CHAPTER 3

Chemical Function Based Virtual Screening: Discovery of Potent Lead Molecules for the \textit{Bcr-Abl} Tyrosine Kinase Using VX-680
3.1 Introduction

Protein phosphorylation is a central regulatory strategy to alter the cellular functions, and protein kinases catalyze the transfer of the γ-phosphate of adenosine triphosphate (ATP) to acceptor proteins. Protein tyrosine kinases (PTKs) are critical regulators of cell proliferation, invasion, metastasis and cell survival (Edward & Sausville, 1999). Two classes of PTKs are present in cells, the receptor protein tyrosine kinases (RTKs) and the non-receptor protein tyrosine kinases (NRTKs). RTKs are transmembrane glycoproteins that are activated by the binding of their cognate ligands, and transduce the extracellular signal to the cytoplasm by phosphorylating tyrosine residues on the receptors themselves (autophosphorylation) and on downstream signaling proteins. NRTKs are integral components of the signaling cascades triggered by RTKs and by other cell surface receptors such as G protein-coupled receptors and receptors of the immune system.

NRTKs lack receptor-like features such as an extracellular ligand-binding domain and a transmembrane spanning region, and most NRTKs are localized in the cytoplasm (Neet & Hunter, 1996). NRTKs are anchored to the cell membrane through amino terminal modification, such as myristoylation or palmitoylation. In addition to a tyrosine kinase domain, NRTKs possess domains that mediate protein-protein, protein-lipid and protein-DNA interactions. The most commonly found protein-protein interaction domains in NRTKs are the Src homology 2 (SH2) and Src homology 3 (SH3) domains (Kuriyan & Cowburn, 1997). The SH2 domain is a compact domain of 100 amino acid residues that binds phosphotyrosine residues in a sequence-specific manner. The smaller SH3 domain (60 residues) binds proline rich containing sequences capable of forming a polyproline type II helix. Some NRTKs lack SH2 and SH3 domains but possess subfamily-specific domains used for protein-protein interactions. For example, members of the Jak...
family contain specific domains that target them to the cytoplasmic portion of cytokine receptors. The NRTK Fak possesses two domains that mediate protein-protein interactions; an integrin-binding domain and a focal adhesion-binding domain. The NRTK Bcr-Abl contains a nuclear localization signal but is found in both the nucleus and the cytoplasm. In addition to SH2 and SH3 domains, Bcr-Abl possesses an F actin–binding domain and a DNA-binding domain (Stevan & Till, 2000).

A number of diseases, including cancer, diabetes and inflammation, are linked to perturbation of protein kinase mediated cell signaling pathways. Therefore, protein kinases are targets for treatment of a number of diseases. Bcr-Abl kinase is NRTK, that is expressed in a wide range of cells and it is localized at several subcellular sites, including the nucleus, cytoplasm, mitochondria, endoplasmic reticulum and cell cortex, where Bcr-Abl interacts with a large variety of cellular proteins, including signaling adaptors, kinases, phosphatases, cell-cycle regulators, transcription factors and cytoskeletal proteins (Pendergast, 2002). The Bcr-Abl gene was first identified as the cellular homolog of the transforming gene of Abelson murine leukaemia and subsequently found to be involved in the Philadelphia chromosome translocation in human leukaemia and to encode a non-receptor tyrosine kinase (Wong & Witte, 2004; Hantschel & Superti-Furga, 2004; Sawyers & Druker, 1999).

Bcr–Abl is an oncogene that arises from fusion of the Bcr (breakpoint cluster region) gene with the c-Abl proto-oncogene. The Philadelphia chromosome involves fusion of the Bcr gene on chromosome 22 at band q11 with the Abl proto-oncogene on chromosome 9 at band q34 (Rowley, 1973). Three different Bcr–Abl variants can be formed, depending on the amount of Bcr gene included: p185, p210 and p230. The three variants are associated with distinct types of human leukemia. P185 is associated with 20–30% of acute lymphocytic leukemia (ALL), p210 with
90% of chronic myelogenous leukemia (CML) and p230 with a subset of patients with chronic neutrophilic leukemia (CNL) (Melo, 1996). The oncogenic ability of \( \text{Bcr-Abl} \) requires deregulated tyrosine kinase activity which leads to the recruitment of adaptor molecules, phosphorylation of signaling molecules and activation of downstream signaling events (Lugo et al., 1990; Daley et al., 1990).

