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Chemical Function Based Virtual Screening: Discovery of Potent Lead Molecules for the *Bcr-Abl* Tyrosine Kinase Using VX-680

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ABSTRACT

In this work we have present new strategy for designing of pharmacophore and filtering potentially massive combinatorial libraries using structural information of a Ligand- Protein binding interactions. Here we describe the discovery, using Chemical function and shape-based virtual screening, of a potent, ATP site-directed inhibitor of the Bcr-Abl tyrosine kinase, an important and novel drug target for chronic myelogenous leukemia (CML). The chemical feature based pharmacophore model was built for Abl tyrosine kinase from Structure of Abl VX-680 Complex using the View Hypothesis workbench, which is implemented in the Catalyst software. This pharmacophore model consists of five features: two hydrogen bond donors, two hydrogen bond acceptor and one hydrophobic function. This pharmacophore model was used as a query to search NCI, Maybridge and Derwent-WDI2005 databases to identify other new lead compounds. A total of **289** compounds were retrieved as hits from virtual screening.The hits obtained were docked into the active site of Bcr-Abl tyrosine kinase crystal structure (PDB_ID: 2F4J) using GOLD software. Based on the molecular docking we have shown the key interactions between the hit molecules and Abl tyrosine kinase are conserved.

Keywords: *BCR-ABL, VX-680, CML, Tyrosine kinase, Pharmacophore*

Introduction

c-Abl is a nonreceptor tyrosine kinase (NRTK) that is expressed in a wide range of cells. -Abl is localized at several subcellular sites, including the nucleus, cytoplasm, mitochondria, endoplasmic reticulum and cell cortex, where it interacts with a large variety of cellular proteins, including signalling adaptors, kinases, phosphatases, cell-cycle regulators, transcription factors and cytoskeletal proteins [1]. The *c-abl* gene was first identified as the cellular homologue of the transforming gene of Abelson murine leukaemia and was found subsequently to be involved in the Philadelphia chromosome translocation in human leukaemia and to encode a non-receptor tyrosine kinase [2-4].

Bcr–Abl is an oncogene that arises from fusion of the *Bcr* (breakpoint cluster region) gene with the *c-Abl* proto-oncogene.

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The Philadelphia chromosome (Ph) involves fusion of the *Bcr* gene on chromosome 22 at band q11 with the *Abl* proto-oncogene on chromosome 9 at band q34 [5]. 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) [6]. The oncogenic ability of *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 [7,8].

Protein phosphorylation is a central regulatory strategy to alter cellular function 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 [9]. Two classes of PTKs are present in cells- the receptor PTKs and the nonreceptor PTKs. Receptor tyrosine kinases (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 [10]. 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 3 (SH3) domains [11]. The SH2 domain is a compact domain of 100 residues that binds phosphotyrosine residues in a sequence-specific manner. The smaller SH3 domain (60 residues) binds proline-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 [12].

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. The PTK *Bcr-Abl* kinase causes chronic myelogenous leukemia (CML). Inhibiting this enzyme might induce the apoptosis of the diseased cells from the patient's body. In 1996, Novartis team [13] reported a successful *Bcr-Abl* inhibitor, CGP57148, later renamed STI-571 or imatinib.

Imatinib (STI-571 or Gleevec) is a specific inhibitor that binds with high affinity to the inactive conformation of the *Bcr-Abl* tyrosine kinase and has been shown to be effective in the treatment of CML with little toxicity, compared to other cancer therapies [13-15]. In addition to its ability to block *Bcr-Abl*, imatinib also inhibits the platelet-derived growth factor (PDGF) receptor and the *c-Kit* receptor [16,17]. *c-Kit* is the cellular homolog of the v-*kit* retroviral oncogene and the c-*Kit* gene product is expressed in hematopoietic progenitor cells, mast cells, germ cells, interstitial cell of cajal, and some human tumors [18]. CML represents the first human malignancy to be successfully treated with a small molecule imatinib. In spite of its several virtues, clinical resistance to imatinib has been reported in small numbers of patients due to *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 [19].

