Characterization of Aurora kinase inhibitors by molecular modeling, docking and virtual screening approaches

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بسم الله الرحمن الرحيم

Dedication

Dedicated to my beloved mama

Acknowledgments

All praises for Almighty Allah, Lord of the Worlds, Who has created a fascinating world and gifted human being with knowledge and wisdom to explore the closed domains and veiled legends of the nature. Without Allah's divine help, I would not have been able to achieve anything in my life. All respects to Holy Prophet Hazrat Mohammad (P.B.U.H), the most perfect among all human beings ever born on the surface of the earth, who is forever a source of guidance and knowledge for humanity as a whole.

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LIST OF ABBREVIATIONS

2D	Two Dimensional
3D	Three Dimensional
AA	Amino Acid
ADT	Autodock Tools
ATP	Adenosine Triphosphate
AR	Aromatic ring
CADD	Computer-aided drug design
DNA	Deoxyribonucleic Acid
DPF	Docking Parameter File
G1 phase	Post-mitotic phase
G2 phase	Pre-mitotic phase
GPF	Grid Parameter File
H3	Histone 3
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
H-Bond	Hydrogen Bond
HD	Hydrophobic region
LIGPLOT	Ligand Plot
MSA	Multiple Sequence Alignment
NIH Server	Structural Analysis and Verification Server
NMR	Nuclear magnetic resonance
PDB	Protein Data Bank
QSAR	Quantitative structure-activity relationship
RMSD	Root Mean Square Deviation
RCSB	Research Collaboratory for Structural Bioinformatics
VMD	Visual Molecular Dynamics
VS	Virtual Screening

List of twenty amino acids found in proteins, along with the single-letter and three-letter code used to represent these amino acids in protein data bases.

Amino Acid	Single Letter Code	Three Letter Code
Isoleucine	I	Iso
Leucine	L	Leu
Valine	V	Val
Phenylalanine	F	Phe
Methionine	M	Met
Cysteine	C	Cys
Alanine	A	Ala
Glycine	G	Gly
Proline	P	Pro
Threonine	T	Thr
Serine	S	Ser
Tyrosine	Y	Tyr
Tryptophan	W	Trp
Glutamine	Q	Gln
Asparagine	N	Asn
Histidine	H	His
Glutamic acid	E	Glu
Aspartic acid	D	Asp
Lysine	K	Lys
Arginine	R	Arg

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Abstract

Standard cytotoxics and radiation therapeutic approaches in cancer are not only highly toxic, but also of limited efficacy in treatment of a significant number of cancer patients. The molecular analysis of many cancer genomes have shown a remarkable complexity and pointed to key genomic and epigenomic alterations in cancer. These discoveries are paving the way for targeted therapy approaches. However, there are a large number of potential targets but only a few can regulate key cellular functions and intersect multiple signaling networks. The Aurora kinase family members (A, B, and C) are a collection of highly related and conserved serine-threonine kinases that fulfill these criteria, being key regulators of mitosis and multiple signaling pathways. Aurora kinase family members are involved in a wide range of cell cycle events including centrosome separation, cytokinesis, kinetochore formation, spindle assembly, chromosomal segregation and microtubule dynamics. Alterations in Aurora kinase signaling are associated with mitotic errors and have been closely linked to chromosomal aneuploidy in cancer cells. Thus, Aurora kinases have emerged as promising targets for cancer therapy because of their critical role in mitosis. In order to find the selective features for Aurora kinase inhibitors, 3D-pharmacophore hypothesis was built using two datasets, a set of novel Aurora specific inhibitors reterived from Enamine database and a set of known Aurora kinase inhibitors. The selected hypothesis was then used for screening Princeton and Uorsy databases in order to retrieve more potent and novel inhibitors. These hits were sorted on the basis of Absorption, Distribution, Metabolism and Excretion (ADME) and drug like properties and then short listed compounds were verified by molecular docking studies in order to gain insights into the structural features of these inhibitors. Our docking results implicate that chemical features of model 1 for both pharmacophore models are in full agreement with enzyme inhibitor specific targeting leading to kinase activity inhibition.

INTRODUCTION

1 Introduction

Cancer cells manifest characteristic abnormal growth properties accompanying clonal evolution of cells displaying progressively increasing genomic instability capable of invasion and metastasis to distant organ sites. With the emerging knowledge about the role of known oncogene and tumor suppressor gene mediated pathways in deregulating the growth of cancer cells, novel chemotherapeutic agents targeting these pathways are being developed. Such therapeutic approaches are designed to disrupt the signaling networks involving the respective target genes which are aberrantly expressed to cause uncontrolled growth of the malignant cells. While these strategies have shown promise in the initial treatment outcomes, their long term efficacy remains questionable in many instances since prolonged exposure to a specific target inhibiting drug often leads to cancer cells rewiring the aberrantly expressing signaling events to continue proliferation in a deregulated manner. This becomes possible since signaling cascades determining the abnormal growth phenotype are not regulated by linear events but result from complex functional networks constituted of cross talking individual signaling pathways. It is therefore logical to expect that for cancer therapeutics to be maximally effective, multiple signaling pathways converging on the fundamental growth regulatory processes such as DNA replication and/or mitosis need to be targeted in a robust manner. It is note-worthy, in this context, that efficacy of most conventional and novel chemotherapeutic agents rests on the premise that cancer cells can be preferentially eliminated due to their persistent cycling nature by interfering with either their replication/repair of DNA or by disrupting the mitotic division process (Katayama H. *et al.*, 2010).

Functional genomic data from tumors is proving helpful in alleviating the problem by identifying the putative therapeutic target proteins regulating cell cycle that are differentially expressed in tumors compared with the normal cells of the adult tissues. It is plausible that pharmaceutical targeting of such proteins would help the development of a new generation of effective therapeutic drugs that will have minimal host toxicity. These drugs while still interfering with the cell proliferation process would be expected to have a more selective effect on the tumors due to a preferential negative response of signal attenuation in the tumor cells compared to their normal counterparts (Sharma *et al.*, 2006). Based on this rationale, proteins involved in cell

cycle (Perez *et al.*, 2007) regulation and cell cycle associated kinases (Keen and Taylor, 2004) expressing at abnormally high levels in tumors, have been proposed as promising novel targets for the development of anticancer drugs. In the recent past, a number of inhibitors against new mitotic targets have indeed been rapidly moving into clinical trials (Garber, 2005). Among the mitosis regulatory kinases, evolutionarily conserved family of serine/ threonine kinases referred to as Aurora kinases has emerged as exceptionally attractive targets for anticancer drug discovery. The interest in designing drugs against Aurora kinase family members stems from the facts that these kinases express at elevated levels in many human cancers. Moreover, they are not only vitally important regulators of mitosis but have also been shown to functionally interact with multiple critical oncoproteins and tumor suppressor proteins. Preclinical studies of Aurora kinase inhibitors have shown promising results and the ongoing phase I and II clinical trials for several of these targets as anticancer molecules have also yielded encouraging results so far.

1.1 Aurora Kinases

Aurora kinases play an important role in cell cycle and belong to the serine/threonine kinase family (Girdler, 2006). In recent years Aurora kinases have been emerged as an important drug targets in several pharmaceutical companies and research industries, since they play a major role in regulating mitosis and cytokinesis (Foote *et al.*, 2008). Mitosis is a vital process for the regeneration of tissues development of an individual as well as for the functional integrity of a cell (Qi, 2005). Three different types of Aurora kinases were reported in mammals, designated as Aurora kinases A, B and C, which share a high similarity in amino acid sequence , however they exhibit distinct functions. For example, Aurora kinase A is ''polar kinase", primarily associated with the centrosomes separation (Dutertre, 2002). Aurora kinase B is ''equatorial kinase", and is a chromosomal passenger protein (Murata-Hori, 2002), while Aurora kinase C appears in the centrosome from anaphase to telophase and is highly expressed in testis. All three kinases influence the cell cycle from its G2 phase through cytokinesis but they appear at specific locations during mitosis. Abnormalities of Aurora kinases have strong link with cancer initiation and serve as guide to the current growth of new classes of anti-cancer drugs which specifically target the ATP-binding domains of Aurora kinases. Aurora kinase B and Aurora

kinase A (Aurora B/A) are over expressed in human tumors (Ditchfield, 2003)and inhibition of these kinases may lead to antitumor effects, hence they have attaineda considerable interest in developing specific and novel anti-cancer drugs to achieve the selectivity between Aurora B/A (Foote *et al.*, 2008).

1.2 Aurora Kinase Protein Structure

Aurora kinases have an amino acid sequence length ranging from 309-403 (Bolanos-Garcia, 2005). They have N-terminal domain, a protein kinase domain and a C-terminal domain. Aurora A and B share 71% identity in their C-terminal catalytic domain. The high percentage of conservation is very important in relation to the specificity of substrates and inhibitors. Structural and motif based comparisons suggested an early divergence of Aurora A from Aurora B and Aurora C (Cheetham *et al.*, 2002). Aurora A, B, and C have been mapped on chromosomes 20q13.2, 17p13.1, and 10q13 respectively.

1.3 Aurora Kinase Function

Aurora kinases are involved in multiple functions of mitosis. Aurora A is involved in mitotic entry, separation of centriole pairs, accurate bipolar spindle assembly, alignment of metaphase chromosomes and completion of cytokinesis (Marumoto *et al.*, 2003). The activity of Aurora A is closely related to centrosomes. It plays a role in bipolar spindle assembly, maturation of duplicated centrosomes by recruiting proteins including D-Tacc24 (Berdnik and Knoblich, 2002), γ-tubulin25 (Hannak *et al.*, 2001), SPD-2 (Kemp *et al.*, 2004), and centromeric ChToh27 (Conte *et al.*, 2003). Recently role of Aurora A in the promotion of nuclear envelop breakdown has been described (Portier *et al.*, 2007). Aurora B is one of the main components of the chromosomal passenger complex, which is a functional mitotic structure. It is involved in chromosomal bi-orientation, regulating kinetochores microtubule association and cytokinesis (Adams *et al.*, 2001). Incorrect attachments of sister chromatid kinetochores to microtubules can be resolved by Aurora B. Inhibition of Aurora B by small molecule inhibitor, Hesperadin, significantly increased syntelic attachment, which may lead to genetic instability (Hauf *et al.*, 2003). Aurora B is specifically enriched at merotelic attachment sites and is involved in the release of improper kinetochore microtubule attachments during chromosomal bi-orientation (Knowlton

et al., 2006). Aurora B phosphorylates histone H3 (Ser 10), which is believed to aid in chromatin condensation and separation (Goto *et al.*, 2002). Aurora C exhibits similar functions to those assigned to Aurora B and is required for cytokinesis. Hence Aurora C may be as important a kinase as other kinases in the regulation of various mitotic events. Figure 1.1 shows role of Aurora kinases in mitosis (Li *et al.*, 2004).

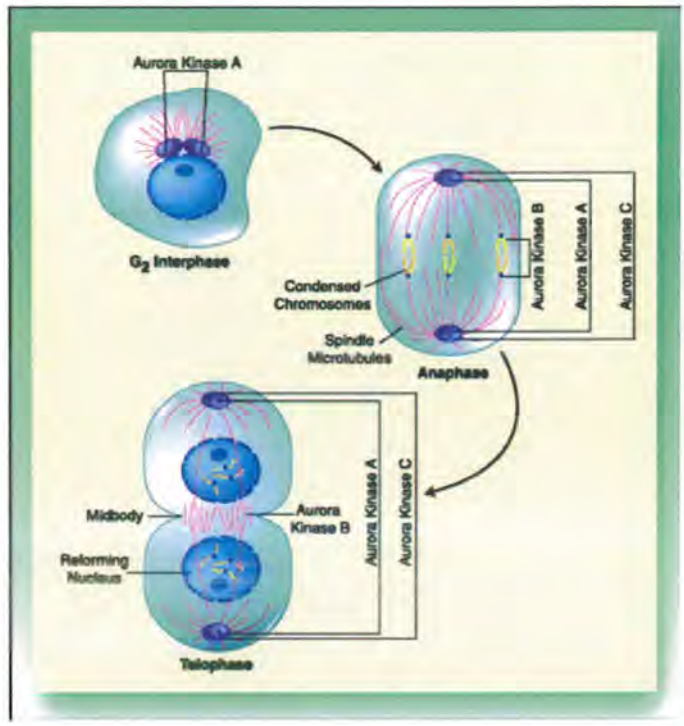


Figure 1.1: A schematic image illustrating the location and expression of Aurora Kinases A, B, and C during mitosis (Adapted from: Teicher, 2008).

1.4 Aurora Kinase Role in Cancer

Aurora kinases perform important functions during mitosis and hence their aberrant expression can lead to cell transformations underlying cancer. In many tissues, Aurora kinase over-expression leads to genetic instability (aneuploidy), which may cause cancer. Aneuploidy is a condition where the cells alter, DNA content may arise from mitotic defects including centrosome duplication, centrosome separation, cytokinesis and chromosomal bi-orientation errors. In all these processes, Aurora kinases are involved. Therefore it is tempting to state that the aberrant expression of Aurora kinases may lead to aneuploidy. Hence Aurora genes have been classified as

bonafide oncogenes (Kollareddy *et al.*, 2008). Table 1.1 shows involvement of Aurora kinases in various types of cancers.

Table 1.1: Over-expression or amplification of Aurora kinases in wide variety of tumors types, making them as attractive targets.

Aurora Kinase	Tumor Type	Reference
Aurora A	Breast Cancer	(Zhou *et al.*, 1998)
	Human Gliomas	(Reichardt *et al.*, 2003)
	Ovarian	(Zhou *et al.*, 1998)
	Prostrate	(Zhou *et al.*, 1998)
	Cervical	(Zhou *et al.*, 1998)
	Colon	(Zhou *et al.*, 1998)
	Pancreatic	(Li *et al.*, 2003)
	Lung Cancer	(Gu *et al.*, 2007)
Aurora B	Colon Cancer	(Katayama *et al.*, 1999)
	Thyroid cancer	(Sorrentino *et al.*, 2005)
	Oral cancer	(Qi *et al.*, 2007)
	Non small cell lung carcinoma	(Smith *et al.*, 2005)
	Breast cancer	(Tchatchou *et al.*, 2007)
Aurora C	Breast cancer	(Kimura *et al.*, 1999)
	Liver cancer	(Kimura *et al.*, 1999)

1.5 Development of Aurora Kinase Inhibitors

Since the discovery of Aurora kinases, much effort has been made by researchers to identify inhibitors. Typically, all three kinases influence by regulating the cell cycle and their malfunction may lead to cancer phenotype. This has increased the possibility of developing new anti-cancer drugs that could inhibit Aurora kinases. Among these inhibitors, AT9283, AZD1152, PHA-739358, MLN8054, Hesperadin, MK-0457 and ZM447439 are of interests with specificities to type of Aurora kinases and are in clinical trials (Mountzios *et al.*, 2008). In this study, we used three known inhibitors of Aurora Kinases: i) Hesperadin ii) MLN8237 and iii) 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline.

1.5.1 Aurora Kinase Specific inhibitors are more potent inhibitors than other targeted cancer therapies

Aurora kinase inhibition maybe a highly significant cancer therapeutic strategy for a number of reasons. First, Aurora kinase activity is essential for the fundamental defect in cancer and the basis of its ability to cause uncontrolled cell proliferation. If a cancer cell is prevented from proliferation, it cannot cause injury. Secondly, Aurora kinase inhibition, by stopping cell proliferation, also prevents a cancer cell from accruing additional mutations, the phenomenon that enables tumors eventually to escape the effects of anti-cancer drugs. Also, inhibition of Aurora kinase activity freezes cancer cells in mitosis, which frequently pushes them into programmed cell death or apoptosis, resulting in reduction in number of cancer cells (Eymin and Gazzeri, 2010).

Furthermore, Aurora kinase activity is necessarily higher in proliferating cells than in resting cells (i.e., cells in the G1 phase of the cell cycle). This provides for some natural degree of selectivity, or targeting of Aurora kinase inhibitors for cancer cells rather than normal or resting cells. Finally, because Aurora kinase acts in the last phases of cell cycle, Aurora kinase inhibitors have the potential to be more applicable and less likely to encounter resistance than other targeted cancer therapies (Park and Lee, 2003).

1.6 Computer-Aided Drug Design

Computer-aided drug design (CADD) is an important part of the rational drug design process, in which extensive computer modeling methods are employed to reduce the costs and speed up the drug developing process (Acharya *et al.*, 2011). Generally speaking, drug developing is still a time-consuming, expensive, difficult, and inefficient process with low rate of novel therapeutic discovery. Based on the most updated estimation in 2009, the average expense on one new drug is about 1.3 to ~1.7 billion and the developing process may take about eight to ten years (Collier, 2009). Among 10 projects, only one of them would be successful. Therefore, computer-aided drug design composes an important part of the drug developing process. CADD can fully utilize the existing knowledge about the current available drugs and structure information of the drug targets. Based on the information utilized, CADD can be

classified into two categories: the ligand based (indirect drug design) methods and the structure based (direct drug design) methods (Taft *et al.*, 2008).

The ligand based methods rely on the knowledge of other existing ligands that can bind to the target (Chang *et al.*, 2006). With the structural information and the binding affinities of the known ligands, the pharmacophore model can be constructed and the quantitative structure-activity relationship (QSAR) may be derived. By these models, researchers can modify the existing ligands or predict new molecules that can efficiently bind to the same target. However, the ligand based methods must start from a set of known high-affinity ligands and thus can only explore limited conformational spaces around the ligands used upon constructing the model. Therefore, these methods are mainly used to improve and optimize existing drugs (Loew *et al.*, 1993).

The structure based methods rely on the knowledge of three dimension structure of the drug target, which can be obtained using experimental methods such as X-ray crystallography and NMR spectroscopy or constructed by homology modeling method (Marrone *et al.*, 1997). Structure based drug design can be further divided into two categories: Virtual screening, which focuses on finding ligands from an existing database that can bind to the active site of a given target; the other one is fragment based method, which aims to design a novel molecule with high affinity and selectivity by joining the fragments that may bind to multiple parts of the active site (Schneider and Fechner, 2005).

The virtual screening method has to screen a large database in order to isolate the top hits. Although the screening algorithms have been optimized and run very fast, the computational costs are still tremendous, especially when taking the flexibility of molecules into account. On the other hand, the fragment based methods can start with a smaller fragments database and generate *denovo* scaffolds by recombination of the limited fragments, and thus been proposed as a time and cost-efficient manner. However, as the fragment based methods are newly emerged and still under developing, results are normally used to "inspire" the medicinal scientists rather than been adopted (Schneider and Fechner, 2005).

The methods mentioned above are complementary to each other and could exist in different phases of drug developing process. For example, when designing a new drug for given target with 3D structure, virtual screening method can be adopted first to give some suggestion.

1.7 Aims and Objectives

In this study, we adopted ligand based as well as structure based virtual screening methods to find more potent inhibitors for Aurora kinases.

The structures of Aurora B and C will be predicted and binding site information for three Aurora kinases is gathered. Two datasets will be included in the study,

i) Dataset of novel inhibitors of Aurora kinases,

ii) Dataset of known inhibitors of Aurora kinases.

Binding modes for both datasets will be studied in detail for Aurora kinase A, B and C. Ligand based drug designing process is used for 3D pharmacophore generation for both datasets and that pharmacophore model is used for screening additional databases to reterieve more Aurora kinase inhibitors. As binding site of Aurora kinases is well studied, structure based drug designing process will be used to screen novel inhibitors that frequently interact with the binding site residues of Aurora kinases. Dataset of known inhibitors will also be used for derivatives generation to find more stable, specific and potent inhibitors for all three members of Aurora kinases.

MATERIALS AND METHODS

2 Materials and Methods

2.1 Software, Servers and Databases Used

Table 2.1 shows the list of software, servers and tools that are used in our research study.