The NRTK \( \text{Bcr-Abl} \) kinase is a causative agent of CML and inhibiting the \( \text{Bcr-Abl} \) kinase enzyme might induce the apoptosis of the diseased cells from the patient’s body. In 1996, Novartis team (Druker et al., 1996) reported a successful \( \text{Bcr-Abl} \) inhibitor, CGP57148, which is later renamed STI-571, Gleevec or imatinib.

Imatinib is a specific inhibitor that binds with high affinity to the inactive conformation of the \( \text{Bcr-Abl} \) tyrosine kinase and has been shown to be effective in the treatment of CML with little toxicity, compared to other cancer therapies (Schindler et al., 2000; Druker et al., 2001a; O’Brien et al., 2003). In addition to its ability to block \( \text{Bcr-Abl} \), imatinib also inhibits the platelet-derived growth factor (PDGF) receptor and the \( \text{c-Kit} \) receptor (Druker et al., 2001b; Buchdunger et al., 2000). \( \text{c-Kit} \) is the cellular homolog of the \( \text{v-kit} \) retroviral oncogene and the \( \text{c-Kit} \) gene product is expressed in hematopoietic progenitor cells, mast cells, germ cells, interstitial cells of Cajal and some human tumors (Nocka et al., 1989). CML represents the first human malignancy to be successfully treated with a small molecule inhibitor, imatinib. In spite of its several virtues, clinical resistance to imatinib has been reported in small number of patients due to \( \text{Bcr-Abl} \) gene mutation or amplification. Although some of these mutations are located close to the imatinib-binding site, most of the mutations occur at distal positions. A plausible mechanism for the induction of resistance by these mutations involves the destabilization of the inactive conformation, with concomitant preservation of the catalytic capabilities of the kinase domain (Shah et al., 2002).
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A significant progress for the treatment of patients with resistance to imatinib is the identification of inhibitors that can bind to both active and inactive conformations of Bcr-Abl kinase and those that bind preferably to the active form and provide a way to oppose the mutation-induced resistance to imatinib. Other mechanisms of imatinib resistance include Bcr-Abl gene amplification (Gadzicki et al., 2005). In order to overcome the resistance to imatinib, a number of new inhibitors have been synthesized. The most effective ATP mimics are AMN107 (Weisberg et al., 2005) and BMS-354825, which inhibit almost all imatinib resistant forms of Bcr-Abl (Shah et al., 2004; Doggrell, 2005) but are not effective against the T315I mutant. The T315I mutation is the most common mutation found in patients undergoing imatinib therapy (Shah et al., 2002) and this is responsible for nearly 15% of resistant cases. A single nucleotide change at the genetic level, replaces threonine with isoleucine in the protein product at position 315 thus causing this mutation (T315I). The side chain hydroxyl group of Thr315 forms critical hydrogen bonds with imatinib (see Figure 3.1a) and Thr315 is located at the center of the imatinib binding site in Bcr-Abl kinase. This residue separates the ATP binding site from an internal cavity that is of variable size in different protein kinases (Bcr-Abl kinase, c-Kit receptor), and this gatekeeper residue plays a vital role in determination of the inhibitor specificity (Liu, et al., 1999) and regulates the binding of inhibitors. Thr315 opens up an auxiliary binding site, which is occupied by the piperazinyl-substituted benzamide moiety of imatinib and participates through the hydroxymethylene side chain, in a crucial H bonding interaction between imatinib and Abl (Schindler et al., 2000), as well as Bcr-Abl (Manley et al., 2002; Nagar et al., 2002). Mutation to isoleucine abrogates the possibility of this H bonding interaction, which, combined with the additional bulk of the isoleucine side chain, sterically hinders imatinib binding and leads to imatinib insensitivity and consequently resistance in patients.
Figure 3.1a. A Schematic View of the Inhibitor, Imatinib Bound to the Bcr-Abl Kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex are Indicated.