A significant progress for the treatment of patients with resistance to imatinib is the identification that inhibitors that can bind to both active and inactive conformations of *Bcr-Abl* kinase, or that bind preferably to the active form and provide a way to oppose the mutation-induced resistance to imatinib. A relatively common mutation in imatinib resistant patients is a single nucleotide change that replaces threonine with isoleucine at position 315 (T315I). Thr315 forms critical hydrogen bonds with imatinib. Other mechanisms of STI-571 resistance include Bcr-Abl gene amplification [20]. In order to overcome the resistance to imatinib, a number of new inhibitors have been synthesized. The most effective ATP mimics are AMN107 [21] and BMS-354825, which inhibit almost all STI- 571-resistant forms of Bcr-Abl [22,23] but are not effective against the T315I mutant. The T315I mutation is the most common mutation found in patients undergoing imatinib therapy [19] and this is responsible for nearly 15% of resistant cases. Thr315 is located in the center of the imatinib binding site in *Bcr-Abl* kinase (Figure 1a) and 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 determinant of inhibitor specificity [24] and regulate 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-bond interaction between imatinib and Abl [14], as well as Bcr-Abl [25,26]. Mutation to isoleucine abrogates the possibility of this H-bond interaction, which, combined with the additional bulk of the isoleucine side-chain, sterically hinders imatinib binding and leads to imatinib insensitivity and consequently resistance.

Under these situations, it is essential to find possible molecules that have been developed as drugs for other protein kinases and might serve to inhibit imatinib resistant forms of *Bcr-Abl* kinase. Similar to the behavior of imatinib, dasatinib (BMS-354825) [22] 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 [27]. The possibility of ATP-competitive compounds is to bind either wild-type 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 [28] 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 is 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 [28]. 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 *Bcr-Abl* kinase, with a Kd of ~20 nM or lower to wild-type *Bcr-Abl*, 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 IC50 value of 10 nM and BCR-ABL (T315I) with an IC50 value of 30 nM [27].

The crystal structure of VX-680 bound to the catalytic domain of *Bcr-Abl* (PDB ID: 2F4J) containing a mutation (H396P) has been solved [29]. 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* activity in cells derived from patients carrying the T315I mutation in the kinase domain of *Bcr-Abl*, and that it retains activity against purified T315I mutation in vitro. These results provide a structural explanation for the retention of activity by VX-680 towards mutant proteins, which are no longer inhibited by imatinib [29]. The structure of the kinase domain *Bcr-Abl* (H396P) bound to VX-680 is shown in Figure. 1b.

This three dimensional crystal structure (PDB ID: 2F4J) is the source for the virtual screening strategy used to discover novel inhibitors. Virtual screening provides assurance to an inexpensive and fast alternative method to high-throughput screening (HTS) for the discovery of useful lead molecules in the drug discovery studies [30]. 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. 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 [31]. Both the shape and the chemical features of the ligand are critical for its biological activity. More recently, several new approaches have been described for pharmacophore screening that enable both shape and pharmacophore information to be included in the search query [32,33].

In this article we have generated a chemical function and shape based pharmacophore of VX-680 using Hypogen module in catalyst software. This pharmacophore was used for the screening of databases NCI, Maybridge and Derwent-WDI2005 and the obtained hits were docked into the *Bcr-Abl* kinase crystal structure using GOLD software [34,35]. Our goal is to search for molecules with alternative leads to the VX-680 in a commercially available database that would inhibit wild type mutant *Bcr-Abl* kinases. The chemical component pharmacophoric hypothesis was built using the "View Hypothesis" workbench within Catalyst [36] using the conformation of VX680- *Bcr-Abl* kinase interactions reported in the X-ray complex with *Bcr-Abl* kinase (PDB ID 2F4J). 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.

Methods

Protein Preparation.
The 3-D coordinates of *Bcr-Abl* kinase complexed with VX-680 (PDB_ID: 2F4J) [29] was downloaded from protein structure databank, www.rcsb.org/. Hydrogen atoms were added to the protein using biopolymer module in InsightII [37] keeping all the residues in their charged form. Initially, all the hydrogen atoms were minimized, keeping the heavy atoms fixed. The 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. Crystallographic waters were retained for docking studies. In addition to this we have mutated T315I in biopolymer module and performed the same methods for energy minimization.

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 Hypothesis" workbench module in Catalyst using the conformation of VX-680 reported in the X-ray complex with *Bcr-Abl* kinase (PDBID: 2F4J). In the hypothesis; two hydrogen bond donors (HD), two hydrogen bond acceptors (HA) and one hydrophobic (HP) were allowed as observed in the protein structure. A maximum of 5 features were selected to construct the pharmacophore hypothesis. This pharmacophore query was used for the virtual screening of small molecule databases

a multi-conformational Catalyst database, which was built using the best option with the MAXCONFS option set to 250 and the energy threshold set to 15 kcal/mol.

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. For each molecule in the database, up to 250 conformers were generated using the "fast fit" method in Catalyst. 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 five [38].

