Discovery of Specific Kinase Inhibitors : Main Focus on Aurora B and cSrc Kinases

by

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This is to certify that I have examined this copy of a master's thesis by

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To my dear Grandparents Kadirye and Mustafa

ABSTRACT

Many cancers have been associated with the deregulation of kinases that have become a prime target for cancer treatment. In this project, we aim to determine Aurora B kinase specific and cSrc kinase specific inhibitors by conducting virtual screening on ZINC database molecules. In our first project, our vision includes different binding site of drugs for the inhibition of Aurora B that have not yet been taken into account in previous reported studies. Indeed not only we aim to find the compounds that inhibit Aurora B but we also want to find drugs that are specific to Aurora B. Those molecules, which are theoretically shown to act as good inhibitors, are also verified experimentally. Promising results are obtained which display that our inhibitors cause reduction in Aurora B activity. As a second project, we identify cSrc specific inhibitors theoretically. The molecules found for cSrc are bound in the ATP pocket and extended into the allosteric site in the inactive conformation of the cSrc kinase. They are bound in conserved ATP pocket by hydrophobic interactions and resided in the allosteric site by making direct hydrogen bonds with residue GLU310. The binding mode of these inhibitors is the same as of type II kinase inhibitors. They bind to less conserved allosteric site and conserved ATP pocket simultaneously.

ÖZET

Hücre içinde faaliyet gösteren kinazların denetim mekanizmalarının bozulması birçok kanser çeşidine sebep olmaktadır. Bu nedenle kinazlar kanser tedavisi için yürütülen araştırmaların ana hedeflerindendir. Bu doğrultuda hücre içi birçok yaşamsal olayı düzenleyen kinazlara özgü küçük molekül inhibitörleri geliştirilmiş ve yeni inhibitörler geliştirilmesine çalışılmaktadır. Bu projede kanser hastalığının tedavisi için Aurora B ve cSrc kinazını inhibe eden yeni ilaçların bulunması ve geliştirilmesi amaçlanmaktadır. Aurora kinazlar sadece mitoz bölünme sırasında rol oynayıp bölünmeyen hücrelerde ifade edilmemesi sebebiyle kanser tedavisi için ideal hedeflerdendir ancak bu kinazını inhibe eden hiçbir ilaç henüz piyasada bulunmamaktadır. Aurora B kinaz için geliştiren ilaçların tümü kinazın aktif kısmına bağlanan ATP rekabetçi ilaçlardır bu ilaçlar az da olsa diğer yaşamsal kinazların da faaliyetini engellemektedir. Bu özgün araştırma projesinde Aurora B kinazın ve diğer 14 önemli kinazın aktif kısmına bağlanmayan ancak Aurora kinazların bu kinazları aktive eden proteinlerle bağlanma yerini hedefleyen moleküller hesaplamalı biyoloji metodları ile bulunmuş ve deneysel yöntemler ile sonuçların ümit vaat edici olduğu gösterilmiştir. İkinci bir proje olarak cSrc kinazın kendine özgü kısmı ilaç hedefi olarak belirlenmiştir. Aurora B projesinde uygulanan hesaplamalı yöntemler uygulanmıştır. Projede moleküller sadece hesaplamalı yöntemlerle bulunmuş deneysel çalışmalar yapılmamıştır. Bulunan ilaçlar özellikle cSrc kinaza bağlanmakta ve bağlanma şekli 2.çeşit kinaz sınıfına aittir.

ACKNOWLEDGEMENTS

The words are mere symbols of symbols and they are pointless if you don't use them to express your feel and opinion. I think now is the perfect time to express my sincere thanks to all the people who helped and made my life at Koç University a joyful experience.

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Chapter 1

INTRODUCTION

The central role of kinases in cellular processes is important. Deregulation of kinase activity in an expanding list of diseases suggest many kinases as potential drug targets [2]. The majority of small molecule kinase inhibitors target the ATP binding site. Kinase drug discovery results in a grand challenge due to significant similarity of kinase pockets. Imatinib (Gleevec; Novartis), the first small molecule kinase inhibitor to be approved for use in humans, has dramatically changed the prognosis for patients with chronic myeloid leukemia. The success of imatinib has demonstrated that targeting kinases can be a very effective therapeutic approach. The approval of additional small molecule kinase inhibitors has shown that imatinib is not unique. The large number of compounds which is currently in preclinical and clinical development progresses towards marketing approval. Therefore, small molecule kinase inhibitors are a new class of drugs that will grow significantly. [3-5]. A first step towards understanding how kinase inhibitor selectivity obtained is to identify unique residues in kinase pockets interacting with their specific inhibitors. Due to the significant similarity of kinase pockets, ATP competitive inhibitors show low kinase specificity. While **GSK461364** is a Polo-like kinase (PLK) inhibitor, it also inhibits Aurora A (AURA) and Cyclin-dependent kinase 2 (CDK2). **SU-6668** inhibits Aurora Kinases, VEGFR2, FGFR and PDGFR. **AT-9283** binds to all Aurora kinases, JAK2, JAK3 and ABL kinases[6]. The drugs have tendency to promiscuously inhibit kinases. We selected fifteen kinases (AURA, AURB, VEGFR2, FGFR, CDK2, cSrc, JAK2, PKA, PTK2, AKT1, ABL, PLK1, PLK4, KIT, and JAK3) to create kinase ligand network.

Natural products are derived from natural sources such as plants, animals or microorganisms. They have become important for pharmaceutical industry to identify potential drug candidates. Penicillin from the mould *Penicillium notatum,* morphine from the opium poppy and the anticancer drug taxol from the Pacific yew tree are important examples to the natural products. That is why in the first of this, the natural products have been chosen in our docking studies and continued with synthetic compounds found in ZINC database. We mainly used OpenEye and GOLD softwares for molecular modeling. Besides targeting kinase pockets, our vision includes targeting different binding site of drugs for the inhibition of Aurora B that have not yet been taken into account in previously reported studies. Indeed not only we aimed to find drugs that inhibit Aurora B but we also wanted to find the drugs that are specific to Aurora B.

This thesis aims to the discovery of specific kinase inhibitors and mainly focuses on inhibition of Aurora B and cSrc kinases.

In Chapter 2, a detailed literature review is given for Aurora B, cSrc and drugs targeting kinase –ligand interactions in literature.

In Chapter 3, detailed explanation of the methods used is given. Chapter 4 focuses on the discovered specific Aurora B kinase inhibitors as a result of virtual screening. Interactions between the kinase and the compounds are displayed. The unique approaches of the project are explained in the Chapter 4. Chapter 5 illustrates the interactions between cSrc and its specific inhibitors. Chapter 6 focuses on novel inhibitors found for other kinases.

The thesis concludes with a short summary of the work performed and future work.

Chapter 2

LITERATURE REVIEW

2.1 Aurora Kinases

Aurora Kinases have important roles in the centrosome cycle, spindle assembly, chromosome condensation, microtubule-kinetochore attachment, spindle checkpoint and cytokinesis. They are regulated through phosphorylation, the binding of specific partners and ubiquitin-dependent proteolysis. Aurora A and Aurora B are paralogue mitotic serine – threonine kinases; however they have distinct localization and functions [7]**.**

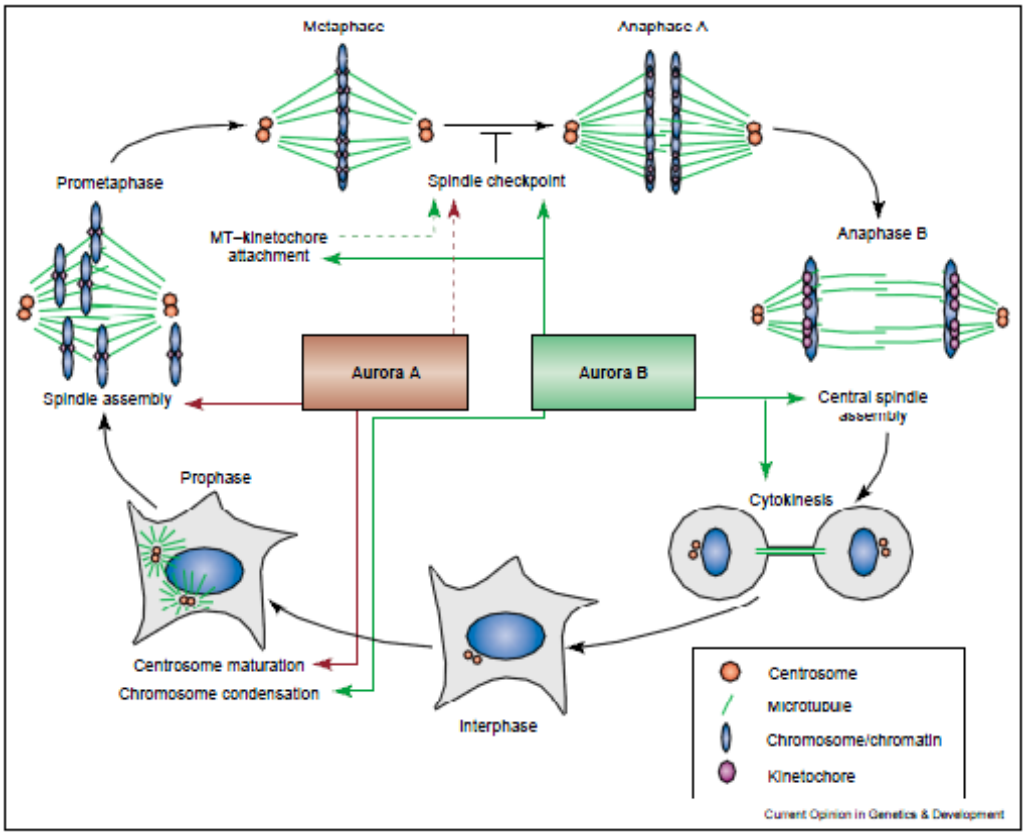

Figure 1 The roles of Aurora A and B in mitotic phase events [7**]**

2.1.1 Function and Regulation of Aurora B Kinases

Aurora B first localizes to centromere during interphase, then appears in the midzone of the central spindle in early mitosis and finally displays activity at the midbody during late mitosis. INCENP, survivin, borealin and Aurora B forms the core unit of Chromosomal Passenger Complex (CPC). [8] The most strongly conserved region of INCENP is the IN-box consisting of residues 798-840. In Figure 2 Aurora B: IN-box interaction was shown (purple and cyan, repectively). It binds and activates Aurora B and is a substrate of this kinase [9]**.** Aurora B phosphorylates histone H3 on Ser10 and has crucial role in during cleavage furrow and cytokinesis since it phosphorylates substrates that include vimentin, desmin, myosin II regulatory light chain, central-spindlin [10].Aurora B directs several proteins to the kinetochore. Furthermore it plays role in sensing and correction pathway of syntelic microtubule/kinetochore attachments in which both sister kinetochores are attached to microtubules from the same spindle pole [11]**.** The activation of Aurora B by INCENP is a two-step process in which INCENP partially activates Aurora B, and then full activation is reached after the phosphorylation of a conserved Thr-Ser-Ser (TSS) motif near the C terminus of INCENP (residues 848-850). The Aurora B: INCENP complex is an intermediately active state of Aurora B when the TSS motif is removed [12]. As we can see in Figure 2 and Figure 3, Aurora B: INCENP complex**,** Aurora B has the classical bilobar protein kinase fold. The N-terminal lobe (N-lobe, residues 86–174) is rich in β strands and functions in nucleotide binding and interacts with kinase regulators. The C-terminal lobe (C-lobe, residues 175–347) is mainly α-helical. The ATP binding pocket lies at the interface between the two lobes and C- terminal lobe has residues for directly phosphate transfer. In Figure 2 and 3c, the IN-box forms a molecular crown extending linearly about 70 Å along the N-lobe of the kinase [13].

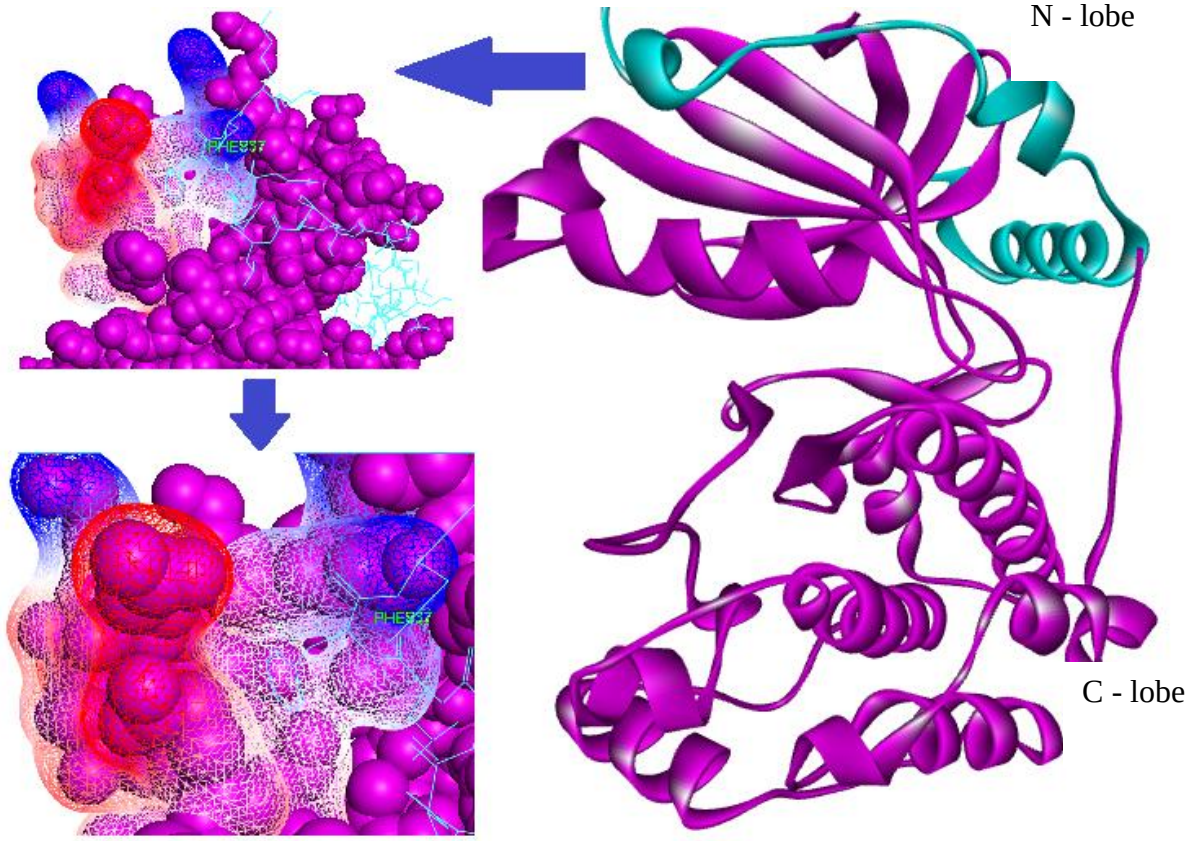

Figure 2 Aurora B: IN - box interaction (diagrams were created with Discovery Studio)

The IN-box residues Glu814, Tyr815, Tyr816, Lys817, Pro818, Ile819 and Asp820 make well ordered contacts with the C-terminal Aurora B residues Pro352, Pro353, Val354, and Tyr355. The equivalent interaction region is disordered in the Aurora A: TPX2 crystals [14]. The IN-box does not contain a hydrophobic core and uses its hydrophobicity to pack against Aurora B.

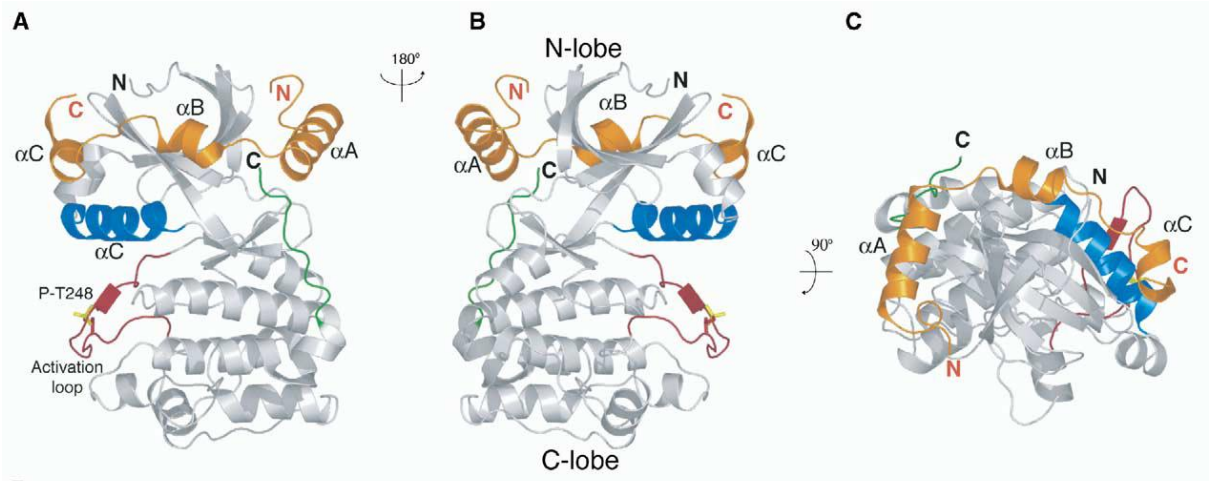

Figure 3 Overall View of the Aurora B : IN-box Complex[13]

Before starting more detailed explanation of Aurora B: INCENP interaction, we need to explain the superscripts. IN, Au-B indicates residues of INCENP and Aurora B, respectively. INCENP$^{790\text{-}847}$ is the INCENP segment lacking of the TSS motif. INCENP$^{790\text{-}856}$ is the segment of INCENP which TSS motif is found. Sessa *et al.*[13] study these segments to show the intermediate and fully active states of Aurora B, respectively. Later we will explain these mechanisms step by step. Glu791^{IN}, Trp801^{IN}, Pro818 ^{IN}, and Phe837^{IN}are only four residues in INCENP$^{790\text{-}847}$ are fully conserved. Pro799^{IN}, Trp801^{IN}, and Ala802^{IN} pack against a hydrophobic pocket of Aurora B consisting of residues Glu96, Leu99, Ile118 (Figure 3b) and the side chain of Trp801^{IN} is piled up the side chain of Arg111$^{Au\text{-}B}$. The helix α A^{IN} is amphipathic and binds Aurora B residues Leu109, Ile118, Phe172 with Leu807, Ala810 and Gln814 hydrophobic side chains (Figure 3c). The side chain of Gln814^{IN} forms hydrogen bonds with the main chain amide and carbonyl groups of Ile118$^{Au\text{-}B}$. This interaction keeps the C-terminal part of α A^{IN} helix closer to Aurora B. The side chains of Tyr815^{IN} and Tyr816^{IN} retain the side chain of Pro353$^{Au\text{-}B}$ in the C-terminal extension of Aurora B (Figure 3d). INCENP turns sharply with Pro818 to follow the surface of the N-lobe and forms α B^{IN} helix. It is also amphipathic (Figure 3e). Finally,

the INCENP chain progresses into αC^{IN} helix. The hydrophobic side chains of $Pro831^{IN}$, $Leu833^{IN}$, $Leu836^{IN}$, and $Phe837^{IN}$ mediate the interaction with Aurora B in this region (Figure 3f)[13]. $Phe837^{IN}$ is fully conserved in INCENP sequences. Its side chain points vertically into a deep pocket on the Aurora B surface composed of, $Leu129^{Au-B}$, $Glu135^{Au-B}$, $Leu136^{Au-B}$, $Arg139^{AuB}$ and $Ile166^{Au-B}$.

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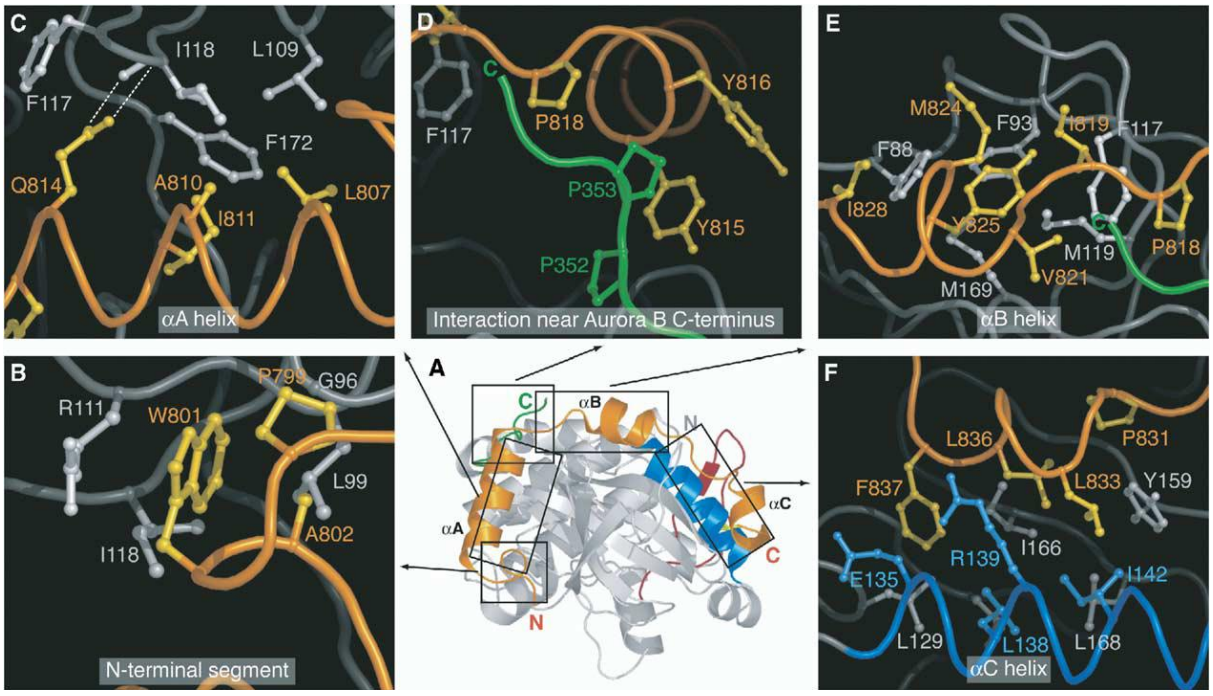


Figure 4 Details of the Aurora B: IN-box Interaction (orange: IN-box, gray: Aurora B, blue: Activation loop of Aurora B) [13]

2.1.2 Activation of Aurora B by INCENP

In the Aurora A:TPX2 complex TPX triggers a movement on the activation loop of Aurora A, which as a consequence adopts a fully extended conformation [13]. The activation loop

of protein kinases plays a critical role in substrate recognition for phosphate transfer, and its fully extended conformation is a hall- mark of active protein kinases [15]. In contrast with the Aurora A: TPX2 complex, INCENP does not make any direct contacts with the activation loop along its winding around the N- lobe of Aurora B (Figure 4f). If the conformation of the activation loop is indicative of an active kinase two more features explain why the Aurora B: INCENP$790^{–847}$ complex represents an intermediate state of activation rather than the fully active state observed in the Aurora A: TPX2 complex. The first feature is the suppression of the interaction between $Lys122^{Au\text{-}B}$ and $Glu141^{Au\text{-}B}$. This is probably the primary reason why the Aurora B: $INCENP^{790–847}$ complex is only partially active relative to the Aurora B: $INCENP^{790–856}$ complex. These residues reveals a buried ion pair that orients the α and β phosphates of ATP for phosphotransfer in active state of kinases [16]. The distance of α amino group of Lys122 and the carboxylate oxygens of Glu141 increases to ~5.4 Å in the Aurora B: INCENP structure. The conformational stability of $Lys122^{Au\text{-}B}$ indicates that $Glu141^{Au\text{-}B}$ does not play role in the function on $Lys122^{Au\text{-}B}$, and the function of $Glu141^{Au\text{-}B}$ may become important for catalysis when the phosphates of ATP are in the active site**.** $Glu141^{Au\text{-}B}$ resides on the αC helix of Aurora B. The change of its position relative to the equivalent residue in Aurora A can be explained by a 15° counterclockwise rotation of the $\alpha C^{Au\text{-}B}$ helix along its axis (Figure 5c). This rotation is likely caused by $Phe837^{IN}$, whose aromatic side chain pushes against the side chain of $Leu138^{Au\text{-}B}$ (Figure 5c). The relatively small rotation imposed by $Phe837^{IN}$ on the αC helix results in the unusual position of $Glu141^{Au\text{-}B}$. N- and C-lobes of Aurora B are opened as a result of a 15° rotation. The C-terminal segment of Aurora B is fully extended. The side chain of $Pro353^{Au\text{-}B}$ fits properly into the pocket formed by $Tyr815^{IN}$ and $Tyr816^{IN}$. The C-terminal tail cannot be stretched further. Aurora B: INCENP complex is partially active. Partially active complex phosphorylates the TSS motive of INCENP via activation loop and Leu138 pushes Phe837 out.