Under these situations, it is essential to find possible molecules that have been developed as drugs for other protein kinases and might also serve to inhibit imatinib resistant forms of Bcr-Abl kinase. Similar to the behavior of imatinib, dasatinib (BMS-354825) (Shah et al., 2004) and other Bcr-Abl inhibitors, exhibit a significant loss of affinity for Bcr-Abl (T315I) relative to other Bcr-Abl variants. This implies that it is particularly difficult to inhibit Bcr-Abl (T315I) with an ATP-competitive compound (Carter et al., 2005). The possibility of ATP competitive compounds is to bind either the wild-type Bcr-Abl or T315I mutant Bcr-Abl, but not both. Imatinib and dasatinib are two clinically valuable Bcr-Abl kinase
inhibitors that serve as a paradigm for the study of emergence of resistance in targeted cancer therapy.

In order to test the existing inhibitors against drug-resistant mutants of Bcr-Abl, Todd and co-workers (von et al., 2003) developed competition binding assays for a panel of clinically important mutants. In this study they have used various types of kinase inhibitors and found that VX-680 is binding with high affinity to Bcr-Abl kinase (T315I) mutant. VX-680 has been previously reported as a potent inhibitor of all three Aurora kinases A, B and C with apparent inhibition constant ($K_i$) values of 0.6, 18 and 4.6 nM for Aurora A, Aurora B and Aurora C respectively (Harrington et al., 2004). VX-680, not only blocks cell proliferation but can also induce cell death by apoptosis in multiple tumor types, both in vitro and in vivo. VX-680 also blocks the phosphorylation of a direct downstream substrate of the Aurora kinases, histone H3, in tumor tissue in vivo. The VX-680 molecule binds tightly to the wild type Bcr-Abl kinase, (Kd of ~20 nM or lower), and most of the Bcr-Abl mutants, including T315I (Kd 5-20 nM). In the enzyme activity assays, VX-680 potently inhibited wild-type Bcr-Abl with an IC$_{50}$ value of 10 nM and Bcr-Abl (T315I) with an IC$_{50}$ value of 30 nM (Carter et al., 2005).

The crystal structure of VX-680 bound to the catalytic domain of Bcr-Abl (PDB_ID: 2F4J) containing a mutation (H396P) has been solved (Young et al., 2006). This mutation confers imatinib resistance in Bcr-Abl kinase but is inhibited by VX-680 in vitro. It has been shown that VX-680 inhibits Bcr-Abl kinase activity in cells derived from patients carrying the T315I mutation in the kinase domain of Bcr-Abl, and that it retains activity towards purified T315I mutation in vitro. These results provide a structural explanation for the retention of inhibitory activity of VX-680 towards mutant proteins, which are no longer inhibited by imatinib. The structure of the kinase domain of Bcr-Abl (H396P) bound to VX-680 is shown in Figure 3.1b.
This 3-D crystal structure (PDB_ID: 2F4J) is the source for the virtual screening strategy used to discover novel inhibitors to Bcr-Abl kinase. Virtual screening provides assurance to an inexpensive and fast alternative, to high throughput screening (HTS) in order to discover useful lead compounds for drug discovery projects (Jürgen, 2002). Drug discovery methods such as structure-based virtual screening focuses on using the protein crystal structure and is exemplified by receptor-based docking methods such as affinity docking, FlexX, Autodock and GOLD.

**Figure 3.1b.** A Schematic View of the Inhibitor, VX-680 Bound to the Bcr-Abl (H396P) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex are Indicated.
Another virtual screening approach is to generate a pharmacophore which represents the 3-D arrangement of a set of chemical features, functional groups from an inhibitor that have critical interactions with the receptor (Mason et al., 2001). The chemical features of the inhibitor are critical for its biological activity. More recently, several new approaches have been described for pharmacophore screening that enable pharmacophore information to be included in the search query (Hahn, 1997; Putta et al., 2002).