Docking. The new lead molecules identified from virtual screening, were docked into the crystal structure of *Bcr-Abl* kinase (PDB_ID: 2F4J) using GOLD software. GOLD (Genetic

Optimization of Ligand Docking) is a genetic algorithm for docking flexible ligands into protein binding sites. For each of the 10 independent GA runs, a maximum number of 100000 GA operations were performed on a set of five groups with a population size of 100 individuals. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10 respectively. Default cutoff values of 2.5 Å (d_{H-X}) for hydrogen bonds and 4.0 Å for Van der Waals were employed. During docking, the default algorithm speed was selected, and the ligand binding site in the Abl kinase, was defined within a 10 Å radius with the centroid as Glu 316 main chain O 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 root mean square deviation (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 analyzed using SILVER to understand the mode of protein-inhibitor binding.

Hardware and Software. InsightII [37] was used for energy minimization of *Bcr-Abl* kinase and Catalyst 4.11 [36] 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 [35] and docking analysis using SILVER (29, 30) were carried out on an Intel P4-based windows system.

Results and Discussion

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

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. Two hydrogen-bond acceptor features were predicted to interact with the main chain N of Met318 and N13 atom of VX-680 with water molecule. This water acts as a bridge molecule between N13 atom of VX-680 and main chain N of Asn322. The positions of 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 majority of kinase inhibitors that have been deposited in the protein databank form interactions at these positions. The cyclopropane ring inVX-680 was selected as the required hydrophobic group.

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. To assess the drug-likeness of these hits, a second screen, incorporating Lipinski's rule of 5 was used. A total of 289 molecules were obtained as hits from this screen. This second screen selects only those molecules that possess drug like properties.

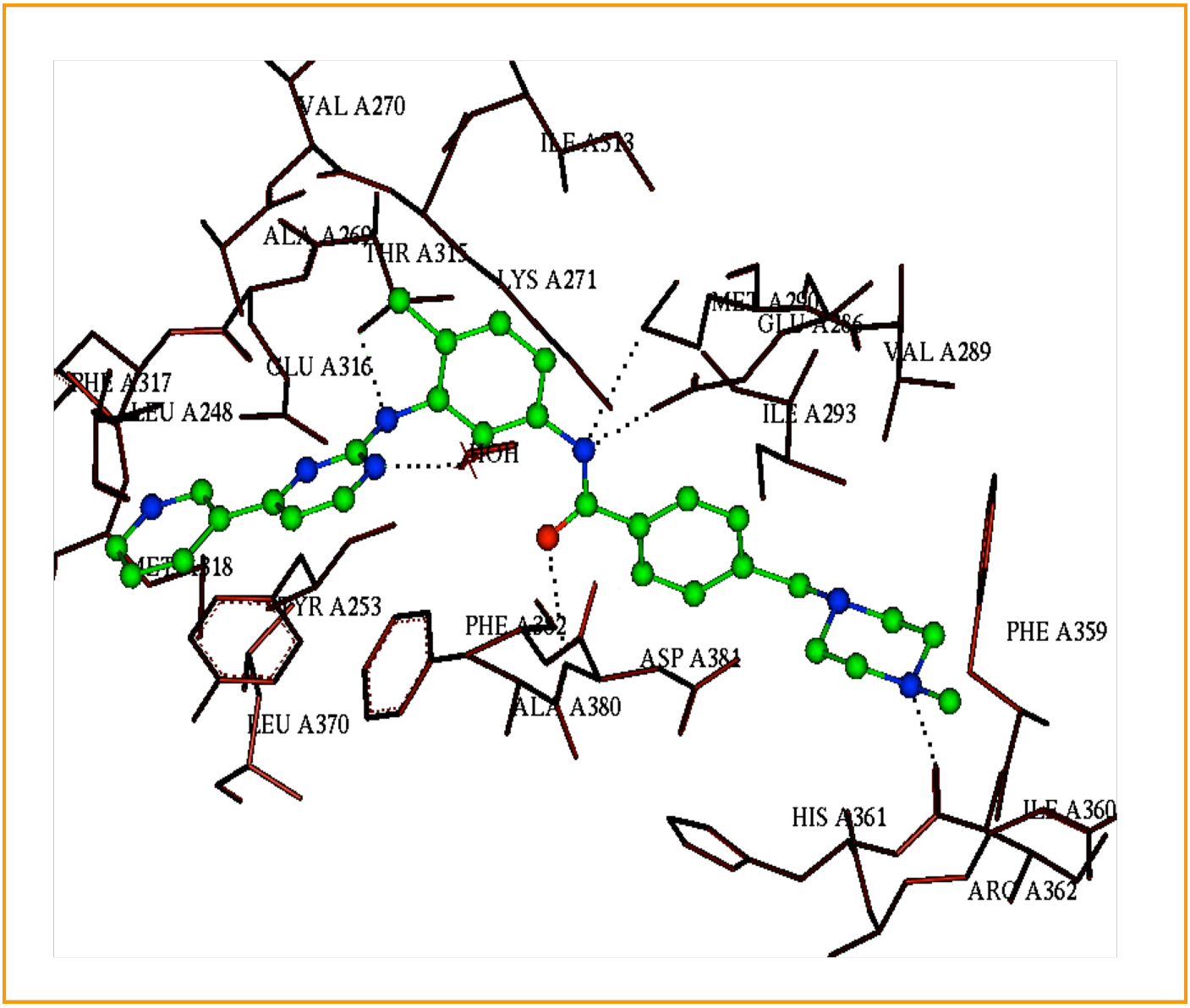
GOLD Docking. The crystal structure of *Bcr-Abl* kinase bound to substrate VX-680 (PDB_ID: 2F4J) was used for the docking studies. All amino acids within 10 Å radius from the Glu316 main chain O atom were considered to comprise the active site. Docking was carried out using GOLD 3.10 software.