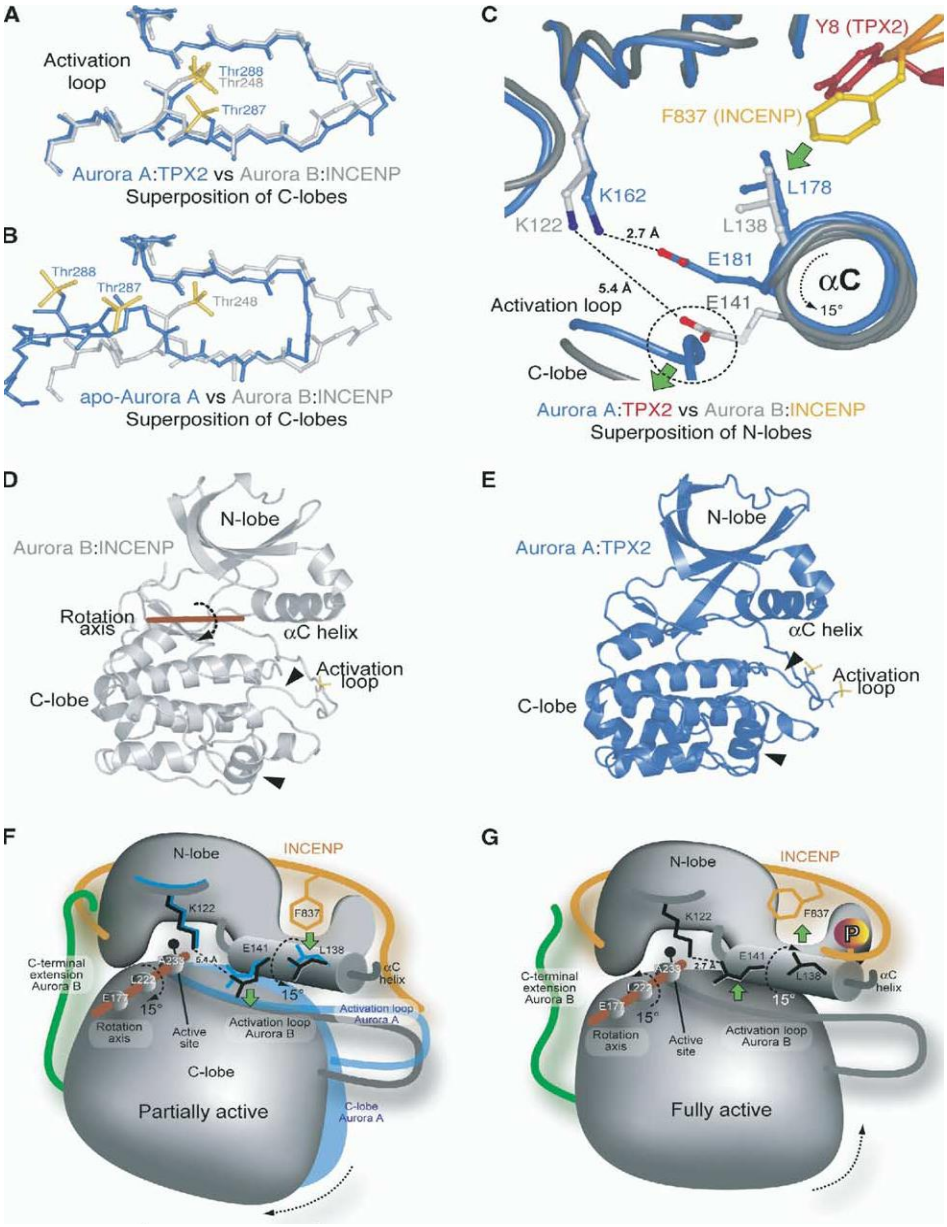


Figure 5 Activation Mechanism of Aurora B [13]

After movement of Phe837 side chain, $\alpha C^{Au\text{-}B}$ rotates back into a position that would restore the interaction of Glu141 with Lys122. It also allows the closure of the catalytic cleft. Aurora B: INCENP complex is fully active. Two hypotheses were proposed to interpret these observations. (1) The opening of the catalytic cleft is a consequence of the rotation of the $\alpha C^{Au\text{-}B}$ helix. $Phe837^{IN}$ causes the rotation, because its side chain pushes against the side chain of $Leu138^{Au\text{-}B}$ in the activation loop (Figure 5c). After rotation, the side chain of $Glu141^{Au\text{-}B}$ pushes on the open portion of the catalytic cleft. (2) The C-terminal tail of Aurora B is key to forcing an open state of the catalytic cleft via the interaction of $Pro353^{Au\text{-}B}$ with $Tyr815^{IN}$ $Tyr816^{IN}$. With an open cleft, αC rotates away from its expected position in an active kinase. In transition from partially active state to fully active state, it is thought that $Phe837^{IN}$ is more likely to take the center stage. First, $Phe837^{IN}$ is fully conserved in INCENP sequences whereas the C-terminal tail of Aurora B is poorly conserved. Second, $Phe837^{IN}$ is physically close to the TSS motif. Based on the comparison of structures with Aurora A: TPX2 complex and Aurora B: INCENP complex, even a relatively small rotation of the side chain of Phe837 can cause the rotation of the αC helix. It is shown that a larger change such as the substitution of Phe837 with alanine results in the complete loss of the interaction between INCENP and Aurora B [13].

2.1.3 Borealin and Survivin bind to INCENP

As mentioned above the core unit of Chromosomal Passenger Complex (CPC) consists of Aurora B, INCENP, Survivin and Borealin (Figure 6). While N-terminal domin is required to target the CPC to the central spindle and midbody, the C-terminal domain of Borealin is needed for targeting to the centromere. Aurora B does not form any contact to Survivin or Borealin. It is incorporated into the CPC via binding to the IN-box of INCENP. Hence localization of Aurora B during cell division is regulated by INCENP interaction [17].

Besides CPC there are other interacting proteins that are required for the activity of Aurora B in centromere and central spindle. [18]. The CPC components act as a single structure, they travel together. Disintegration of this complex ends up with failure of CPC targeting. [19].

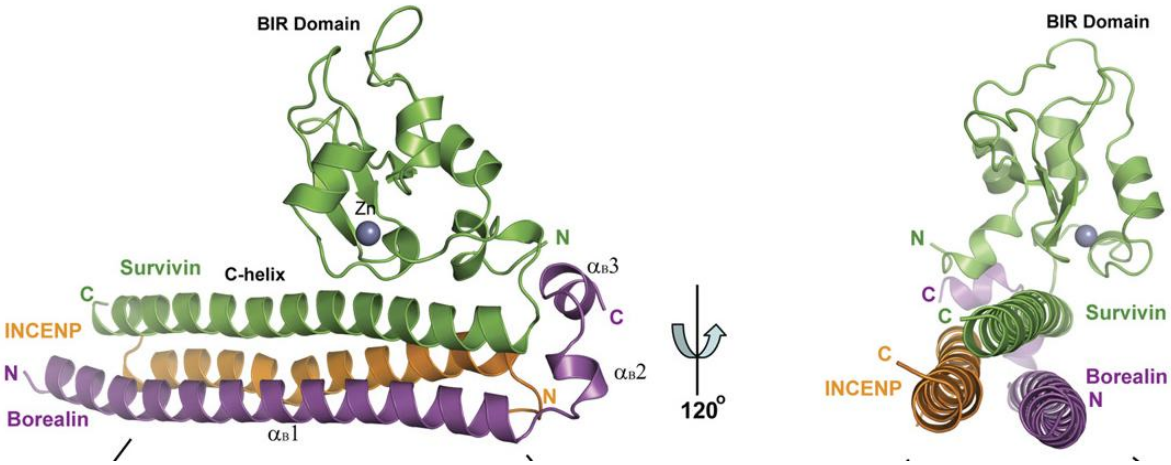


Figure 6 Survivin interacts with Borealin and INCENP [19]

2.2 cSrc Kinases

The Src kinase family modulates the terminal phosphate transfer from ATP molecule to the tyrosine residue in substrate protein. The Src family of protein tyrosine kinases is comprised of nine members which are Src, Fyn, Yes, Blk, Yrk, Fgr, Hck, Lck and Lyn [20].

The cellular proto – oncogene cSrc is a non receptor tyrosine kinase. They are highly conserved allosteric enzymes since they control the critical points of signal transduction pathways in cell growth and proliferation [21].

2.2.1 Function and Regulation of cSrc Kinases

cSrc plays a crucial role in the controlled disassembly of cell–cell adhesions during their dynamic regulation[22]. By having this role, its over expression can promote the epithelial tumor progression since disruption of epithelial cell-cell adhesion elevates cellular invasion [23]. cSrc also takes place in hypoxia-induced vascular endothelial growth factor (VEGF) production. Since VEGF induces angionesis, cSrc becomes important for endothelial cell survival signaling [24-26]. Platelet-derived growth factor receptor (PDGFR) activity is also regulated by cSrc because inhibition of the cSrc resulted in decreased PDGFR activity. It is found that cSrc regulates PDGFR-induced DNA synthesis via the Phosphatidylinositol 3(PI3)/Akt and mitogen-activated protein kinase (MAPK) signalling mechanism [27]. Moreover, cSrc activity modulation affects integrin signaling and focal adhesion kinase (FAK) signaling pathway. These studies illustrate the impact of cSrc interacting proteins on signal transduction pathways. Obviously, deregulation of kinase control results in problems with activation of downstream signaling pathways which are mostly seen in cancer development. cSrc kinase activity increased ubiquitously in many human cancers through over-expression or up-regulation of kinase. cSrc is aberrantly active in prostate, colon, pancreatic cancers and glioblostoma, as a result of which it became an important target for cancer treatment. [28-32]. There are three important structural regulations that keep the cSrc in its inactive state: (1) keeping the Tyr416 residue (Tyr419 for human) as unphosphorylated in the activation loop, (2) having the proline containing – linker between SH2 and SH3 modules occupied by SH3 module and (3) binding of SH2 module to the phosphorylated Tyr527 residue (Tyr530 for human) in catalytic domain [33] (Figure 7).Dephosphorylation of Tyr527 and phosphorylation of Tyr416 are needed for the activation of the cSrc kinase. Thus phosphorylation of Tyr416 and Tyr527 residues are the stimulatory and the inhibitory phosphorylation sites, respectively [21, 34].

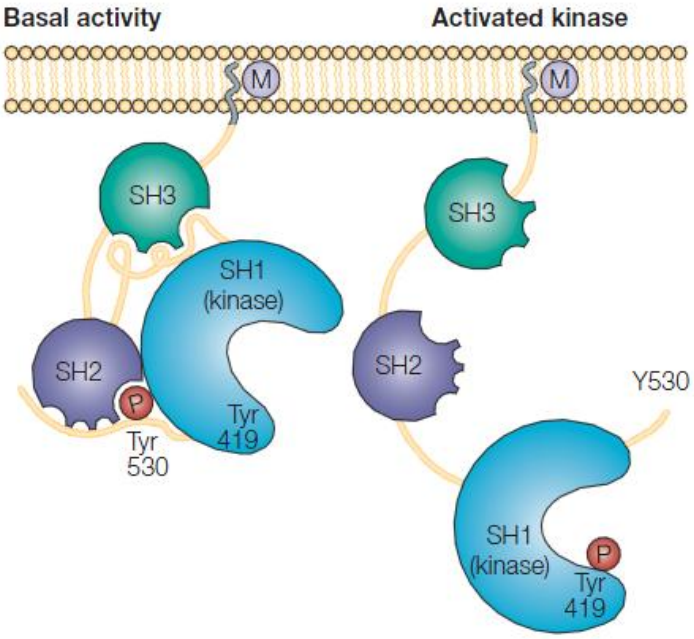


Figure 7 Structure and Activation of Human cSrc kinase [1]

The inactive state of the cSrc kinase catalytic domain is stabilized by the activation loop which forms a short alpha helix that buries into the catalytic site with the side chain of Tyr416. In the inactive state, formation of the Lys295–Glu310 salt bridge is prevented by an outward movement of alpha C helix (the residues 304-316) which is pushed out by the activation loop. This is called DFG-out conformation which is the main property of inactive kinases. The alpha helix loop is important for maintaining the cSrc kinase in its inactive state. Tyr416 can be autophosphorylated by another cSrc kinase molecule as a result of dephosphorylation of the Tyr527 residue and this event results in the active state of cSrc kinase [35, 36]

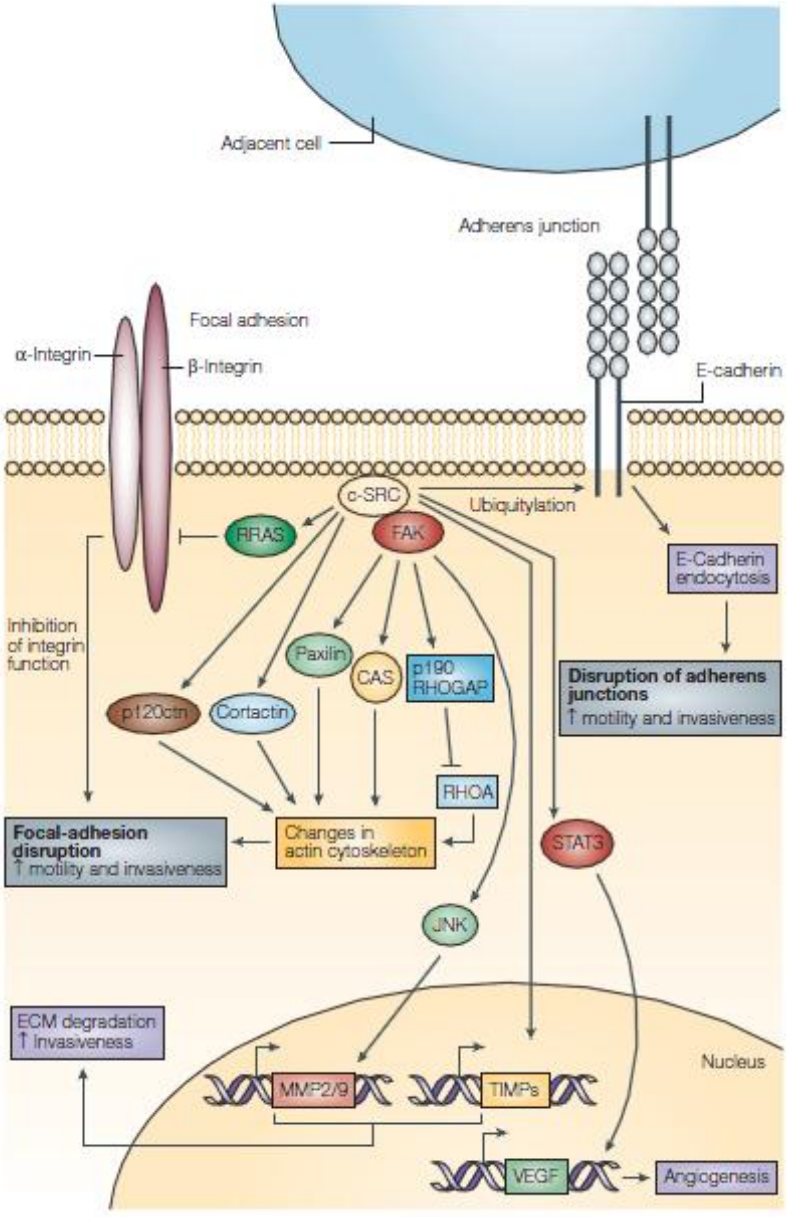
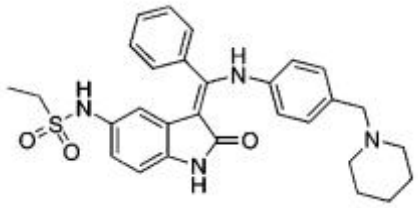


Figure 8 Effects of cSrc on Tumour-cell behavior [1]

Motility and invasiveness result in the loss of cell-cell adhesion. E-cadherin regulates the cell-cell adhesions. cSrc enhances the loss of adhesion by triggering the ubiquitylation of E-cadherins. E-cadherins removed by endocytosis and it causes the disruption of adherens junctions. cSrc stimulates RRAS and it inhibits integrin function hence it results in focal-adhesion disruption. Focal-adhesion disruption causes to increase mobility and invasiveness (Figure 8).

2.3 Small molecule inhibitors for Aurora B and cSrc in literature



Compound **Hesperadin** is reported as an inhibitor of Aurora-B (IC50 of 250 nM) with significant cross-reactivity against six other kinases [37].

Figure 9 Hesperadin, Aurora B inhibitor [38]

The inhibition of histone H3 phosphorylation (Ser10) results in endoreduplication and large polyploid cells in the absence of cell division.

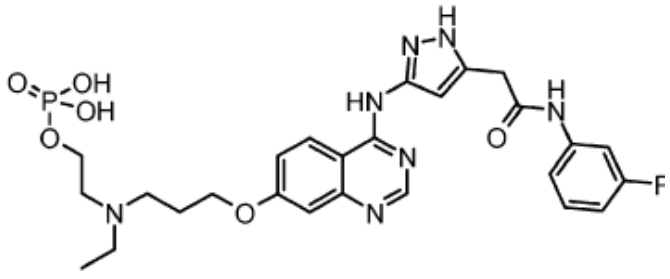


Figure 10 AZD1152–hydroxyquinazoline pyrazol anilide (HQPA)[39**]**

AZD1152 is a dihydrogen phosphate prodrug of a pyrazoloquinazoline Aurora kinase inhibitor [AZD1152–hydroxyquinazoline pyrazol anilide (HQPA)] and is converted rapidly to the active AZD1152-HQPA (Figure 10) in plasma [38]. AZD1152-HQPA is a highly potent and selective inhibitor of Aurora B (IC50; 0.36 nmol/L) compared with Aurora A (IC50; 1,369 nmol/L) and has a high specificity compared to 50 other kinases [39]. After administering AZD1152 to animals with human tumor xenografts, an inhibition of histone H3 phosphorylation was indicated following by a failure of tumor cell division. Also endoreduplication and induction of tumor cell death by apoptosis were observed. Moreover, these findings are consistent with the observed effects of AZD1152-HQPA in vitro [40]. The phenotype of AZD1152 treated animals are distinct from the phenotype associated with antimitotic/ anti-tubulin agents, such as paclitaxel where cells usually arrest in mitosis [39].Analyses of tissue from AZD1152-treated tumors displayed a failure of cell division leading ultimately to apoptosis which is the indication of the inhibition of Aurora B activity. The suppression of histone H3 phosphorylation was followed by accumulation of 4N cells, with subsequent accumulation of polyploid cells. Increased apoptosis in AZD1152-treated tumors were observed compared to control. It supports a mechanism by which induction of endoreduplication leads to polyploidy and eventual apoptosis [39]. Hence histone H3 phosphorylation was found to be a sensitive and highly dynamic marker of Aurora kinase inhibition.

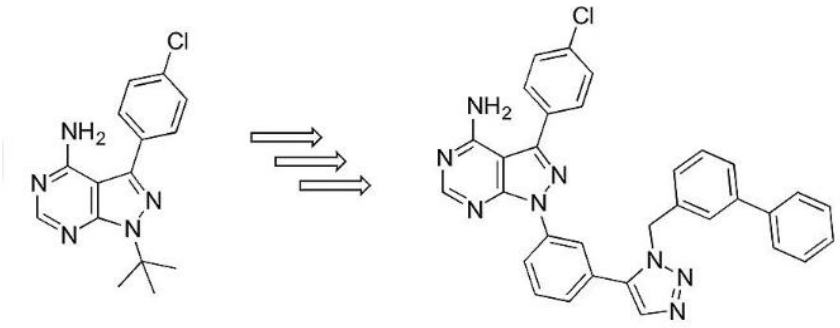


Figure 11 Highly selective cSrc inhibitor design [41]

Compound in Figure 11 targets the phosphate binding loop (P-loop) of the cSrc kinase. Compound in left is modified and highly selective compound is obtained.

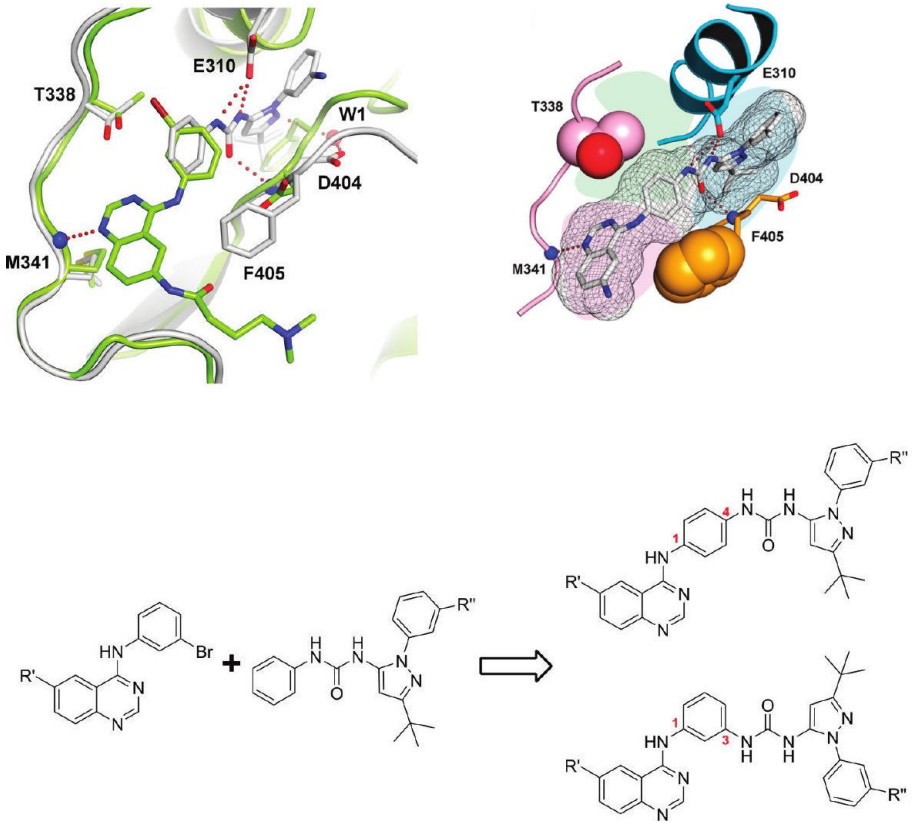


Figure 12 Mechanism of rationally designed type II inhibitors based on the binding modes of type I 4-aminoquinazolines and type III pyrazoloureas bound to cSrc [42]

Fragment-based drug discovery suggests the way to obtain molecules that have higher binding affinities. Combining the two weak binders should come up with molecules having higher binding affinities. [42]. Getlik et al. performed that combination and they obtained RL45 which binds to wild type cSrc kinase in the DFG-out conformation (Figure 12). The cocrystallized structure showed that RL45 displays type II inhibitor binding mode which it spans from the allosteric site to the kinase hinge region. Hence RL45 binds to cSrc in its

DFG-out conformation and locks the kinase in inactive state forming the critical interactions [42].

Chapter 3

METHODS

3.1 Computational methods for drug design and discovery

OpenEye scientific software (OpenEye Scientific Software, Inc., Santa Fe, NM, USA) was mainly used for the molecular modeling. Kinase receptor preparation and docking were performed by using FRED (Fast Rigid Exhaustive Docking) v2.2.5 (OpenEye)[43, 44] and Discovery Studio 3.0 Visualizer (Accelrys, http://accelrys.com/). Ligands were downloaded from the ZINC database (ZINC version 12)[45] and were prepared for docking by using OMEGA v2.4.3 (OpenEye)[46-48].

3.1.1 Kinase receptor preparation

The ABL2 (PDB code 2XYN[49]), AKT1 (PDB code 3MVH[50]), AURA (PDB code 3HA6 [51]), AURB (PDB code 2VRX[52]), CDK2 (PDB code 3LFQ[53]), cSrc (PDB code 3F3V[42]), FGFR (PDB code 3RHX[54]), JAK2 (PDB code 3KRR[55], JAK3 (PDB code 3PJC[56], KIT (PDB code 3G0E[41]), PKA (PDB code 3BWJ[57]), PLK1 (PDB code 3KB7[58]), PLK4 (PDB code 3COK), PTK2 or FAK (PDB code 2JKK[59]), VEGFR2 (PDB code 3C7Q[60] X-ray structures were downloaded from PDB (Protein Data Bank[61]). The crystal structures were visualized using Discovery Studio 3.0 Visualizer and active sites were determined by selecting the cocrystalized molecule (known active site inhibitor) in the structure and finding the close residues to the selected molecule. Discovery Studio 3.0 Visualizer calculates the minimum distance of all residues to the selected molecule and chooses residues within 3.5 Å. Those residues within 3.5 Å are the binding site or active site of our kinase and they are saved in a new pdb file which will be called as active site box in FRED receptor preparation. Crystallographic waters and any

active molecules bound to the protein active site were stripped because FRED treats any molecule in the active site as a part of the protein and they can block the ligand binding to the active site. We removed crystallographic waters and any active molecules bound to the protein active site using Discovery Studio 3.0 and saved as new protein pdb file. Receptor files were created with new protein file and active site file to define the active site of protein in FRED which creates its own receptor file to use in docking. We used default parameters to create receptor in FRED. The correct protonation state of the protein was checked by FRED and 4 Å was added from the center of the active site to every side (x, y, z coordinate axes). The size of the active site box, the size of the outer contour and the size of the inner contour are three important three properties of the receptor. Reasonable values for the inner and contour volumes are 50-100 and 500-2000 cubic Angstroms, respectively. We optimized and confirmed these properties by docking known inhibitor to the receptor using FRED.

3.1.2 Ligand Preparation

Ligands were downloaded as mol2 files from the ZINC natural products database and conformers were generated taking these files as input structures with OMEGA (OpenEye Scientific Software). OMEGA is the program designed for use with large libraries for computer – aided drug design. It generates multi – conformer structure databases with speed and reliability. OMEGA performs rapid conformational expansion of drug-like molecules, yielding a throughput of tens of thousands of compounds per day per processor. OMEGA creates conformational databases and they are used as input in FRED. Default parameters were used in conformer generation. OMEGA generated additional conformers by taking our structures in database as input. It enumerated the ring conformations and invertible nitrogen atoms. Firstly, OMEGA removed exocyclic substituents from the input model and added them after generation of every possible ring conformations. As a next

step, OMEGA enumerated invertible nitrogens. Invertible nitrogens have pyramidal geometry, no specified stereochemistry and no more than one hydrogen. Finally, torsion driving is the last step and we used default torsion library which is stored in OMEGA.

3.1.3 Docking and Virtual screening

FRED docks molecules using an exhaustive search algorithm that systematically searches rotations and translations of each conformer of the ligand within the active site at a specified resolution. During the exhaustive search, unrealistic poses are filtered, and those that survive are scored. Following the exhaustive search, the 100 top scoring poses are subject to systematic solid body optimization (a local exhaustive search at a finer resolution than the global exhaustive search). The best scoring pose is then used to rank the ligand against other ligands in the screening database. The protein is held rigid during the docking process, as are the conformers of the ligand. Ligand flexibility, however, is implicitly included by docking a conformer ensemble of each molecule. FRED treats each conformer of a molecule as rigid during the docking process, although the docking process is effectively flexible with respect to the ligand because multiple conformers of each ligand are docked into the site. Generating conformers prior to running FRED reduces runtime (because conformation generation is done independent of the active site, it needs only to be done once for any given ligand database, rather than for every docking run).