Table 2.1: List of software, tools and servers.

No.	Tools	Purpose	URL
1.	Swiss Model	3D Structure generation	*http://swissmodel.expasy.org/*
2.	Wincoot	Structure Refinement	*http://www.ysbl.york.ac.uk/~lohkamp/coot/wincoot.html*
3.	NIH sever, Molprobity	Structure Validation	*http://nihserver.mbi.ucla.edu* *http://molprobity.biochem.duke.edu/*
4.	WHATIF	Structure Validation	*http://swift.cmbi.ru.nl/servers/html/index.html*
5.	PROCHECK	Structure Validation	*http://nihserver.mbi.ucla.edu/PROCHECK/*
6.	VERIFY-3D	Structure Validation	*http://nihserver.mbi.ucla.edu/Verify_3D/*
7.	ERRAT	Structure Validation	*http://nihserver.mbi.ucla.edu/ERRATv2/*
8.	Enamine Database	Aurora kinase focused library is taken for ligands screening	*http://www.enamine.net/*
9.	LigandScout	Pharmacophore Generation	*http://www.inteligand.com/ligandscout/*
10.	PyRx	Virtual Screening	*http://pyrx.sourceforge.net*
11.	Autodock	Dockings	*http://autodock.scripps.edu*
12.	Princeton, Uorsy Databases	Libraries are taken to perform virtual Screening	*http://www.princetonbio.com* *http://www.ukrorgsynth.com*
13.	Pubchem	Known inhibitors are taken	*http://pubchem.ncbi.nlm.nih.gov/*

No.	Tools	Purpose	URL
14.	Chem T	Analogs generation of known inhibitors and for 2D profiles generation	*http://www.esa.ipb.pt/biochemcore/index.php/ds/c*
15.	Others	Discovery Studio, Ligplot, Orisis Property Explorer, Pymol, VMD	*http://accelrys.com/products/discovery-studio, www.biochem.ucl.ac.uk/bsm/ligplot, http://www.organicchemistry.org/prog/peo/, www.pymol.org/, http://www.ks.uiuc.edu/Research/vmd/*

2.2 3D Structure Prediction

In the absence of a well-defined structure, homology modeling provides a rational alternative to develop a reasonable 3D model. Notably, homology modeling is currently the most accurate method for 3D structure prediction yielding models suitable for a wide spectrum of applications, such as investigations into mechanism, structure-based drug development and virtual screening (Li and Wang, 2007). This approach can produce a reasonable structural model for any given protein sequence that has related templates having more than 25% amino acid sequence identity (Tramontano, 1998) .

The construction of protein models by homology modeling normally proceeds along a series of well-defined and commonly accepted steps: (1) sequence alignment between the target and the template; (2) building an initial model; (3) refining the model; and (4) evaluating the quality of the model (Ginalski, 2006). Figure 2.1 shows steps of homology modeling.

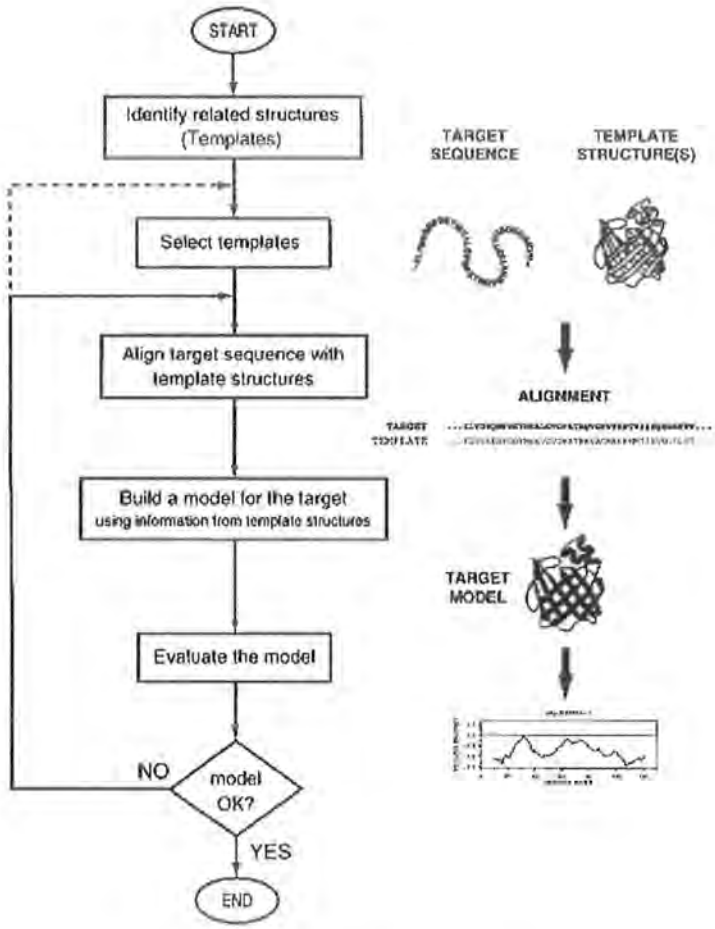


Figure 2.1: Steps of Homology Modeling. First of all template is identified using target sequence, then model of taget sequence is build using template structure and finally model is evaluated (Adapted from Eswar *et al.*, 2007).

2.2.1 Swiss Model

In order to elucidate the 3D structures of Aurora B and C, we used template models having PDB IDs 2VGP and 2NP8, respectively. Swiss-Model program was used to predict the structures.

Swiss Moel was initiated in 1993 by Manuel Peitsch. Swiss Model is a fully automated protein structure homology modeling server accessible via the ExPASy Web server (Schwede *et al.*, 2003; http://swissmodel.expasy.org/). It takes a sequence alignment and a PDB file as input for the template. These are submitted over a server, and the knowledge-based homology model is constructed using the ProModII program (Peitsch, 1996). Model construction includes complete backbone and side chain building, loop building, and verification of model quality including packing.

The model thus built is energy minimized using the Gromos96 force field (Christen *et al.*, 2005). The model coordinates are returned in PDB format. Very little or no user intervention is needed.

2.3 Structure Validation

The models were optimized by Wincoot (Emsley *et al.*, 2010) and validated by Ramachandran plot (Laskowski *et al.*, 1996), PROCHECK (Laskowski *et al.*, 1996), Errat (Colovos and Yeates, 1993), Verify 3D (Eisenberg *et al.*, 1997) and WHAT-if (Vriend and Sander, 1993).

2.3.1 Wincoot

The structures were optimized and refined using wincoot (Emsley *et al.*, 2010). Wincoot was used for geometry analysis in order to check for improbable bond lengths, angles, etc. Similarly, peptide omega analysis was also performed using Wincoot to check non-planar peptide bonds. Problematic rotamers were then corrected by Wincoot, using its rotamer library. Rotamer analysis by Wincoot checks for unusual protein side-chain conformations.

2.3.2 PROCHECK

PROCHECK (Laskowski *et al.*, 1993) was used to estimate the stereo-chemical quality of a model. Overall, PROCHECK program finds covalent geometry, planarity, dihedral angles, chirality, non-bonded interactions, main-chain hydrogen bonds, disulphide bonds, stereo chemical, parameters, parameter comparisons and residue-by-residue analysis.

2.3.3 Ramachandran Plot

Ramachandran's plot is a protein structure validation tool for checking the detailed residue-by-residue stereo-chemical quality of a protein structure. The phi and psi distribution of Ramachandran's plot of non-glycine, non-proline residues are summarized in Ramachandran Plot. A good homology model should have >90% of the residues in the favorable region. Ramachandran plot was constructed for each protein model using PROCHECK web-server.

2.3.4 ERRAT

ERRAT (Colovos and Yeates, 1993) is a so-called "overall quality factor" for non bonded atomic interactions, and higher scores mean higher quality. The normally accepted range is >50 for a high quality model.

2.3.5 Verify 3D

VERIFY 3D (Eisenberg *et al.*, 1997) uses energetic and empirical methods to produce averaged data points for each residue to evaluate the quality of protein structures. Using this scoring function, if more than 80% of the residue has a score of >0.2 then the protein structure is considered of high quality.

2.3.6 WHAT-IF

WHAT-IF (Vriend and Sander, 1993) is used to check the normality of local environment of amino acids. For the WHAT-IF evaluation, quality of the distribution of atom types is determined around amino fragments. For a reliable structure, WHAT-IF packing scores should be above −5.0.

Figure 2.2 shows the workflow diagram of research study.

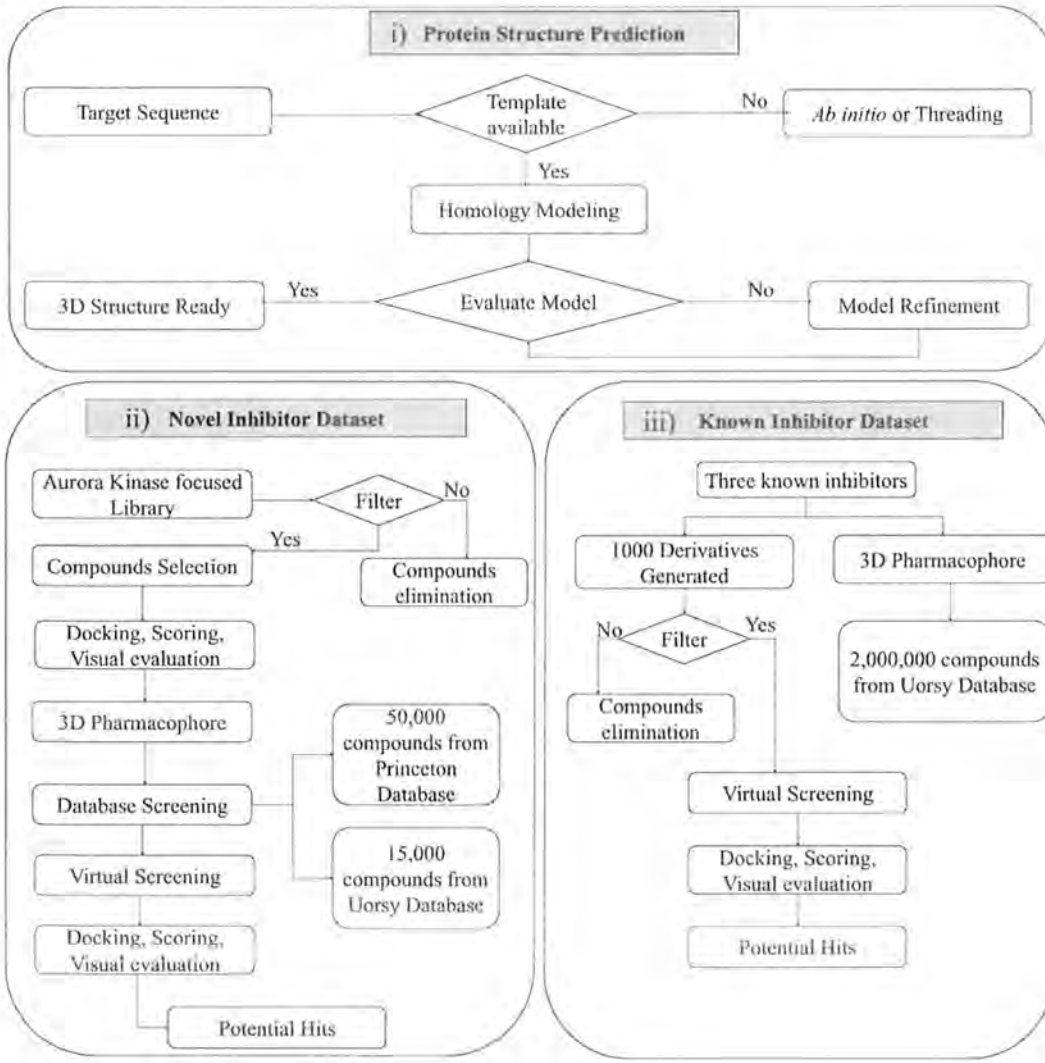


Figure 2.2: Workflow diagram, i) Protein structure prediction methods, If template is available homology modeling is used otherwise ab initio or threading strategy is used, the predicted structure is also refined and evaluated, **ii)** Novel Inhibitors Dataset is generated from Aurora kinase focused library, a pharmacophore model is generated from the novel inhibitors dataset that is used further for database screening to find more Aurora kinase inhibitors, **iii)** Known inhibitors Dataset is also used for derivatives and pharmacopore generation that is also used to find Aurora kinase inhibitors by database screening strategy.

2.4 Data Collection

Two datasets are prepared for our study.

i) Compounds extracted from Enamine (*www.enamine.net*) Aurora Kinase Focused Library.
ii) Known inhibitors of Aurora Kinases.

2.4.1 Virtual Screening of Aurora Kinase Focused Library

Virtual screening (VS) is usually described as a cascade of filter approaches to narrow down a set of compounds to be tested for biological activity against the intended drug target. Starting with a fast evaluation of the drug-likeness of compounds, VS is often followed by ligand-based and/or structure-based approaches if the target structure is available (Muegge, 2008). The computational methods employed in VS can be divided into three classes, with increasing complexity and computational requirements. In practice they are often used in combination. These include, evaluation based on two-dimensional (2D) property profiles (Zheng *et al.*, 1998); evaluation based on a target-specific pharmacophore, which is a reduced representation of the key features in the target system or ligand and evaluation based on detailed 3D structure modeling of receptor-ligand interaction (van Drie *et al.*, 1989).

In this study 2,575 compounds were selected for virtual screening from Aurora focused library taken from Enamine Database. 2D property profiles were generated to check whether a compound is "drug like" and to check whether it is synthetically accessible or orally bio-available. A simplest way to verify is by the "rule of five" (Lipinski *et al.*, 2001). Compounds that fulfill the "drug like" criteria were selected for VS.

The most detailed and time consuming, but perhaps the most relevant VS technique involves objective docking of 3D models of both ligand and receptor targets. The quality of the fit between a given ligand and target is used to rank the ligand, or to predict the actual binding affinity by calibration with the known ligand receptor complexes. The molecular docking approach requires 3D model of both target and library of compound structures (Subramanian *et al.*, 2006). In this study, Autodock 4.2 (Morris *et al.*, 1998) integrated in pyrx was used to perform VS. Compounds were short-listed on the basis of binding energies and were subsequently checked for toxicity, solubility, mutagenicity and tumorgenic effects by OSIRIS Property Explorer (Korff *et al.*, 2012). Hits showing high risks of undesired effects like mutagenicity or a poor intestinal absorption were eliminated while compounds exhibiting low toxicity values were further selected. The compounds were then filtered on the basis of binding affinities to the potential ATP binding site of Aurora kinase (Li *et al.*, 2008)

by molecular docking. These compounds were subsequently ranked and grouped on the basis of their specificity, selectivity and affinity properties. Moreover, further families were identified for selected compounds which included quinazoline, carboxamide and benzoic acid. Later using these families, more compounds were selected on the basis of binding and docking energies. The test set compounds included in this study were 2-(thiophen-2-yl) quinazoline (A); N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide (B); 2-{[(1E)-(2-hydroxyphenyl) methylidene]amino}benzoic acid (C) and a known inhibitor 2-(1H-pyrazol-5-yl)-1H-benzimidazole (D) (Figure 2.3).

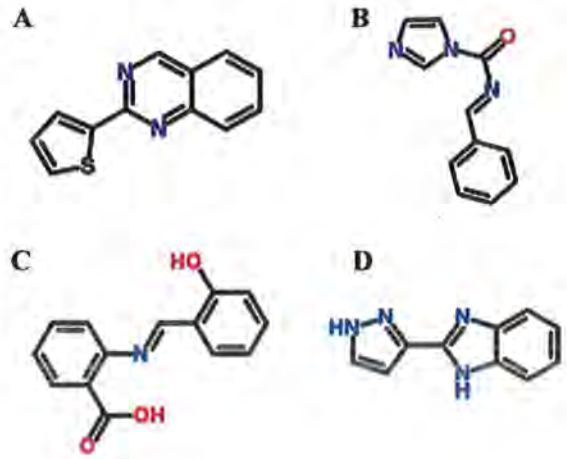


Figure 2.3: Basic structures of compounds isolated from Aurora focused library, (A) 2-(thiophen-2-yl) quinazoline, **(B)** N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide, **(C)** 2-{[(1E)-(2-hydroxyphenyl)methylidene]amino}benzoic acid and **(D)** 2-(1H-pyrazol-5-yl)-1H-benzimidazole.

2.4.2 Known Inhibitors Dataset

Three known inhibitors, MLN8237, Hesperadin and 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline (Figure 2.4) are used in this study and taken from pubchem database (*http://pubchem.ncbi.nlm.nih.gov/*). MLN8237 (Alisertib) is a selective Aurora kinase A inhibitor with median IC50 of 61 nM. MLN8237 (Alisertib) is a second-generation, orally bioavailable, highly selective small molecule inhibitor of the Aurora A kinase (serine/threonine protein kinase) with potential antineoplastic activity. MLN8237 (Alisertib) binds to and inhibits Aurora A kinase, which may

result in disruption of the assembly of the mitotic spindle apparatus, disruption of chromosome segregation, and inhibition of cell proliferation (Maris *et al.*, 2010).

Hesperadin is a human Aurora B inhibitor with an IC50 of 40 nM for the prevention of the phosphorylation of substrate (Jetton *et al.*, 2009).

The third compound 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline (Kollareddy *et al.*, 2012) is reported in pubchem database for inhibition of Aurora A and Aurora B. It acts as a potent, selective, and ATP-competitive inhibitor of Aurora kinases (IC_{50} = 310 nM and 240 nM for Aurora A and B, respectively.

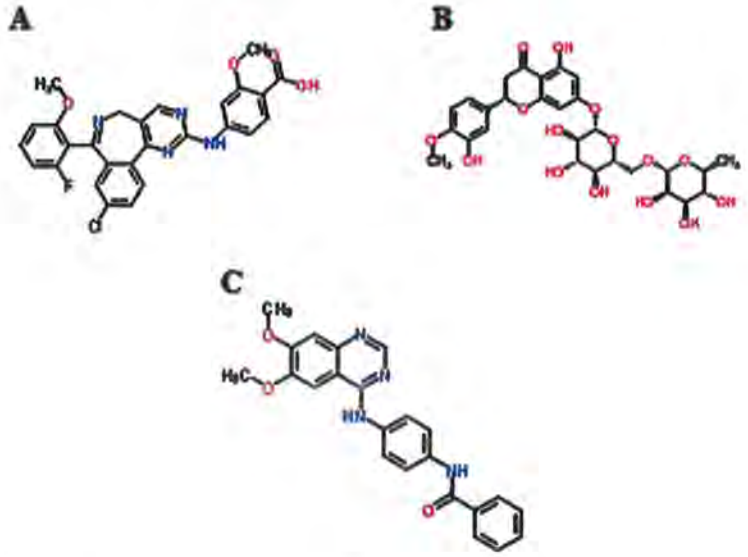


Figure 2.4: Basic Structures of A) MLN8237, **B)** Hesperadin and **C)** 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline.

2.5 Pharmacophore Generation

A pharmacophore is defined as an ensemble of universal chemical features (hydrogen bonds, charge interactions and hydrophobic areas) that characterizes a specific mode of action of a ligand in the active site of the macromolecule in 3D space. This pharmacophoric pattern is the condition for ligand-macromolecule interaction. Searching these chemical patterns in large molecule databases allows finding new scaffolds for developing lead structures. In this study, we have developed two three dimensional pharmacophore hypotheses using Ligand Scout tool (Wolber and Langer, 2005), i) set of four novel compounds (A) 2-(thiophen-2-yl) quinazoline. (B) N-[(E)-

phenylmethylidene]-1H-imidazole-1-carboxamide. (C) 2-{[(1E)-(2-hydroxyphenyl)methylidene]amino}benzoic acid and (D) 2-(1H-pyrazol-5-yl)-1H-benzimidazole (A-D) taken from Aurora kinase focused library ii) three known inhibitors of Aurora kinases i.e., A) MLN8237, B) Hesperadin and C) 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline (A-C).