The inhibitor VX680 was docked into the Abl kinase and the following interactions between VX680 and the Abl kinase have been observed. (i) A hydrogen bond interaction between the pyrazole ring $N_{19}H$ and Glu316 carbonyl oxygen ($N_{19}H\cdots O{=}C$, 2.32 Å). (ii) A hydrogen bond between pyrazole ring N_{20} and the main-chain NH of Met318 (N20$\cdots$NH, 3.17 Å). (iii) A hydrogen bond between N30 and the side-chain carbonyl oxygen of Asp381 (N30$\cdots$O=C, 2.35 Å). (iv) One bridge water molecule (H_2O) is in between N13of VX680 and main-chain NH of Asn322 (N13$\cdots H_2O\cdots$NH). (V). Ahydrogen bond between S_{23} and the main chain NH of Gly249 ($S_{23}\cdots$H-N, 3.66 Å). The RMSD between the docked pose of VX680 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.

The molecules obtained from virtual screening were docked into the Abl kinase crystal structure. All molecules fit into the VX680 binding site of the enzyme. The binding of some molecules to Abl kinase is described below. The interaction of Hit NCI0166619 is shown in Figure 2a. In the molecule NCI0166619, O11 makes hydrogen bonding interactions with the main chain NH of Met318 (O11 $\cdots$HN, 3.28 Å). Further, the N19 makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N19$\cdots$O=C, 2.48 Å). A bifurcated hydrogen bond between O3 and N20 with the side-chain oxygen of Thr315 (O3$\cdots$H-O, 2.94, N20$\cdots$O 2.63 Å). The O_6H makes hydrogen bonding interactions with the side-chain carbonyl oxygen of Asp381 (O_6H $\cdots$O=C, 2.51 Å), O7 makes hydrogen bonding interactions with the main-chain NH of Asp381 (O7 HN, 2.58 Å).

The interaction of Hit NCI0210892 is shown in Figure 2b. In the molecule NCI0210892, N5 makes hydrogen bonding interactions with the main chain NH of Gly321 (N5 $\cdots$HN, 3.39 Å) and N_5H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Met318 ($N_5H\cdots O{=}C$, 2.07 Å). The N20 makes hydrogen bonding interactions with the main chain NH of Met318 (N20 $\cdots$HN, 2.83 Å). The $N_{17}H_2$ makes hydrogen bonding interactions with the main chain

Figure 1a: A schematic view of the inhibitor, Imatinib bound to the *Bcr-Abl* kinase. Hydrogen bonding interactions in the protein-inhibitor complex are indicated.