FRED was used to dock the conformers created by OMEGA in the active site of the fifteen kinases to determine the best conformations. We preferred to use chemgauss 3 scoring function for exhaustive search which is also default scoring function for FRED. It is a fast scoring function which is one of the important criteria in exhaustive search. It describes the shape and chemistry of compounds by using smooth Gaussian functions. The conformations obtained from exhaustive docking were scored with Chemscore scoring

function by FRED. The well oriented ligand poses after enumeration had lowest energy with Chemscore. Chemscore scoring function is a sum of 5 crucial components: (1) lipophilic interactions (2) hydrogen bonds (3) metalic interactions (4) clash penalty and (5) frozen rotatable bond penalty.

3.1.4 LigandScout

Hydrogen bond formation and lipophilic side chain interactions which reveal the hydrophobic nature of the active site characterize the binding site of protein. The interactions of docked kinase - ligand complexes were visualized and analyzed using LigandScout 2.03 software [62]. LigandScout 2.03 software depicts the interactions between protein and ligand. It generates structure-based pharmacophores with exclusion volume spheres which define the borders of the active site. The ligand interactions with critical residues in the active site of protein reveal binding mechanism of ligand. The ligand interactions found using LigandScout was correlated with Chemscore scoring components. Hydrogen bonds and lipophilic interactions are almost same in both softwares.

3.1.5 BROOD

BROOD is a software application designed to help project teams in drug discovery explore chemical and property space around their hit or lead molecule. BROOD generates analogs of the lead by replacing selected fragments in the molecule with fragments that have similar shape and electrostatics, yet with selectively modified molecular properties. BROOD fragment searching has multiple applications, including lead-hopping, side-chain enumeration, patent breaking, fragment merging, property manipulation, and patent protection.

3.2 Experimental methods

3.2.1 Cell Culture

HeLa PC3 cells were cultured in low glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) with 1% penicillin streptomycin. Cells were cultured at 37 °C in humidified air containing 5 % CO_2.

3.2.2 Synchronization of HeLa PC3 cells

Before treating cells with our kinase inhibitors, it is necessary to synchronize cells at G1/S cell cycle stage. To synchronize cells at this stage we used double thymidine block method. When the cells reached %70-80 confluency, first 200µL thymidine was added to fresh medium. After 20 hours, cells were washed three times with 37 °C warmed phosphate-buffered saline (PBS) and fresh medium added. After 8 hours, second 200µL thymidine was added to fresh medium. After 17 hours, the cells in the interphase were taken. After 10 hours, the cells were taken in mitosis. Drug treatment was made 17 hours after the second thymidine treatment. The cells in the interphase and mitosis were taken with a protocol: Medium was sucked and the cells were washed with 5ml PBS. Then 1.5ml tyripsin was added and 5-6 minutes incubated at 37 °C. 5 mL cold PBS was added into plates and transferred to the falcons. They were centrifuged at 1200 rpm at 4 °C for 4 min. Then the supernatants were sucked and pellets were resuspended with 5 ml cold PBS. Again they were centrifuged at the same conditions. After resuspending the pellet with 1ml cold PBS, they were transferred to the eppendorfs. They were centrifuged at 7500 rpm at 4 °C for 4 min. Supernatants were sucked and pellets were resuspended with PBS - Triton X lysis buffer. They were centrifuged at 7500 rpm at 4°C for 5 min. Supernatants were transferred to new eppendorfs. Laemmli sample – DTT was added into samples with 1:1 ratios and heated at 75 °C for 5 min.

3.2.3 Immunoblot Analysis of Aurora B

Protein concentrations were measured with BCA Assay. Equal amounts of protein were separated by SDS-PAGE and transferred to nitrocellulose membranes. Blots were blocked for 30 min in TBS Tween - 20 supplemented with 4% nonfat milk and subsequently incubated overnight at 4 °C in primary antibodies, namely, anti-phospho-H3 Ser10, anti-actin. After being washed, blots were incubated with secondary antibodies and then detected on film using enhanced chemiluminescence (ECL) detection system.

Chapter4

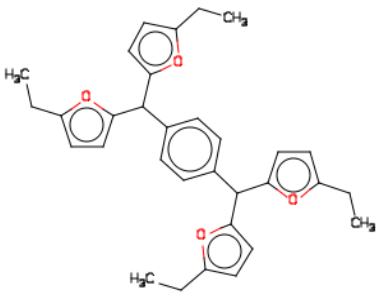
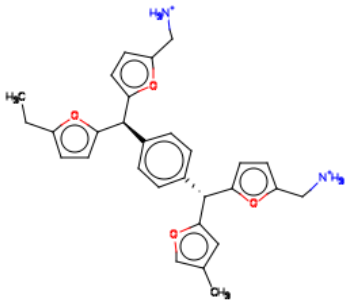
Results and Discussion for Aurora B

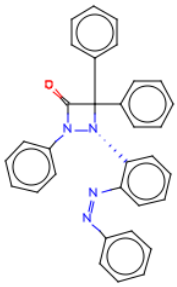
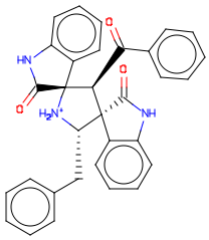
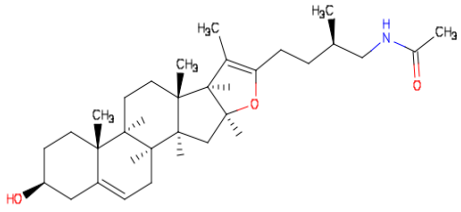
In preclinical *in vivo* studies, aurora kinase inhibitors suppressed tumor growth regardless of their specificity profiles, highlighting the therapeutic potential of Aurora A and Aurora B as candidate targets for anticancer drugs. Inhibition of Aurora B activity in vivo has profound effects on tumor growth and there are several drugs in clinical phases that have the potential for efficacy in multiple tumor types; however, these candidates also have the potential to bind and inhibit the other kinases because of the similarity of the kinase pockets. Our vision includes different binding site for the inhibition of Aurora B that have not yet been taken into account in previous reported studies. Indeed, the goal of this study not only to find compounds that inhibit Aurora B, but also to find drugs that are specific to Aurora B. Since Aurora B is inside the cell, the potential inhibitor has to obey Lipinski's rule of 5. These rules ensure that a drug has good permeation and adsorption if there are less than 5 H-bond donors and less than 10 H-bond acceptors. Also the molecular mass of the drug should not exceed 500 daltons and LogP should not be greater than 5. Function of Aurora B is regulated by phosphorylation, binding of specific partners and ubiquitin dependent proteolysis. Localization of Aurora B is provided by the monoubiquitination. In our project we were interested in the regulation of Aurora B by binding of the specific partner, INCENP. The most strongly conserved region of INCENP, the IN-box interacts and activates Aurora B. INCENP is also substrate of Aurora B. When we look at the activation mechanism of Aurora B in Figure 5, it is two step processes in which INCENP partially activates Aurora B, whereas full activation requires the phosphorylation of a TSS motif near the C terminus of INCENP.

In this project, Aurora B: INCENP interaction site has been chosen as a target region. The compounds were docked to the pocket where Phe837^{IN} side chain points vertically into a deep. 200,000 natural products have been docked through OpenEye scientific software. Ten natural products shown in Table 1 (OpenEye and GOLD Scores) have been determined as possible drug candidates specifically bind to the Aurora B:INCENP interaction site in a first step. After completion of docking for natural products, approximately 5 million compounds were screened using OpenEye scientific software. We obtained twenty three compounds which specifically bind to the Aurora B:INCENP interaction site. All thirty three hit molecules do not bind to other fourteen kinases (AURA, VEGFR2, FGFR, CDK2, cSrc, JAK2, PKA, PTK2, AKT1, ABL, PLK1, PLK4, KIT, and JAK3) and kinase pocket of Aurora B. In Table 1 and Figure 13, we could not give ZINC ID numbers and structures of the five compounds due to ongoing experiments. Figure 14 displays the twenty three compounds found as a result of the virtual screening with NIH small molecules and Princeton molecules which were downloaded from ZINC database. Some of them have low molecular weights which were in the range between 200 and 350 g/mol. They could be further modified but due to the time limitation we did not perform it. Figure 15 and Table 3 show important hit molecules arising between compounds in Figure 14. Figures 16, 17, 18, 19, 20 display the binding mode of five natural products in the Aurora B: INCENP interaction site. The molecules interact with the critical residues on the surface of Aurora B where the side chain of Phe837^{IN} points into vertically. Leu129, Glu135, Arg139, Ile142, Ile166 and Leu168 are the residues which molecules form the hydrophobic interactions or hydrogen bonds. Molecule revealed in Figure 20 was modified structure of molecule ZINC03847564. Modification was performed with BROOD software. While ZINC03847564 does not form hydrogen bond, modified ZINC03847564 forms two hydrogen bonds with Arg139 and Glu143. Binding of the molecule to Aurora B: INCENP

interaction site was enhanced as a result of the modification, while binding of it to Aurora B kinase pocket was diminished (Table1).

Table 1 OpenEye and GOLD Scores

Drug	Molecular Weight (g/mol)	OpenEye Kinase Site [kcal/mol]	OpenEye Incenp Site [kcal/mol]	Gold Kinase Site [kcal/mol]	Gold Incenp Site [kcal/mol]
Compound 1	465	-3,55	-6,02	-4,50	-8,40
Compound 2	495	-3,24	-6,60	-5,50	-9,60
Compound 3	449	-3,60	-6,70	-5,00	-8,40
ZINC03847564	482	-4,47	-6,77	-6,21	-9,10
					
ZINC038447564mod.	472	-3,35	-7,41	-5,70	-9,60
					

Drug	Molecular Weight [g/mol]	OpenEye Kinase Site [kcal/mol]	OpenEye Incenp Site [kcal/mol]	Gold Kinase Site [kcal/mol]	Gold Incenp Site [kcal/mol]
ZINC13402472	480	-3,27	-6,0	-4,06	-9,60
					
ZINC12878305	500	-3,30	-6,20	-4,30	-9,60
					
Compound 4	463	-3,30	-6,20	-4,30	-9,60
Compound 5	496	-3,16	-6,0	-5,0	-7,20
ZINC12654725	455	-3,60	-6,14	-5,50	-7,20
					

Drugs	ABL	CDK1	VEGFR2	PTK2	PLK4	PLK1	PKA	KIT	FGFR	AKT1	JAK2	JAK3	CSRC	AURA	AURB Kin	AURB Phen
ZINC12654725	-3.30	-3.98	-4.5	-3.44	-2.95	-4.06	-4.49	-3.1	-3.55	-4.61	-3.65	-7.17	-4.04	-3.92	-3.6	-6.14
Compound 5	-2.86	-3.41	-4.77	-4.28	-3.95	-3.35	-3	-3.99	-4.88	-4.17	-2.53	-4.78	-4.32	-3.25	-3.14	-6
Compound 4	-2.77	-2.66	-5.08	-2.39	-3.87	-2.99	-2.36	-3.09	-4.86	-2.15	-3.49	-4.17	-2.86	-3.59	-3.3	-6.2
Compound 1	-2.63	-1.91	-4.38	-0.68	-3.43	-1.83	-4.38	-2.89	-3.48	-2.59	-1.53	-2.87	-1.61	-3.7	-3.55	-6.02
ZINC03847564	-2.29	-3.99	-5.22	-4.78	-4.43	-3.79	-4.3	-2.83	-5.52	-4	-4.75	-5.45	-3.67	-4.78	-4.47	-6.77
ZINC13402472	-2.22	-1.94	-5.12	-3.35	-4.23	0	-1.02	-0.3	-3.05	-3.37	-1.3	-2.72	-3.1	-4.54	-3.27	-6
Compound 3	-1.97	-1.84	-4.95	-2.92	-3.59	-2.22	-2.31	-3.19	-4.14	-3.83	-3.18	-4.45	-2.79	-3.64	-3.6	-6.7
Compound 2	-1.77	-2.75	-5.25	-1.7	-4.93	-2.73	-3.98	-2.61	-3.18	-4.75	-3.45	-5.98	-1.48	-3.23	-3.24	-6.6
ZINC03847564mod.	-1.69	-3.74	-4.54	-5.02	-5.4	-4.37	-4.67	-3.36	-5.19	-4.06	-3.65	-4.22	-4.67	-5.15	-3.35	-7.41
ZINC12878305	-0.48	-1.84	-4.67	-2.39	-3.55	-1.33	-3.79	-0.96	-3.3	-3.35	-1.49	-4.41	-1.38	-4.74	-3.77	-6.78

Table 2 Binding Energies of ten compounds for fifteen kinases

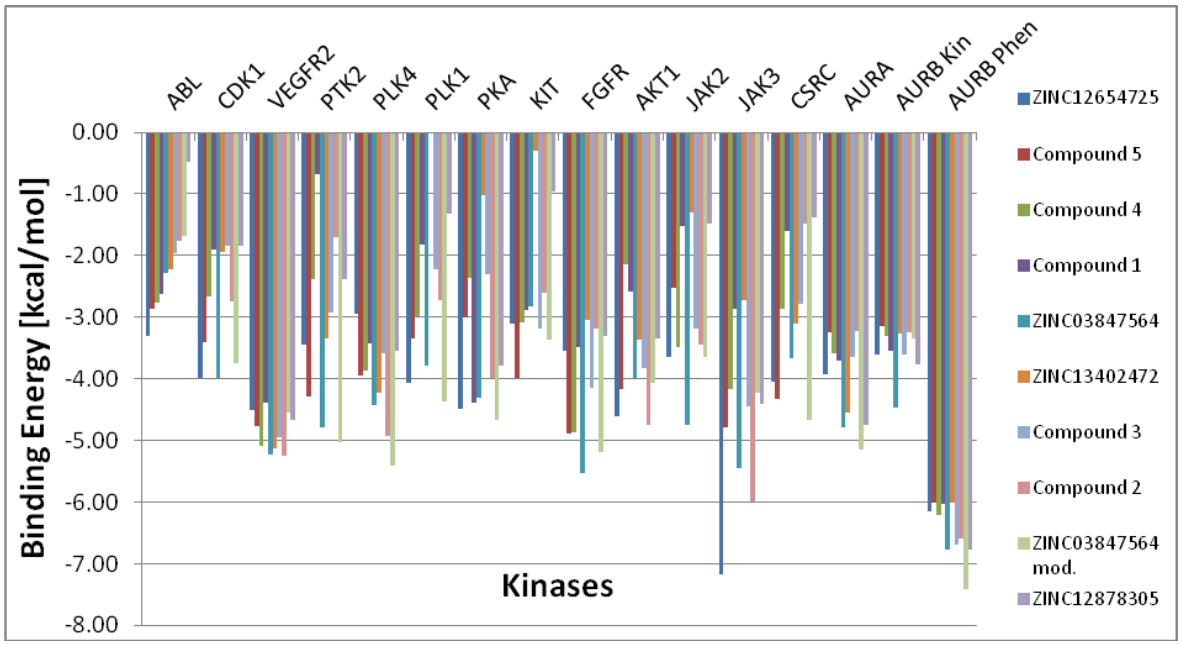


Figure 13 Binding energies of ten compounds for fifteen kinases

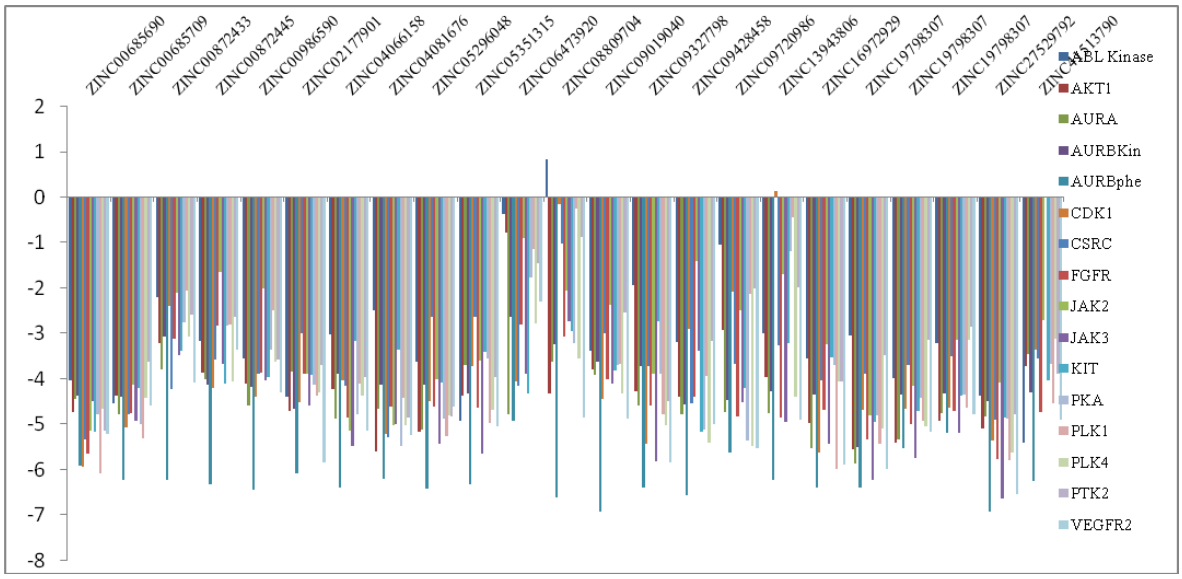


Figure 14 Binding energies of twenty three compounds for fifteen kinases

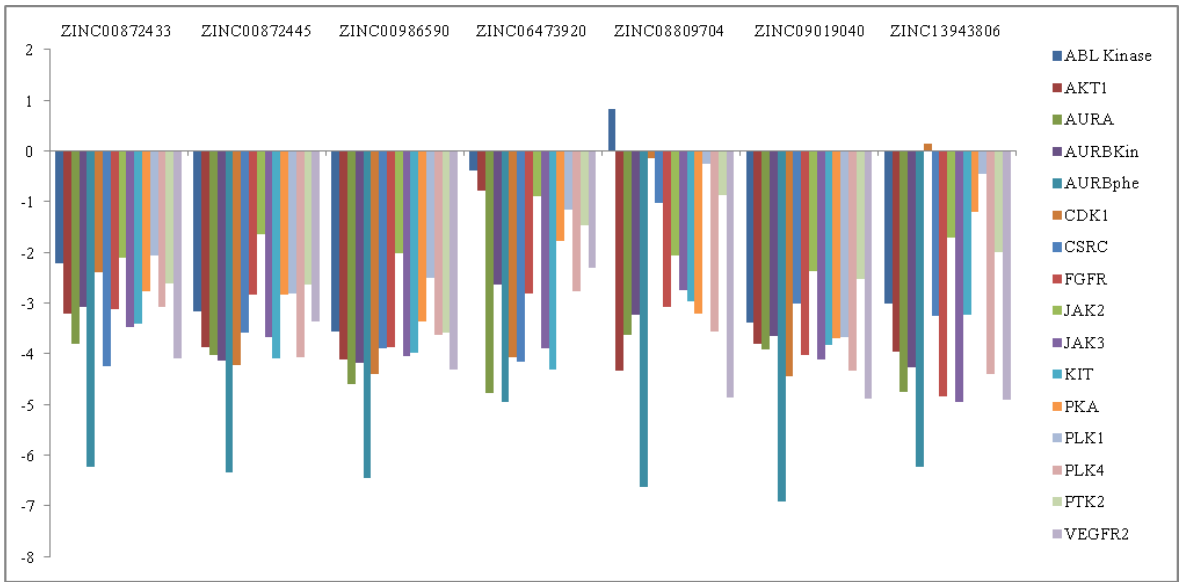


Figure 15 Binding energies of seven compounds for fifteen kinases

Table 3 Binding energies of seven compounds for fifteen kinases

Kinases	ZINC00872433	ZINC00872445	ZINC00986590	ZINC06473920	ZINC08809704	ZINC09019040	ZINC13943806
ABL	-2,22	-3,17	-3,56	-0,38	0,84	-3,39	-3,00
AKT1	-3,22	-3,88	-4,12	-0,78	-4,33	-3,81	-3,96
AURA	-3,80	-4,01	-4,60	-4,78	-3,62	-3,91	-4,76
AURBKin	-3,08	-4,12	-4,18	-2,64	-3,24	-3,64	-4,27
AURBphe	**-6,23**	**-6,34**	**-6,44**	**-4,94**	**-6,63**	**-6,92**	**-6,23**
CDK1	-2,40	-4,21	-4,41	-4,06	-0,15	-4,44	0,14
CSRC	-4,24	-3,58	-3,89	-4,16	-1,02	-3,01	-3,25
FGFR	-3,13	-2,83	-3,86	-2,80	-3,08	-4,02	-4,85
JAK2	-2,11	-1,65	-2,01	-0,90	-2,06	-2,38	-1,71
JAK3	-3,48	-3,67	-4,04	-3,89	-2,73	-4,11	-4,95
KIT	-3,40	-4,10	-3,97	-4,32	-2,96	-3,83	-3,22
PKA	-2,75	-2,84	-3,37	-1,78	-3,22	-3,69	-1,20
PLK1	-2,07	-2,81	-2,50	-1,16	-0,24	-3,67	-0,44
PLK4	-3,08	-4,06	-3,63	-2,77	-3,56	-4,34	-4,39
PTK2	-2,60	-2,64	-3,57	-1,46	-0,87	-2,53	-1,99
VEGFR2	-4,08	-3,37	-4,31	-2,30	-4,86	-4,88	-4,90