In this work we have generated a chemical function based pharmacophore of VX-680 using “View Hypotheses” module in catalyst software. This pharmacophore was used for the screening of databases such as, NCI, Maybridge and Derwent-WDI2005 and the obtained hits were docked into the Bcr-Abl kinase crystal structure using GOLD software (Jones et al., 1997). Our goal is to search for molecules with alternative leads to the VX-680 in a commercially available database that would inhibit wild type and mutant Bcr-Abl kinases. The chemical component pharmacophoric hypotheses was built using the “View Hypotheses” workbench within Catalyst using the conformation of VX-680- Bcr-Abl kinase interactions reported in the X-ray complex. This pharmacophoric query was used to search a multi-conformational databases using Catalyst. Finally, the molecules identified from virtual screening were docked into the Bcr-Abl kinase using GOLD software to observe the key interactions between the screened molecules and Bcr-Abl kinase and thus validate the hits as useful and novel Bcr-Abl kinase inhibitors.
3.2 Methods

3.2.1 Protein preparation:

The 3-D co-ordinates of Bcr-Abl kinase complexed with VX-680 (PDB_ID: 2F4J) (Young et al., 2006) was downloaded from protein structure databank, (http://www.rcsb.org/). Hydrogen atoms were added to the protein using Biopolymer module in InsightII 2005 (InsightII 2005, Accelrys) keeping all the residues in their charged form. In the first step all the hydrogen atoms were minimized, keeping the other atoms fixed. In the second step whole protein complex including crystal water was energy minimized by the steepest descent followed by conjugate gradient methods to achieve a convergence gradient of 0.01 kcal/mol using CVFF force fields in InsightII 2005. Crystallographic waters were retained for docking studies. In addition to this, we have mutated T315I in Biopolymer module and used the same methods for energy minimization in order to study the binding of these molecules to Bcr-Abl kinase (T315I) mutant.

3.2.2 Pharmacophore model generation:

From the crystal structure (PDB_ID: 2F4J), a ligand-based (VX-680) pharmacophore query was generated for Bcr-Abl kinase using View Hypotheses workbench module in Catalyst (Catalyst 4.11, Accelrys) using the conformation of VX-680 reported in the X-ray complex with Bcr-Abl kinase. In the hypotheses, two hydrogen bond donors (HD), two hydrogen bond acceptors (HA) and one hydrophobic interaction (HP) were allowed as observed in the protein structure. A maximum of 5 features were selected to construct the pharmacophore hypotheses. This pharmacophore query was used for the virtual screening of small molecule databases and we identified 289 molecules form the NCI, Maybridge and Derwent-
WDI2005 databases. These databases are multi-conformational Catalyst databases, which were built using the best option with the MAXCONFS option set to 250 and the energy threshold set to 15 kcal/mol.

3.2.3 Virtual screening:

The Pharmacophore query was used as a 3-D structural query in the screening of NCI, Maybridge and Derwent-WDI2005 databases. NCI, Maybridge and Derwent-WDI2005 databases comprise 2,38,819, 59,652 and 67,050 molecules respectively. The chemical function based pharmacophore model was used for database searching by the best flexible search method in Catalyst. The molecules obtained were further filtered using Lipinski’s rule of 5 (Lipinski et al., 1997).

3.2.4 Docking:

The new lead molecules identified from virtual screening, were docked into the crystal structure of Bcr-Abl kinase (PDB_ID: 2F4J) using GOLD (GOLD 3.10, CCDC, UK) software. GOLD (Genetic Optimization of Ligand Docking) is a genetic algorithm for docking flexible ligands into protein binding site. The details were discussed in the section 2.2.5. During docking, the default algorithm speed was selected, and the ligand binding site in the Bcr-Abl kinase, was defined within a 10 Å radius with the centroid as Glu 316 main chain carbonyl oxygen atom. For docking, the number of poses for each inhibitor was set to ten, and early termination was allowed if the top five bound conformations of a ligand were within 1.5 Å (RMSD). After docking, the individual binding poses of each ligand were re-ranked according to the GOLD score. The top ranked conformation of each ligand was selected and analysed using SILVER (SILVER 1.1.1, CCDC, UK) to examine the mode of protein-inhibitor binding.
3.2.5 **Hardware and software:**

InsightII 2005 was used for energy minimization of *Bcr-Abl* kinase, and Catalyst 4.11 was used for pharmacophore generation and virtual screening on SGI Octane2 workstation equipped with 2600 MHz MIPS R14000 processors. The docking calculations using GOLD software and docking analysis using SILVER (Nissink *et al.*, 2002) were carried out on an Intel P4-based windows system.
3.3 Results and Discussion

The aim of the present work is to identify novel lead molecules as inhibitors for \( Bcr-Abl \) kinase and its mutant (T315I). We have achieved this using pharmacophore model generation, virtual screening of small molecule databases and molecular docking studies.