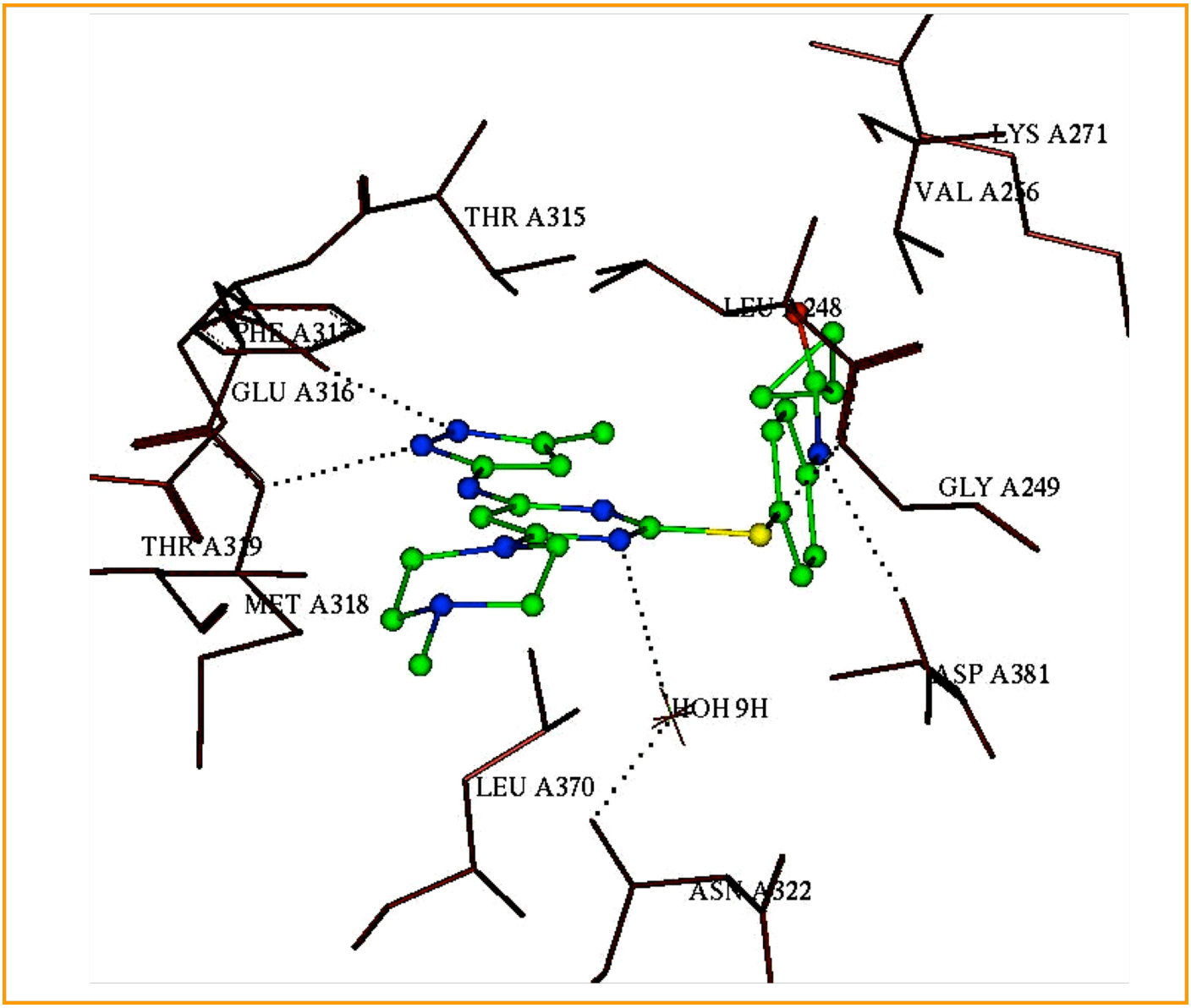


carbonyl oxygen of Glu316 ($N_{17}H_2$····O=C, 2.32 Å) and The $N_{17}H_1$ makes hydrogen bonding interactions with the side chain oxygen of Thr315 ($N_{17}H_1$····O, 3.28 Å). The O12 makes hydrogen bonding interactions with the main chain HN of Asp381 (O12····HN, 2.59 Å).

The interaction of Hit HTS07964 is shown in Figure 2c. In the molecule HTS07964, N13 makes hydrogen bonding interactions with the main chain HN of Met318 (N13 ····HN, 3.01 Å) and $N_{13}H$ makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 ($N_{13}H$ ····O=C, 3.09 Å). The O28 makes hydrogen bonding interactions with the side chain OH of Thr315 (O28····H-O, 2.48 Å). The $N_{18}H$ makes hydrogen bonding interactions with the main chain carbonyl oxygen of Asp381 ($N_{18}H$····O=C, 3.46 Å). Further, A bifurcated hydrogen bond between O_{23} and side chain oxygen of Glu286 , side chain NH of Lys271 (O_{23}···HO, 3.47 Å; O_{23}···NH 2.87Å).

The interaction of Hit RJF00578 is shown in Figure 2d. In the molecule RJF00578, N3 makes hydrogen bonding interactions with the main chain NH of Met318 (N3 ····HN, 3.48 Å) and N_3H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N_3H ····O=C, 3.11 Å) . A bifurcated hydrogen bond between RJF00578 N_1H and side chain oxygen of Thr315 (N_1H····O, 2.58 Å), N5 and side chain oxygen hydrogen of Thr315 (N_5····HO, 3.28 Å). The O_7 makes hydrogen bonding interactions with main chain NH of Asp381 (O_7 ····HN, 3.45 Å). One bridge water molecule (H_2O) is in

Figure 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.



between O7 of RJF00578 and side-chain OH of Glu286 (O7···H_2O···HO 2.95, 2.88 Å).