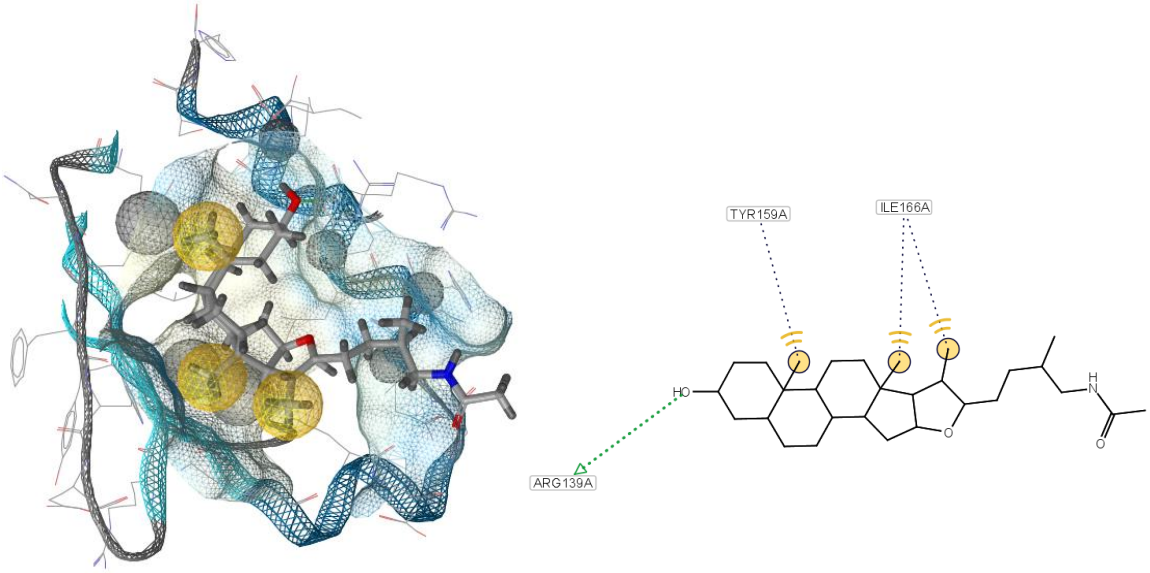


Figure 16 ZINC12654725 binds to the Aurora B:INCENP interaction site

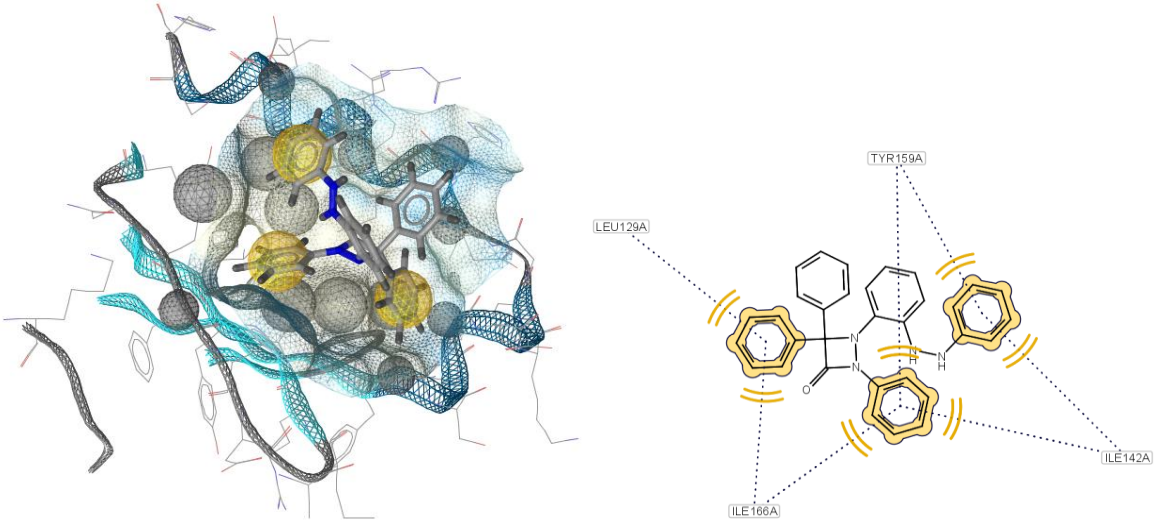


Figure 17 ZINC13402472 binds to the Aurora B:INCENP interaction site

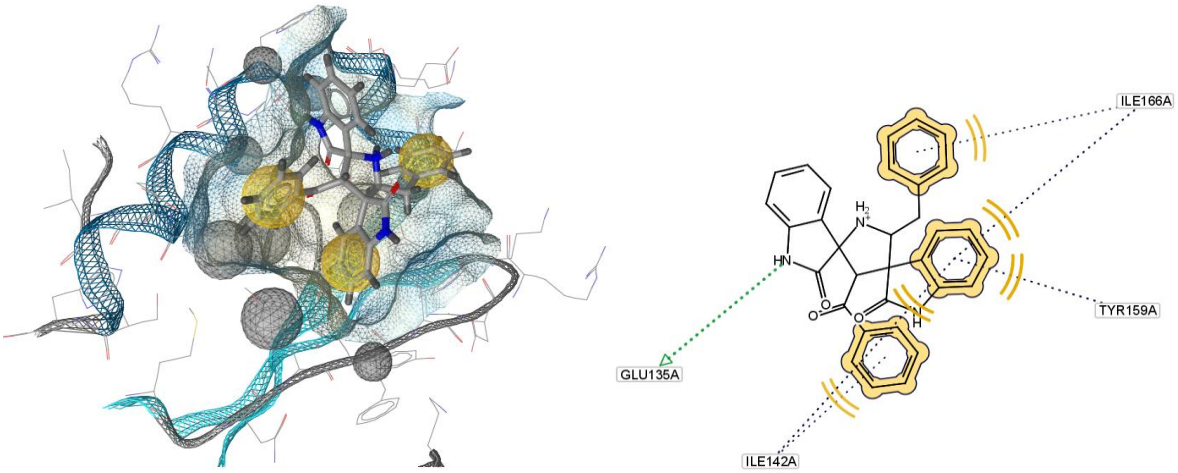


Figure 18 ZINC12878305 binds to the Aurora B:INCENP interaction site

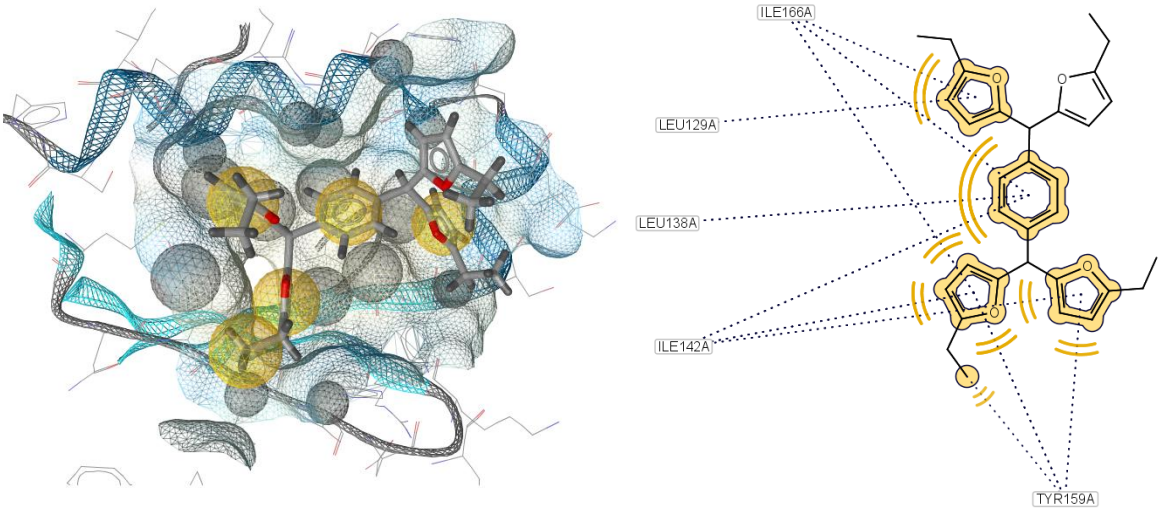


Figure 19 ZINC03847564 binds to the Aurora B: INCENP interaction site

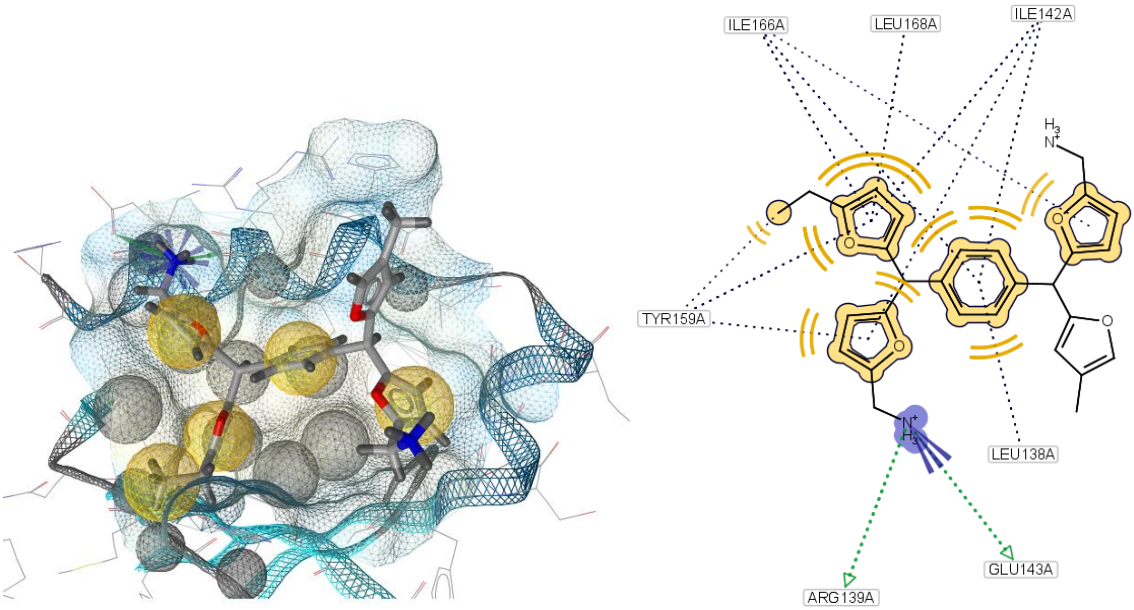


Figure 20 ZINC03847564Modified binds to the Aurora B:INCENP interaction site

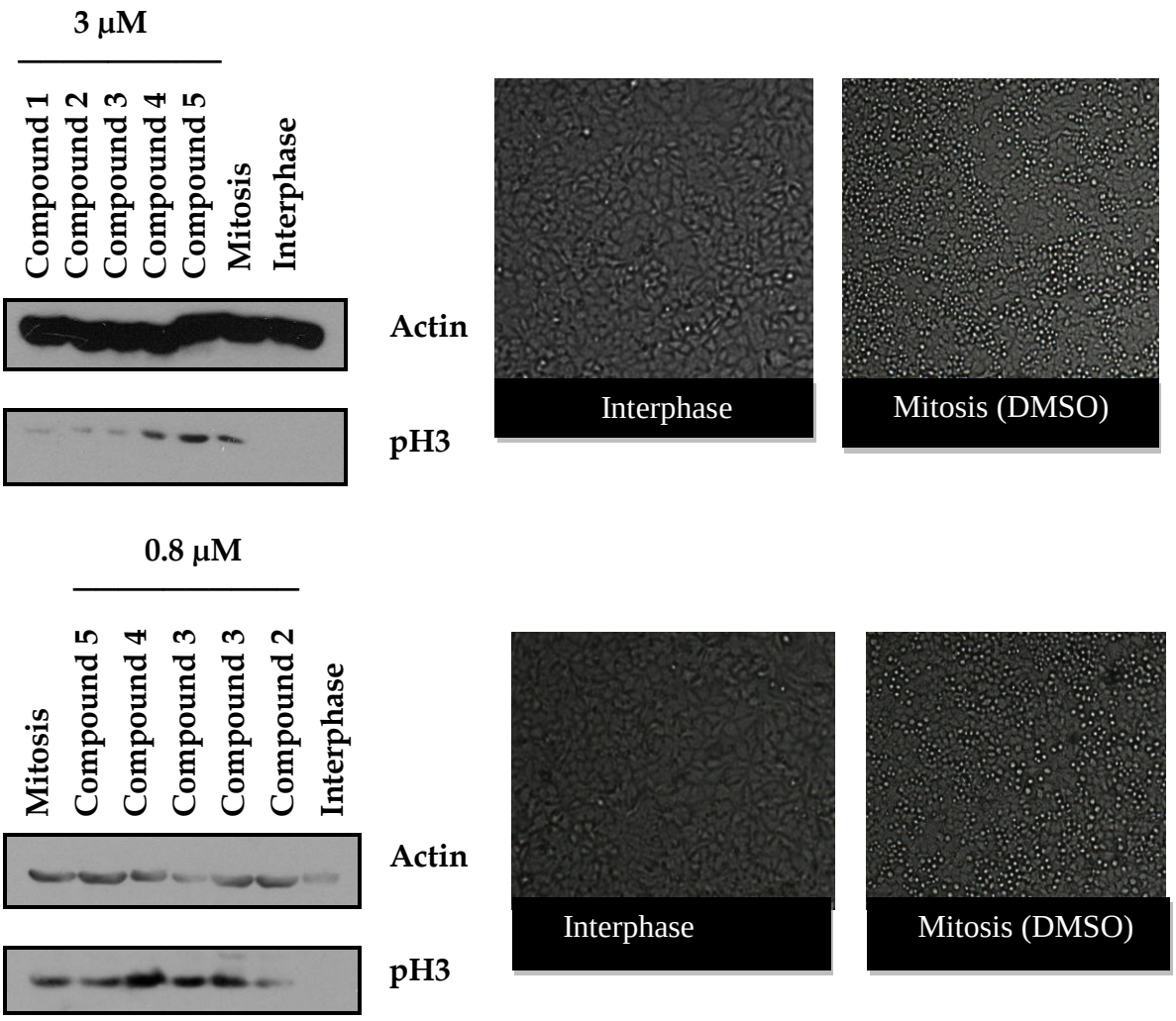


Figure 21 HeLa PC3 cells were synchronized and then they were treated with 0.8 μM and 3 μM compounds for 10 h

In order to investigate the effect of several compounds on HeLa cells, several experiments were performed.

The cells were lysed and blotted for Actin and Phospho histone H3 proteins. The blotting analysis was performed for the cells treated with compound 1, compound 2, compound 3,

compound 4 and compound 5 (Figure 21). We synchronized the cells at interphase and the cells were treated with the compounds when the cells released from interphase. We obtained promising results with compound 1, compound 2 and compound 3 for 3 μM treated cells. For 0.8 μM concentration of compound 2 promising result was obtained in terms of reduction in phosphorylation of histone H3. Actin protein was chosen as a control loading. As can be observed in figure 21, it is clear that cells were synchronized perfectly. As expected any band for phoshoH3 could not be observed in interphase cells because histone H3 is phosphorylated during mitosis. The images of cells taken before drug treatment and after 10 h treatment demonstrate the synchronization. The bands of pH3 for cells treated with 3 μM compound 1, 2 and 3 are weak when compared to control mitosis (DMSO treated only). This shows that phosphorylation of histone H3 was partially inhibited. Next the cells will be treated with higher concentration of compounds (10 μM). If results suggest any changes in phosphorylation, immunoprecipitation assays for INCENP localization will be applied. This will ultimately give significant information about binding of the compounds to the correct interaction site of Aurora B: INCENP but not to the kinase pocket.

Chapter5

Results and Discussion for cSrc

The goal of this study is to find novel inhibitors that specifically bind the cSrc kinase in its inactive state. Wild type crystal structure of cSrc kinase was used (PDB codes 3F3V)[42] in which the inhibitor bind to the kinase with the DFG-out conformation. ZINC natural products database which is composed of about 200,000 compounds was used for virtual screening. Virtual screening aims to identify compounds from a database that fit into the target site of the protein. We performed virtual screening for fifteen kinases that we indicated above and we identified wild type cSrc kinase specific molecules which only bind to the cSrc kinase. Eventually, cSrc kinase specific eight substantial hit molecules were identified. Six of them share the similar structures. Table 4 displays the chemical properties of eight molecules and Figure 22 exhibits their structures. Table 5 demonstrates the binding energies of the molecules with the components of the Chemscore scoring function. In addition to eight molecules, three closest following molecules to each hit molecule were also indicated in Table 5. This information is useful to distinguish the differences in modes of binding for different molecules.

Table 4 The properties of eight molecules which were calculated using LigandScout

Compound	H-bond donors	H-bond acceptors	Molecular weight (g/mole)	Rototable bonds
ZINC08300039	3	1	342.51	8
ZINC08635951	4	2	397.57	6
ZINC08635285	4	3	402.54	7
ZINC08635693	4	3	496.73	9
ZINC08635694	4	3	453.66	6
ZINC08635798	4	2	417.62	6
ZINC12654246	2	4	490.64	15
ZINC04236491	2	4	475.57	10

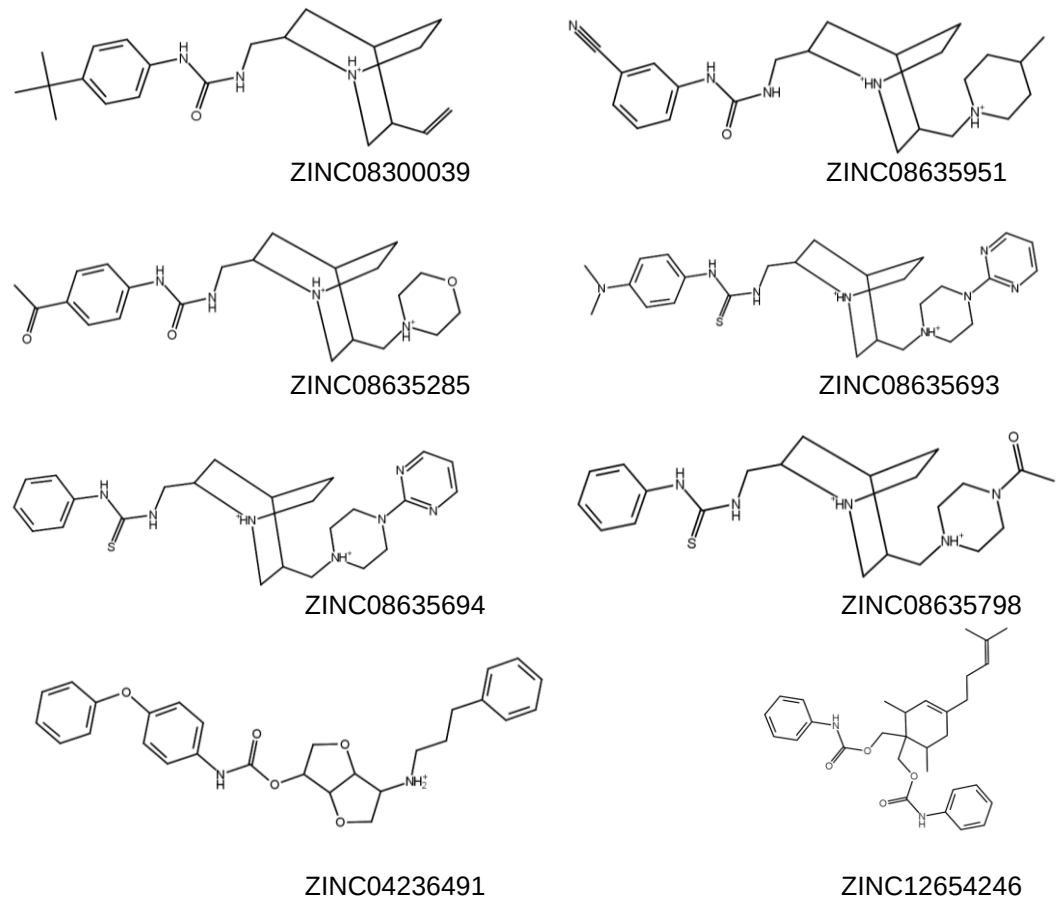


Figure 22 The structures of eight inhibitors obtained as a result of virtual screening

ZINC08300039 binds to the target site of cSrc with -30.54 kjoule/mole binding free energy; hence it inhibits cSrc with exceptional specificity over the other kinases **(**Table 5 and Appendix A1 Figure 1). The amide group of methylurea forms two direct hydrogen bonds with GLU 310. 5-vinyluinuclidine makes hydrogen bond with Asp404 (Figure 23b). Thus, molecule is sandwiched between the allosteric site residues by making N-H ··· O hydrogen bonds. They are classified as strong hydrogen bonds and constitute -7.07 kjoule/mole binding energy component of Chemscore scoring function. Notably, 4-tert-butylphenyl moiety is captured by the hydrophobic residues Met 314, Leu317, Leu322,

Val323 and Val 402 in the allosteric site of the kinase domain (Figure 23a). Besides this, vinyl group attached to quinuclidin contributes to total hydropobicity by interacting Met341, Leu393 and Phe405 in the hinge region of the kinase domain.

All these hydrophobic interactions comprise -26.74 kjoule/mole, LIPO component of Chemscore. The high specificity of molecule for cSrc over other kinases is unusual because they also contain very similar hydrophobic kinase pockets and this can be observed from the similar lipophilic interactions with the molecule (Appendix A1 Figures 1a, 1b, 1c). Distinctively, specificity is ensured with Frozen Rotatable Bonds (RB) and clashes. Frozen Rotatable Bond is a penalty for the loss of entropy due to rotatable bonds that can no longer rotate upon binding of the molecule to the target site of the kinase and clash is penalty for clashes between ligand and protein. These penalties are very low for cSrc when compared to the other kinases. Hence, low penalty score makes the binding of the molecule to the cSrc favorable addition to other enhancing interactions.

Table 5 Binding energy description of molecules with one bound three unbound kinases

Drug Name	Kinase	Binding Energy (kjoule/mole)	LIPO	RB	H-Bond	Clash
ZINC12654246	**CSRC**	**-31.11**	**-33.29**	**3.57**	**-3.34**	**1.96**
	AURB	-18.65	-25.35	5.31	-0.00	1.38
	JAK3	-18.69	-25.14	3.57	-0.00	2.88
	VEGFR2	-18.38	-29.54	5.91	-0.00	5.25
ZINC08635951	**CSRC**	**-29.98**	**-27.12**	**4.24**	**-9.45**	**2.35**
	PLK4	-15.16	-19.35	4.24	-2.63	2.58
	PLK1	-17.84	-27.13	4.24	-0.65	5.71
	FGFR	-19.80	-19.57	4.24	-5.02	0.55
ZINC08635798	**CSRC**	**-28.54**	**-27.75**	**4.14**	**-6.68**	**1.74**
	CDK1	-17.09	-18.42	4.24	-3.34	0.42
	ABL	-17.41	-26.66	4.14	-3.59	8.69
	PLK1	-18.22	-24.58	4.14	-0.00	2.21
ZINC08635694	**CSRC**	**-31.29**	**-30.91**	**3.69**	**-6.68**	**2.59**
	FGFR	-17.75	-24.32	3.69	-3.34	6.21
	AKT1	-18.24	-21.49	4.33	-1.36	0.28
	JAK2	-15.14	-20.37	1.81	-0.00	3.42
ZINC08635693	**CSRC**	**-30.22**	**-34.78**	**5.36**	**-6.68**	**5.88**
	CDK1	-21.20	-23.37	2.49	-2.54	2.23
	AURB	-17.42	-24.75	5.36	-0.00	1.97
	PTK2	-14.28	-18.61	2.81	-0.29	1.80
ZINC08300039	**CSRC**	**-30.54**	**-26.74**	**2.29**	**-7.07**	**0.99**
	KIT	-19.43	-23.49	2.29	-4.36	6.13
	PLK4	-19.36	-30.25	2.53	-6.65	15.02
	PTK2	-19.72	-17.71	2.29	-6.68	2.38
ZINC04236491	**CSRC**	**-30.21**	**-30.53**	**3.83**	**-6.56**	**3.06**
	AKT1	-18.23	-27.22	4.61	-0.26	4.64
	FGFR	-17.67	-20.59	2.81	-0.00	0.12
	AURB	-19.73	-27.12	4.61	-0.88	3.65
ZINC08635285	**CSRC**	**-28.21**	**-26.59**	**4.24**	**-8.29**	**2.44**
	AURA	-18.02	-15.50	4.17	-6.68	0.00
	ABL	-14.93	-21.65	4.24	-2.33	4.82
	KIT	-13.65	-20.56	5.93	-0.00	0.98

The ChemScore score (**Binding Energy**) is a sum of the following components in **OpenEye**: **LIPO**; Interaction between lipophilic atoms, **RB**; **Frozen Rotatable Bond** Penalty for loss of entropy due rotatable bonds that can no longer rotate upon binding to the active site, **H-Bond**; **Hydrogen Bond** Interactions between donors and acceptors, **Clash;** Penalty for clashes between ligand and protein.

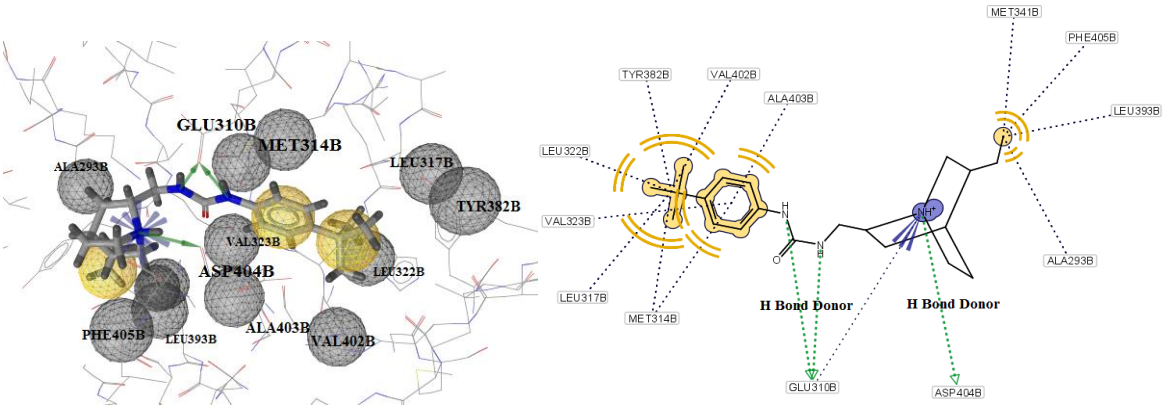


Figure 23 cSrc kinase with ZINC08300039. (a) 3D representation of cSrc with bound ZINC08300039. (b) 2D representation of interaction of ZINC08300039 with cSrc kinase specific residues

In Figure 24 demonstrates the binding of compound ZINC08635951 to cSrc. The binding of compounds ZINC08635951 and ZINC08300039 is similar where amide moiety of methylurea in compound ZINC08635951 makes additional hydrogen bond with Asp404 and has higher RB and clash penalties. The compound binds to kinase with -29.98 kjoule/mole binding energy. 3-cyanophenyl group contacts to hydrophobic residues Val313 and Met314 in the allosteric site of kinase domain. Methyl moiety attached to piperidyl interacts with Leu 273, Ala 293 and Tyr340 in the hinge region of the kinase domain. In addition to hydrophobic interactions, hydrogen bonds also provide the selectivity of ZINC08635951 to cSrc. It can be observed that the binding mode of ZINC08635951 to other kinases in supplementary data (Appendix A1 Figures 2a, 2b, 2c).

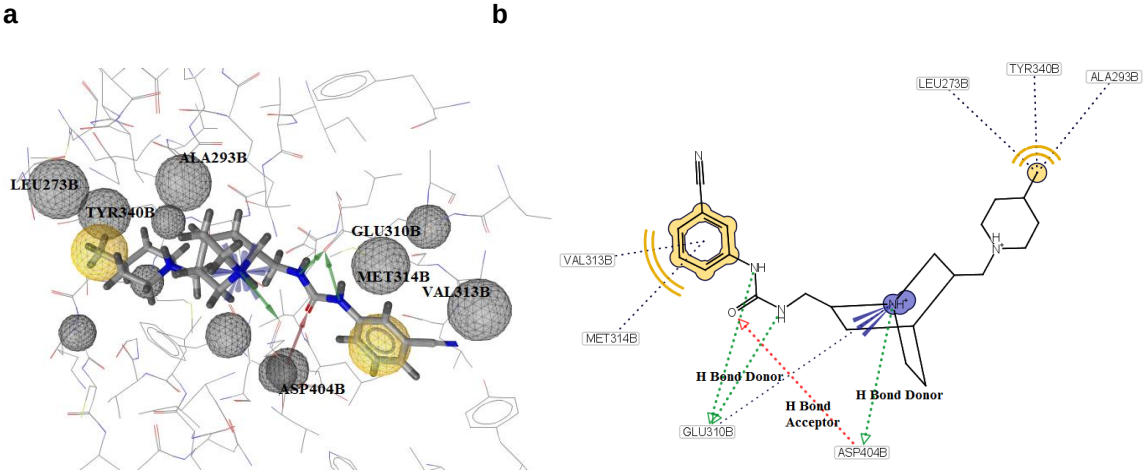


Figure 24 cSrc kinase with ZINC08635951. (a) 3D representation of cSrc with bound ZINC08635951. (b) 2D representation of interaction of ZINC08635951 with cSrc kinase specific residues

In Figure 25, compound ZINC08635285 fits into the hinge region and allosteric site of the kinase domain. Acetylphenyl moiety interacts with Met 314 and Val323 hydrophobic residues. Amide groups make hydrogen bonds with Glu310. 5-quinuclidin also forms hydrogen bond with Asp404. Although morpholinomethyl does not interact with any residues, compound has -26.59 kjoule/mole binding energy components which indicates the existence of hydrophobic interactions. The compound forms far less interaction with other kinases as illustrated in Appendix A1 Figures 3a, 3b, 3c. Total Chemscore binding energy is -28.21 kjoule / mole for cSrc while it is higher for other kinases (Table 5).