2.5.1 Ligand Scout

Ligand Scout (Wolber and Langer, 2005) generates structure-based as well as ligand-based pharmacophore models. The ligand-based strategy derives pharmacophore models from a set of ligands in the absence of the macromolecule structure by considering the conformational flexibility of the ligands. It is based on the principle that common structures containing small molecules provide similar biological activity. Thus, this approach searches a common feature pattern that is shared in an active ligand-set (Wolber *et al.*, 2006).

2.6 Docking and Virtual Screening Studies

Automated docking was used to locate the appropriate binding orientations and conformations of various inhibitors in the Aurora kinase binding pocket.

2.6.1 Auto Dock 4.2

To perform the task, genetic algorithm in program Auto Dock 4.2 (Morris *et al.*, 1998) was used. Working with Auto Dock 4.2 includes 3 steps:

1. Preparation of receptor and ligand files.
2. Calculation of affinity maps by using a 3D grid around the receptor and ligand.
3. Defining the docking parameters and running the docking simulation.

Polar hydrogen atoms and Kollman charges were assigned to the receptor proteins. For ligands, Gasteiger partial charges were assigned and non-polar hydrogen atoms were merged. All torsions for ligands were allowed to rotate during docking.

The program Auto Grid was used to generate the grid maps. Each grid was centered at the structure of the corresponding receptor. The grid dimensions were 80*80*80 $Å^3$ with points separated by 0.375 Å. For all ligands, random starting positions, random

orientations and torsions were used. The translation, quaternion and torsion steps were taken from default values in Auto Dock. The Lamarckian genetic algorithm method was used for minimization using default parameters. The standard docking protocol for rigid and flexible ligand docking consisted of 50 runs, using an initial population of 150 randomly placed individuals, with $2.5*10^6$ energy evaluations, a maximum number of 27000 iterations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1. Cluster analysis was performed on the docked results using an RMS tolerance of 1.0 Å. The clusters were ranked by the lowest energy representative of each cluster.

2.6.2 PyRx

PyRx (*http://pyrx.scripps.edu*) is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables Medicinal Chemists to run Virtual Screening from any platform and helps users in every step of this process, from data preparation to job submission and analysis of the results. While it is true that there is no magic button in the drug discovery process, PyRx includes docking wizard with easy to use user interface which makes it a valuable tool for Computer-Aided Drug Design. PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for Rational Drug Design.

RESULTS

3 Results

3.1 Structure Prediction and Refinement

3.1.1 Prediction of human Aurora B and C 3D Structures

In order to predict the human Aurora B structure, crystal structure of *Xenopus laevis* Aurora B (PDB ID: 2VGP; resolution 1.70 Å) was obtained from PDB (Berman *et al.*, 2000) and used as template. MSA analysis indicated 80% sequence identity between the target and template sequences and RMSD score was found to be 0.055Å. Alignment between target and template is shown in Figure 3.1.

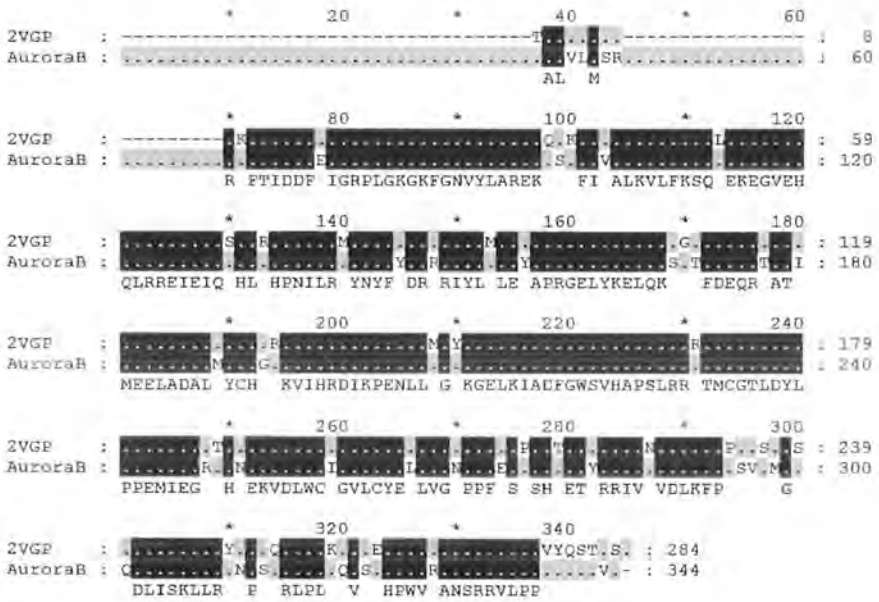


Figure 3.1: Sequence Alignment of target and template for Aurora kinase B.

Aurora C model prediction was carried out using human Aurora A (PDBID: 2NP8, resolution 2.25 Å) as best template, which exhibited a sequence identity of 77%. The backbone RMSD between the 2NP8 and Aurora C modeled structure was 0.079Å, indicating a high homology shown in Figure 3.2.

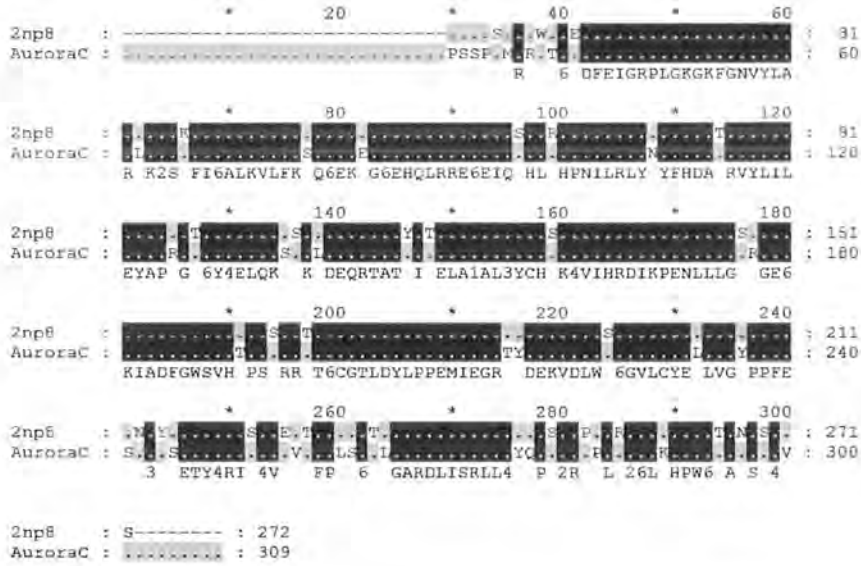


Figure 3.2: Sequence Alignment of template and target for Aurora kinase C.

3.1.2 Structure Validation

Ramachandran's plot indicated that 99.1% and 99.3% residues lie in allowed regions for both Aurora B and C models, respectively as shown in Figure 3.3. None of the active site residues were present in the disallowed region. Moreover, parameters like peptide bond planarity, non-bonded interactions, Cα tetrahedral distortion, main chain H-bond energy and overall G factor for both the structures lie within favorable range as shown in Figure 3.4.

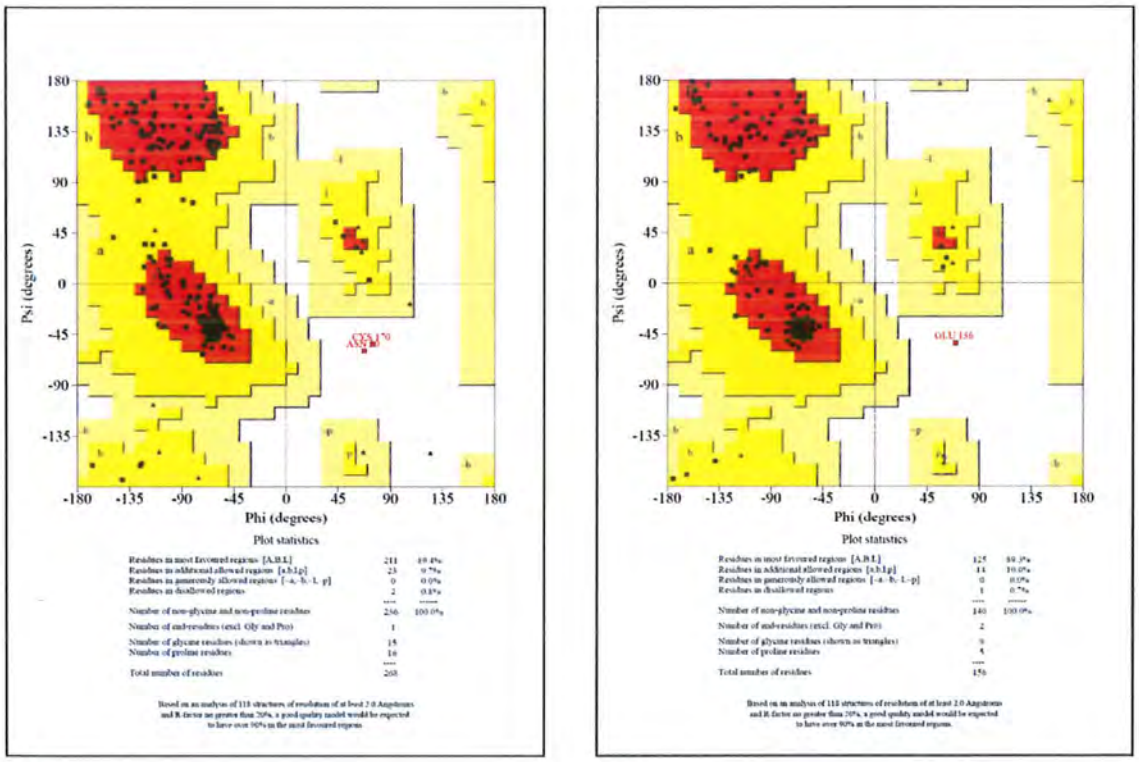


Figure 3.3: Ramachandran plot for homology modeled A) Aurora kinase B, it showed that 99.2% of residues lie in favored and allowed regions, **B)** Aurora kinase C, it shows that 99.3% of residues lie in favored and allowed regions.

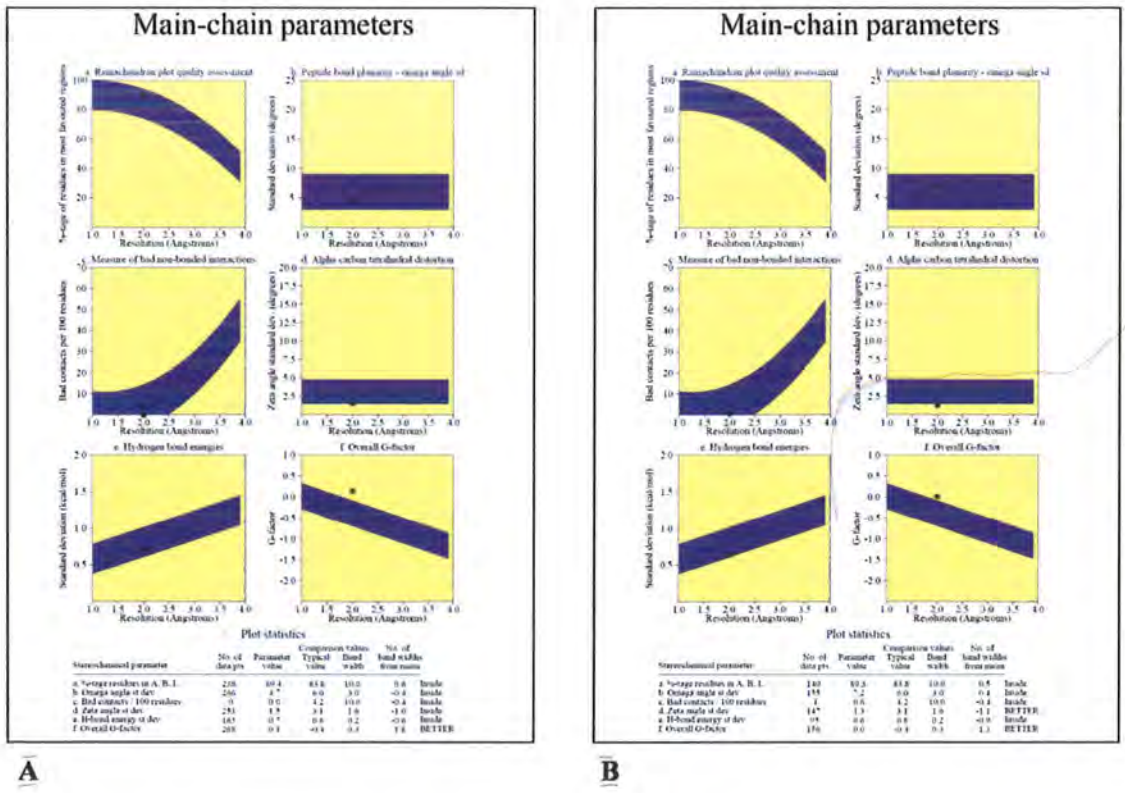


Figure 3.4: Main chain parameters plotted by PROCHECK for (A) Aurora kinase B and **(B)** Aurora kinase C.

The homology models were further verified using ERRAT, Verify3D and WHAT-IF tools. ERRAT measures the overall quality factor for non bonded atomic interactions and an accepted range is above 50 for a high quality model. The ERRAT scores were 95.2 and 87.7 for Aurora B and C, respectively.

Verify3D employs energetic and empirical methods to produce averaged data points for each residue to evaluate the quality of structures. A score above 0.2 for more than 80% residues suggests a considerable high model quality. In both Aurora B and C, 92.19% and 98.73% residues had score above 0.2.

WHAT-IF is used to check the normality of local environment for amino acids. In this evaluation, the quality atomic distribution is determined around amino fragments. For a reliable structure, WHAT-IF packing scores should be above −5.0. In case of Aurora B and C models, none of the scores for any residue was found less than −5.0. These

data indicated that Aurora B and C models are of good quality to allow for further study.

3.1.3 Binding Sites for Aurora B and C

Generally, at ATP binding pocket of Aurora kinase A, Glu211 and Ala213 residues were found to be crucial for the potent ligand binding and kinase selectivity (Talele and McLaughlin, 2008). In case of Aurora B protein, Lys106, Glu155 and Ala157 were the critical amino acids with respect to interaction (Fu *et al.*, 2008). These residues were found to be highly conserved structurally among Aurora proteins. For identifying ATP binding pocket of Aurora kinase C an alignment between Aurora A and C is carried out, it showed that for Aurora kinase C Glu 121 and Ala 123 residues were the critical amino acids. Binding site information for Aurora kinases was also gathered from PDB (Berman *et al.*, 2000) database. All enteries reported in PDB for Aurora kinases were gathered and their binding mode (residues) was studied using Ligplot. For Aurora kinase A the residues reported in PDB enteries include Ala213 and Glu211, Leu139, Val147, Lys 162 and Glu217. For Aurora kinase B the residues include Ala157, Glu155, Leu83, Val91, Lys106 and Glu161. Likewise binding site information for Aurora kinase C is gathered from alignment of Aurora C with Aurora A and B. The binding site residues are also conserverd in Aurora C and includes Ala123, Glu121, Leu39, Val47, Lys62 and Glu 127 respectively. PDB enteries included in the study include 2DWB, 2WTV, 2WTW, 2C6D, 4AF3, 3QBN, 3P9J, 2W1D, 2W1G, 2W1C, 3HOY, 3H1O, 2X6E, 2X6D, 2X81, 3MYG, 3NRM, 3UO4, 3UOD, 3UPW, 3M11, 2VGP, 2VRX, 3VAP, 3K5U, 2BFY, 2NP8, 2BFX, 2VGO, 3AMA, 3AMB, 2F4J, 2W1H.

3D structure of Aurora B along with the template structure and its superimposition results is shown in Figure 3.5 A-C. 3D structure of Aurora C along with its template and superimposition results is shown in Figure 3.6 D-F.

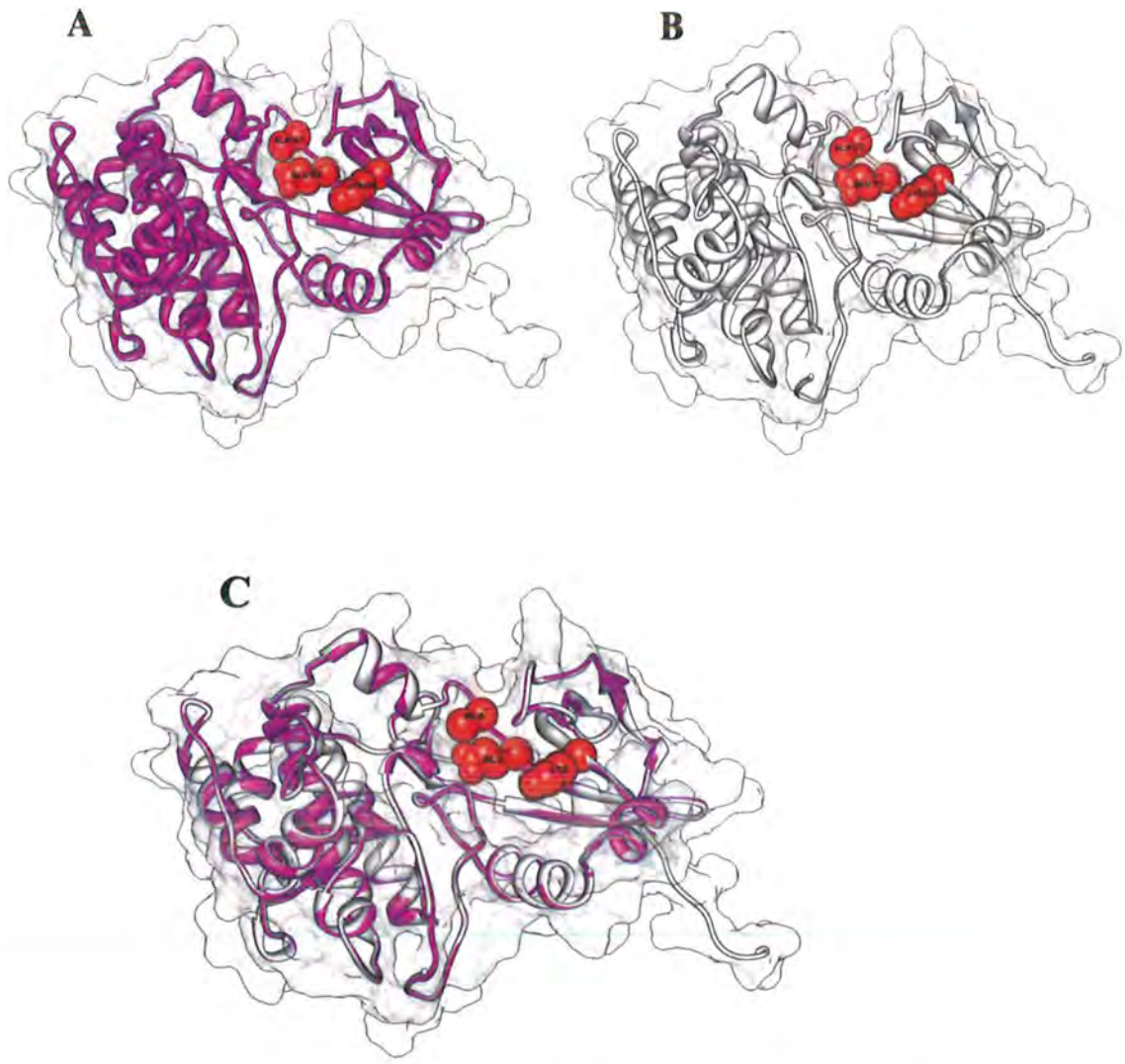


Figure 3.5: Surface and ribbon representations of Aurora Kinase B model in pink color (A) and template model (2VGP) in gray, (B) are superimposed well (RMSD C_α = 0.055Å) indicated in (C).