3.3.1 Generation of pharmacophore model:

Our choice of pharmacophore features was based upon the conformation of VX-680 reported in the X-ray complex with \( Bcr-Abl \) kinase. Two hydrogen bond donor features were predicted to interact with the side chain amino group of Asp381 and the main chain carbonyl oxygen of Glu316. The pyrazole group \( N_{20} \) of HD1 accepts a hydrogen bond from Met318 and \( N_{30} \) atom of VX-680 as HD2 accepts a hydrogen bond from Asp381. Two hydrogen bond acceptor features were predicted to interact with the main chain nitrogen of Met318 and \( N_{13} \) atom of VX-680 with water molecule. This water acts as a bridge molecule between \( N_{13} \) atom of VX-680 and main chain nitrogen of Asn322. The pharmacophore feature HA1 is complementary to the pyrazole group \( N_{19} \) of Glu316, while pyrimidine group of \( N_{13} \) as HA2 accepts a hydrogen bond from Wat9 that in turn forms a hydrogen bond with the main chain NH of Asn322. The positions of these glutamic acid and metionine are strictly conserved across the \( Bcr-Abl \) kinase family and are involved in binding to the adenine moiety of ATP. The cyclopropane ring in VX-680 was selected as the required group for hydrophobic interaction (HP) (Figure 3.1c).

The majority of kinase inhibitors that have been deposited in the protein databank form interactions with amino acid residues at these positions. These interactions are shown in Figure 3.1b. A Schematic representation of
pharmacophore model is shown in Figure 3.1c. The mapping of Pharmacophore with VX-680 molecule is shown in Figure 3.1d.

**Figure 3.1c.** A Schematic Representation of Pharmacophore Model. Distances Between the Characters are in Å Units.
3.3.2 Database screening:

The pharmacophore query generated above was used to screen NCI, Maybridge and Derwent-WDI2005 databases. In all, about 5000 molecules were obtained as hits from in silico screening (screen 1). To assess the drug-likeliness of these hits, a second screen, incorporating Lipinski’s rule of 5 was used. A total of 1100 molecules were obtained as hits from this screen (Screen 2). To further increase the probability of the hit to be a lead, a fitness score >7.00 was used as the third screen (Screen 3). The fitness score indicates how well the features in the pharmacophore overlap with the chemical features in the ligand. A total of 289 molecules were obtained as hits from this screen. In Figure 3.1d shows a Schematic representation of VS strategy.
Figure 3.1d. Schematic Representation of VS Strategy.

3.3.3 GOLD docking:

The crystal structure of Bcr-Abl kinase bound to substrate VX-680 (PDB_ID: 2F4J) was used for the docking studies. All amino acids within 10 Å radius from the Glu316 main chain carbonyl oxygen atom were considered to comprise the active site. Docking was carried out using GOLD 3.10 software.

The inhibitor VX-680 was docked into the Bcr-Abl kinase and the following interactions between VX-680 and the Bcr-Abl kinase have been observed. (i) A hydrogen bond interaction between the pyrazole ring N_{19}H and Glu316 carbonyl oxygen (N_{19}H—O=C, 2.32 Å). (ii) A hydrogen bond between pyrazole ring N_{20} and the main chain NH of Met318 (N_{20}—NH, 3.17 Å). (iii) A hydrogen bond between N_{30} and the side chain carbonyl oxygen of Asp381.
(N\textsubscript{30} \cdots \text{O}=\text{C}, 2.35 \text{ Å}). (iv) One bridge water molecule (H\textsubscript{2}O) is in between N13 of VX-680 and main chain NH of Asn322 (N\textsubscript{13} \cdots H\textsubscript{2}O \cdots NH). (V) A hydrogen bond between S\textsubscript{23} and the main chain NH of Gly249 (S\textsubscript{23} \cdots H-N, 3.66 Å). The RMSD between the docked pose of VX-680 and its bound conformation in the crystal structure 2F4J is 0.56 Å, indicating that GOLD software was able to reproduce the correct pose and is a reliable method for these docking studies.