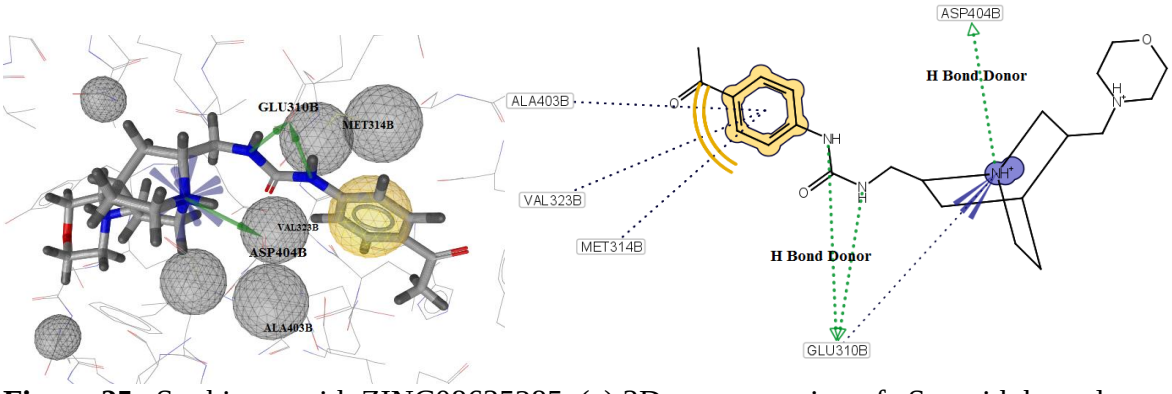


Figure 25 cSrc kinase with ZINC08635285. (a) 3D representation of cSrc with bound ZINC08635285. (b) 2D representation of interaction of ZINC08635285 with cSrc kinase specific residues

4-dimethylaminophenyl moiety of ZINC08635693 enhanced the compound to bind cSrc with favorable selectivity profile. The moiety interacts with highly hydrophobic residues Met314, Leu317, Leu322, Met374, Val377 and Val402 in the allosteric site of the kinase domain. These interactions constitute -34.78 kjoule/mole binding energy component arising from lipophilic interactions. Amide group of thiourea and quinuclidine make hydrogen bonds with Glu 310. Quinuclidin and pyrimidine ring interact with the side chain of Phe405 which is one of the important residues of DGF motif (Figure 26).

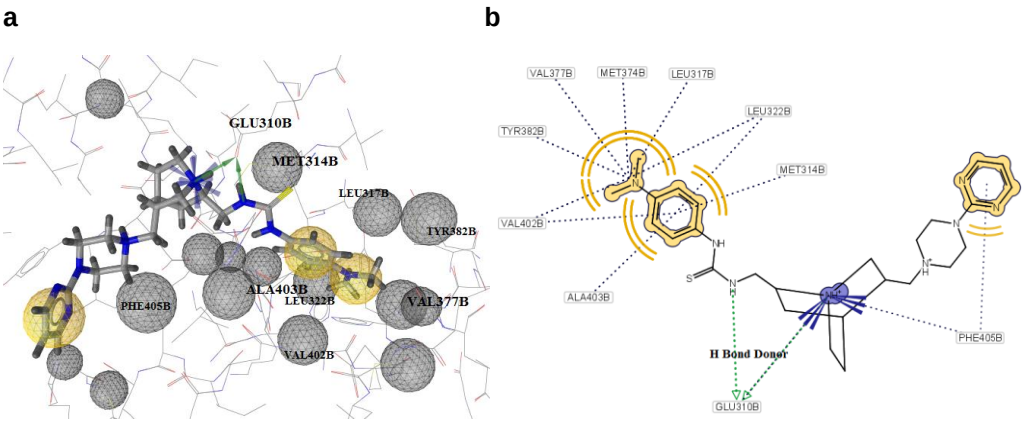


Figure 26 cSrc kinase with ZINC08635693. (a) 3D representation of cSrc with bound ZINC08635693. (b) 2D representation of interaction of ZINC08635693 with cSrc kinase specific residues

Dimethylamino group is missing in compound ZINC08635694 when compared to ZINC08635693 (Figure 26). Notice that the missing group decreases the hydrophobic interactions with allosteric site in the kinase domain; on the other hand, it causes the decline in RB and clash penalties (Table 5). Hence ZINC08635694 is equipotent with ZINC08635693 against cSrc. Pyrimidine ring of ZINC08635694 has contact to Leu273 in addition to Phe405 (Figure 27).

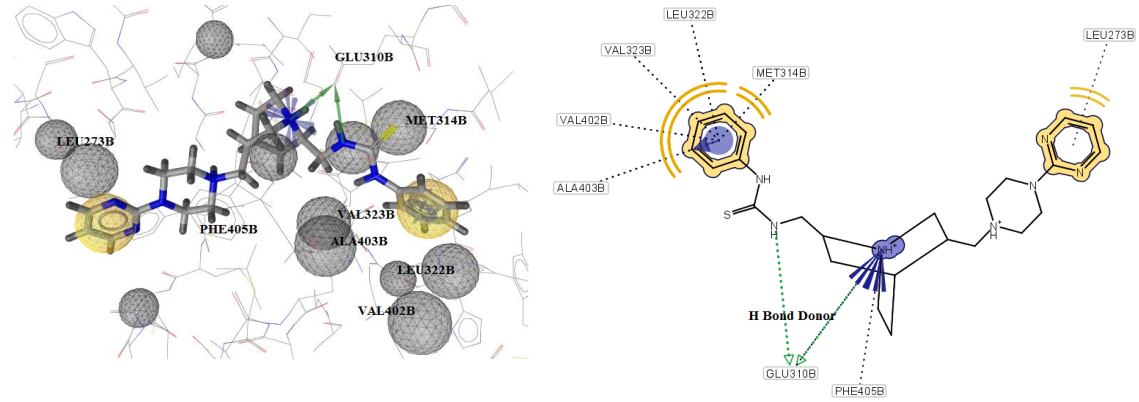


Figure 27 cSrc kinase with ZINC08635694. (a) 3D representation of cSrc with bound ZINC08635694. (b) 2D representation of interaction of ZINC08635694 with cSrc kinase specific residues

Compound ZINC08635798 with a phenyl group attached to thiourea interacts well with Met314, Leu322 and Val402 hydropobic residues in the allosteric site of kinase domain (Figure 28). Moreover, amide group and quinuclidine fit properly forming hydrogen bonds with Glu310 in the allosteric site. Quinuclidine also contacts to Phe405 which keeps the compound – kinase interaction in the transition part from allosteric site to hinge region of kinase domain. Notably, ZINC08635798 forms far less hydrophobic interactions with CDK1. Despite it displays equipotent hydrophobic interactions with cSrc, ABL and PLK1, hydrogen bonds and clash penalties make it selective to cSrc (Appendix A1 Figures 6a, 6b, 6c).

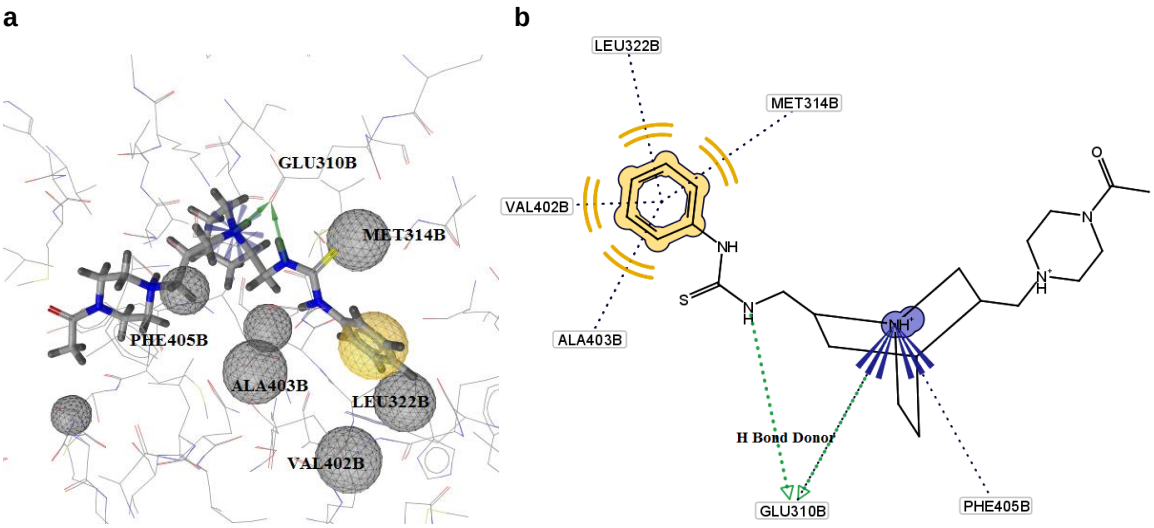


Figure 28 cSrc kinase with ZINC08635798. (a) 3D representation of cSrc with bound ZINC08635798. (b) 2D representation of interaction of ZINC08635798 with cSrc kinase specific residues

Molecule ZINC12654246 binds the active site of cSrc kinase domain with -31.11 kjoule/mole binding free energy. It binds cSrc with exceptional specificity over the other kinases forming 2 direct hydrogen bonds with Glu310 and Asp404 and important

hydrophobic interactions (Figure 29). Notably, ZINC12654246 binds to cSrc in a different fashion to above mentioned molecules. It shows quite high interactions with kinase domain residues. The high specificity of the molecule for cSrc over other kinases is unusual because the binding sites of them are very similar geometrically. They also contain very similar hydrophobic kinase pockets and we can see the similar lipophilic interactions with the molecule (Appendix A1 Figures 7a, 7b, 7c). So specificity comes from entropy and clash penalties. These penalties are very low for cSrc with respect to the other kinases. That makes the binding of the molecule to the cSrc favorable.

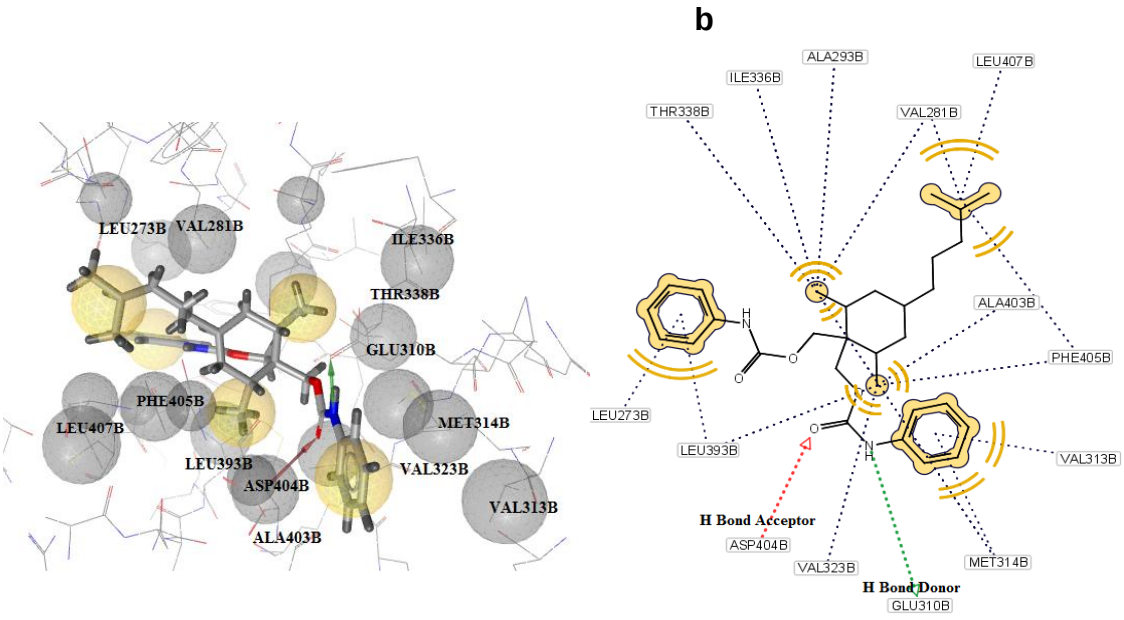


Figure 29 cSrc kinase with ZINC12654246. (a) 3D representation of CSRC with bound ZINC12654246. (b) 2D representation of interaction of ZINC12654246 with CSRC kinase specific residues

Compound ZINC04236491 forms four hydrogen bonds with residues Glu310, Thr338, Asp404. Nitrogen and oxygen moieties of 3-phenylpropylamine and furan ring constitute strong hydrogen bonds, respectively. What is more to the point is hydrophobic interaction which compound forms with Leu273, Met314, Leu322, Leu393, Val402 and Ala403. It associates with them through aromatic rings of 3-phenylpropylamine and phenoxyphenylcarbamate moieties (Figure 30).

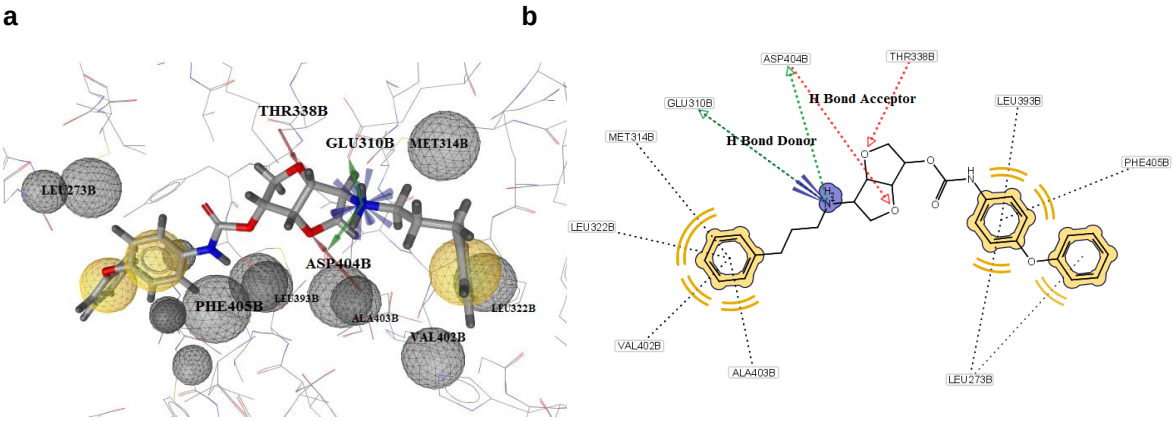


Figure 30 cSrc kinase with ZINC04236491. (a) 3D representation of cSrc with bound ZINC04236491. (b) 2D representation of interaction of ZINC04236491 with cSrc kinase specific residues

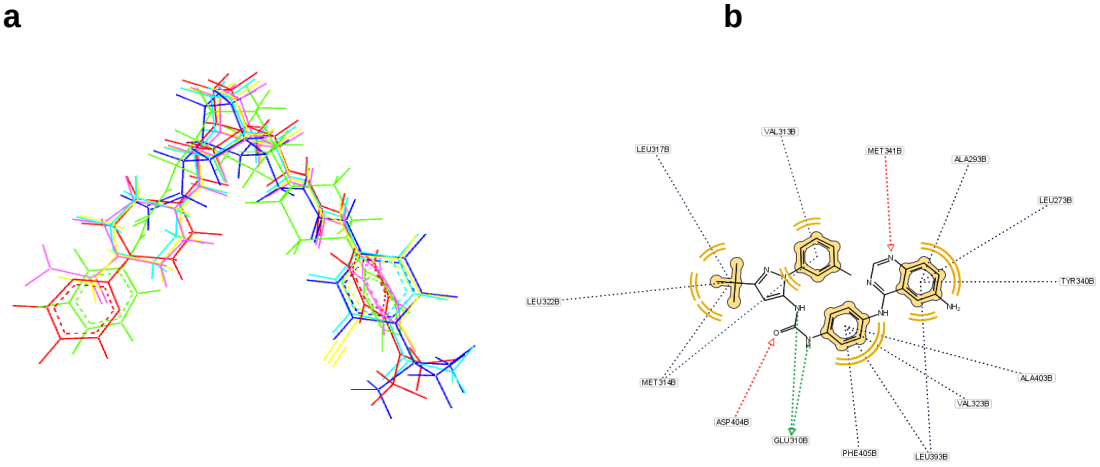


Figure 31 (a) Overlay of six compounds (b) InhibitorRL45 (PDB code: 3F3V)

Table 6 Overlay Similarities of six molecules calculated using the Discovery Studio

Molecule	ZINC08300039	ZINC08635285	ZINC08635693	ZINC08635694	ZINC08635798	ZINC08635951
ZINC08300039	1	0.83	0.78	0.73	0.76	0.79
ZINC08635285	0.83	1	0.92	0.86	0.93	0.97
ZINC08635693	0.78	0.92	1	0.89	0.94	0.92
ZINC08635694	0.73	0.86	0.89	1	0.88	0.87
ZINC08635798	0.76	0.93	0.94	0.88	1	0.95
ZINC08635951	0.79	0.97	0.92	0.87	0.95	1

In this thesis, novel compounds which bind into the kinase domain of the cSrc protein are discovered. The similarity ratios of those six inhibitors described in previous sections were calculated using Discovery Studio (Table 6). It is now clear that they share very similar structures and consist of phenyl, urea and quinuclidine derivatives. The fact that these compounds share similar structures suggests that this is not coincidence. Furthermore, it was noticed that phenyl and urea derivatives exist in the structure of inhibitor RL45 cocrystalized with the cSrc kinase which was used in this study. Figure 31b displays the residues in the kinase domain of cSrc interacting with RL45. The aromatic rings have hydrophobic contacts to the side chain of the residues Leu273, Ala293, Val313, Met314, Leu317, Leu322, Val323, Tyr340, Met341, Leu393, Ala403 and Phe405 in the hinge region and allosteric site of the kinase domain. Most notably, it forms hydrogen bonds with Glu310, Met341 and Asp404. Similar interactions between cSrc kinase and six compounds obtained as a result of virtual screening were observed. We consider that the interactions between them deserve special attention. Getlik et al. stressed that RL45 binds to wild type cSrc kinase in the DFG-out conformation [42]. The cocrystallized structure confirmed the claimed type II inhibitor binding mode which RL45 spans from the allosteric site into the

kinase hinge region. RL45 binds to cSrc in its DFG-out conformation and locks the kinase in inactive state forming the critical interactions.

Chapter 6

RESULTS FOR OTHER KINASES

During virtual screening for Aurora B: INCENP interaction region and cSrc kinase, we have also performed screening for thirteen other kinases. As a result of the screening we recognized five JAK3 specific and one Aurora B kinase pocket specific compounds.

Janus Kinase (JAK) family consists of four tyrosine kinase members which are JAK1, JAK2, JAK3 and TYK2. JAK3 is activated by cytokines including IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. It transmits the signals from the cγ receptors (Figure 32). The kinase is only expressed in lymphoid and myeloid cells. Binding of cytokines to the cγ receptors causes the cross phosphorylation and activation of JAK1 and JAK3. Then Signal Transducer and Transcription Activator (STAT) complexes are activated. [63]

a **b**

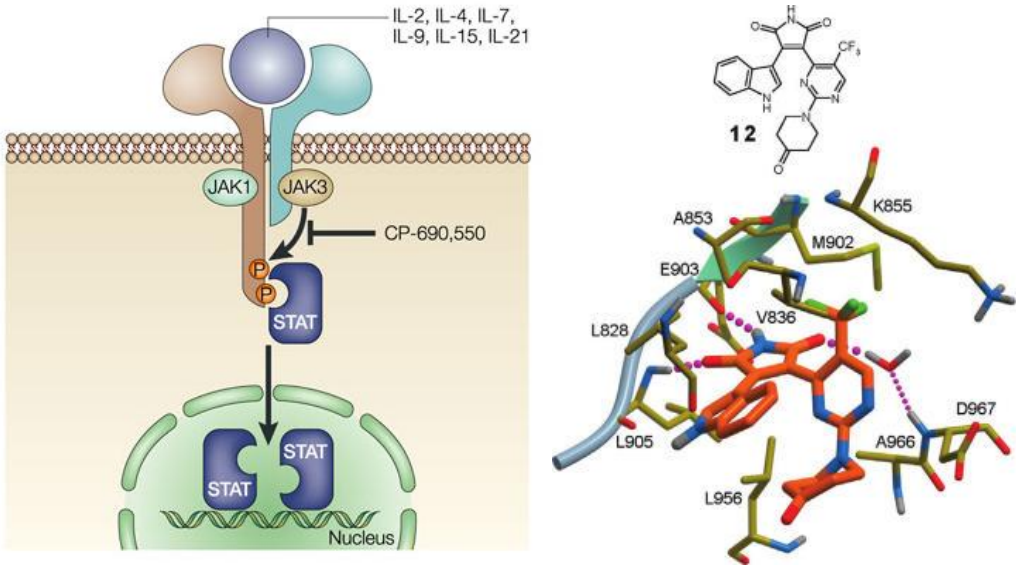


Figure 32 (a) Specific JAK3 inhibitor CP-690,550 (b) Compound 12 [63] [56]

Thoma et al. displayed the highly selective JAK3 inhibitor, compound 12 (Figure 32b) within Janus kinase family [56]. The compound makes direct hydrogen bonds with Glu903 and Leu905 residues. It has hyrophobic interactions with Leu828, Val836, Ala853, Leu956, Ala 966 and Asp967 (Figure 32b).

Table 7 Binding energies of six compounds

	AURB	FGFR	JAK3	KIT	PLK1	PLK4	VEGFR2	ABL	AKT1	AURA	CDK1	CSRC	JAK2	PKA	PTK2
ZINC00645825	-4.9	-4.3	**-6.7**	-4.2	-5.1	-4.6	-4.4	-4	-4	-4	-4	-4	-4	-4	-4
ZINC01102714	-4.8	-4.4	**-6.9**	-4	-4	-4	-4	-4.1	-4.1	-4	-4	-4	-4	-4	-4
ZINC02092599	-4.3	-4	**-6.5**	-4.1	-4	-4.1	-4.1	-4	-4.1	-4	-4	-4.4	-4	-4.6	-4
ZINC03841622	-4	-4.6	**-7.7**	-4	-4.2	-5.3	-4.8	-4	-4	-4	-4.8	-4	-4	-4	-4
ZINC12654725	-4	-4	**-7.4**	-4	-4	-4	-4.6	-4	-4.4	-4	-4.8	-4.1	-4	-4.5	-4
ZINC12887928	**-6.8**	-4	-4.7	-4	-4.4	-5.2	-5	-4	-4.8	-4	-4.3	-4	-4	-4.4	-4

In Figure 33 ZINC12654725 makes three hydrogen bonds with residues Phe833, Lys855 and Asp967. It has important hydrophobic interactions with residues Leu828, Phe 833, Val836, Ala853, Val884, Leu900, Met902 and Leu956. It binds to JAK3 with -7.4 kcal/mole and when we compare the binding affinities to other kinases, it is clear that ZINC12654725 has specificity to JAK3 over the other kinases (Table 7 and Appendix A2 Figures 9a, 9b, 9c)

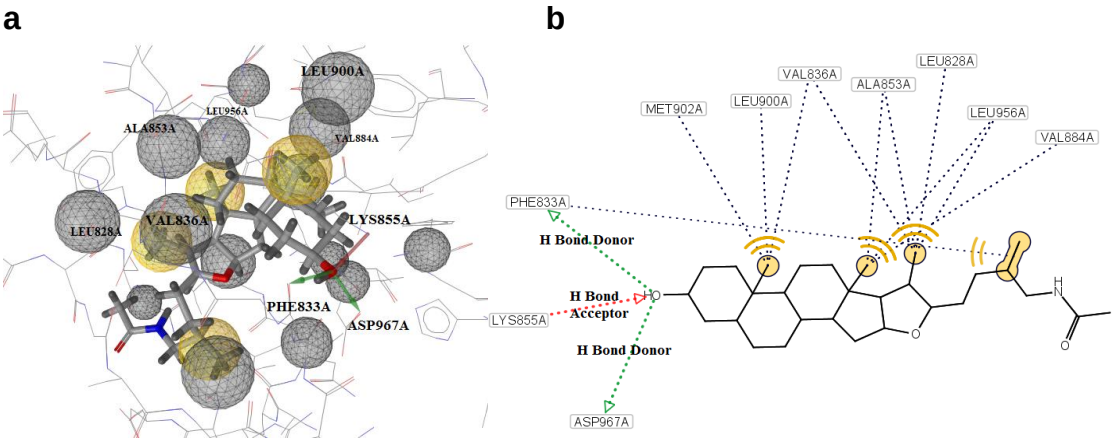


Figure 33 JAK3 kinase with ZINC12654725. (a) 3D representation of JAK3 with bound ZINC12654725. (b) 2D representation of interaction of ZINC12654725 with JAK3 kinase specific residues.

ZINC03841622 form three hydrogen bonds with residues Phe833, Lue905 and Asp912. It shows different binding mode when compared to ZINC12654725 (Figure 34). It has hydrophobic contacts to Leu828, Val836, Ala853, Leu956 and Ala966. With -7.7 kcal/mole binding energy, it has high binding affinity to JAK3 than other kinases (Table 7 and Appendix A2 10a, 10b, 10c).

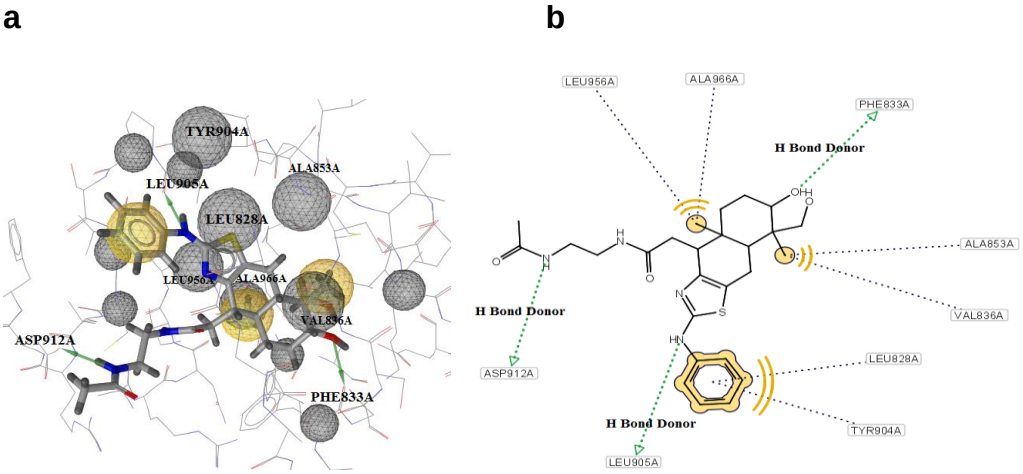


Figure 34 JAK3 kinase with ZINC03841622. (a) 3D representation of JAK3 with bound ZINC03841622. (b) 2D representation of interaction of ZINC03841622 with JAK3 kinase specific residues

In Figure 35, while ZINC02092599 forms one hydrogen bond with Leu828, It has exceptionally high hydrophobic interactions with residues Leu828, Phe833, Val836, Ala853, Leu900, Met902, Tyr904, Leu905, Leu956 and Ala966. It binds to JAK3 with -6.6 kcal/mole energy. Since it forms less hydrogen bond than ZINC03841622 and ZINC12654725 with JAK3, its binding affinity is also less. However, ZINC02092599 has high selectivity to JAK3 within other kinases (Table 7, Appendix A2 Figures 11a, 11b, 11c).