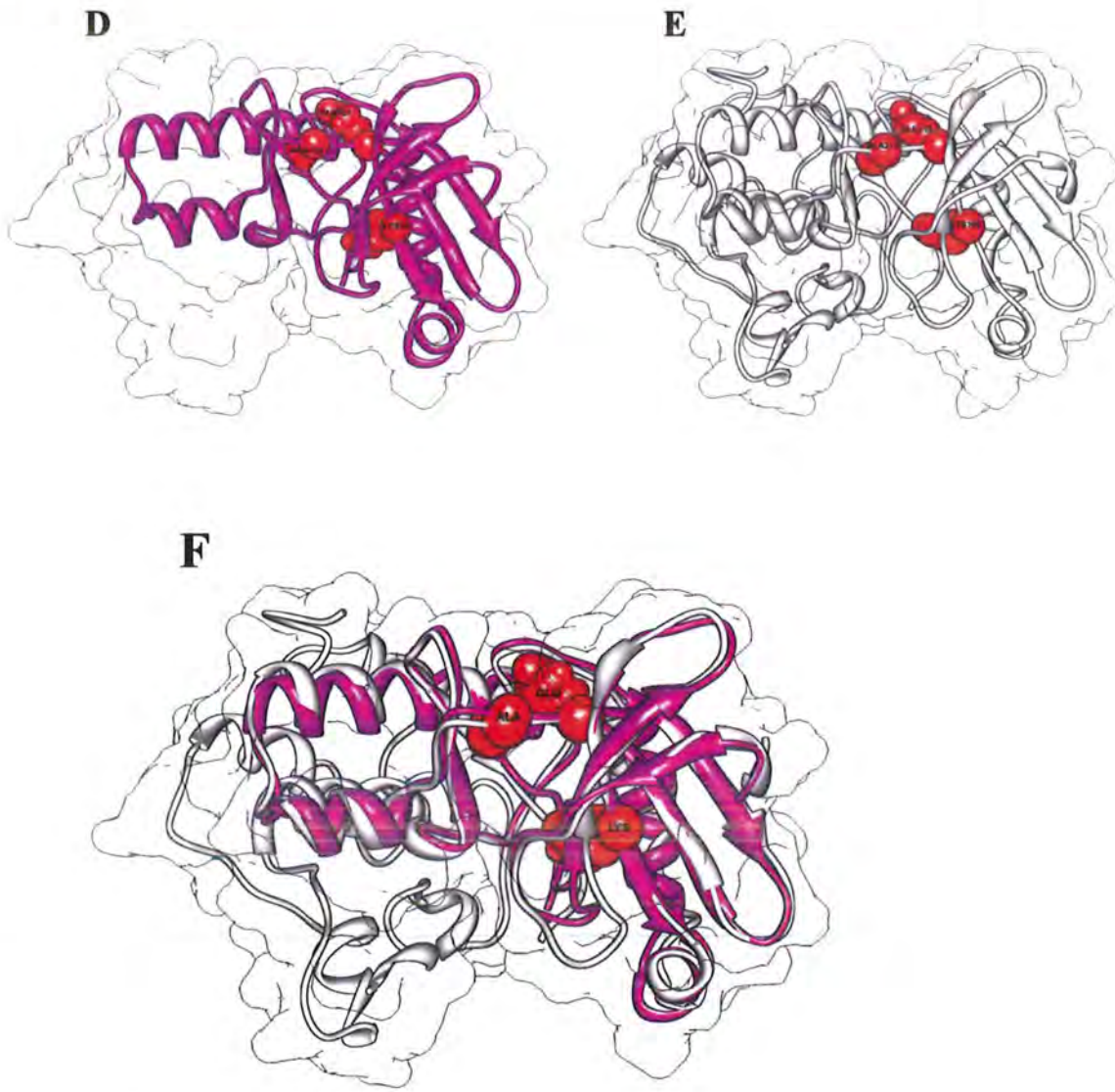


Figure 3.6: Ribbon representation of Aurora Kinase C target structure (D) in pink and template (E) in gray color (2NP8), (F) Superimposition of Aurora C and 2NP8, Amino acid residues involved in binding with ATP are indicated by red color.

3.2 Docking Studies, Pharmacophore Generation and Database Screening for Novel Inhibitors Dataset

2D profiles of selected compounds from Aurora kinase focused libraray were generated from Orisis Property Explorer (Korff *et al.*, 2012) as shown in Table 3.1.

Table 3.1: The 2D profiles of test-set compounds, (A) 2-(thiophen-2-yl) quinazoline, **(B)** N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide, **(C)** 2-{[(1E)-(2-hydroxyphenyl)methylidene] amino}benzoic acid and **(D)** 2-(1H-pyrazol-5-yl)-1H-benzimidazole.

Ligand	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Molecular Weight	C Log P	No. of rotatable bonds	Solubility	Drug Score
A	3	0	212.27	3.47	1	-4.18	0.41
B	4	0	190.137	1.69	1	-1.64	0.81
C	4	3	241.242	2.16	3	-2.88	0.74
D	4	2	184.197	1.31	1	-2.24	0.59

3.2.1 Molecular Docking of Selected Inhibitors

Protein ligands docking were performed using AutoDock 4.2 and each docked pose was analyzed individually to monitor the interactions. Binding energy is the sum of the intermolecular energy and the torsional free energy penalty and docking energy is the sum of the intermolecular energy and the ligand's internal energy. Inhibition constant in AutoDock is calculated using the expression Ki= exp((ΔG*1000)/(Rcal*TK), where ΔG is docking energy, Rcal is 1.98719 and TK is 298.15. Docking energy is the sum of the intermolecular energy and the ligand internal energy (Morris *et al.*, 1998). The values of binding energies, docking energies and inhibition constant values for the selected compounds docked against Aurora kinases are listed in Table 3.2.

Table 3.2: Binding energies, docking energies and inhibition constant values for (A) 2-(thiophen-2-yl) quinazoline, **(B)** N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide, **(C)** 2-{[(1E)-(2-hydroxyphenyl)methylidene]amino}benzoic acid and **(D)** 2-(1H-pyrazol-5-yl)-1H-benzimidazole.

	Aurora Kinase								
	A	B	C	A	B	C	A	B	C
Compounds	**Binding Energy (Kcal/mol)**			**Docking Energy (Kcal/mol)**			**Ki (μM)**		
A	-6.18	-7.1	-6.06	-7.43	-7.99	-6.96	16.08	6.28	36.04
B	-6.14	-7.27	-6.03	-6.85	-7.87	-6.65	26.95	4.67	37.9
C	-6.91	-7.74	-7.63	-6.91	-7.74	-7.63	8.62	2.1	2.55
D	-5.79	-6.79	-6.37	-6.23	-7.23	-6.8	57.0	10.59	21.57

Figure 3.7 shows binding energies of these compounds is graphically represented as

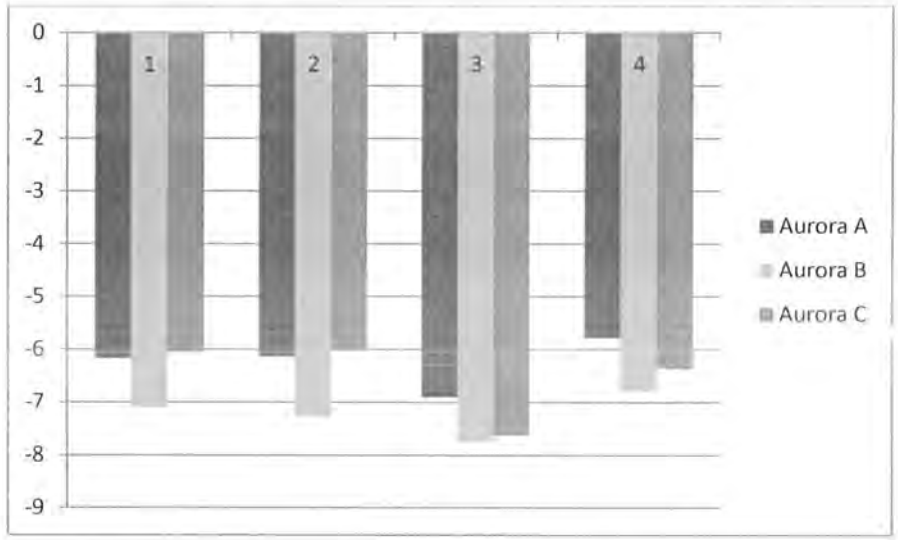


Figure 3.7: Binding energies of (1) 2-(thiophen-2-yl) quinazoline, **(2)** N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide, **(3)** 2-{[(1E)-(2-hydroxyphenyl)methylidene]amino}benzoic acid and **(4)** 2-(1H-pyrazol-5-yl)-1H-benzimidazole against three Aurora kinases.

Figure 3.8 shows docking energies graphically

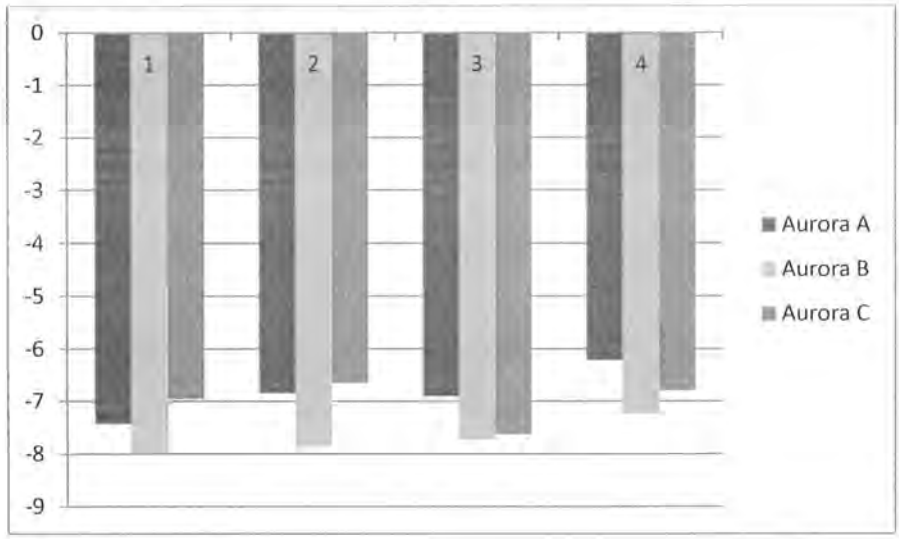


Figure 3.8: Docking energies of (1) 2-(thiophen-2-yl) quinazoline, **(2)** N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide, **(3)** 2-{[(1E)-(2-hydroxyphenyl)methylidene]amino}benzoic acid and **(4)** 2-(1H-pyrazol-5-yl)-1H-benzimidazole against three Aurora kinases.

3.2.2 Binding Mode of 2-(thiophen-2-yl) quinazoline

Altogether, binding energies of all four compounds range from -7.74 to -6.03, while docking energies range from -7.99 to -6.23. Figure 3.9 shows a docked model of 2-(thiophen-2-yl) quinazoline into the active sites of Aurora kinases. Quinazoline -N- ring atom shows H-bonding with Ala213 of Aurora kinase A (-N...HN, distance; 1.9 Å). In addition, it exhibited hydrophobic interactions with Lys162, Glu211, Ala213 and Val147. Likewise, quinazoline -S- atom showed H-bonding with Ala157 of Aurora kinase B (-S...HN, distance; 2.3 Å) and hydrophobic interactions with residues Leu83, Val91, Glu155 and Glu161. It formulated pi-pi interactions with Tyr156 residue. For Aurora kinase C, quinazoline -S- ringed atom showed H-bonding with Ala123 of Aurora kinase C (-S...HN, distance; 2.5 Å) whereas hydrophobic interactions were found with residues Leu49, Ala70 and Glu121 residues. However, pi-pi interactions were seen with Leu173.

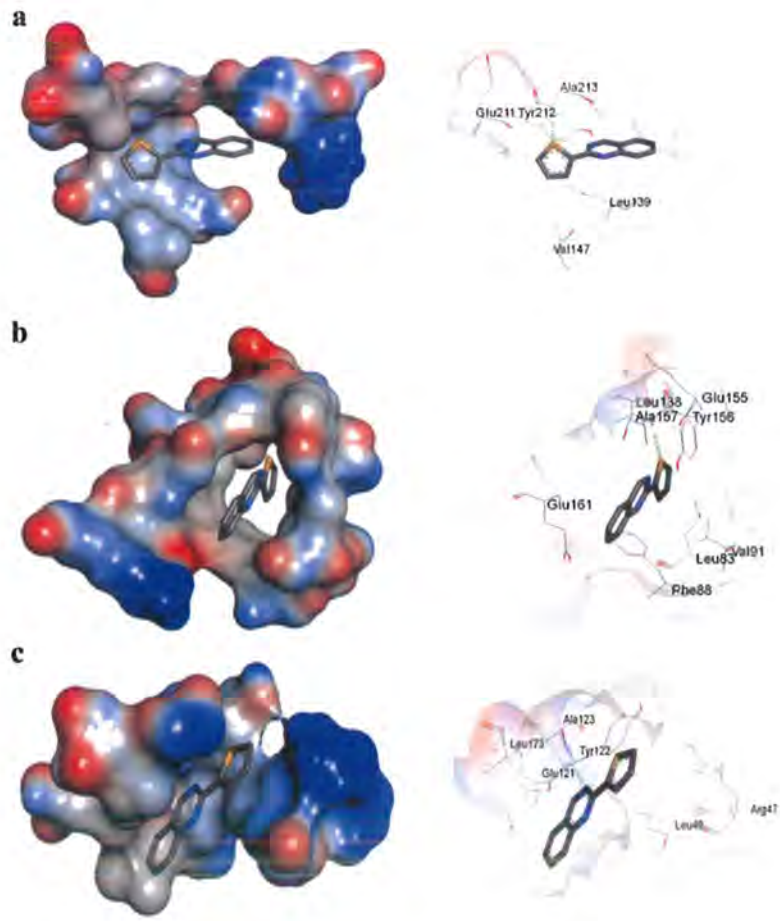


Figure 3.9: 2-(thiophen-2-yl) quinazoline interactions (a) Aurora kinase A binding pattern, hydrogen bonding is shown with with Ala213, **(b)** Aurora kinase B binding pattern, hydrogen bonding is shown with Ala157, **(c)** Aurora kinase C binding pattern, hydrogen bonding is shown with Ala 123.

3.2.3 Binding Mode of N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide

Figure 3.10 shows docked model of N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide in the Aurora kinases binding sites. N-[(E)-phenyl methylidene]-1H-imidazole-1-carboxamide formulate H-bond with Ala213 of Aurora kinase A by its -O- atom (-O...HN, distance; 2.4 Å). In addition, its hydrophobic interactions were examined with residues Leu139, Val147, Lys162, Leu194 and Glu211. The O atom makes H-bond with Ala157 of Aurora kinase B(-O...HN, distance; 2.4 Å). Furthermore, it showed hydrophobic interactions with Leu83, Val91, Glu155, Glu161.

For Aurora Kinase C, it represented two hydrogen bonds with Ala123 with -O- and -N- atoms (-O...HN, distance; 2.1 Å and N...HN, distance; 3.1 Å) whereas hydrophobic interactions were found with Leu49, Val57, Tyr122, Glu121 and Leu173 residues.

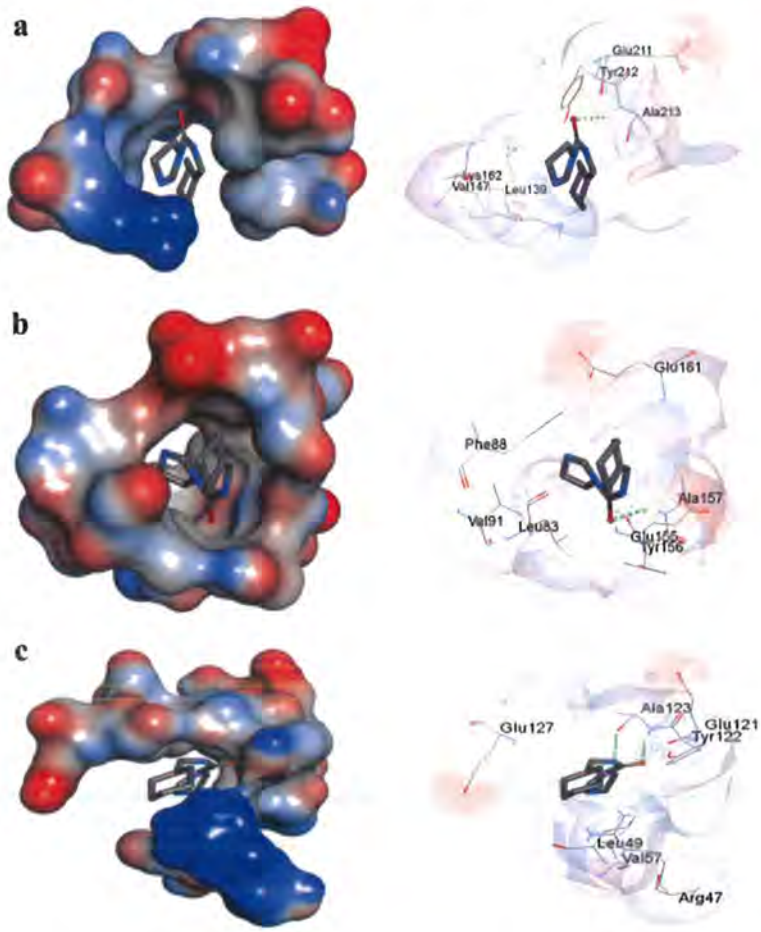


Figure 3.10: N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide interactions (a) Aurora kinase A binding pattern, hydrogen bonding is shown with Ala 213, **(b)** Aurora kinase B binding pattern, hydrogen bonding is shown with Ala 157, **(c)** Aurora kinase C binding pattern, two hydrogen bonds are shown with Ala 123.

3.2.4 Binding Mode of 2-{[(1E)-(2-hydroxyphenyl)methylidene]amino}benzoic acid

Figure 3.11 shows docked model of 2-{[(1E)-(2-hydroxyphenyl)methylidene] amino}benzoic acid in Aurora kinases binding sites. It makes two H-bonds with its two -O- atoms(-O...HN, distance; 2 Å and -O...HN, distance; 2.5 Å) whereas hydrophobic interactions were found with, Ala160, Leu210, Glu211, Pro214,

Gly216, Lys162 and Leu263. The pi-pi interaction was examined with Tyr212. For Aurora Kinase B, this compound showed hydrogen bonding with Ala157 with its -O- atom (-O...HN, distance; 2 Å) and it showed hydrophobic interactions with Leu83, Val91, Gly160, Glu155, Glu161. Two pi-pi interactions were detected with residues Phe88 and Tyr156. For Aurora Kinase C, this compound showed hydrogen bonding with Ala123 with its -O- atom(-O...HN, distance ; 1.8 Å) and it showed hydrophobic interactions with residues Val57, Leu49 Tyr122, Pro124, and Gly126 residues. The pi-pi interaction was found with Arg47.