The interaction of Hit LY186826 is shown in Figure 2e. In the molecule LY186826, O28 makes hydrogen bonding interactions with the main chain NH of Met318 (O28 ····HN, 2.63 Å). The $O_{28}H$ makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 ($O_{28}H$ ····O=C, 3.59 Å). A bifurcated hydrogen bond between LY186826 N_{20} and side chain oxygen of Glu286 (N_{20}····O, 3.58 Å), N20 and side chain Nitrogen hydrogen of Lys271 (N_{20}····HN, 2.96 Å). Further, a bridge water molecule (H_2O) is in between $N_{18}H$ of LY186826 and main-chain NH of Gly383 ($N_{18}H$ ···H_2O···HN 2.92, 2.87 Å).

The interaction of Hit Vibsanol is shown in Figure 2f. In the molecule Vibsanol, O14 makes hydrogen bonding interactions with the main chain NH of Met318 (O14 ····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 O25 makes hydrogen bonding interactions with the main chain NH of Asp381 (O25 ····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 O21 makes hydrogen bonding interactions with the side chain NH of Lys271 (O_{21}

Figure 2: Schematic representation of the interaction between new lead molecules and *Bcr-Abl* (H396P) kinase. Hydrogen bonding interactions in the protein-inhibitor complex. Inhibitor is indicated in ball and stick. (a) NCI0166619 (b) NCI0210892 (c) HTS07964 (d) RJF00578 (e) LY186826 (f) Vibsanol