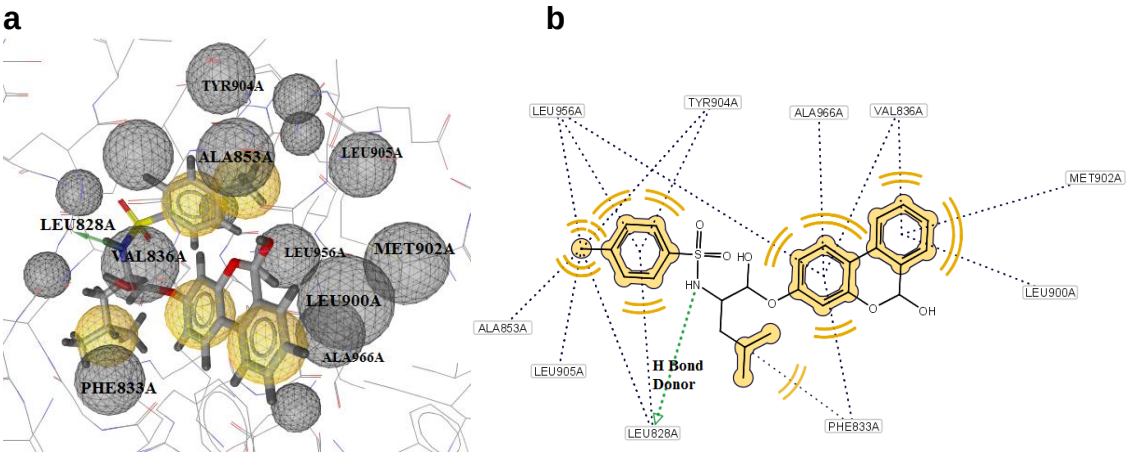


Figure 35 JAK3 kinase with ZINC02092599. (a) 3D representation of JAK3 with bound ZINC02092599. (b) 2D representation of interaction of ZINC02092599 with JAK3 kinase specific residues

ZINC01102714 (Figure 36) and ZINC00645825 (Figure 37) display similar binding modes with ZINC02092599 to JAK3. They form hydrogen bonds with residue Leu905 and have hydrophobic interactions with adjacent residues. ZINC01102714 has higher binding affinity to JAK3 than ZINC00645825 (Table 7). The difference results from hydrophobic interactions. ZINC01102714 has more contacts to hydrophobic residues.

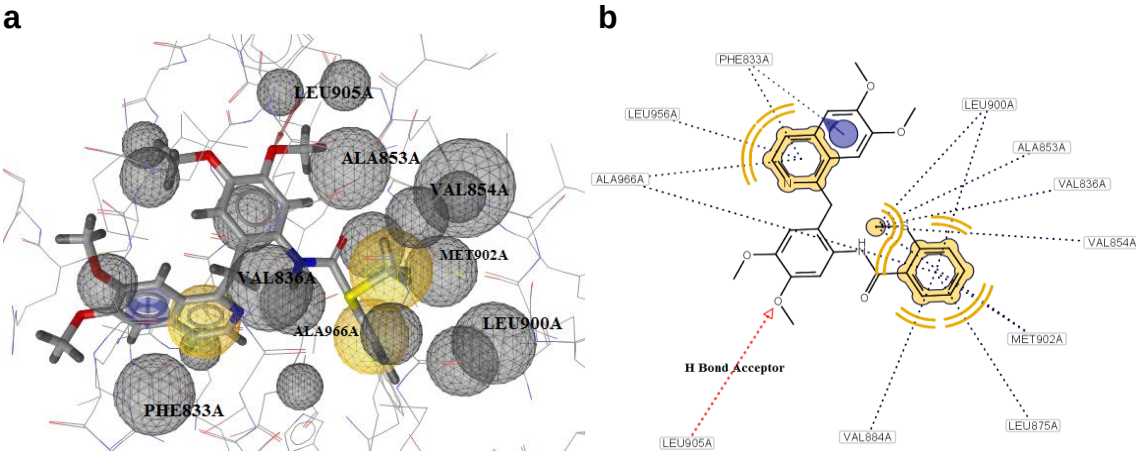


Figure 36 JAK3 kinase with ZINC01102714. (a) 3D representation of JAK3 with bound ZINC01102714. (b) 2D representation of interaction of ZINC01102714 with JAK3 kinase specific residues.

a **b**

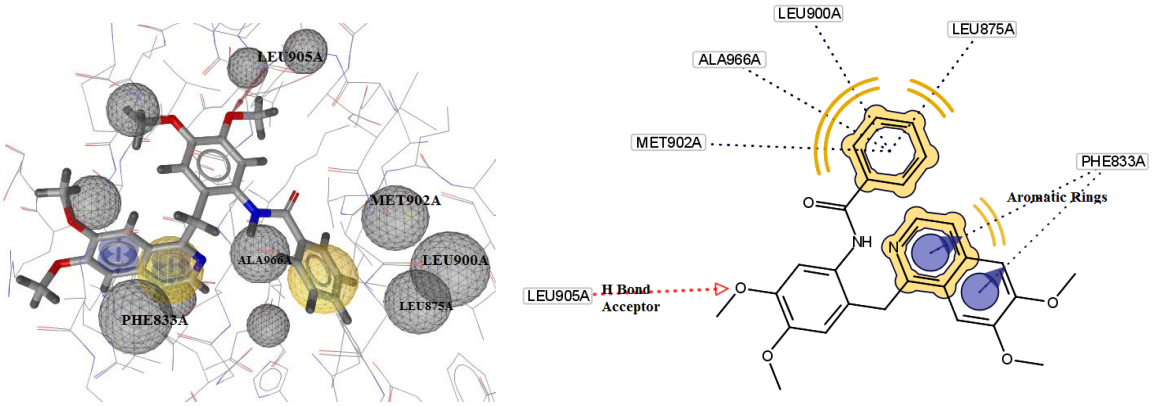


Figure 37 JAK3 kinase with ZINC00645825. (a) 3D representation of JAK3 with bound ZINC00645825. (b) 2D representation of interaction of ZINC00645825 with JAK3 kinase specific residues

In Figure 38, ZINC12887928 binds to Aurora B kinase pocket with -6.8 kcal/mole energy. It forms hydrogen bond with Gln145 and has hydrophobic interactions with Leu99, Val107, Ala120, Leu124, Leu138, Leu154, Met156, Leu168, Phe172 Leu223 and Ala233.

a **b**

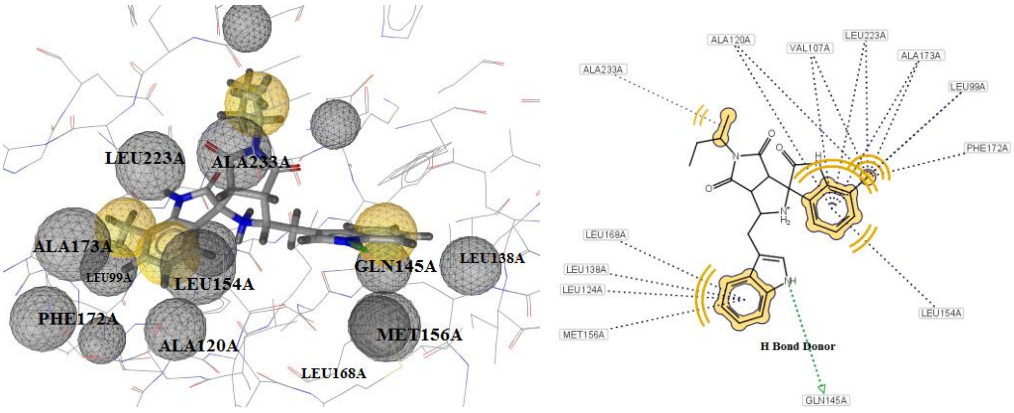


Figure 38 AURB kinase with ZINC12887928. (a) 3D representation of AURB with bound ZINC12887928. (b) 2D representation of interaction of ZINC12887928 with AURB kinase specific residues.

CONCLUSION

In this thesis, we have conducted two projects which aimed to identification of small molecule kinase inhibitors specifically targeting Aurora B: INCENP interaction and cSrc hinge - allosteric region. The mechanism of Aurora B: INCENP interaction is not exactly known. The study of Sessa et al.[13] proposes the activation mechanism of Aurora B by INCENP interaction. Small hydrophobic pocket where IN-box residue Phe837 points out deeply and interacts with Aurora B was chosen as a target site. As a result of the structure and the ligand-based virtual screening with 5 million compounds using the docking softwares OpenEye and GOLD, thirty three important molecules which are specifically bound to the Aurora B:INCENP interaction site were determined as potential drug candidates. Five of those molecules, which were theoretically shown to act as good inhibitors, were tested experimentally and three of them were displayed as promising drug candidates. Two criteria would fulfill the requirements: i) Reduced Aurora B kinase activity ii) Inhibition of Aurora B and INCENP interaction (INCENP-Aurora B interaction activates Aurora B kinase). Experimental study demonstrated that the potential drug molecules could reduce Aurora B kinase activity. The identification of new small molecule inhibitors of Aurora B kinase which would target a different part of the kinase would shed light into the unknown parts of the molecular mechanism of Aurora B kinase during cell division. It would also open the door to new approaches for the treatment of cancer. As an important point, molecules not only target the Aurora B: INCENP interaction but also do not bind to other kinases. They are selective to the Aurora B: INCENP interaction site within kinases.

As a second project, we identified eight inhibitors which specifically bound to cSrc kinase theoretically. The molecules found for cSrc were bound in the ATP pocket and extended into the allosteric site in the inactive conformation of the cSrc kinase. They were bound in

conserved ATP pocket by hydrophobic interactions and resided in the allosteric site by making direct hydrogen bonds with residue GLU310. The binding mode of these inhibitors is the same as of type II kinase inhibitors. They bind to a less conserved allosteric site and a conserved ATP pocket simultaneously.

When the two projects were concluded, huge amount of docking results for fifteen kinases ere obtained. The results were examined and five specific JAK3 inhibitors and one Aurora B inhibitor targeting the kinase pocket were identified. JAK3 inhibitors show similar binding mode to Compound 12 which was discovered by Thoma et al [56]. They target the JAK3 kinase pocket and form hydrogen bonds and hydrophobic interactions with adjacent residues.