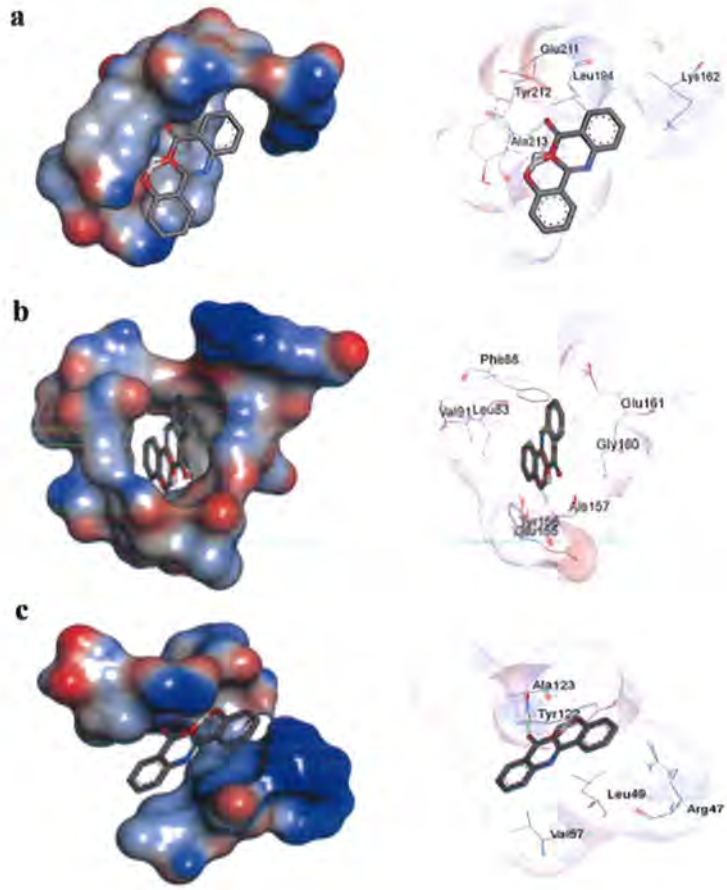


Figure 3.11: 2-{[(1E)-(2-hydroxyphenyl)methylidene]amino}benzoic acid interactions, (a) Aurora kinase A binding pattern, two hydrogen bonds are shown with Ala 213, **(b)** Aurora kinase B binding pattern, hydrogen bonding is shown with Ala 157, **(c)** Aurora kinase C binding pattern, hydrogen bonding is shown with Ala 123.

3.2.5 Binding Mode of 2-(1H-pyrazol-5-yl)-1H-benzimidazole

Figure 3.12 shows docked model of 2-(1H-pyrazol-5-yl)-1H-benzimidazole with Aurora kinases binding sites. 2-(1H-pyrazol-5-yl)-1H-benzimidazole makes two hydrogen bonds with Ala213 with its -N- and -NH- atoms (-N...HN, distance; 2.1 Å and -NH...O, distance; 1.8 Å) and one hydrogen bond with Glu211 with its -NH- atom (-NH...O, distance; 2 Å) whereas it showed hydrophobic interactions with residues Arg137, Leu139, Ala160 and Leu194. For Aurora Kinase B, this compound formulated two bonds with Ala157 with its -N- and -NH- atoms (-N...HN, distance; 2.1 Å -NH...O and distance; 2 Å) and one hydrogen bond with Glu155 with its -NH- atom (-NH...O, distance;1.9 Å) and it showed hydrophobic interactions with Leu83, Ala104, Leu138, Leu207 and Gly160. The pi-pi interaction was detected with Tyr156. For Aurora Kinase C, this compound formulated two hydrogen bonds with Ala123 with its -N- and -NH- atoms (-N...HN, distance; 2 Å and -NH...O, distance; 1.9 Å) and one hydrogen bond with Glu121 with its -NH- atoms (-NH...O, distance; 2.1 Å) whereas hydrophobic interactions were found with residues Glu121, Tyr122 and Gly126 residues. The pi-pi interactions were identified with residue Arg47.

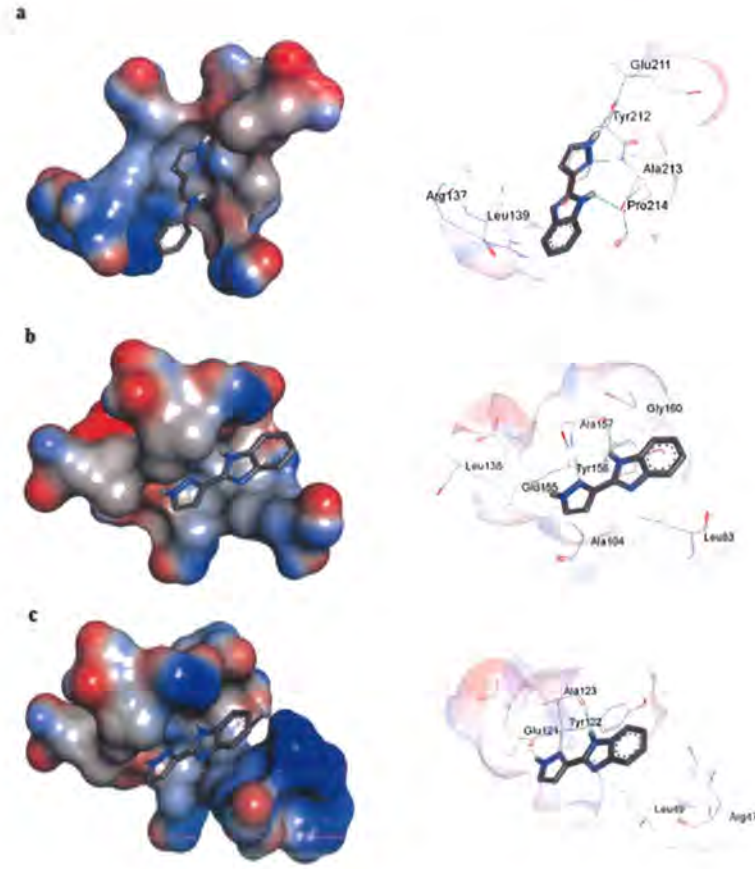


Figure 3.12: 2-(1H-pyrazol-5-yl)-1H-benzimidazole interactions, (a) Aurora A binding pattern, two hydrogen bonds are shown with Ala 213 and one bond is shown with Glu 211, **(b)** Aurora B binding pattern, two hydrogen bonds are shown with Ala 157 and one hydrogen bond is shown with Glu 155, **(c)** Aurora kinase C binding pattern, two hydrogen bonds are shown with Ala 123 and one hydrogen bond is shown with Glu 121.

3.2.6 Evaluation of Pharmacophore model and Analysis

An alignment of four selected compounds was done and ten pharmacophore models are generated using ligand based pharmacophore modeling strategy. Table 3.3 shows score of generated ten pharmacophore models and their corresponding features.

Table 3.3: Scores of ten pharmacophore models along with their features. HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; AR, aromatic ring; HD, hydrophobic region.

Models	Score	HBA	HBD	AR	HD
1	0.6503	+	+	++	+
2	0.6200	+	_	++	+
3	0.6180	+	_	++	+
4	0.6169	++	_	+	+
5	0.6168	++	_	+	+
6	0.6166	+	+	+	+
7	0.6166	+	+	+	+
8	0.6157	++	_	+	_
9	0.6121	++	_	+	_
10	0.6118	++	+	_	+

Among them the first model shows the highest score of 0.6503. For this model five features were identified that includes one hydrogen acceptor, one hydrogen donor, one hydrophobic region and two aromatic rings. This model 1 (Figure 3.13) was used as 3D query to screen the Princeton (*www.princetonbio.com*) and Uorsy (*www.ukrorgsynth.com*) chemical databases consisting of 50,000 and 15,000 structurally diversified molecules, to retrieve new compounds which could be a selective and novel scaffold of Aurora kinase inhibitors.

Figure 3.13: Alignment of 2-(thiophen-2-yl) quinazoline, N-[(E)-phenylmethylidene]-1H-imidazole-1-carboxamide, 2-{[(1E)-(2-hydroxyphenyl)methylidene]amino}benzoic acid and 2-(1H-pyrazol-5-yl)-1H-benzimidazole with pharmacophore model 1, green region shows hydrogen bond donor, red region shows hydrogen bond acceptor.

The specified pharmacophore features for the selected compounds are described in Figure 3.14.

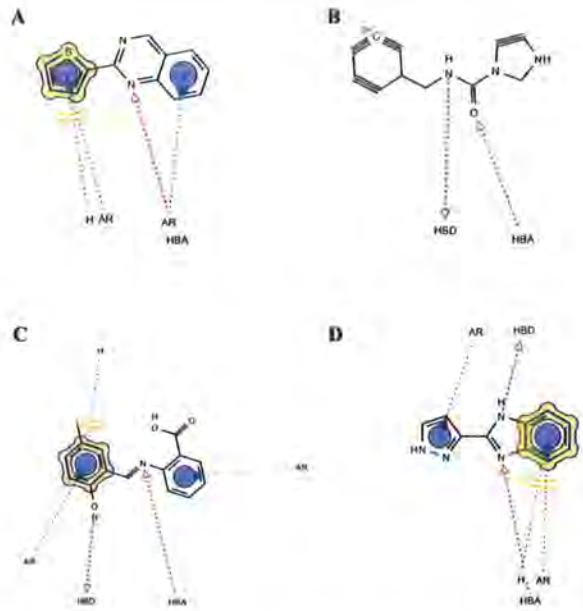


Figure 3.14: 2D Depiction pharmacophore models: (A) compound A **(B)** compound B **(C)** compound C **(D)** compound D. HBA, hydrogen bond acceptor shown by red line; HBD, hydrogen bond donor shown by green line; AR, aromatic ring shown by blue region; HD, hydrophobic shown by yellow region.

In correlation with the sharing features, pharmacophore fit score was calculated for the four compounds. 2D depictions clearly indicate that compounds C and D share all five features of the selected pharmacophore model with score values of 47 and 48, respectively; while compound A shared four features with score of 41 and compound B shared two pharmacophore features having score of 26.

3.2.7 Princeton Results

A library of 50,000 compounds was screened using the generated pharmacophore. 13,982 Compounds were identified that shared the pharmacophore like features. Out of 13,982 compounds, 180 compounds were further extracted on the basis of exact pharmacophore features including one H-acceptor, one H-donor, two rings and one hydrophobic region. These 180 compounds were subsequently used for docking. Receptor and ligand complementarities were checked and 160 compounds were filtered based on these criteria. Later, compounds showing the positive binding energies were eliminated leading to 130 compounds. Interactions were mapped for these compounds with Aurora family members. 30 compounds were short listed based

on frequent interaction with binding sites. 2D structures of these selected compounds are shown in Figure 3.15.

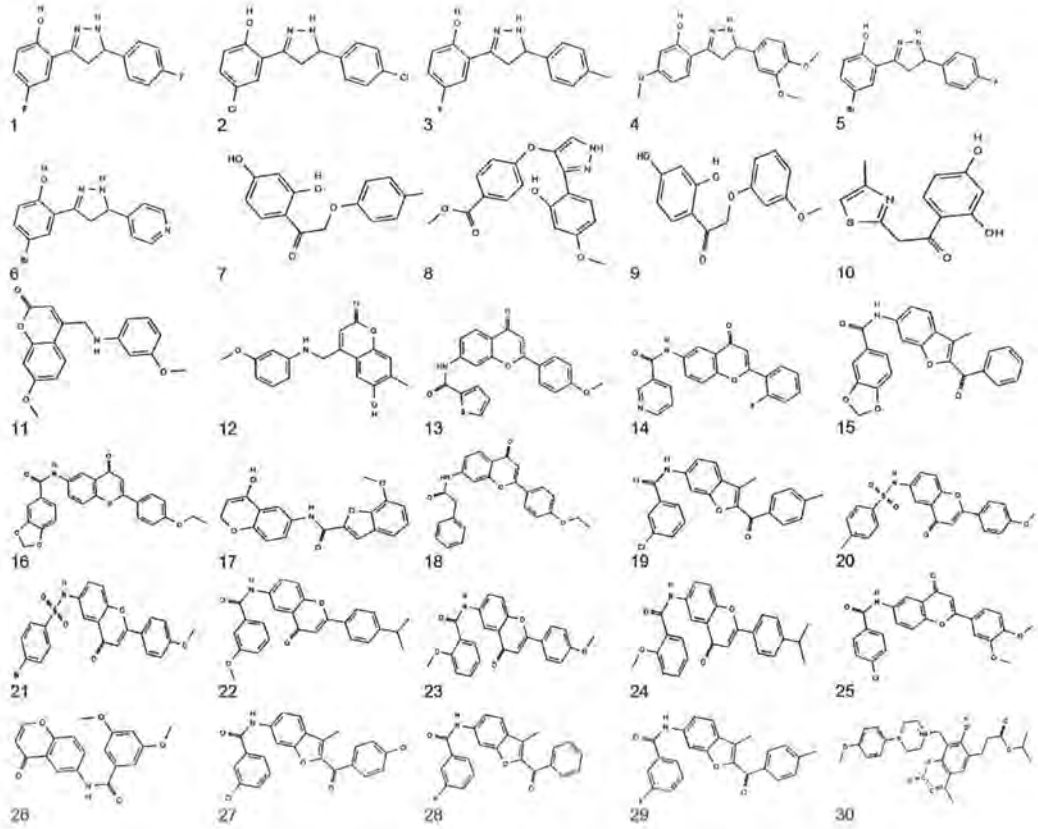


Figure 3.15: 2D structures of 30 compounds extracted from Princeton showing frequent interaction with binding site of Aurora kinases.

Binding, docking and inhibition constant values for these selected compounds is shown in Table 3.4.

Table 3.4: Binding energies, docking energies and inhibition constant values for Aurora kinase A,B and C for Princeton selected compounds.

	Aurora kinase								
	A	B	C	A	B	C	A	B	C
	Binding Energy (Kcal/mol)			Docking Energy (Kcal/mol)			Inhibition Constant (μM)		
1	-8.42	-8.13	-7.48	-9.87	-9.52	-8.75	0.668	1.11	3.28
2	-8.55	-9.33	-8.82	-9.99	-10.35	-9.01	0.538	0.143	0.342
3	-8.21	-8.85	-7.55	-9.35	-10.34	-9.11	0.958	0.324	2.92
4	-7.35	-8.8	-7.77	-9.83	-11.09	-10.54	4.13	0.356	2.03

5	-8.96	-8.47	-8.48	-10.5	-9.77	-9.46	0.269	0.620	0.612
6	-8.18	-10.36	-9.15	-10.18	-12.38	-11.17	1.01	0.25	0.196
7	-6.27	-7.37	-6.31	-8.92	-9.99	-9.12	29.8	3.95	11.28
8	-7.88	-8.78	-9.59	-9.54	-10.37	-10.02	1.67	0.365	0.93
9	-7.08	-7.33	-6.66	-9.25	-9.84	-9.17	6.5	4.23	13.2
10	-6.41	-7.17	-6.16	-7.57	-9.07	-7.78	20.17	5.53	0.30
11	-7.66	-7.95	-8.96	-8.39	-8.67	-9.7	2.42	1.5	0.270
12	-8.06	-8.76	-8.93	-8.29	-9.33	-9.44	1.23	0.381	0.286
13	-9.29	-10.29	-8.88	-10.17	-11.79	-10.34	0.154	0.28	0.307
14	-8.45	-9.04	-9.3	-9.91	-10.59	-10.81	0.639	0.236	0.152
15	-8.74	-9.73	-10.01	-8.97	-9.55	-9.26	0.392	0.73	0.45
16	-8.01	-10.11	-8.8	-10.22	-12.41	-11.15	1.34	0.38	0.355
17	-7.71	-8.78	-7.56	-9.04	-9.96	-8.68	2.22	0.366	2.86
18	-8.3	-10.2	-9.08	-10.59	-12.62	-11.15	0.827	0.33	0.222
19	-9.2	-9.96	-10.18	-10.22	-10.96	-10.9	0.181	0.50	0.34
20	-9.3	-10.96	-9.87	-10.2	-12.4	-11.13	0.15	0.9	0.58
21	-8.21	-9.86	-8.55	-10.33	-12.14	-10.81	0.957	0.59	0.536
22	-9.1	-10.66	-9.09	-11.17	-12.63	-10.05	0.213	0.15	0.216
23	-8.09	-9.12	-8.56	-9.96	-10.84	-10.5	1.17	0.205	0.534
24	-9.04	-10.39	-9.5	-9.94	-11.5	-9.89	0.238	0.24	0.109
25	-7.59	-9.2	-9.07	-10.13	-11.36	-11.49	2.73	0.181	0.223
26	-7.23	-8.64	-7.63	-8.96	-10.37	-9.29	4.99	0.466	2.57
27	-8.92	-9.6	-9.46	-9.11	-9.85	-9.7	0.288	0.92	0.117
28	-8.57	-9.49	-9.35	-8.67	-9.48	-9.07	0.521	0.110	0.139
29	-8.76	-9.68	-10.02	-9.77	-10.58	-10.48	0.381	0.80	0.45
30	-15.3	-16.3	-15.02	-16.1	-16.94	-15.83	$6X10^{-6}$	1.13	0.985

Graphically binding energies are shown in Figure 3.16 and docking energies in Figure 3.17.

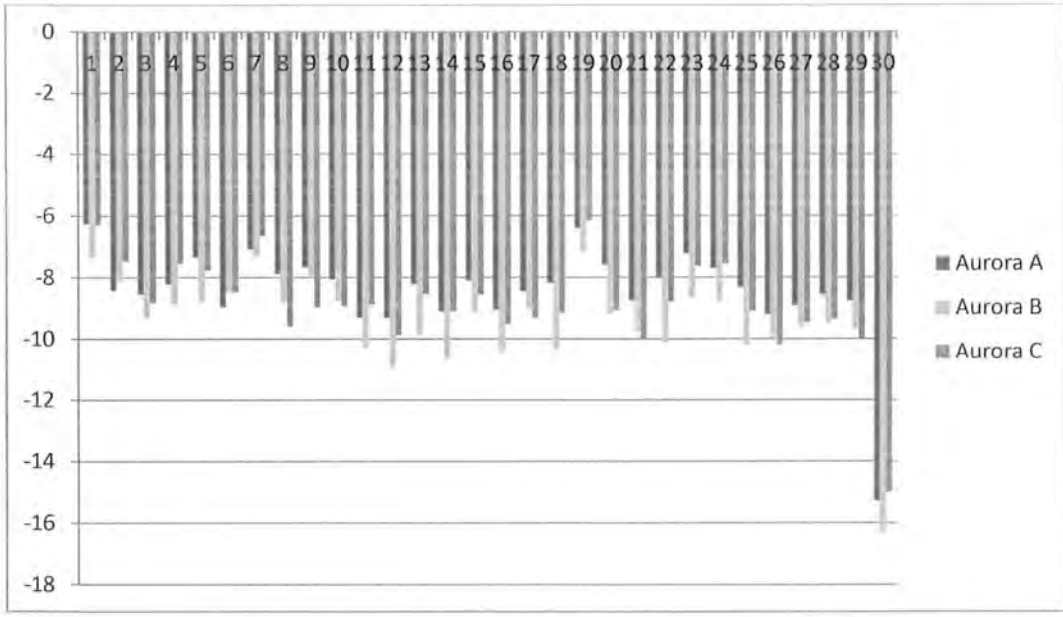


Figure 3.16: Binding Energies for 30 compounds extracted from Princeton database showing frequent interaction with Aurora kinases binding site.

