs was narrowed down to 222 drugs after superimposition which were further visualized for interaction between the targeted protein and the narrowed-down drugs with the help of Discovery Studio. Along with interaction, Discover Studio also observed the amino acid sequence, distance, category and type of bonds between the targeted protein and the selected drugs. The interaction between targeted protein and reference drug showed 3 amino acid which are LYS271, GLU282, and ASP381. Drugs which had at least two amino acid sequences in common with reference drug were shown in Table 4. Finally, six drugs were proposed which had all three amino acids in common with the reference drug. The six drugs were acarbose (anti-diabetic), amrubicin (anti-neoplastic), cetirizine (antihistamine), dabrafenib (anti-neoplastic), streptomycin (anti-mycobacterial) and teniposide (anti-neoplastic).

Next, the non-bonding interaction of the selected six drugs and reference drug with the amino acid sequence, distance, category, and type of bond were shown from Table 5 to Table 11. The different amino acid sequences, distance, categories, and types of bonds of all six drugs and reference drug were also discussed after each table. Dasatinib, reference drug formed two conventional hydrogen bond, one electrostatic pi-cation, and two electrostatic pi-anion bonds in the non-bond binding interaction of the drug and targeted protein. Acarbose formed one conventional and two carbon-hydrogen bonds. Amrubicin gave one conventional, one carbon-hydrogen bond, and one electrostatic pi-anion bonds. Cetirizine formed one conventional and seven carbon hydrogen bonds which is better than dasatinib as it contains all hydrogen bonds. Dabrafenib formed one conventional hydrogen bond and two electrostatic pi-anion. Streptomycin formed four conventional and three carbon-hydrogen bonds which is also better than dasatinib. Lastly, teniposide formed two conventional, one carbon-hydrogen bond, one electrostatic pi-cation and one electrostatic pi-anion bonds.

Chapter 5

Conclusion

The study proposes six potential candidates - acarbose (anti-diabetic), amrubicin (anti-neoplastic), cetirizine (antihistamine), dabrafenib (anti-neoplastic), streptomycin (anti-mycobacterial) and teniposide (anti-neoplastic) which have significant potential to inhibit SRC/BCR-ABL protein, responsible for Philadelphia chromosome-positive ALL with limited treatment options or resistance issues. The selected drugs were identified through molecular docking, superimposition and protein-ligand interaction analysis. This highlights their potential for further investigations.

While this in silico analysis has identified promising potential candidates, experimental validation is essential to ensure the safety and efficacy of the selected drugs in inhibiting the targeted protein. Future studies should include molecular dynamics simulations over a specified duration, usually 100ns to assess the stability and interactions of the proposed six drugs. Additionally, further in vitro and in vivo studies are necessary to evaluate the efficacy and validate the findings of these candidates.

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