Docking calculations were carried out using two types of mutant proteins; these are Bcr-Abl (H396P) kinase and Bcr-Abl (T315I) kinase.

### 3.3.3.1 Bcr-Abl (H396P) kinase docking:

The molecules obtained from virtual screening were docked into the Bcr-Abl kinase (H396P) crystal structure. All molecules fit into the VX-680 binding site of the enzyme. The binding of these docked molecules to Bcr-Abl kinase was examined on graphics. Based on the values of GoldScore, and the protein-ligand binding interactions, some molecules were selected to have better binding and these are described below. We have given the numbering of atoms according to those databases.
The interaction of Hit NCI0166619 is shown in Figure 3.2a. In the molecule NCI0166619, O\textsubscript{11} makes hydrogen bonding interactions with the main chain NH of Met318 (O\textsubscript{11}···HN, 3.28 Å). Further, the N\textsubscript{19} makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N\textsubscript{19}···O=C, 2.48 Å). A bifurcated hydrogen bond between O\textsubscript{3} and N\textsubscript{20}, with the side chain oxygen of Thr315 (O\textsubscript{3}···HO, 2.94, N\textsubscript{20}···O 2.63 Å). The O\textsubscript{6}H makes hydrogen bonding interactions with the side chain carbonyl oxygen of Asp381 (O\textsubscript{6}H ···O=C, 2.51 Å), O\textsubscript{7} makes hydrogen bonding interactions with the main chain NH of Asp381 (O\textsubscript{7}···HN, 2.58 Å).

**Figure 3.2a.** A Schematic View of the Inhibitor, NCI0166619 Bound to the Bcr-Abl (H396P) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit NCI0210892 is shown in Figure 3.2b. In the molecule NCI0210892, \( N_5 \) makes hydrogen bonding interactions with the main chain NH of Gly321 (\( N_5 \cdots\text{HN}, 3.39 \text{ Å} \)) and \( N_5H \) makes hydrogen bonding interactions with the main chain carbonyl oxygen of Met318 (\( N_5H \cdots\text{O=C}, 2.07 \text{ Å} \)). The \( N_{20} \) makes hydrogen bonding interactions with the main chain NH of Met318 (\( N_{20} \cdots\text{HN}, 2.83 \text{ Å} \)). The \( N_{17}H_2 \) makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (\( N_{17}H_2 \cdots\text{O=C}, 2.32 \text{ Å} \)) and The \( N_{17}H_1 \) makes hydrogen bonding interactions with the side chain oxygen of Thr315 (\( N_{17}H_1 \cdots\text{O}, 3.28 \text{ Å} \)). The \( O_{12} \) makes hydrogen bonding interactions with the main chain HN of Asp381 (\( O_{12} \cdots\text{HN}, 2.59 \text{ Å} \)).

**Figure 3.2b.** A Schematic View of the Inhibitor, NCI0210892 Bound to the Bcr-Abl (H396P) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit HTS07964 is shown in Figure 3.2c. In the molecule HTS07964, N_{13} makes hydrogen bonding interactions with the main chain HN of Met318 (N_{13} \ldots \text{HN}, 3.01 \, \text{Å}) and N_{13}H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N_{13}H \ldots \text{O=C}, 3.09 \, \text{Å}). The O_{28} makes hydrogen bonding interactions with the side chain OH of Thr315 (O_{28} \ldots \text{HO}, 2.48 \, \text{Å}). The N_{18}H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Asp381 (N_{18}H \ldots \text{O=C}, 3.46 \, \text{Å}). Further, O_{23} forms a bifurcated hydrogen bond with the side chain oxygen of Glu286 and the side chain NH of Lys271 (O_{23} \ldots \text{HO}, 3.47 \, \text{Å}; O_{23} \ldots \text{NH} 2.87\, \text{Å}).