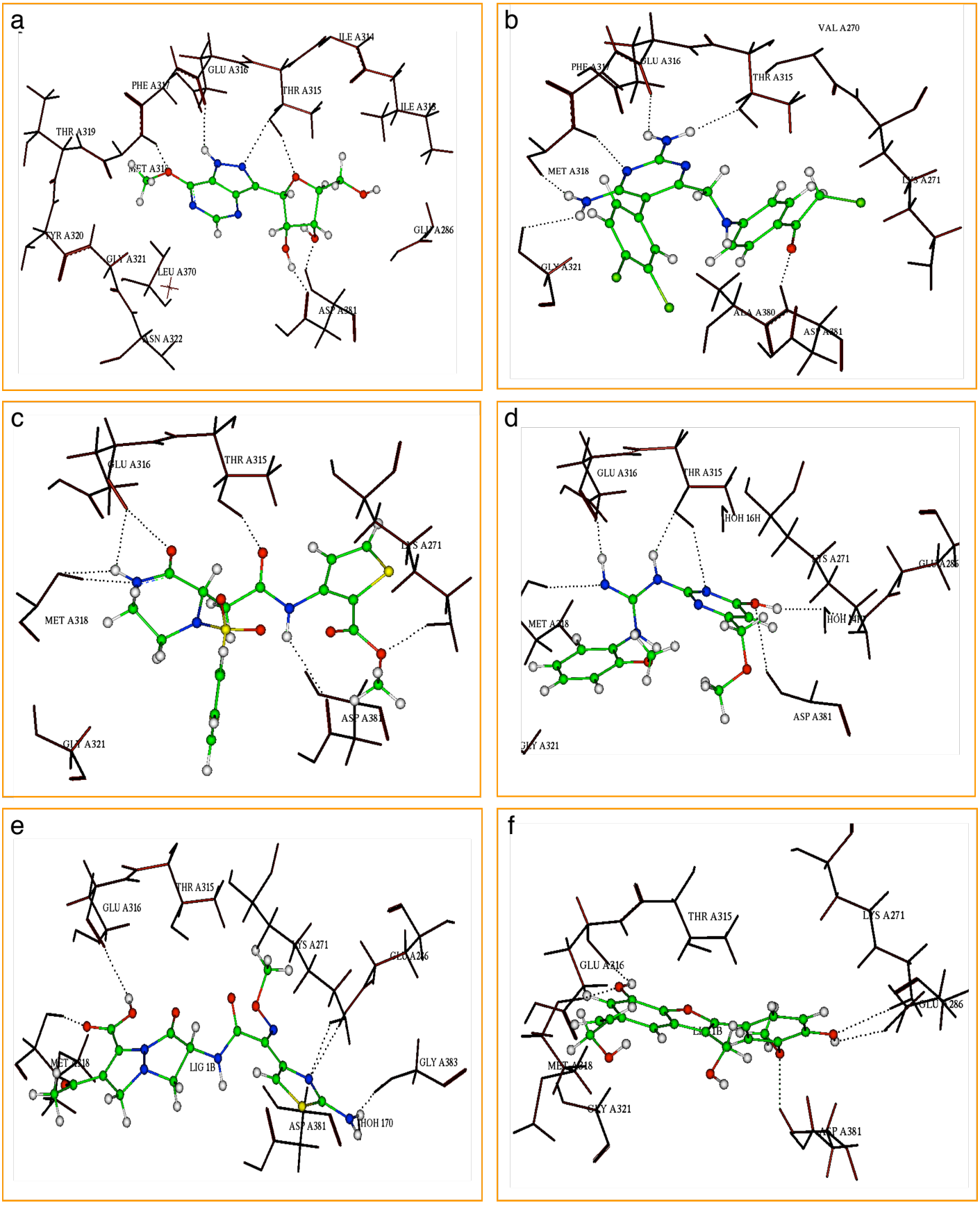
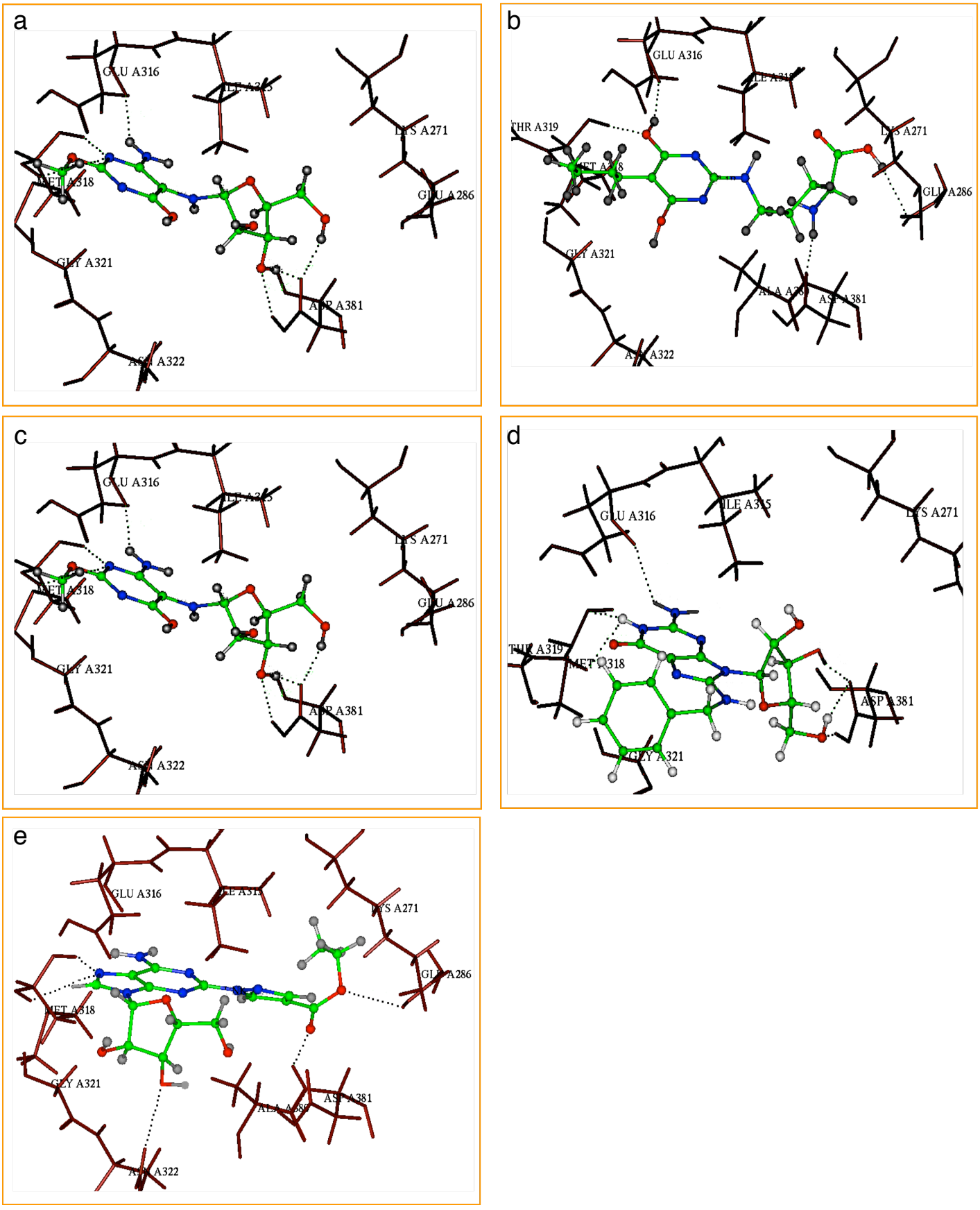


Figure 3: Schematic representation of the interaction between new lead molecules and *Bcr-Abl* (T396I) kinase. Hydrogen bonding interactions in the protein-inhibitor complex. Inhibitor is indicated in ball and stick. (a) NCI0046391 (b) NCI0132917 (c) NCI0694766 (d) HTS 11169 (e) CVT-3127



····HN, 3.59 Å).