APPENDIX A1

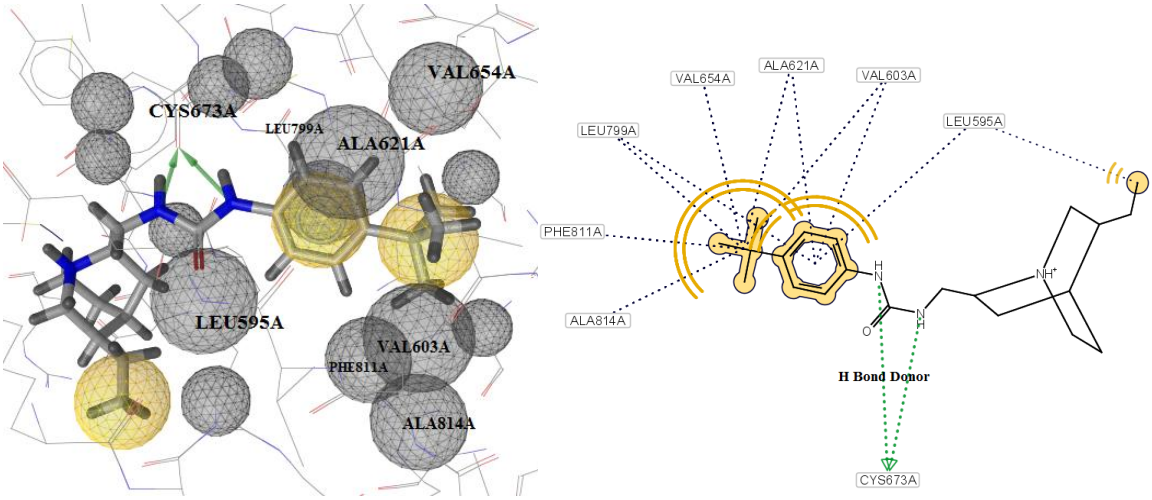


Figure 1a KIT with ZINC08300039

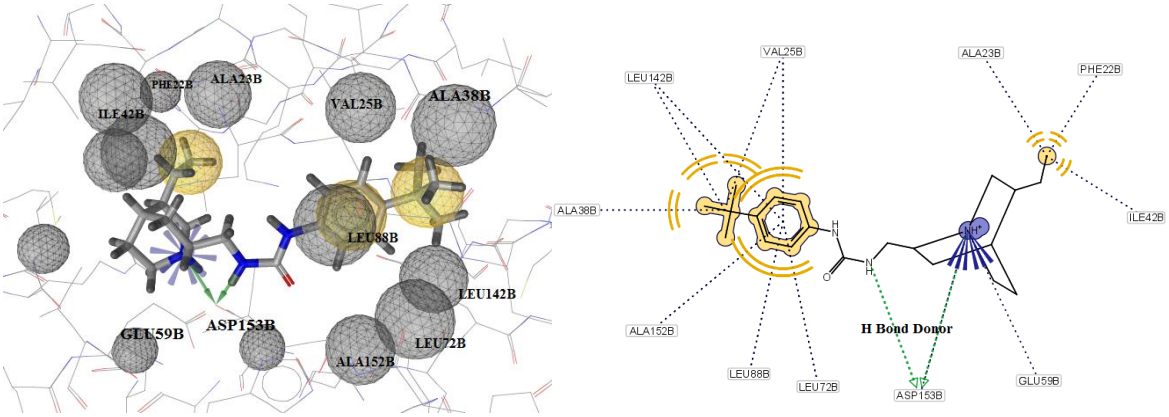


Figure 1b PLK4 with ZINC08300039

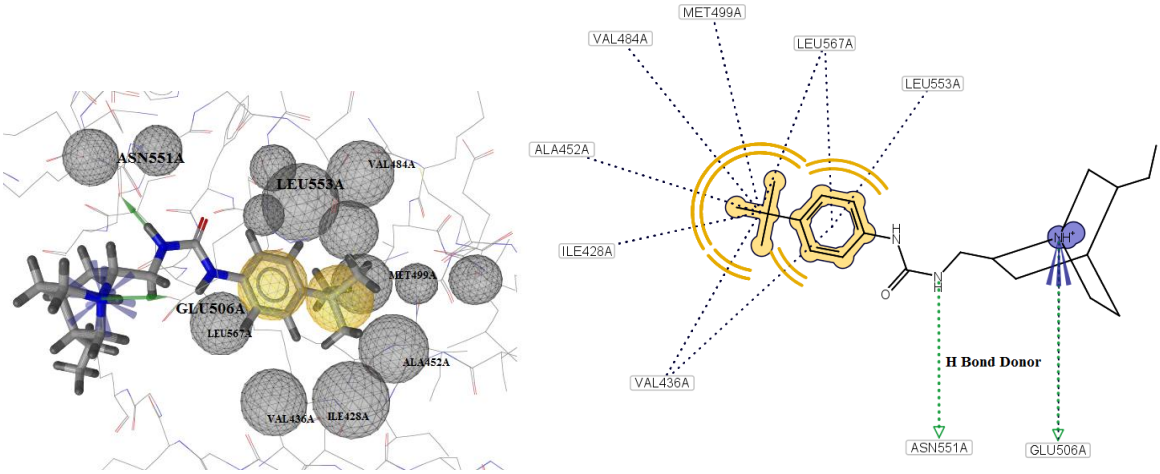


Figure 1c PTK2 with ZINC08300039

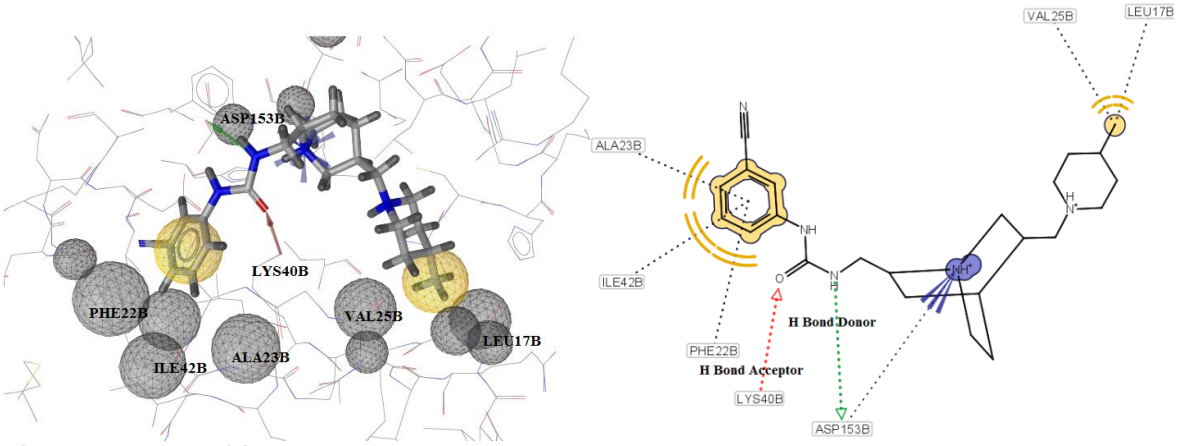


Figure 2a PLK4 with ZINC08635951

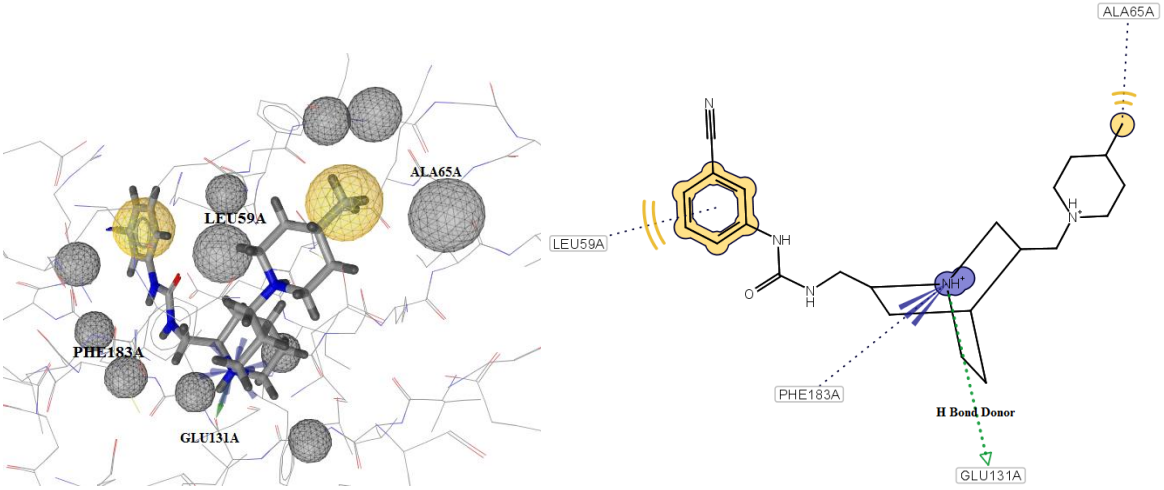


Figure 2b PLK1 with ZINC08635951

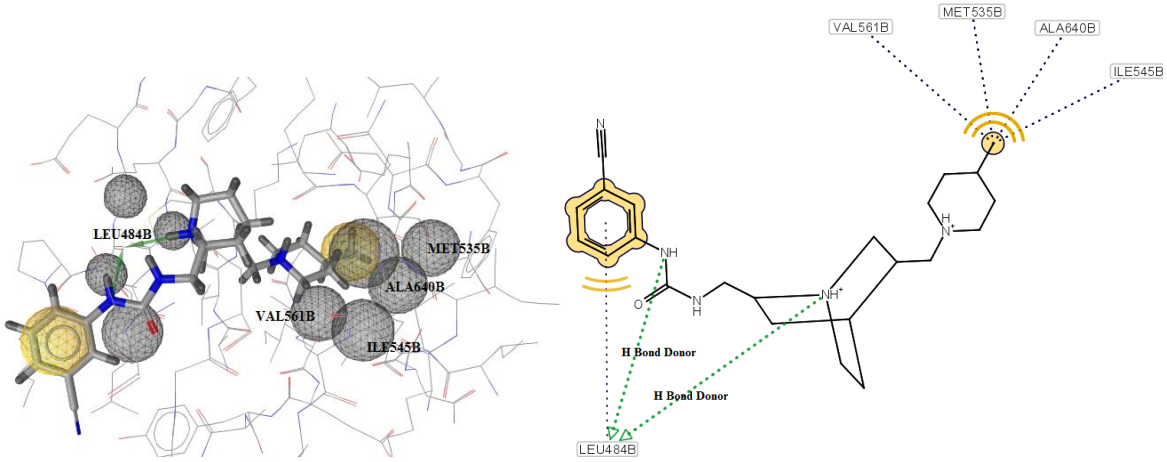


Figure 2c FGFR with ZINC08635951

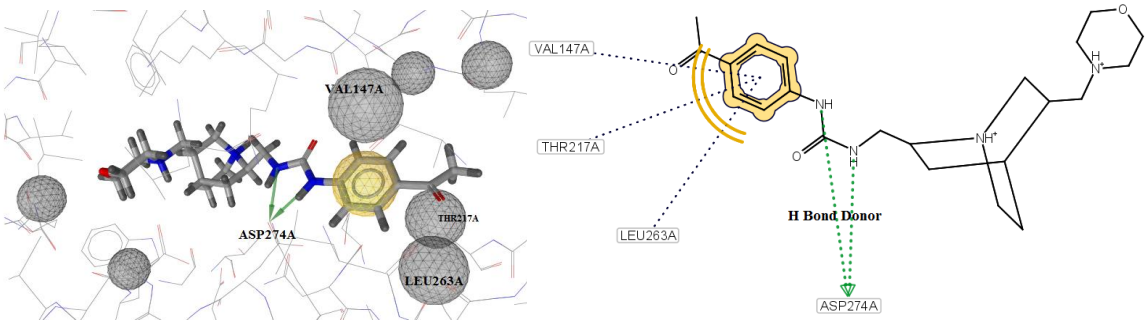


Figure 3a AURA with ZINC08635285

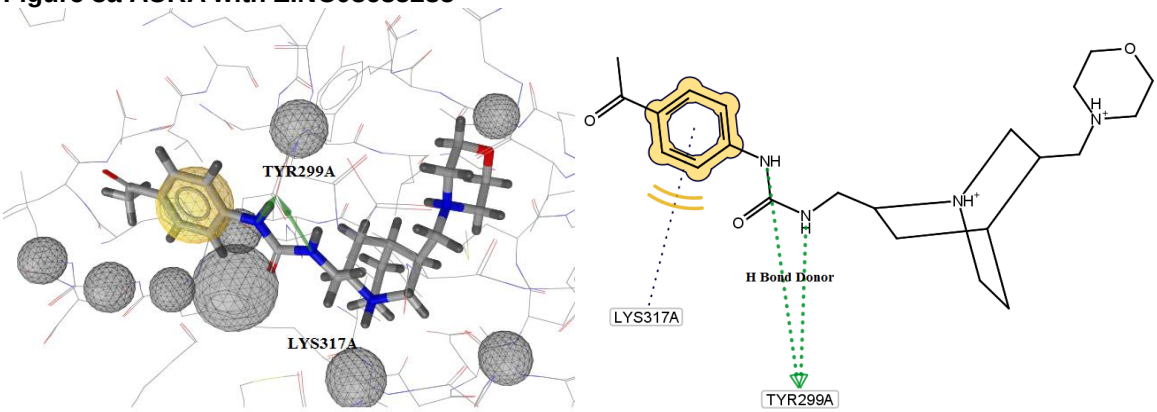


Figure 3b ABL with ZINC08635285

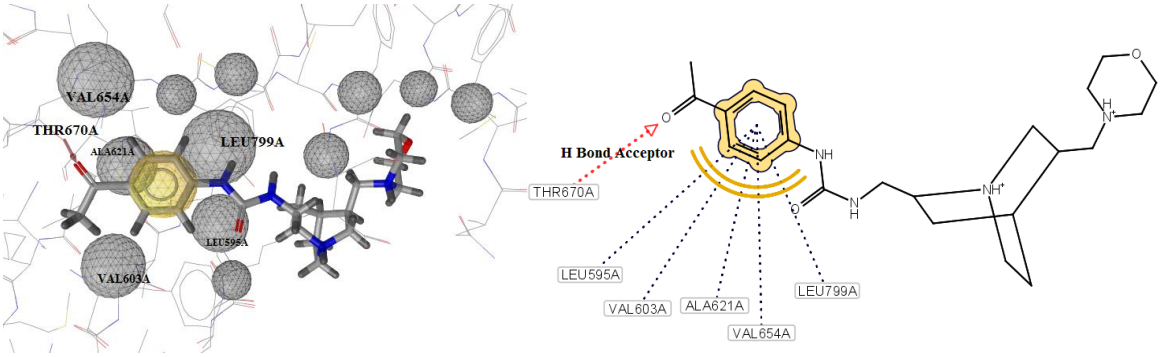


Figure 3c KIT with ZINC08635285

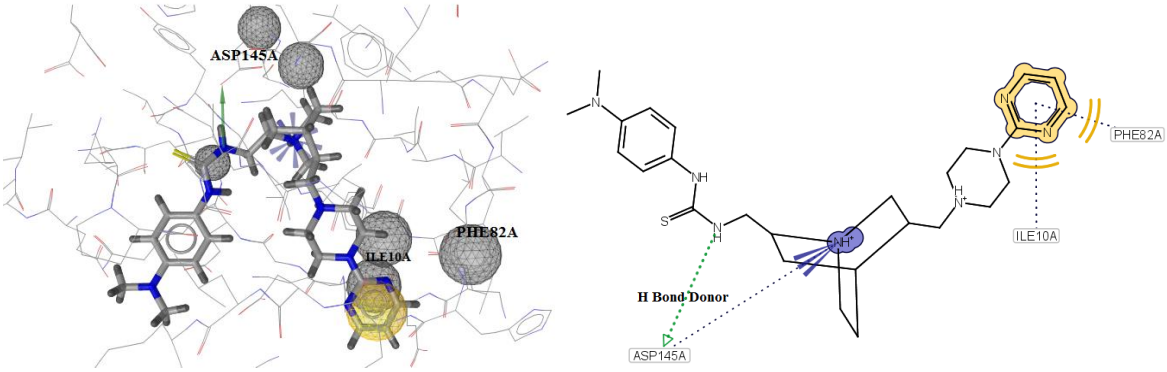


Figure 4a CDK1 with ZINC08635693

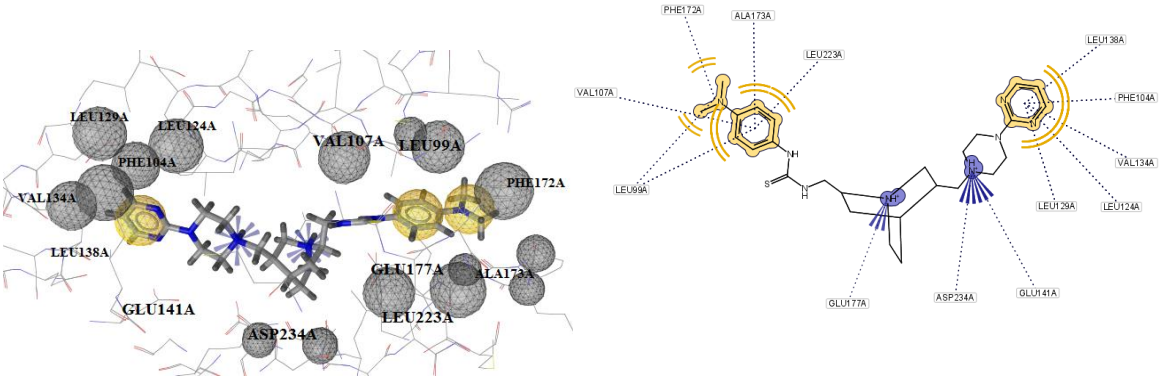


Figure 4b AURB with ZINC08635693

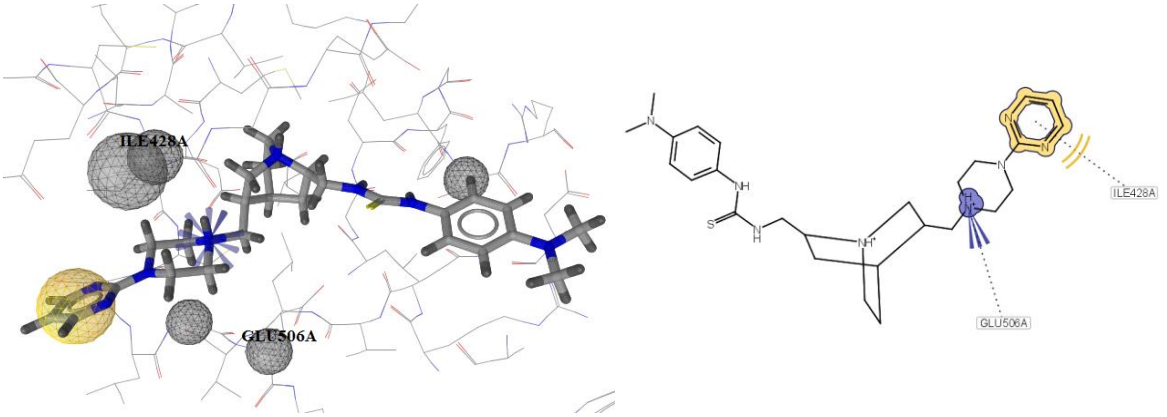


Figure 4c PTK2 with ZINC08635693

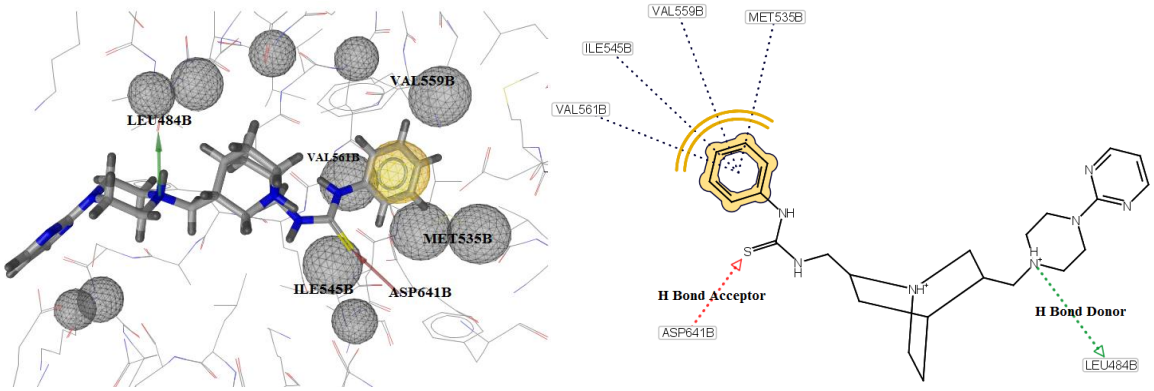


Figure 5a | FGFR with ZINC08635694

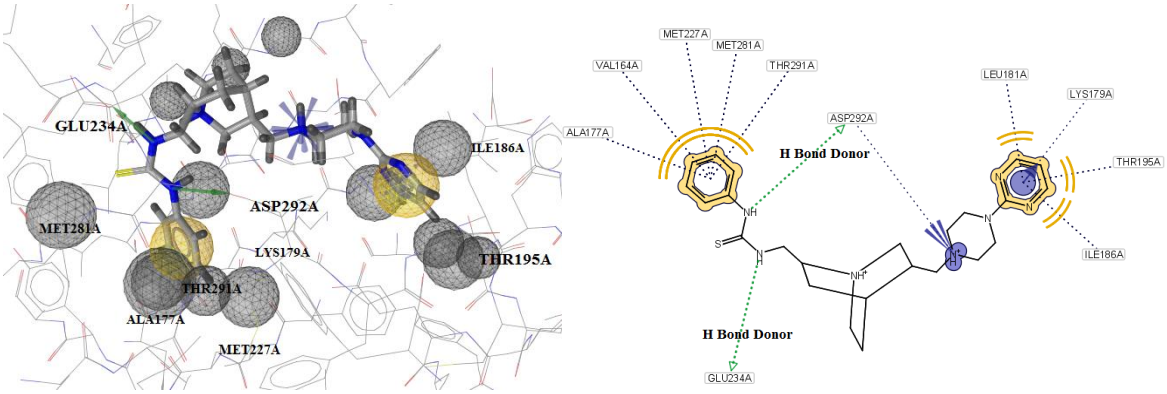


Figure 5b | AKT1 with ZINC08635694

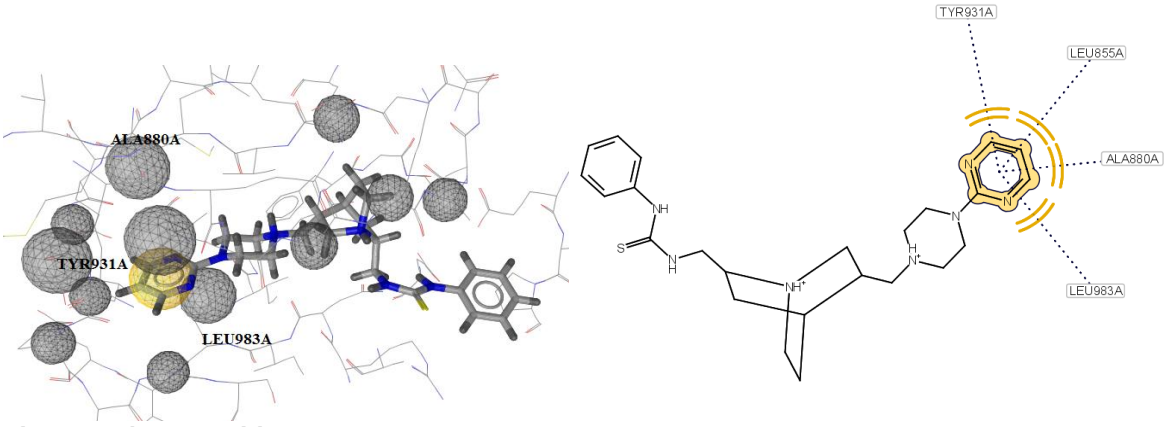


Figure 5c | JAK2 with ZINC08635694

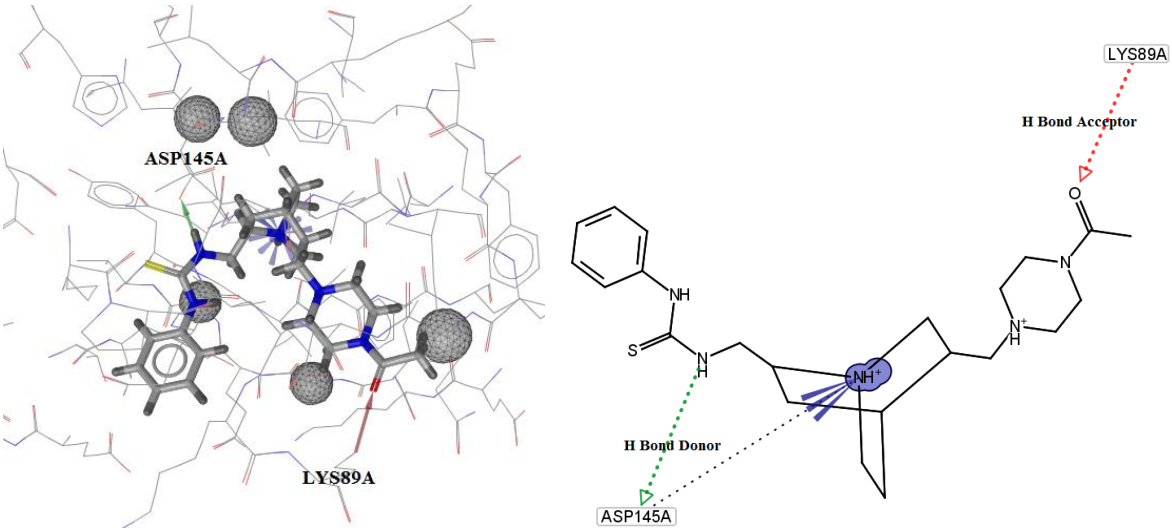


Figure 6a CDK1 with ZINC08635798

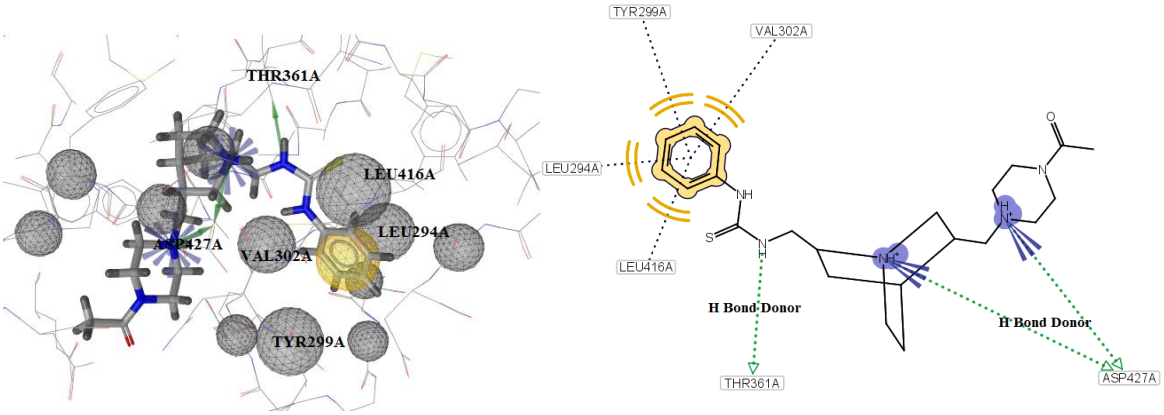


Figure 6b ABL with ZINC08635798

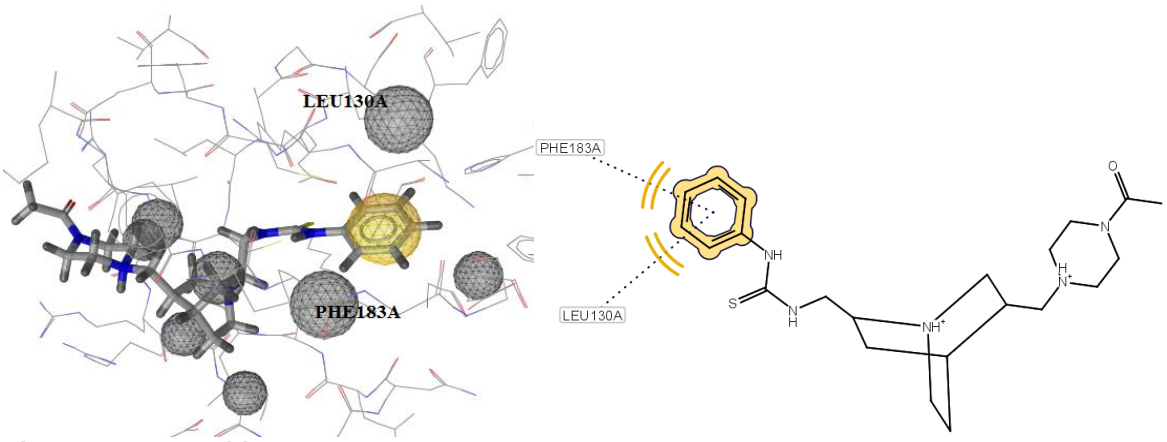


Figure 6c PLK1 with ZINC08635798

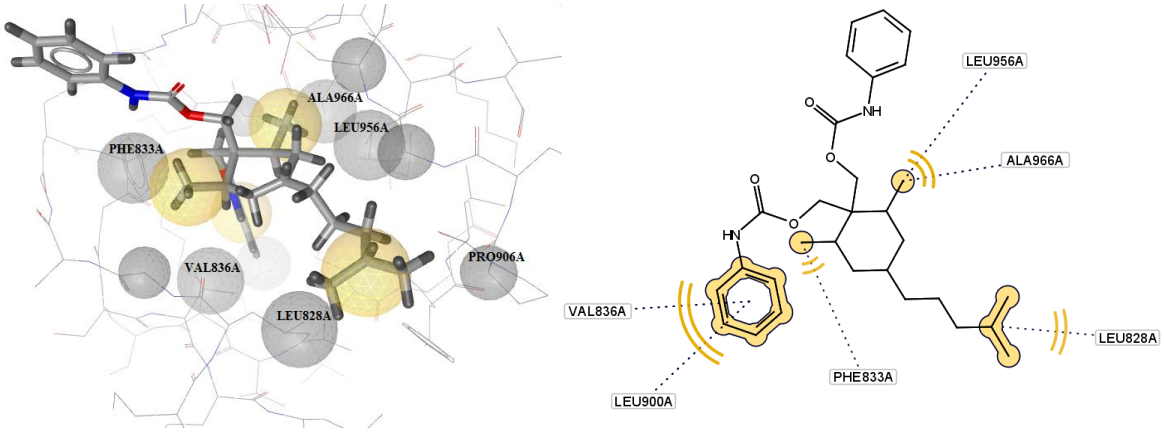


Figure 7a JAK3 with ZINC12654246

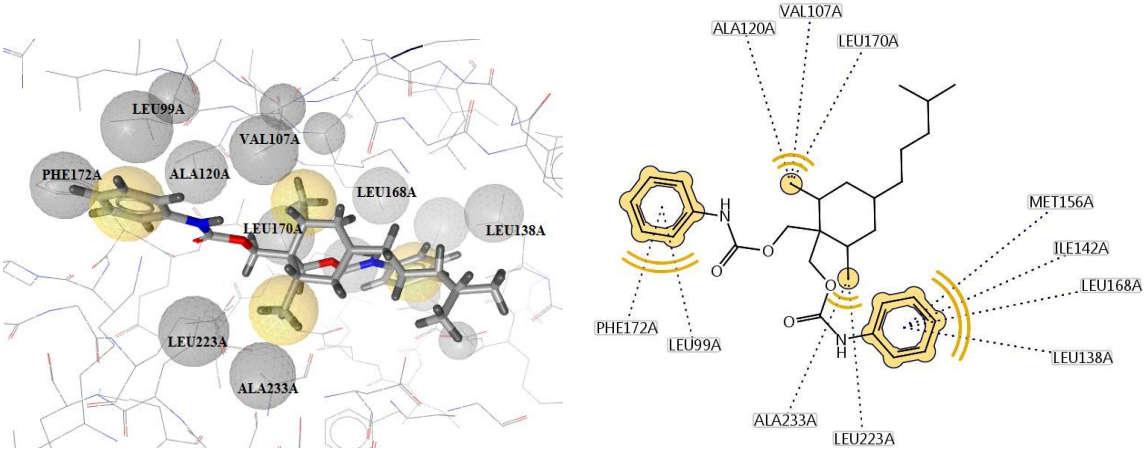


Figure 7b AURB with ZINC12654246

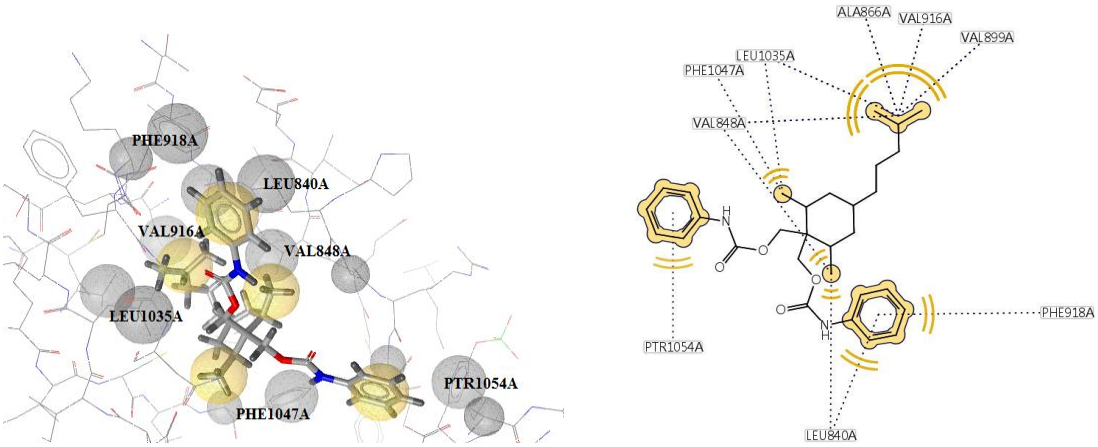


Figure 7c VEGFR2 with ZINC1265424

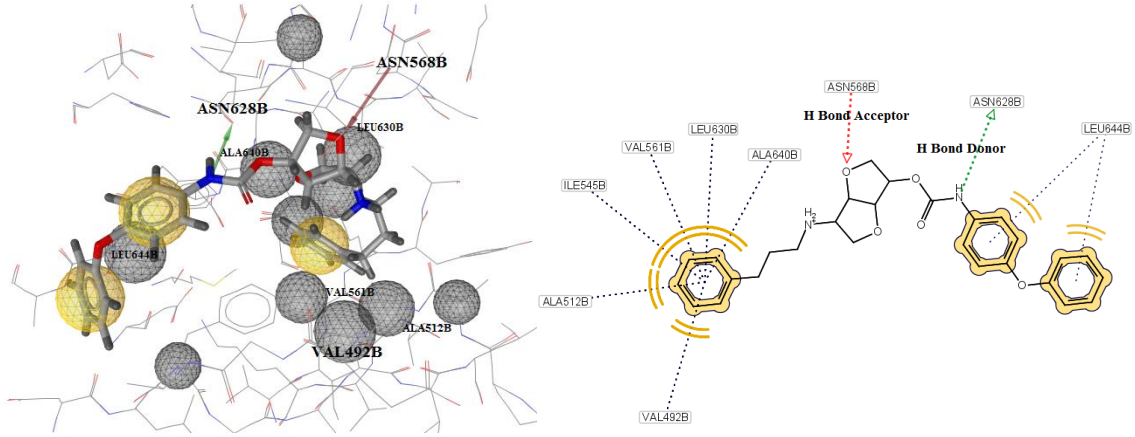


Figure 8a FGFR with ZINC04236491

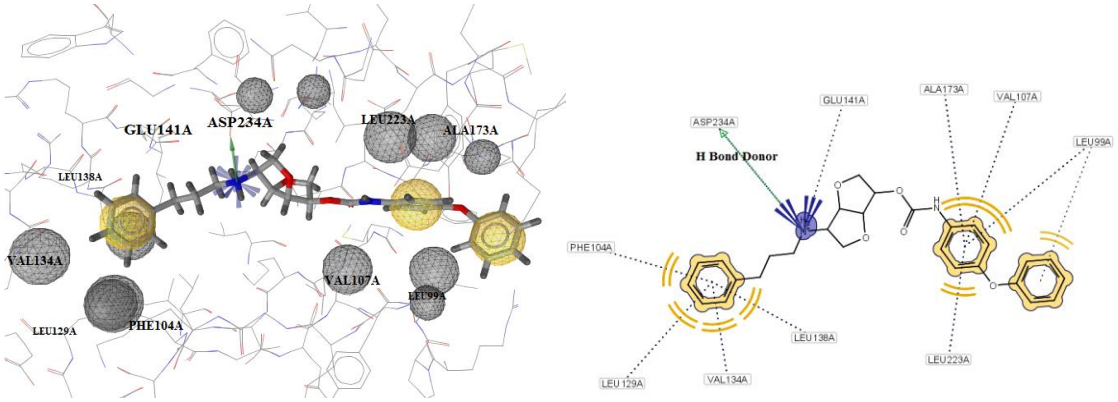


Figure 8b AURB with ZINC04236491

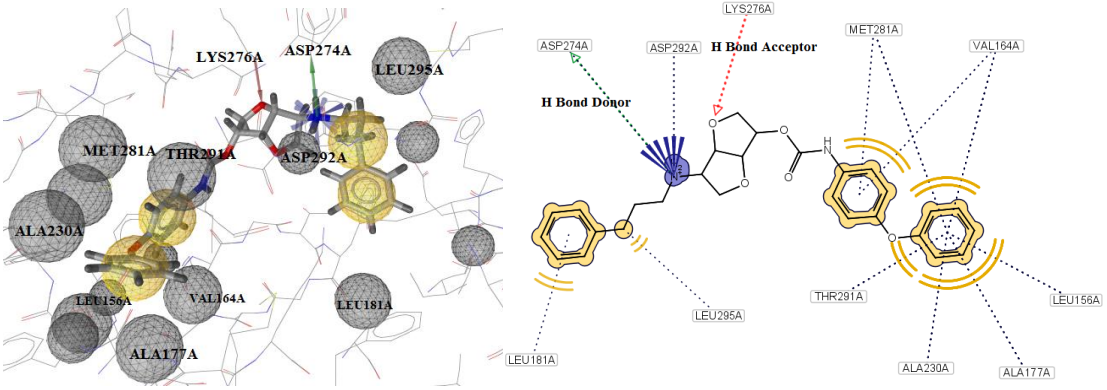