**Figure 3.2c.** A Schematic View of the Inhibitor, HTS07964 Bound to the *Bcr-Abl* (H396P) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit RJF00578 is shown in Figure 3.2d. In the molecule RJF00578, N₃ makes hydrogen bonding interactions with the main chain NH of Met318 (N₃ ...HN, 3.48 Å) and N₃H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N₃H ...O=C, 3.11 Å). A bifurcated hydrogen bond between RJF00578 N₁H and side chain oxygen of Thr315 (N₁H ...O, 2.58 Å), N₅ and side chain oxygen hydrogen of Thr315 (N₅ ...HO, 3.28 Å). The O₇ makes hydrogen bonding interactions with main chain NH of Asp381 (O₇ ...HN, 3.45 Å). One bridge water molecule (H₂O) is in between O₇ of RJF00578 and side chain OH of Glu286 (O₇ ...H₂O ...HO 2.95, 2.88 Å).

**Figure 3.2d.** A Schematic View of the Inhibitor, RJF00578 Bound to the *Bcr-Abl* (H396P) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit LY186826 is shown in Figure 3.2e. In the molecule LY186826, O_{28} makes hydrogen bonding interactions with the main chain NH of Met318 (O_{28} \cdots \text{HN}, 2.63 \text{ Å}). The O_{28}H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (O_{28}H \cdots \text{O=C}, 3.59 \text{ Å}). A bifurcated hydrogen bond between LY186826 N_{20} and side chain oxygen of Glu286 (N_{20} \cdots \text{O}, 3.58 \text{ Å}), N_{20} and side chain NH of Lys271 (N_{20} \cdots \text{HN}, 2.96 \text{ Å}). Further, a bridge water molecule (H_{2}O) is in between N_{18}H of LY186826 and main chain NH of Gly383 (N_{18}H \cdots H_{2}O \cdots \text{HN} 2.92, 2.87 \text{ Å}).

**Figure 3.2e.** A Schematic View of the Inhibitor, LY186826 Bound to the *Bcr-Abl* (H396P) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit Vibsanol is shown in Figure 3.2f. In the molecule Vibsanol, O$_{14}$ makes hydrogen bonding interactions with the main chain NH of Met318 (O$_{14}$····HN, 2.66 Å). The O$_{14}$H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (O$_{14}$H····O=C, 3.12 Å). The O$_{25}$ makes hydrogen bonding interactions with the main chain NH of Asp381 (O$_{25}$····HN, 3.07 Å). Further, O$_{21}$H makes hydrogen bonding interactions with the side chain OH of Glu286 (O$_{21}$H····HO, 3.27 Å) and O$_{21}$ makes hydrogen bonding interactions with the side chain NH of Lys271 (O$_{21}$····HN, 3.59 Å).

**Figure 3.2f.** A Schematic View of the Inhibitor, Vibsanol Bound to the *Bcr-Abl* (H396P) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
3.3.3.2 *Bcr-Abl* (T315I) kinase docking:

In order to test the docking of screened molecules against drug-resistant mutants of *Bcr-Abl* kinase (T315I), we have mutated T315I by using Biopolymer module in *in silico* modeling. We observed that the side chains of Thr315 and mutated Ile315 are well superimposed. So we consider that, it is a good model to carry out the docking studies. Although we mutated T315I we observe that some molecules have formed very good interactions with the protein active site.
The interaction of Hit NCI0046391 is shown in Figure 3.3a. In the molecule NCI0046391, N₃ makes hydrogen bonding interactions with the main chain NH of Met318 (N₃ ⋯HN, 3.16 Å). The N₄H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N₄H ⋯O=C, 2.71 Å). The N₄H makes hydrogen bonding interactions with the main chain N of Met318 (N₄H ⋯NH, 2.42 Å). Further, O₃H forms a bifurcated hydrogen bond with the side chain carbonyl oxygen of Asp381 and the side chain hydroxyl oxygen of Asp381 (O₃H ⋯O=C, 2.65 Å; O₃H ⋯OH, 3.20 Å).

**Figure 3.3a.** A Schematic View of the Inhibitor, NCI0046391 Bound to the Bcr-Abl (T396I) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit NCI0132917 is shown in Figure 3.3b. In the molecule NCI0132917, O$_3$ makes hydrogen bonding interactions with the main chain NH of Met318 (O$_3$ \(\cdots\)HN, 2.68 Å). The O$_3$H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (O$_3$H \(\cdots\)O=C, 3.22 Å). The N$_1$H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Asp381 (N$_1$H \(\cdots\)O=C, 2.60 Å). Further, O$_2$H makes hydrogen bonding interactions with the side chain OH of Glu286 (O$_2$H \(\cdots\)OH, 3.26 Å).