In order to test the docking of screened molecules against drug-resistant mutants of *Bcr-Abl* kinase (T315I) we have mutated T315I in *insilico* modeling. 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 3a. In the molecule NCI0046391, N3 makes hydrogen bonding interactions with the main chain NH of Met318 (N3 ····HN, 3.16 Å). The N_4H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N_4H ····O=C, 2.71 Å). The N_4H makes hydrogen bonding interactions with the main chain N of Met318 (N_4H ····NH, 2.42 Å). Further, A bifurcated hydrogen bond between O_3H with the side chain carbonyl oxygen of Asp381, side chain hydroxyl oxygen of Asp381 (O_3H ····O=C, 2.65 Å; O_3H ····OH, 3.20 Å).

The interaction of Hit NCI0132917 is shown in Figure 3b. In the molecule NCI0132917, O_3 makes hydrogen bonding interactions with the main chain NH of Met318 (N3 ····HN, 2.68 Å). The O_3H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (O_3H ····O=C, 3.22 Å). The N_1H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Asp381 (N_1H ····O=C, 2.60 Å). Further, O_2H makes hydrogen bonding interactions with the side chain OH of Glu286 (O_2H ····OH, 3.26 Å).

The interaction of Hit NCI0694766 is shown in Figure 3c. In the molecule NCI0694766, N2 makes hydrogen bonding interactions with the main chain NH of Met318 (N2 ····HN, 2.96 Å). N_2H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Met318 (N_2H ····O=C, 2.85 Å). The N_3H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N_4H ····O=C, 3.48 Å). Further, A bifurcated hydrogen bond between O_6H with the side chain carbonyl oxygen of Asp381, side chain hydroxyl oxygen of Asp381 (O_6H ····O=C, 2.72 Å; O_6H ····OH, 3.04 Å) and O_1H makes hydrogen bonding interactions with the side chain carbonyl oxygen of Asp381 (O_1H ····O=C, 3.06 Å).

The interaction of Hit HTS 11169 is shown in Figure 3d. In the molecule HTS 11169, N5 makes hydrogen bonding interactions with the main chain NH of Met318 (N5 ····HN, 2.79 Å). The N_5H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Met318 (N_5H ····O=C, 2.75 Å). The N_6H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Glu316 (N_6H ····O=C, 3.45 Å). A bifurcated hydrogen bond between O_1H with the side chain carbonyl oxygen of Asp381, side chain hydroxyl oxygen of Asp381 (O_1H ····O=C, 3.30 Å; O_1H ····OH, 2.33 Å) and O_5H makes hydrogen bonding interactions with the side chain carbonyl oxygen of Asp381 (O_5H ····O=C, 3.42 Å).

The interaction of Hit CVT-3127 is shown in Figure 3e. In the molecule CVT-3127, N7 makes hydrogen bonding interactions with the main chain NH of Met318 (N7 ····HN, 2.69 Å). The N_6H makes hydrogen bonding interactions with the main chain carbonyl oxygen of Met318 (N_6H ····O=C, 2.28 Å). The O6 makes hydrogen bonding interactions with the side chain NH of Asn322 (O6 ···· HN, 3.61 Å). A hydrogen bond between O_5 with the main chain NH of Asp381 (O_5 ····HO, 2.62 Å) and O_4 makes hydrogen bonding interactions with the side chain hydroxyl hydrogen of Glu286 (O_4H····HO, 2.83 Å).

These results shows that the new molecules obtained from virtual screening form several non bonding interactions, and bind Abl kinase in the VX680 binding site. Further modifications of these lead molecules will generate inhibitors that bind Abl kinase with high specificity.

Conclusion

Using pharmacophore modeling and virtual screening, we have identified new lead molecules as Abl kinase inhibitors. We have studied the binding of these inhibitors to Abl kinase using docking methods and confirm that these molecules bind the VX680 binding site of the enzyme. Further modifications and addition of suitable functional groups to these new scaffolds will generate high affinity Abl kinase specific inhibitors.

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