Figure 8c AKT1 with ZINC04236491

APPENDIX A2

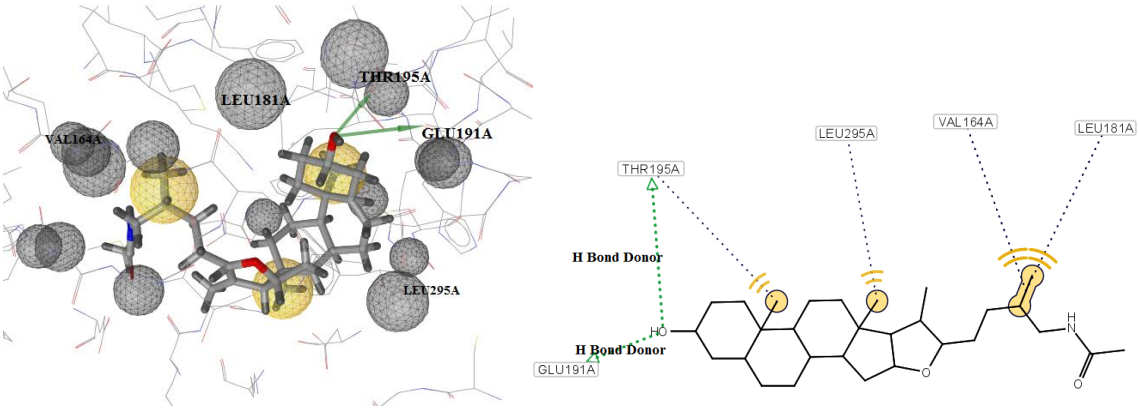


Figure 9a AKT1 with ZINC12654725

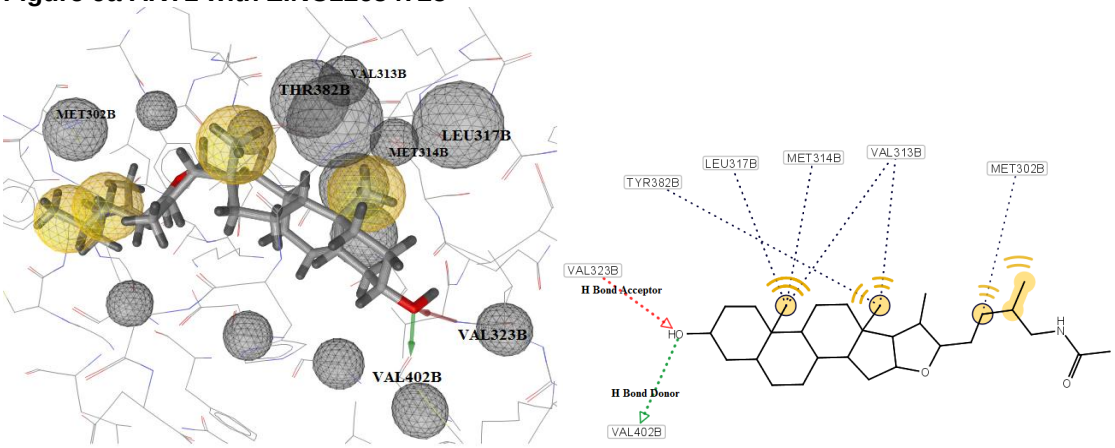


Figure 9b CSRC with ZINC12654725

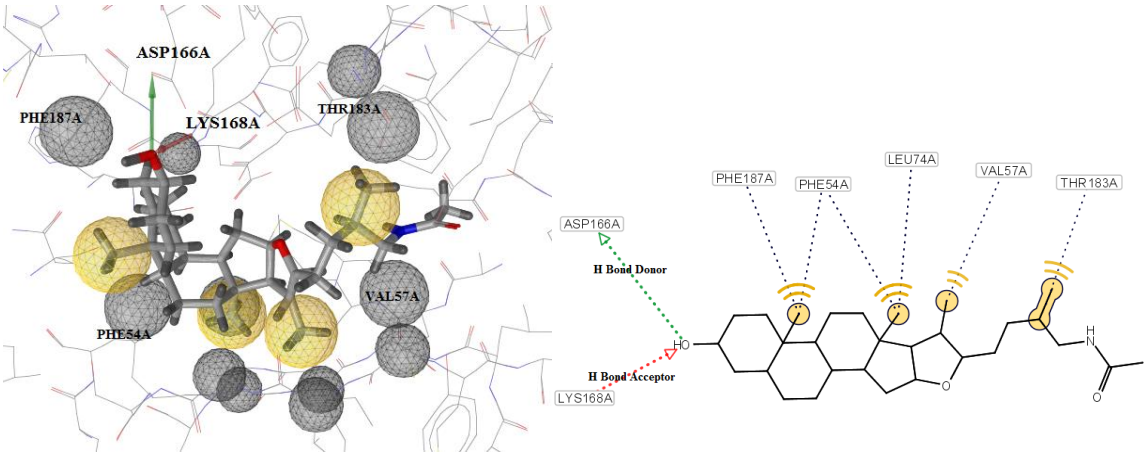


Figure 9c PKA with ZINC12654725

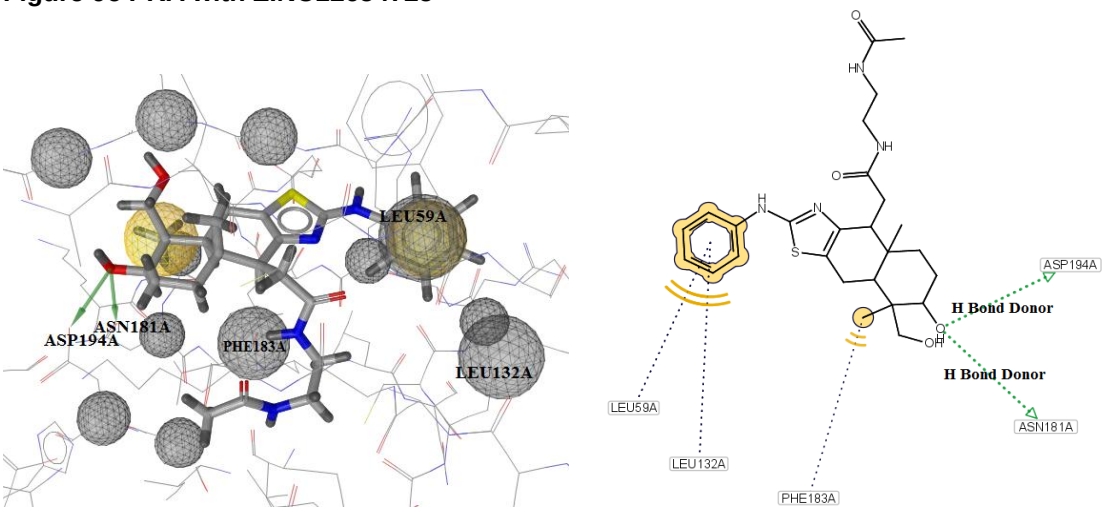


Figure 10a PLK1 with ZINC03841622

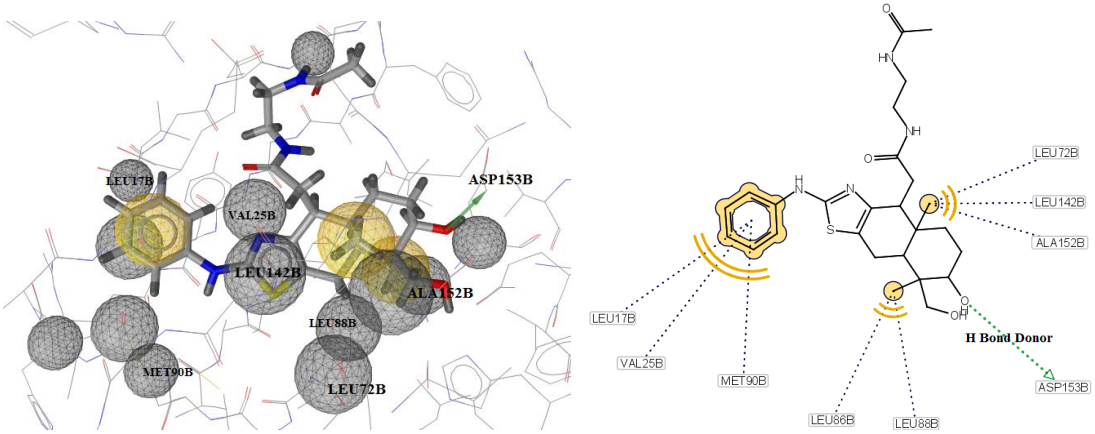


Figure 10b PLK4 with ZINC03841622

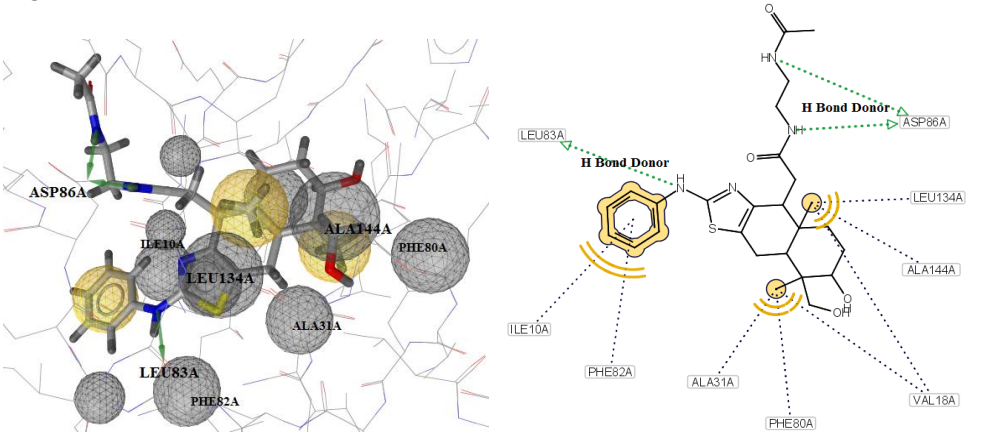


Figure 10c CDK1 with ZINC03841622

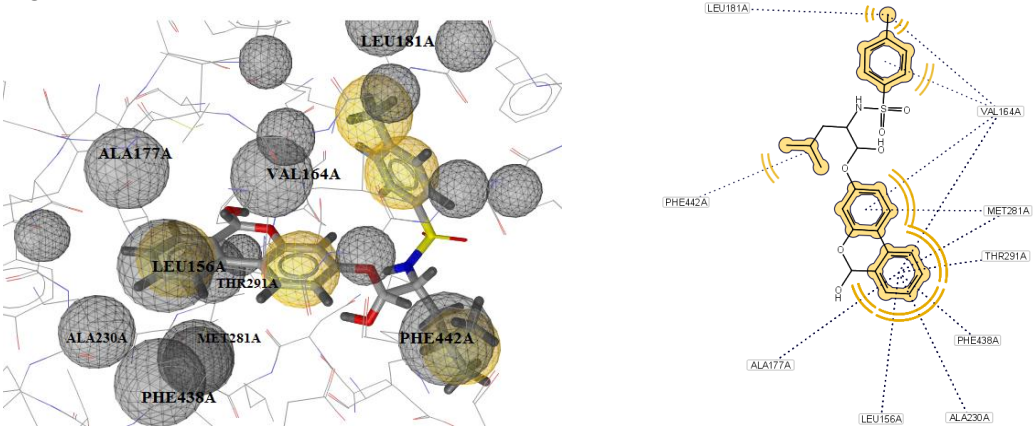


Figure 11a AKT1 with ZINC02092599

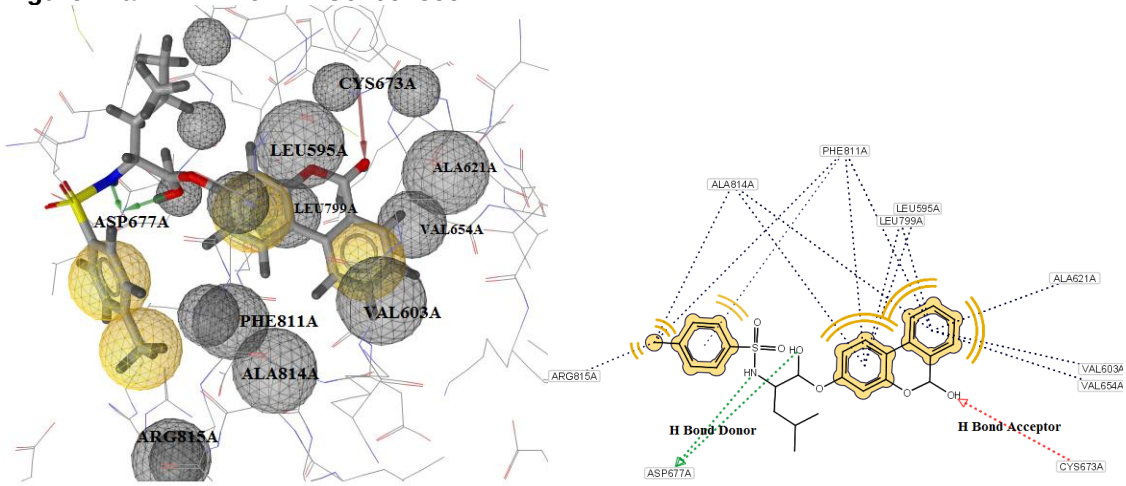


Figure 11b KIT with ZINC02092599

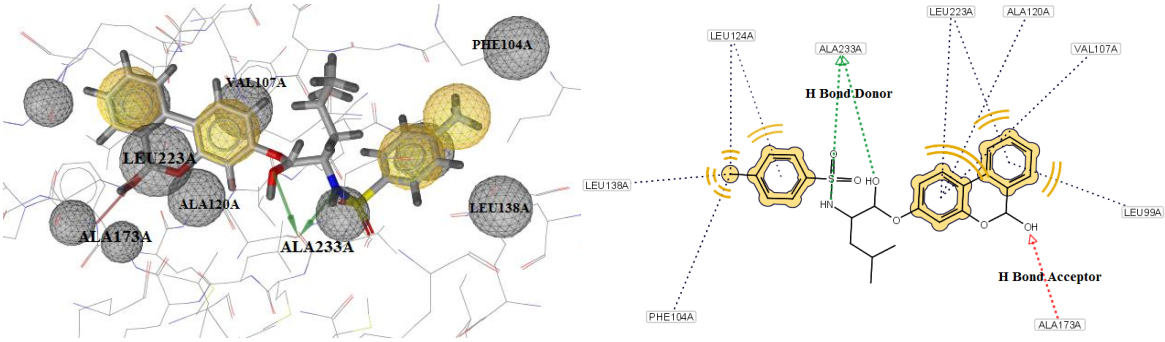


Figure 11c AURB with ZINC02092599

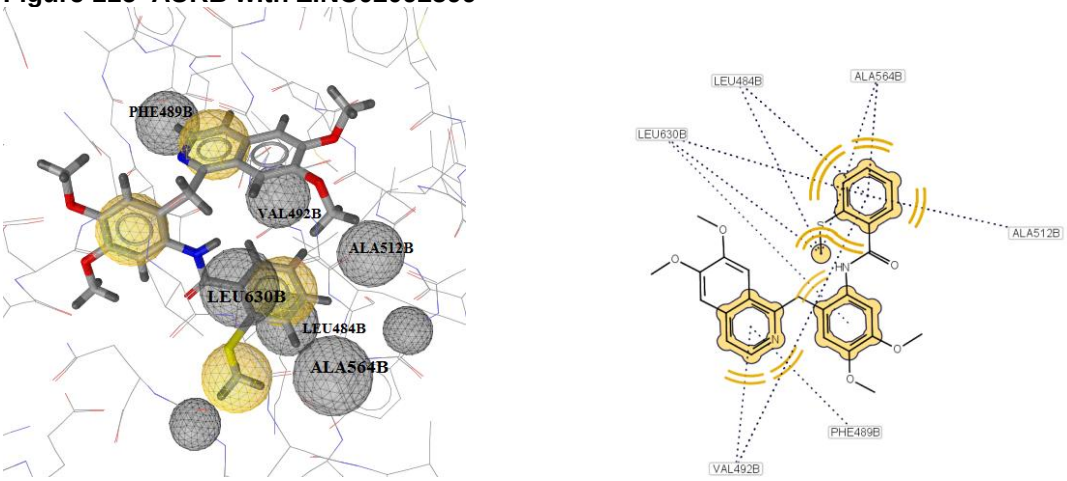


Figure 12a FGFR with ZINC01102714

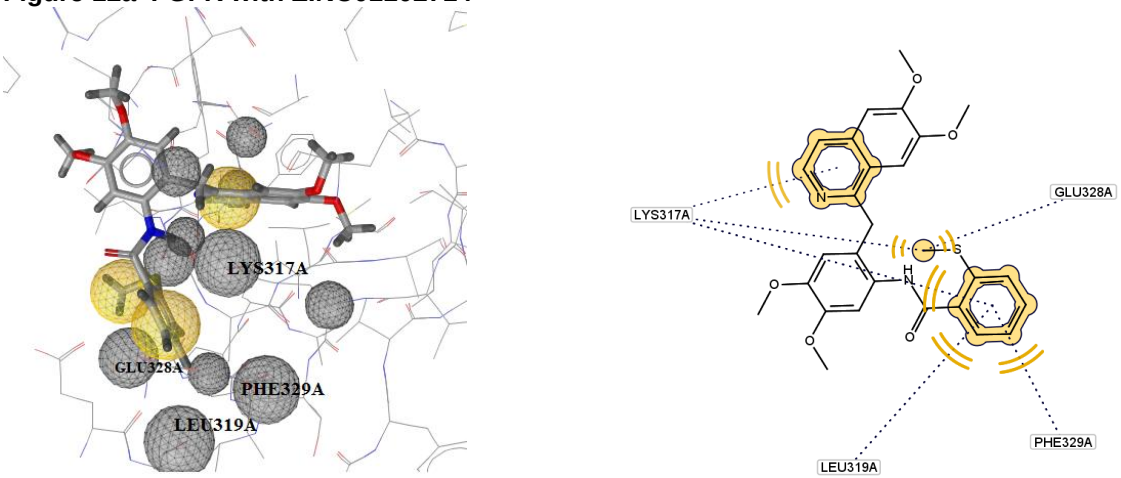
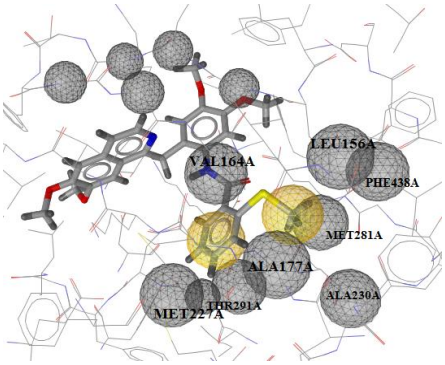


Figure 12b ABL with ZINC01102714



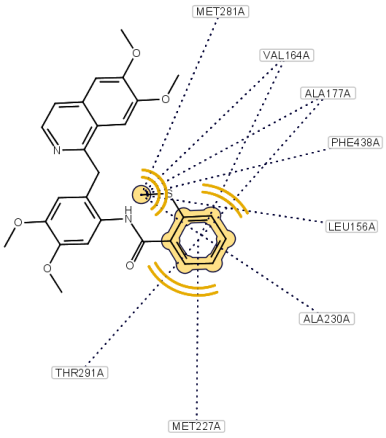
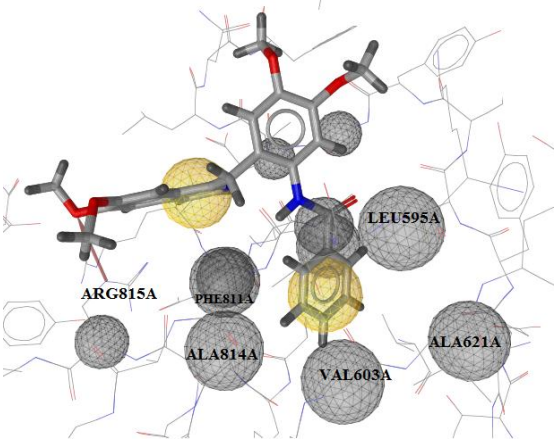


Figure 12c AKT1 with ZINC01102714



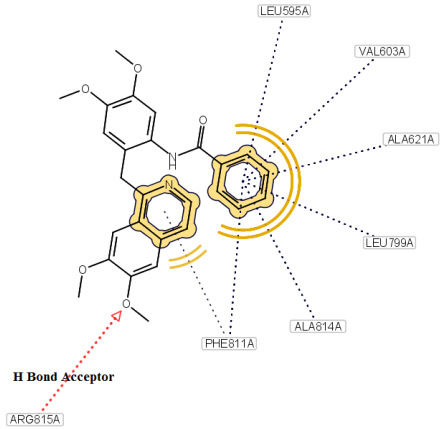
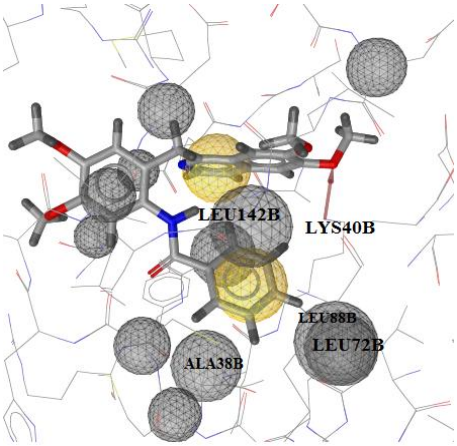


Figure 13a KIT with ZINC00645825



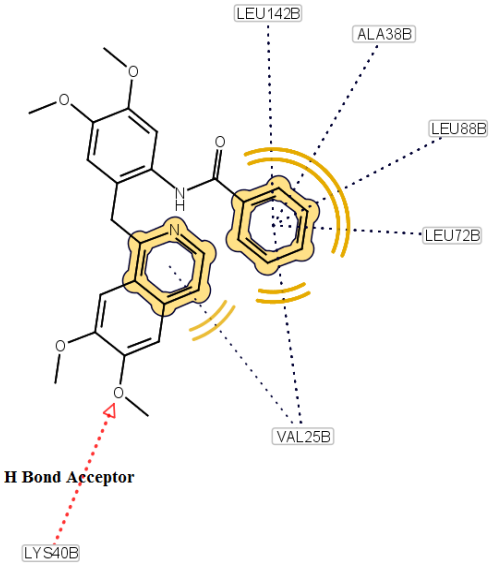
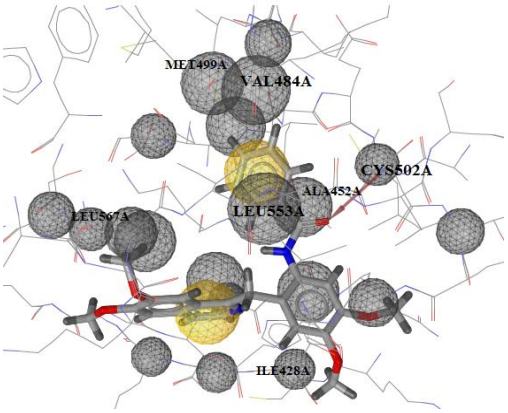


Figure 13b PLK4 with ZINC00645825



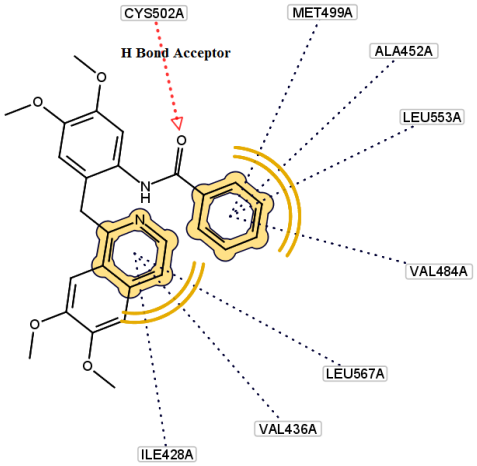
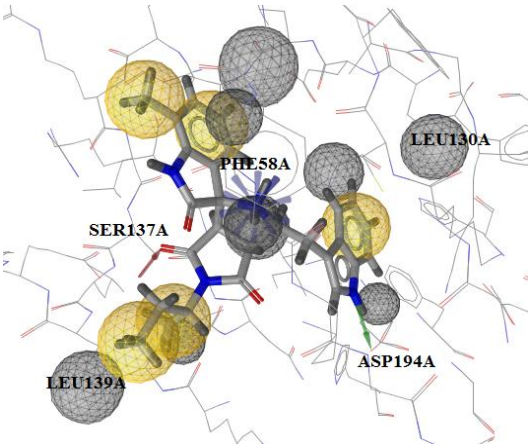


Figure 13c PTK2 with ZINC00645825



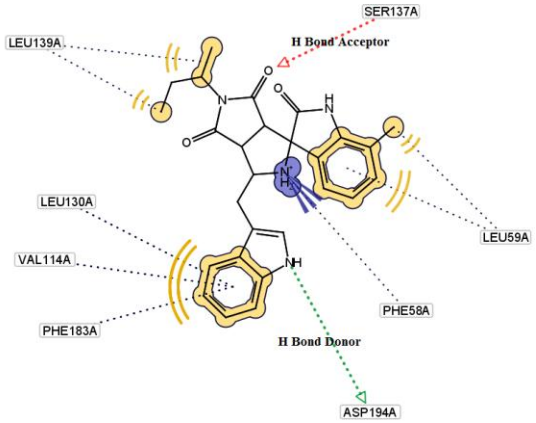
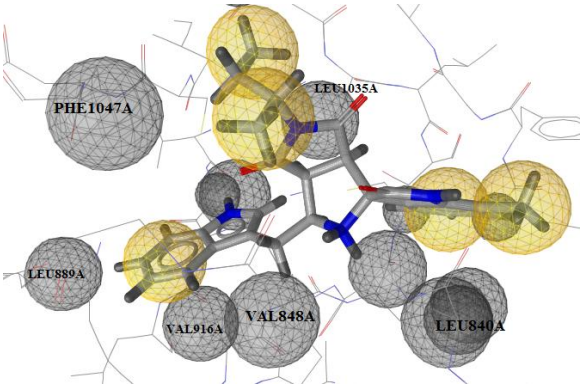


Figure 14a PLK1 with ZINC12887928



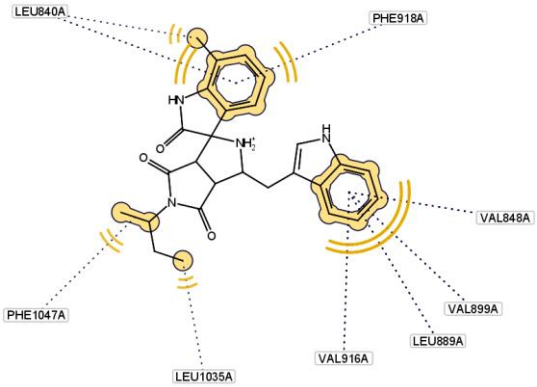
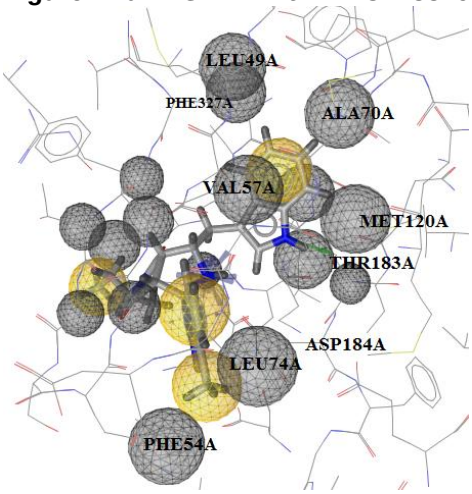


Figure 14b VEGFR2 with ZINC12887928



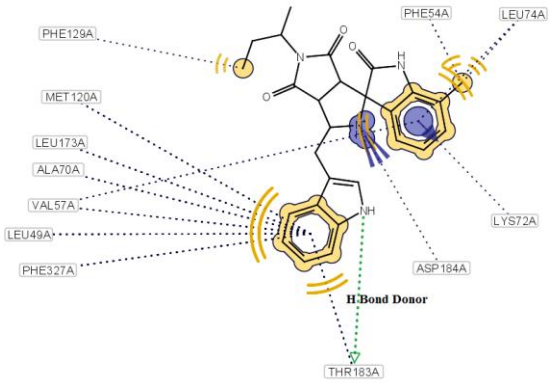


Figure 14c PKA with ZINC12887928

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