**Figure 3.3b.** A Schematic View of the Inhibitor, NCI0132917 Bound to the Bcr-Abl (T396I) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit NCI0694766 is shown in Figure 3.3c. In the molecule NCI0694766, N₂ makes hydrogen bonding interactions with the main chain NH of Met318 (N₂ ⋯HN, 2.96 Å). N₃H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Met318 (N₃H ⋯O=C, 2.85 Å). The N₃H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N₃H ⋯O=C, 3.48 Å). Further, O₆H froms a bifurcated hydrogen bond with the side chain carbonyl oxygen of Asp381 and the side chain hydroxyl oxygen of Asp381 (O₆H ⋯O=C, 2.72 Å; O₆H ⋯OH, 3.04 Å) and O₁H makes hydrogen bonding interactions with the side chain carbonyl oxygen of Asp381 (O₁H ⋯O=C, 3.06 Å).

**Figure 3.3c.** A Schematic View of the Inhibitor, NCI0694766 Bound to the Bcr-Abl (T396I) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit HTS 11169 is shown in Figure 3.3d. In the molecule HTS 11169, N₅ makes hydrogen bonding interactions with the main chain NH of Met318 (N₅ → HN, 2.79 Å). The N₅H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Met318 (N₅H → O=C, 2.75 Å). The N₆H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N₆H → O=C, 3.45 Å). O₁H forms a bifurcated hydrogen bond with the side chain carbonyl oxygen of Asp381 and the side chain hydroxyl oxygen of Asp381 (O₁H → O=C, 3.30 Å; O₁H → OH, 2.33 Å) and O₅H makes hydrogen bonding interactions with the side chain carbonyl oxygen of Asp381 (O₅H → O=C, 3.42 Å).

**Figure 3.3d.** A Schematic View of the Inhibitor, HTS 11169 Bound to the *Bcr-Abl* (T396I) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
The interaction of Hit CVT-3127 is shown in Figure 3.3e. In the molecule CVT-3127, N\textsubscript{7} makes hydrogen bonding interactions with the main chain NH of Met318 (N\textsubscript{7} $\cdots$HN, 2.69 Å). The N\textsubscript{6}H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Met318 (N\textsubscript{6}H $\cdots$O=C, 2.28 Å). The O\textsubscript{6} makes hydrogen bonding interactions with the side chain NH of Asn322 (O\textsubscript{6} $\cdots$HN, 3.61 Å). A hydrogen bond between O\textsubscript{5} with the main chain NH of Asp381 (O\textsubscript{5} $\cdots$HN, 2.62 Å) and O\textsubscript{4} makes hydrogen bonding interactions with the side chain hydroxyl hydrogen of Glu286 (O\textsubscript{4} $\cdots$HO, 2.83 Å).

Figure 3.3e. A Schematic View of the Inhibitor, CVT-3127 Bound to the Bcr-Abl (T396I) kinase. Hydrogen Bonding Interactions in the Protein-Inhibitor Complex. Inhibitor is Indicated in Ball and Stick.
These results show that the new molecules obtained from virtual screening form several non bonding interactions, and bind \textit{Bcr-Abl} kinase in the VX-680 binding site. Further modifications of these lead molecules will generate inhibitors that bind \textit{Bcr-Abl} kinase with high specificity.
3.4 Conclusions

1. Using pharmacophore modeling and virtual screening, we have identified new lead molecules as Bcr-Abl kinase inhibitors.

2. We have studied the binding of these inhibitors to Bcr-Abl kinase using docking methods and confirm that these molecules bind the VX-680 binding site of the enzyme.

3. Further modifications and addition of suitable functional groups to these new scaffolds will generate high affinity Bcr-Abl kinase specific inhibitors.

4. Using two mutant Bcr-Abl kinase proteins for docking, we have identified some useful molecules for drug resistant Bcr-Abl kinase protein.

5. Our results confirm chemical function based virtual screening as a powerful tool to discover novel inhibitors of the protein kinase family, and further validate virtual screening as an inexpensive and efficient means for lead discovery.
3.5 References


Catalyst; Accelrys Inc., San Diego, CA.


Chapter 3


SILVER 1.1.1, CCDC, UK.