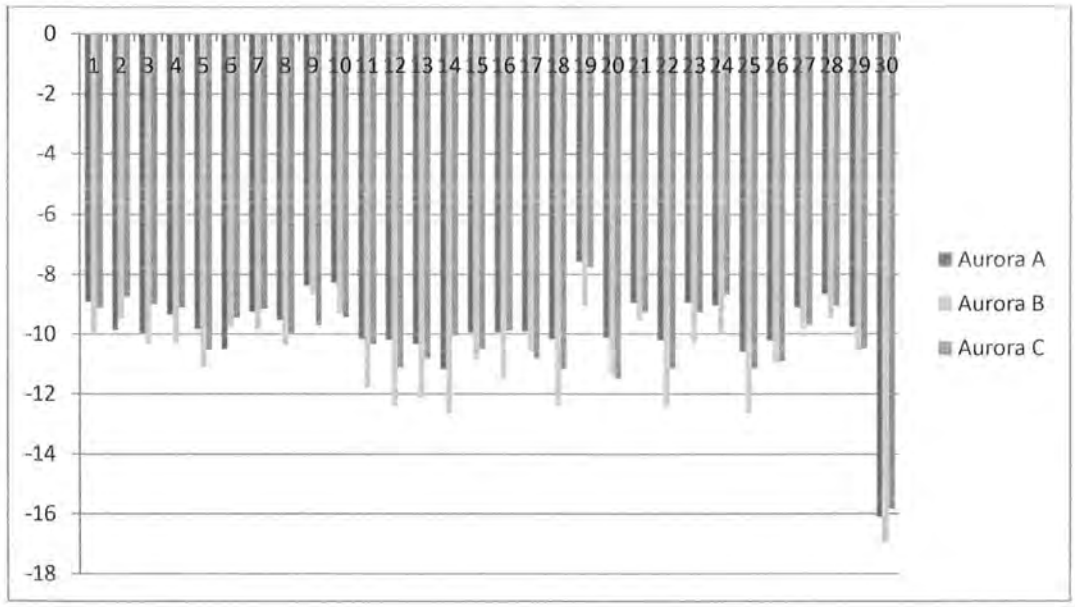


Figure 3.17: Docking Energies for 30 compounds extracted from Princeton database showing frequent interaction with Aurora kinases binding site.

3.2.8 Binding Mode of Princeton extracted compounds

The binding energy values for the Aurora A binding compound derived from Princeton database ranged from -6.41 to -15.3 Kcal/mol, while the docking energies

ranged from -7.57 to -16.1 Kcal/mol. Intermolecular energy values ranged from -7.6 to -16.19 Kcal/mol. Unbound energy values ranged from 0 to -1.05 Kcal/mol. Aurora B binding compounds exhibited binding energies ranging from -7.17 to - 6.3 Kcal/mol. The corresponding docking energies ranged from -8.67 to -16.94 Kcal/mol. Furthermore, intermolecular energies were from -8.37 to -17.9 Kcal/mol. For Aurora C, binding energies ranged from -6.16 to -15.02 Kcal/mol, whereas docking energies ranged from -7.78 to -15.83 Kcal/mol. However, intermolecular energies ranged from -7.35 to -15.91 Kcal/mol. For Princeton selected compounds 1-6 compounds belong to phenol family, 7-8 compounds from benzoate family, 9-10 compounds from ethanone family, 11-12 compounds from chromen-2-one family, 13-19 compounds from carboxamide family, 20-22 compounds from sulfonamide family, 23-29 compounds from benzamide family and compound 30 from propanoate family. All these compounds exhibit frequent interaction with binding sites of Aurora kinases. Among these compounds 30 showed the highest values for binding and docking energies. The interaction patterns of compound 30 with Aurora A, B and C are described in Figure 3.18. For Aurora Kinase A, compound 30 showed hydrogen bonding with Ala213 with its -O- atom (-O...HN, d;2Å), while it formed hydrophobic interactions with Val147, Lys162 Glu211 residues. In addition, 3 pi-pi interactions were detected with Try212, Arg137, Leu139 residues. In contrast, for Aurora B, compound 30 showed hydrogen bonding with Ala157 (-O...HN, distance; 1.8Å) with its -O- atom, hydrophobic interactions with Arg81, Phe88, Val91, Lys106 and Glu155 residues. The pi-pi interaction was found with Try156. In case of Aurora C, compound 30 bonding was with Ala123 with its -O- aom (-O...HN, distance; 2Å) and it formulated hydrophobic interactions with Lys72, Glu121, Gly12 and Leu173 residues. The pi-pi interactions were identified with Arg47 and Leu173.

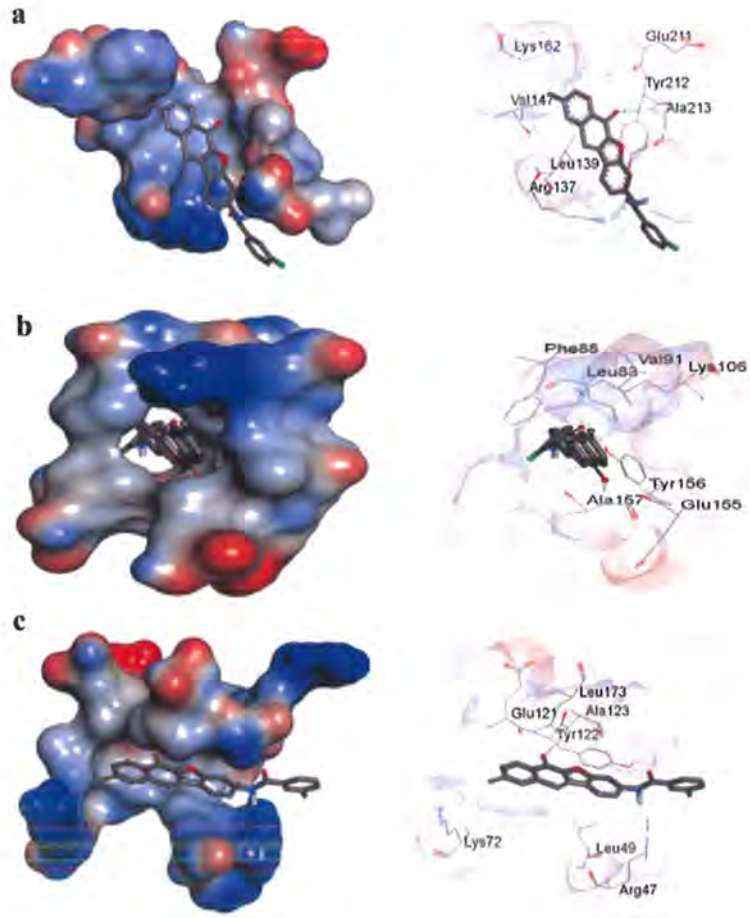


Figure 3.18: Princeton compound 30 mode of interactions. **(a)** Aurora A binding site pattern, it showed hydrogen bonding with Ala 213 **(b)** Aurora B binding site pattern, it showed hydrogen bonding pattern with Ala 157 **(c)** Aurora C binding site interaction showing interaction with Ala 213.

3.2.9 Uorsy database results

A library of 15,000 compounds derived from Uorsy database was screened using the generated pharmacophore hypothesis and 10,330 compounds were short listed based on common pharmacophore like features. Subsequently, 380 compounds having the exact pharmacophore features were selected for docking studies. Furthermore, on the basis of receptor and ligand complementarities, 250 compounds were filtered. Finally, 50 compounds were tested for binding with Aurora kinases and 12 compounds were refined on the basis of binding energy values. Structures of these selected compounds are shown in Figure 3.19. Binding energies, docking energies and inhibition constant for these compounds are shown in Table 3.5.

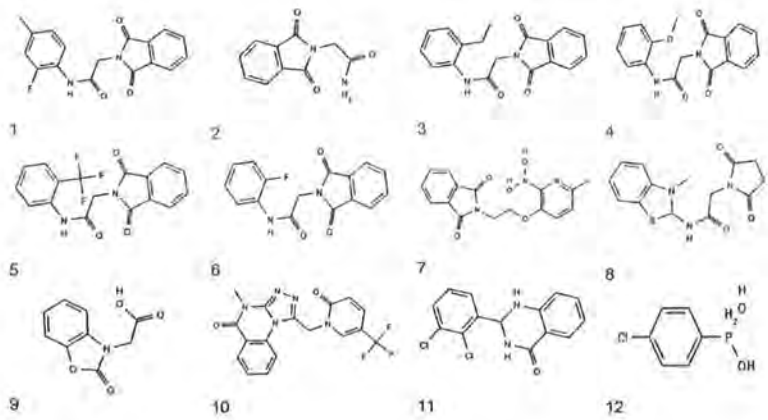


Figure 3.19: 2D structures of 12 compounds extracted from Uorsy showing frequent interaction with binding site of Aurora kinases.

Table 3.5: Binding Energies, docking energies and inhibition constant values for Aurora kinases A, B and C for Uorsy selected compounds.

	Aurora Kinase								
	A	B	C	A	B	C	A	B	C
	Binding Energy (Kcal/mol)			Docking Energy (Kcal/mol)			Inhibition Constant (μM)		
1	-8.87	-9.9	-9.05	-9.46	-10.49	-9.65	0.317	0.055	0.232
2	-7.1	-7.21	-6.46	-8.95	-9.07	-8.3	6.23	5.21	18.42
3	-9.76	-11.45	-10.44	-10.06	-11.75	-10.74	0.07	0.004	0.022
4	-10.37	-11.54	-10.56	-10.67	-11.84	-10.85	0.025	0.004	0.018
5	-9.3	-10.89	-9.83	-9.6	-11.19	-10.13	0. 152	0.01	0.062
6	-9.24	-10.45	-9.58	-9.54	-10.74	-9.88	0.167	0.022	0.094
7	-9.5	-10.79	-9.94	-9.8	-11.09	-10.24	0.109	0.012	0.051
8	-10.02	-10.55	-9.5	-10.02	-10.55	-9.5	0.045	0.019	0.110
9	-6.81	-6.61	-6.03	-7.73	-7.52	-6.95	10.21	14.35	38.08
10	-8.04	-8.55	-8	-8.93	-9.45	-8.89	1.27	0.536	1.37
11	-7.71	-7.33	-6.59	-7.71	-7.33	-6.59	2.25	4.27	14.87
12	-8.77	-9.84	-9.2	-8.77	-9.84	-9.2	0.374	0.061	0.181

Graphically Binding and docking energies are shown in Figure 3.20 and Figure 3.21.

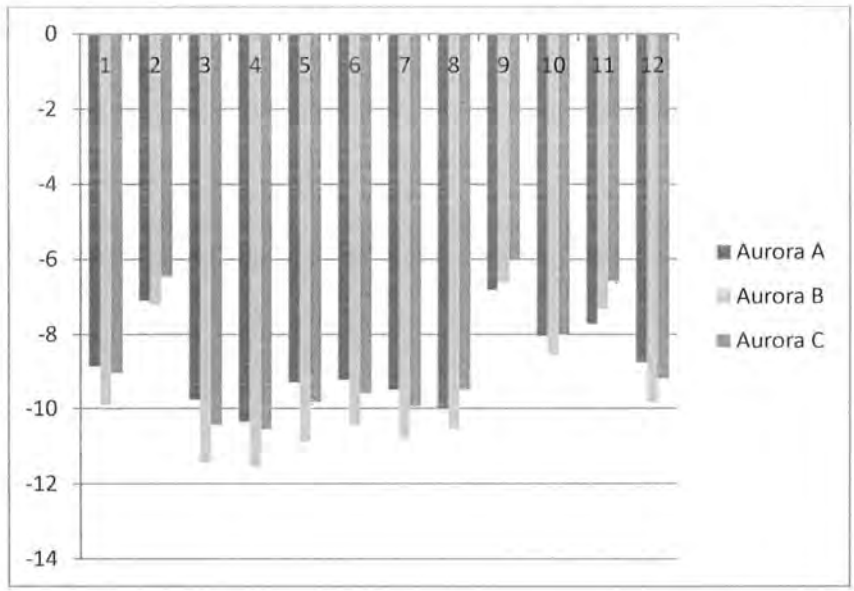


Figure 3.20: Binding energies for 12 Uorsy extracted compounds showing frequent interaction with Aurora kinases binding site.

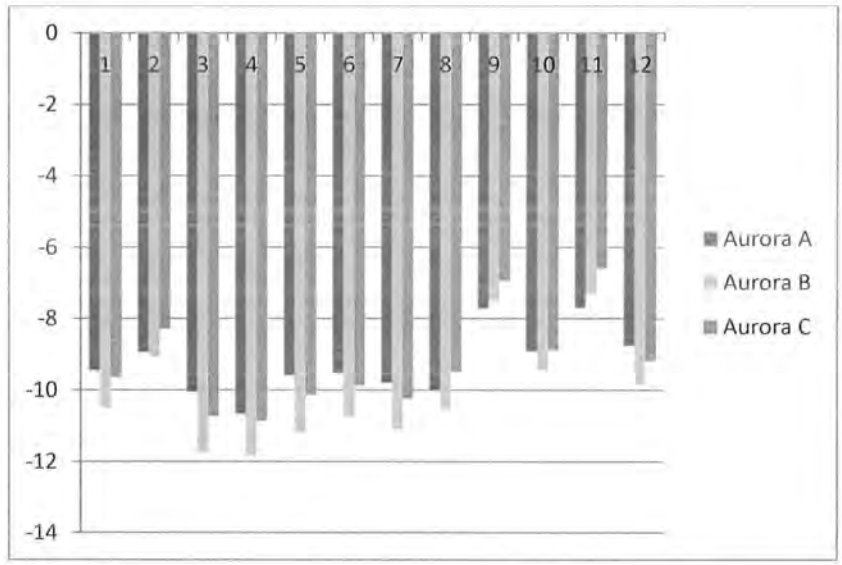


Figure 3.21: Docking Energies for 12 compounds extracted from Uorsy database showing frequent interaction with Aurora kinases binding site.

3.2.10 Binding Mode of Uorsy Screened Compounds

Binding energy values for Uorsy screened compounds involved in Aurora A binding ranged from -6.81 to -10.37 Kcal/mol, while docking energies ranged from -7.71 to -10.67 Kcal/mol. Moreover, intermolecular energies ranged from -7.11 to -10.67

Kcal/mol. In case of Aurora B binding compounds, binding energies ranged from -6.16 to -11.54 Kcal/mol, docking energies ranged from -7.33 to -11.84 Kcal/mol, while intermolecular energies ranged from -6.91 to -11.84 Kcal/mol. For compounds interacting with Aurora C, binding energies ranged from -6.03 to -10.56 Kcal/mol, docking energies ranged from -6.59 to -10.85 Kcal/mol, while intermolecular energies ranged from -6.33 to -10.85 Kcal/mol. For Uorsy screened compounds 1-8 compounds belong to acetamide family, compound 9 belongs to acetic acid family, 9-11 compounds belong to quinazoline family and compound 12 belongs to phosphane family. All these selected compounds show frequent interaction with Aurora kinases binding sites. Compound 4 belongs to acetamide family and shows lowest binding and docking energies among all selected compounds. Its interaction with binding site is analyzed in more detail as shown in Figure 3.22. For Aurora kinase A it makes H-Bond with its -O- atom (-O...HN, distance;1.9Å), hydrophobic interactions with Lys162, Glu211, Val147and Leu139. For Aurora kinase B it makes two hydrogen bonds with its -O- atom with Ala157 and Glu161, i.e., (-O...HN, distance; 2.7Å) , Glu161 (-O...HN, distance; 3.7Å) and one H-Bond with its -H- atom Leu83 (-H...O, distance; 2.7Å), hydrophobic interactions are with Phe88 and Tyr156. For Aurora kinase C it makes H-Bond with Ala123 with its -O- atom (-O...HN, distance; 2.1Å) and hydrophobic interactions with Leu49, Val57, Lys72 and Arg47.

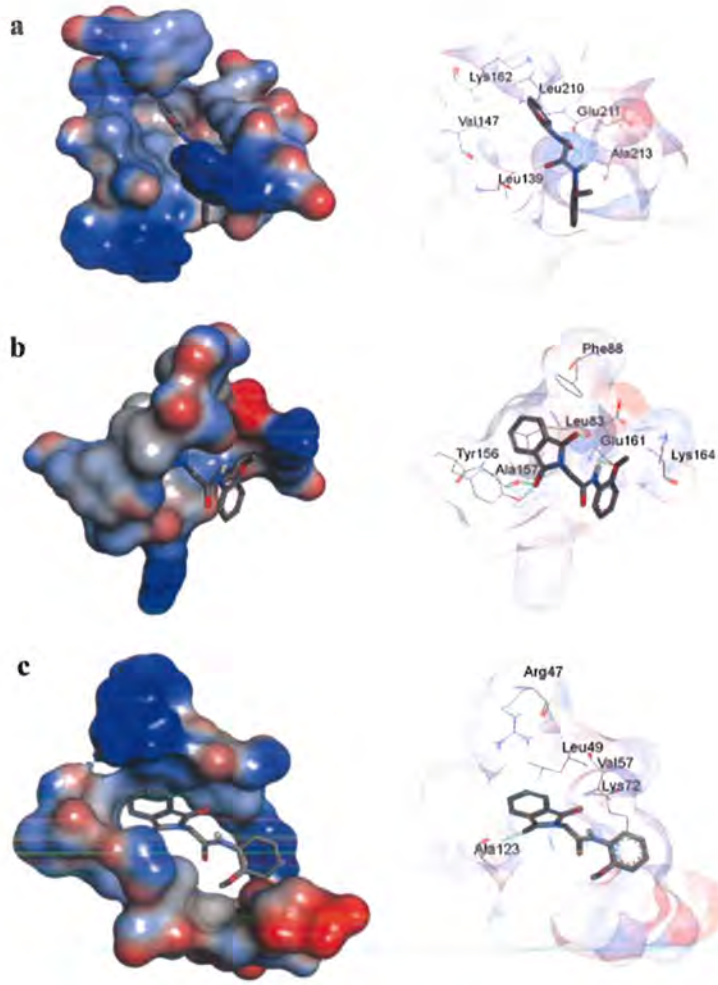


Figure 3.22: Uorsy compound 4 interactions (a) Aurora A binding site pattern, it showed hydrogen bonding with Ala 213 **(b)** Aurora B binding site pattern, it showed hydrogen bonding with Ala 157 and Glu 161 **(c)** Aurora C binding site pattern, it showed hydrogen bonding with Ala 123.

3.3 Docking Studies, Analogs Generation, Pharmacophore Generation and Database Screening for Known Inhibitors Dataset

2D profiles of MLN8237, Hesperadin and 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline compounds is shown in Table 3.6.

Table 3.6: The 2D profiles of A) MLN8237, B) Hesperadin and C) 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline.

Ligand	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Molecular Weight	C Log P	No. of rotatable bonds	Solubility	Drug Score
A	6	2	500	5.558	5	-4.18	0.6
B	6	3	512	-1.2	6	-1.64	0.8
C	7	2	400	2.955	5	-2.88	0.74

3.3.1 Molecular Docking Studies on MLN8237, Hesperadin and 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline

Binding energy, docking energy and inhibition constant for MLN8237, Hesperadin and 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline are shown in Table 3.7.

Table 3.7: Docking energies, binding energies and inhibition constant values for A) MLN8237, B) Hesperadin and C) 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline.

	Aurora Kinase								
	A	B	C	A	B	C	A	B	C
	Binding Energy (Kcal/mol)			Docking Energy (Kcal/mol)			Inhibition Constant (nM)		
A	-8.67	-8.68	-10.02	-10.16	-10.18	-11.51	442.99	431.23	45.01
B	-5.09	-6.02	-9.64	-9.57	-10.5	-14.11	184850	38360	86.19
C	-7.63	-7.83	-7.27	-9.42	-9.62	-9.06	25700	18200	46700

Graphically binding and docking energies of these compounds are shown in Figure 3.23 and Figure 3.24.

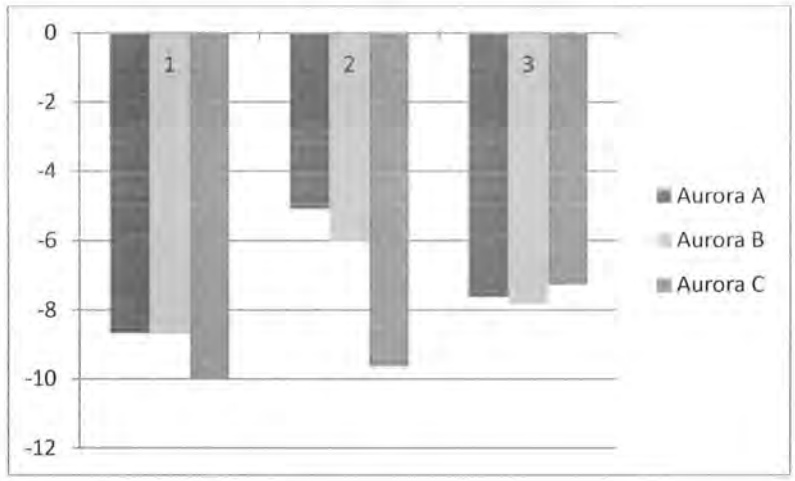


Figure 3.23: Binding energies for A) MLN8237, **B)** Hesperadin and **C)** 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline.

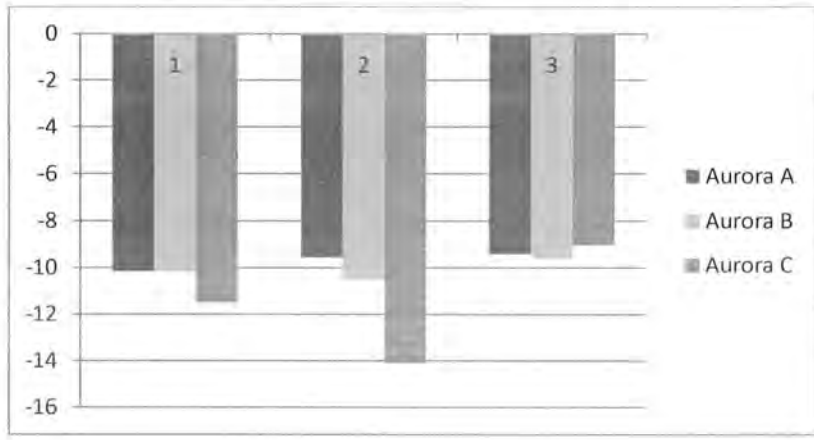


Figure 3.24: Docking Energies for A) MLN8237, **B)** Hesperadin and **C)** 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline.

3.3.2 Analogs Generation for Known Inhibitors

For each inhibitor three R groups were introduced to generate 1000 derivatives generated for each inhibitor by using ten different functional groups for every R group (Figure 3.25). Some functional groups are not compatible with ligand so derivatives are checked for functional group complementarity. Derivatives showing complementarity with selected functional groups were further selected, lipinski rule of five was applied for further filtration process, derivatives obeying rule of five were further selected for docking studies. Derivatives that lacked complementarity with receptor were eliminated. Compounds which showed lower binding and docking

energies than parent analog were further selected. Finally interactions were checked for selected compounds with binding site. Compounds which showed frequent interaction with binding site were short listed.

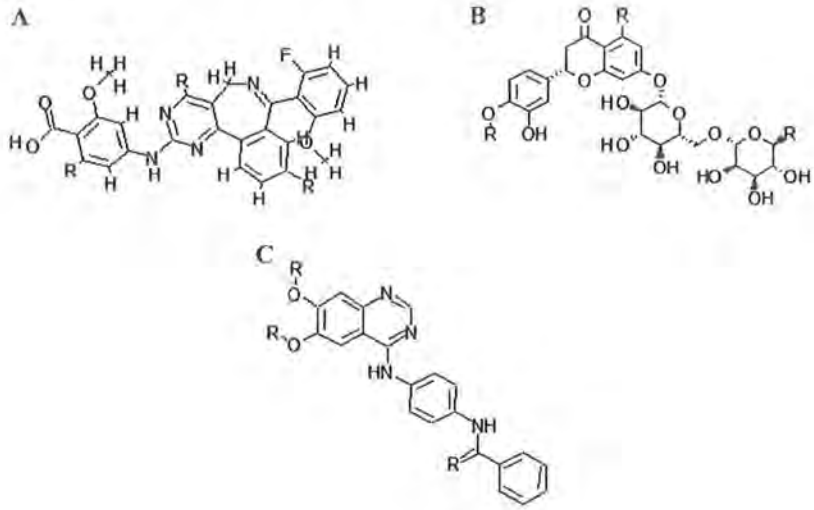


Figure 3.25: R groups introduced in A) MLN8237, **B)** Hesperadin, **C)** 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline.

3.3.3 Binding Mode of MLN8237

In Aurora A MLN8237 showed two H Bond with Ala213 at a distance of 2.1 Å and Glu211 at a distance of 2.1 Å respectively with its -O- atom (-O...HN), hydrophobic interactions with Leu139, Lys162, Thr217, no pi-pi interactions are detected. In Aurora B this compound makes H Bond with Ala157 at a distance of 2.2 Å and Glu125 at a distance of 2.1 Å with its -O- atom (-O...HN) , hydrophobic interactions with Phe88, Glu155, Tyr156 and Glu161, no pi-pi interactios were detected. In Aurora C MLN8237 showed two H Bonds with Ala123 at a distance of 2 Å and 2.3 Å and one H Bond bond with Glu121 at a distance of 3 Å with its -O- atom (-O...HN), hydrophobic interactions with Leu49, Lys72 , two pi-pi interactions were detected with Gly52 and Lys53 residues as shown in Figure 3.26.

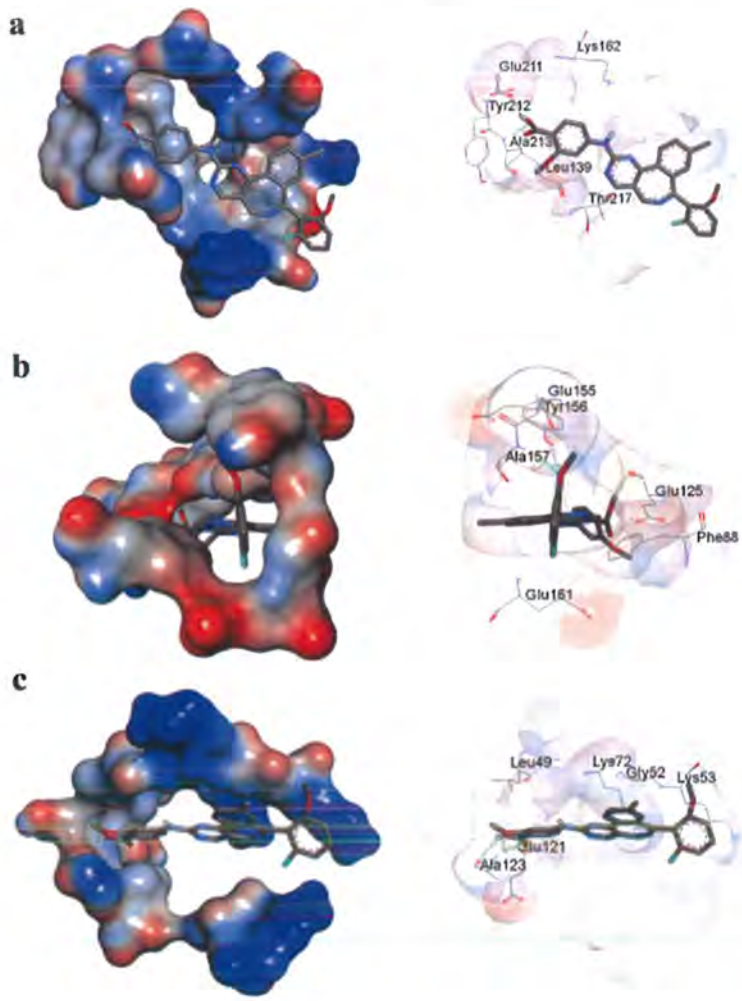
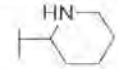
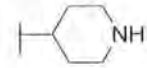
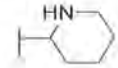
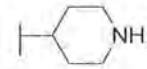


Figure 3.26: MLN8237 interactions a) Aurora A binding site pattern, two hydrogen bonds are shown with Ala 213 and Glu 211 **b)** Aurora B binding site pattern, two hydrogen bonds are shown with Ala 157 and Glu 125 **c)** Aurora C binding site pattern, two hydrogen bonds are shown with Ala 123 and one hydrogen bond is shown with Glu 121.

Derivatives of MLN8237 which shows lower binding and docking energies and frequent interaction with binding site of Aurora kinases are shown in Table 3.8.

Table 3.8: Derivatives of MLN8237.

	R1		R2		R3	
1		Aniline	Br	Bromine		2-Piperidine
2		Aniline	Br	Bromine		4-Piperidine
3		Aniline	Cl	Chlorine		2-Piperidine
4		Aniline	Cl	Chlorine		4-Piperidine
5	Br	Bromine		Hydroxyethyl	Br	Bromine
6	Br	Bromine		Hydroxyethyl	Cl	Chlorine
7	Br	Bromine		Hydroxyethyl		Hydroxyethyl
8	Br	Bromine		Hydroxyethyl		Aminoethyl
9	Br	Bromine		Aminoethyl	Br	Bromine
10	Br	Bromine		Aminoethyl		Methyl

Binding and Docking energies of these derivatives is shown in Table 3.9.

Table 3.9: Binding energies, docking energies and inhibition constant values for MLN8237 Derivatives.

	Aurora Kinase								
	A	B	C	A	B	C	A	B	C
	Binding Energy (Kcal/mol)			Docking Energy (Kcal/mol)			Inhibition Constant (nM)		
1	-14.21	-17.33	-13.91	-20.47	-23.6	-19.36	0.03837	0.000197	0.25236
2	-13.26	-17.25	-13.16	-21.15	-23.52	-19.43	0.0122	0.000226	0.22403
3	-15.31	-17.57	-11.72	-23.92	-23.84	-19.5	0.00599	0.000132	0.19909
4	-14.79	-17.37	-13.23	-21.05	-23.64	-20.05	0.01448	0.000185	0.07893
5	-11.49	-11.69	-13.03	-14.75	-12.88	-15.35	0.11577	2.69	0.04142

6	-13.43	-11.77	-14.16	-14.63	-12.96	-15.28	0.14263	2.34	0.0476
7	-14.04	-13.31	-14.08	-14.04	-13.61	-15.98	0.08415	0.17478	0.00321
8	-13.5	-13.31	-15.68	-13.8	-13.61	-15.91	0.12776	0.17583	0.00357
9	-11.88	-11.37	-15.62	-14.4	-12.57	-14.98	0.2076	4.61	0.0780
10	-13.65	-12.19	-13.69	-14.24	-12.79	-14.87	0.09806	1.15	0.03462

Graphically binding and docking energies for MLN8237 derivatives are shown in Figure 3.27 and Figure 3.28.

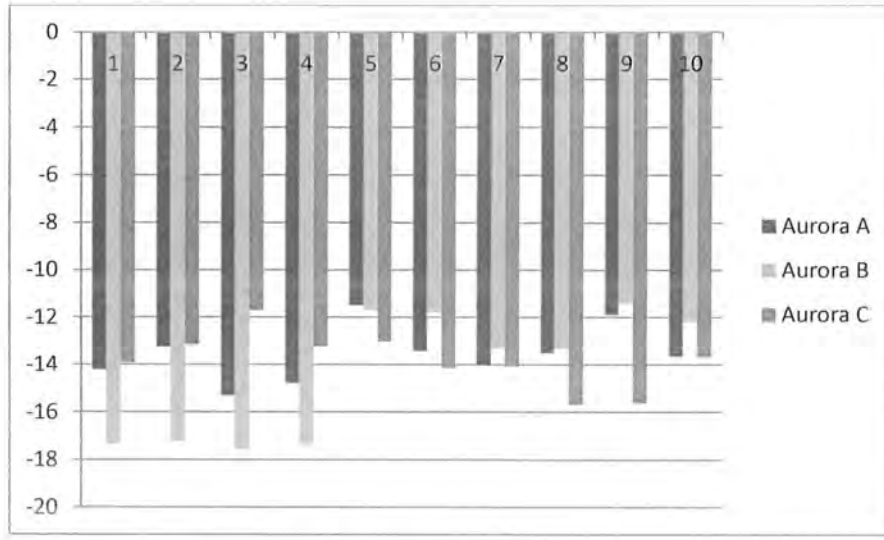


Figure 3.27: Binding Energies for MLN8237 Derivatives.

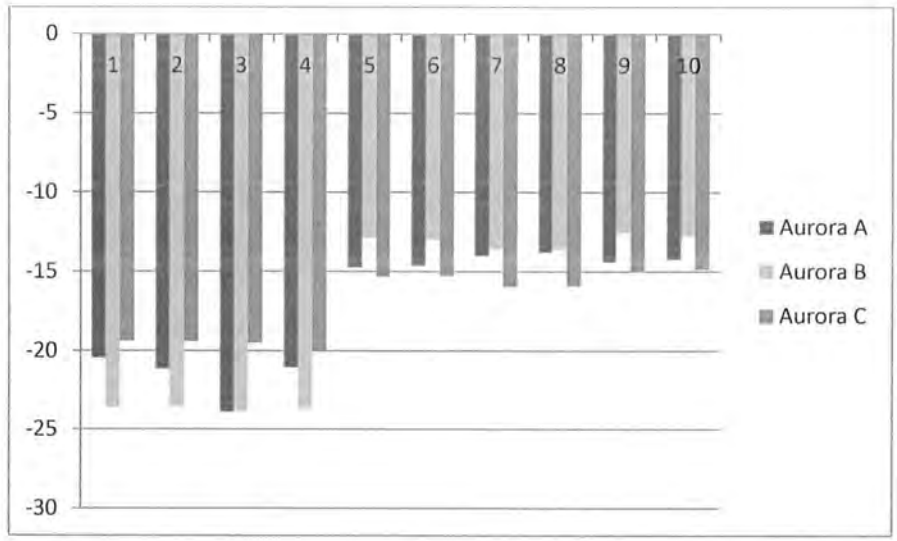


Figure 3.28: Docking Energies for MLN8237 Derivatives.

3.3.4 Binding Mode for Hesperadin

In case of Aurora A Hesperadin makes H Bond with Ala213 at a distance of 2.2 Å with its -O- atom, Glu211 at distance of 2.3 Å with its -O- atom and 2 bonds with Lys141 at a distance of 1.9 Å and 2.4 Å respectively with its -H- atom, it makes hydrophobic interaction with Leu139 and Glu260. In case of Aurora B Hesperadin makes H Bond with Ala157 at a distance of 2.1 Å with its -H- atom, Pro158 at distance of 2.3 Å with its -H- atom, pi-pi interaction Arg176 and hydrophobic interaction with Phe88, Leu83 residues. In case of Aurora C Hesperadin makes H Bond with Ala123 at a distance of 1.8 Å with its -O- atom, Glu127 at distance of 2.2 Å with its -H- atom, Gly52 at a distance of 2.5 Å with its -O- atom, pi-pi interactions with Arg47 and hydrophobic interaction with Glu121, Leu49, Lys72 and Val57 residues as shown Figure 3.29.

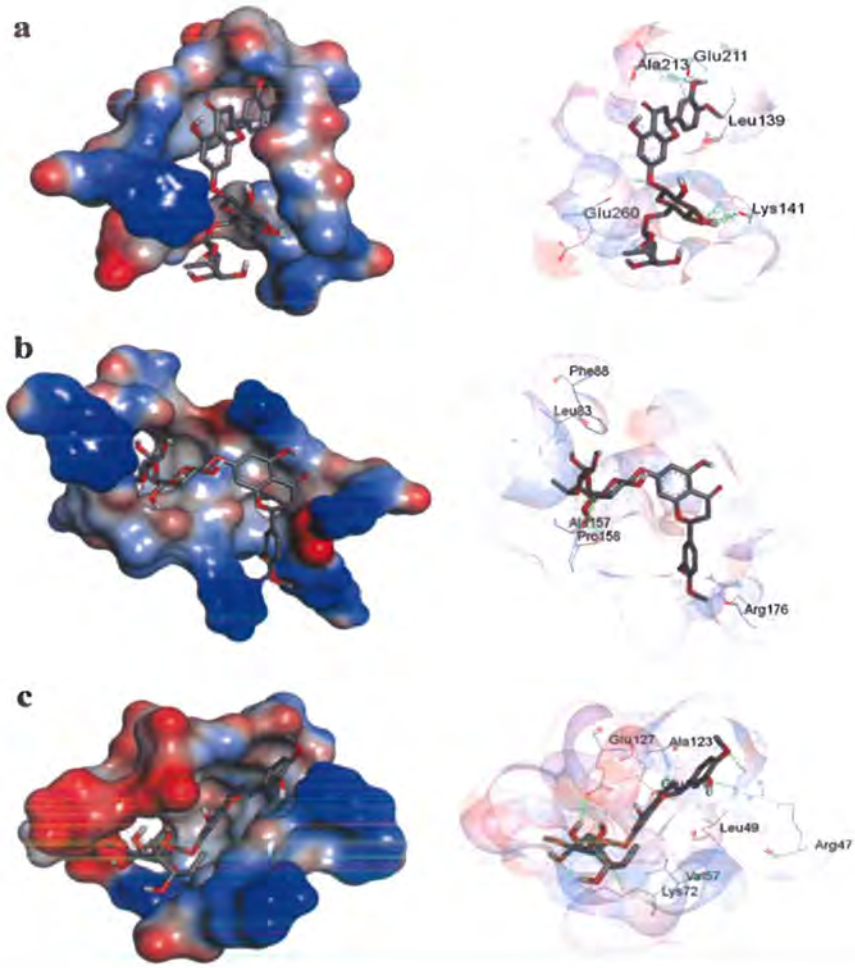
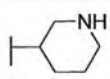
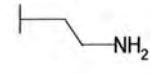
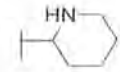
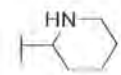
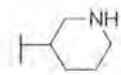
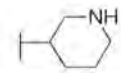


Figure 3.29: Hesperadin interactions a) Aurora A binding site pattern, two hydrogen bonds are shown with Ala 213 and Glu 211, also two hydrogen bonds are shown with Lys 141, **b)** Aurora B binding site pattern, hydrogen bonding is shown with Ala 157 and Pro 158, **c)** Aurora C binding site pattern, hydrogen bonding is shown with Ala 123, Glu 127 and Gly 52. H-Bond is indicated by green dashed line.

Table 3.10: Derivatives of Hesperadin.

	R1		R2		R3	
1		3-Piperidine	H	Hrdrogen		4-Piperidine
2		Methyl	H	Hrdrogen		Aminoethyl

3		Hydroxyethyl		Hydroxyethyl	H	Hrdrogen
4		Hydroxyethyl		Hydroxyethyl		2-Piperidine
5		Hydroxyethyl	H	Hrdrogen	H	Hrdrogen
6		Aminoethyl	H	Hrdrogen		2-Piperidine
7		Aminoethyl	H	Hrdrogen		3-Piperidine
8		Aminoethyl	H	Hrdrogen	H	Hrdrogen
9	H	Hrdrogen		Aniline		3-Piperidine

Derivatives of hesperadin showing lower binding and docking energies then hesperadin and showed frequent interaction with Aurora kinases binding site are shown in Table 3.11.

Table 3.11: Binding energies, docking energies and inhibition constant values for Hesperadin Derivatives

	Aurora Kinase								
	A	B	C	A	B	C	A	B	C
	Binding Energy (Kcal/mol)			Docking Energy (Kcal/mol)			Inhibition Constant (nM)		
1	-21.53	-18.78	-21.17	-24.52	-21.77	-24.15	0.0000002	0.0000057	0.0000003
2	-20.98	-18.21	-21.23	-22.42	-21.19	-23.29	0.0000057	0.000019	0.000001
3	-19.42	-15.98	-20.39	-19.2	-17.77	-21.34	0.000173	0.00194	0.0000047
4	-17.73	-16.17	-19.89	-19.22	-17.96	-21.42	0.000166	0.0014	0.000004
5	-17.91	-16.54	-19.9	-19.18	-17.73	-21.11	0.000065	0.000747	0.0000025
6	-18.61	-16.48	-20.51	-19.82	-18.27	-21.75	0.000061	0.000826	0.000002
7	-18.03	-16.55	-19.96	-19.84	-18.34	-21.79	0.0000022	0.000739	0.0000022
8	-10.62	-16	-10.68	-20.09	-18.38	-22.28	0.0001047	0.00187	0.0000026
9	-17.94	-14.76	-18.86	-20.6	-17.14	-21.24	0.000044	0.01524	0.000015

Graphically binding and docking energies for hesperadin derivatives are shown in Figure 3.30 and Figure 3.31.

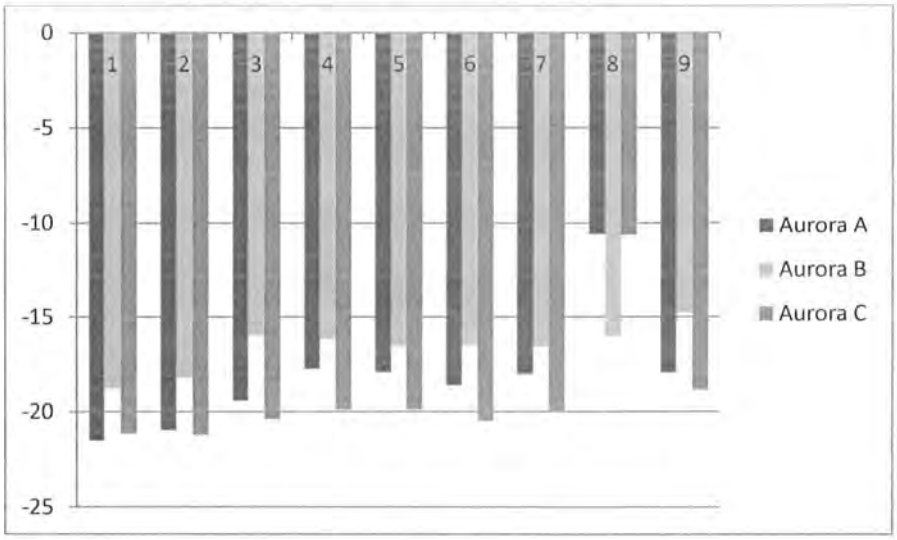


Figure 3.30: Binding Energies for Hesperadin Derivatives.

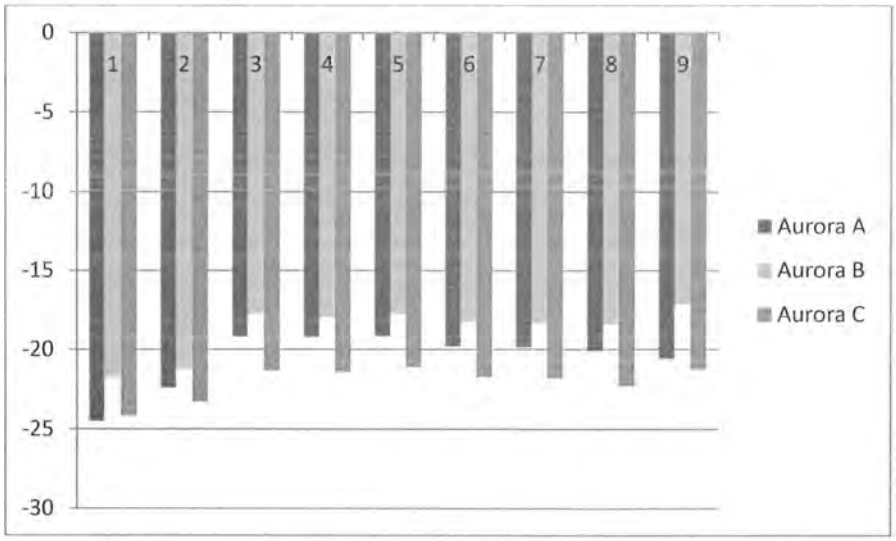


Figure 3.31: Docking Energies for Hesperadin Derivatives.

3.3.5 Binding Mode of 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline

In case of Aurora A 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline makes H Bond with Ala213 at a distance of 3 Å with its -O- atom, pi-pi interactions with Arg137 and Tyr212 and hydrophobic interaction with Val147, Ala160, Leu139, Thr217 residues. In case of Aurora B this compound makes H Bond with Ala157 at a

distance of 3.1 Å with its -O- atom and Glu161 at a distance of 2.5 Å with its -N- atom, pi-pi interaction with Gly160 and hydrophobic interaction with Phe88, Leu83, Val91, Glu155 residues. In case of Aurora C it makes two H Bonds with Ala123 at a distance of 2.2 Å and 3.2 with its -N- atom, pi-pi interactions with Leu49 and hydrophobic interaction with Leu49, Val57, Lys72 and Glu121 residues shown in Figure 3.32.

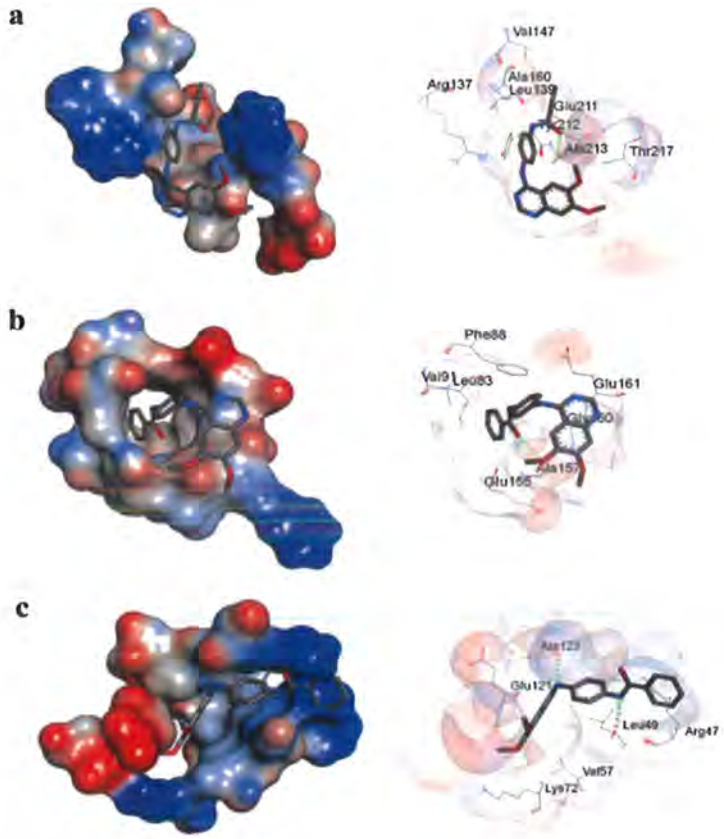
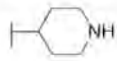


Figure 3.32: 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline interactions a) Aurora A binding site, hydrogen bonding is shown with Ala 213, **b)** Aurora B binding site pattern, hydrogen bonding is shown with Ala 157 and Glu 161, **c)** Aurora C binding site, two hydrogen bonds are shown with Ala 123. H-Bond is indicated by green dashed line.

Table 3.12: Derivatives of 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline

	R1		R2		R3	
1		3-Piperidine	Cl	Chlorine		Methyl
2		4-Piperidine	Cl	Chlorine	Cl	Chlorine
3		Aniline	Cl	Chlorine		Methyl

Derivatives of 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline that show lower binding and docking energies then 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline are shown in Table 3.13.

Table 3.13: Binding energies, docking energies and inhibition constant values for 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline Derivatives.

	Aurora Kinase								
	A	B	C	A	B	C	A	B	C
	Binding Energy (Kcal/mol)			Docking Energy (Kcal/mol)			Inhibition Constant (nM)		
1	-11.64	-10.05	-12.13	-13.43	-11.84	-13.92	2.94	42.75	1.27
2	-11.89	-10.13	-12.29	-13.68	-11.92	-14.08	1.92	37.25	0.9742
3	-14.13	-11.21	-10.56	-15.62	-12.7	-12.05	0.04383	6.06	18.15

Graphically binding and docking energies of 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline derivatives is shown in Figure 3.33 and Figure 3.34.

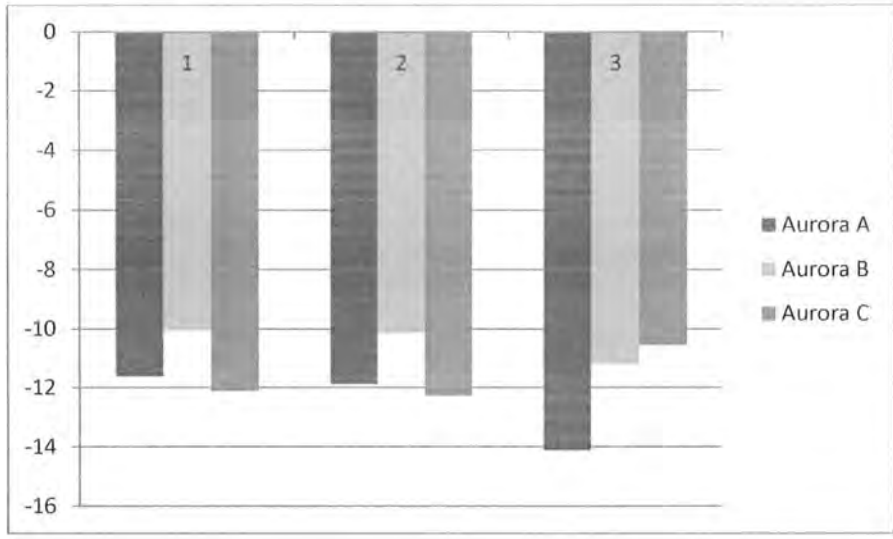


Figure 3.33: Binding Energies for 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline Derivatives.

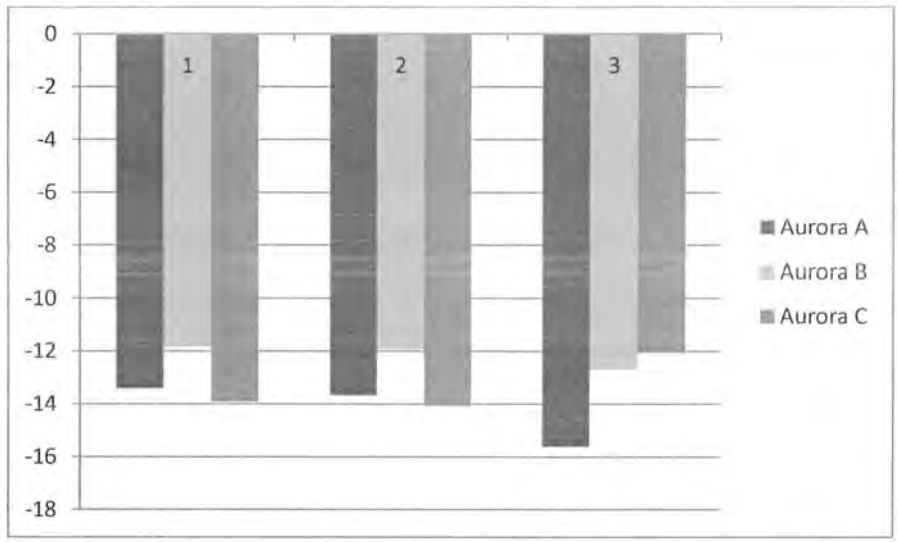


Figure 3.34: Docking Energies for 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline Derivatives.

3.3.6 Evaluation of Pharmacophore model and Analysis

An alignment of three known inhibitors A) MLN8237, B) Hesperadin and C) 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline was carried out and ten pharmacophore models were generated. Table 3.14 shows the score of models along with corresponding features.

Table 3.14: Scores of ten pharmacophore models along with their features. HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; AR, aromatic ring; HD, hydrophobic.

Models	Score	HBA	HBD	AR	HD
1	0.75	+++++++	++	+	-
2	0.73	+++++++	+	-	-
3	0.72	++++++++	+	-	-
4	0.68	+++++	++	-	-
5	0.66	++++++	+	-	-
6	0.65	++++++	+	-	-
7	0.65	+++	++	+	-
8	0.64	+++++	++	-	-
9	0.61	+++++	+	-	-
10	0.60	++++++	+	-	-

Among them the first model shows the highest score of 0.75. For this model ten features were identified which include seven hydrogen acceptors, two hydrogen donors and one aromatic ring. This model 1 was used as 3D query to screen the Uorsy chemical databases consisting of 2,00,000 compounds structurally diversified molecules, to retrieve new compounds which could be a selective and novel scaffold of Aurora kinase inhibitors. Figure 3.35 shows alignment of three inhibitors along with the corresponding features of pharmacophore model.

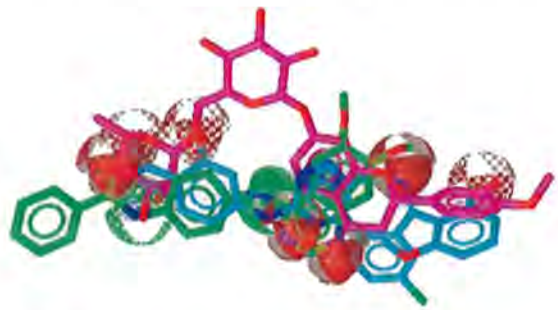


Figure 3.35: Alignment of MLN8237, Hesperadin and 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline with pharmacophore model 1, green region shows hydrogen bond donor, red region shows hydrogen bond acceptor.

2D depiction of these compounds is shown in Figure 3.36.

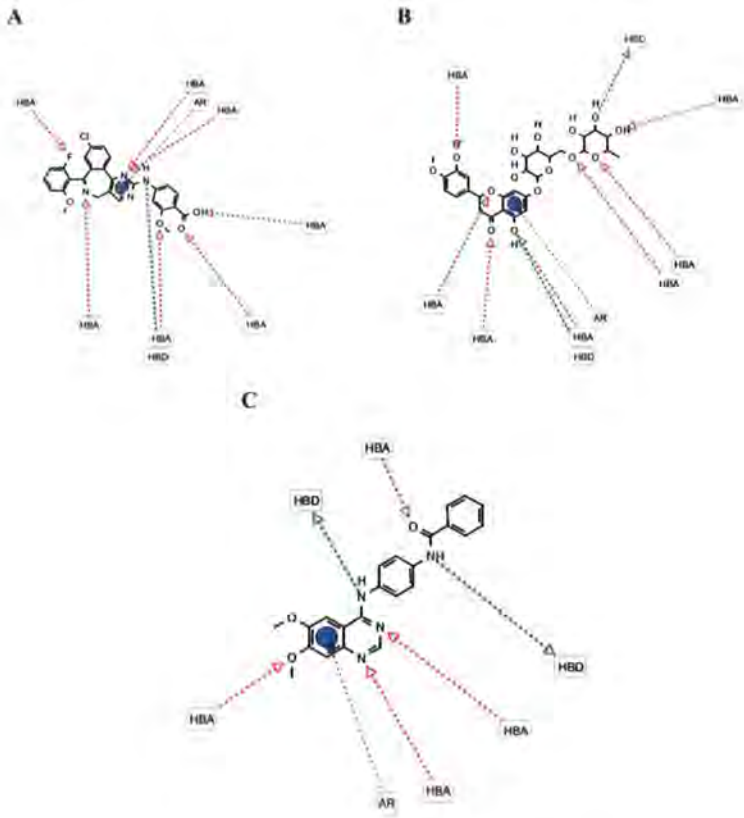


Figure 3.36: 2D Depiction pharmacophore models: (A) MLN8237 **(B)** Hesperadin **(C)** 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline. HBA, hydrogen bond acceptor shown by red line; HBD, hydrogen bond donor shown by green line; AR, aromatic ring shown by blue region.

3.3.7 Uorsy Results

A library of 20,00,00 compounds was screened using the generated pharmacophore. 15000 Compounds were identified that shared the pharmacophore like features. Out of 15000 compounds, 200 compounds were further extracted on the basis of exact pharmacophore features including one aromatic ring, seven hydrogen bond acceptors and two hydrogen bond donors.

These 200 compounds were subsequently used for docking. Receptor and ligand complementarities were checked and 120 compounds were filtered based on these criteria. Later, compounds showing positive binding energies were eliminated leading to 90 compounds. Interactions were mapped for these compounds with Aurora family

members. 10 compounds were short listed based on frequent interaction with binding sites. Structures of these selected compounds are shown in Figure 3.37.

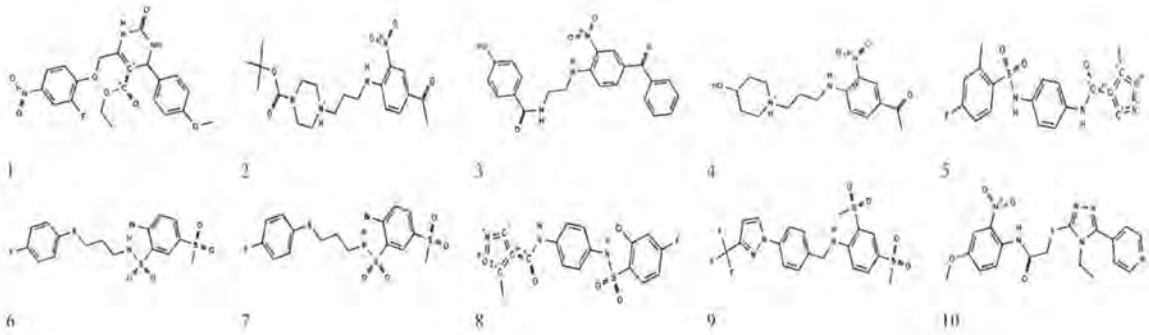


Figure 3.37: 2D structures of Uorsy Extracted Compounds showing frequent interaction with Aurora kinases binding site.

Binding, docking and inhibition constant values for these uorsy extracted compounds is shown in Table 3.15.

Table 3.15: Binding energies, docking energies and inhibition constant values for Uorsy selected compounds.

	Aurora Kinase								
	A	B	C	A	B	C	A	B	C
	Binding Energy (Kcal/mol)			Docking Energy (Kcal/mol)			Inhibition Constant (nM)		
1	-8.73	-8.57	-4.25	-10.52	-10.36	-11.88	397.08	521.69	39.97
2	-5.59	-6.96	-10.09	-7.98	-9.35	-6.64	79440	7900	766020
3	-6.1	-6.29	-6.05	-8.78	-8.97	-8.74	33930	24640	36650
4	-6.55	-6.25	-5.37	-8.94	-8.64	-7.76	15760	26230	115710
5	-10.65	-12.45	-10.29	-10.39	-13.34	-11.18	15.71	0.7490	28.76
6	-11.23	-10.79	-10.66	-12.42	-11.98	-11.86	5.87	12.38	15.3
7	-11.8	-12.59	-12.09	-12.7	-13.49	-12.98	2.23	0.59058	1.38
8	-10.79	-12.32	-10.75	-11.69	-13.21	-11.64	12.22	0.93451	13.24
9	-10.59	-12.15	-10.56	-12.09	-13.64	-12.05	17.14	1.25	18.3
10	-8.79	-8.11	-8.37	-8.79	-8.11	-8.37	7410	23170	14920

Graphically binding and docking energies of uorsy extracted compounds are shown in Figure 3.38 and Figure 3.39.

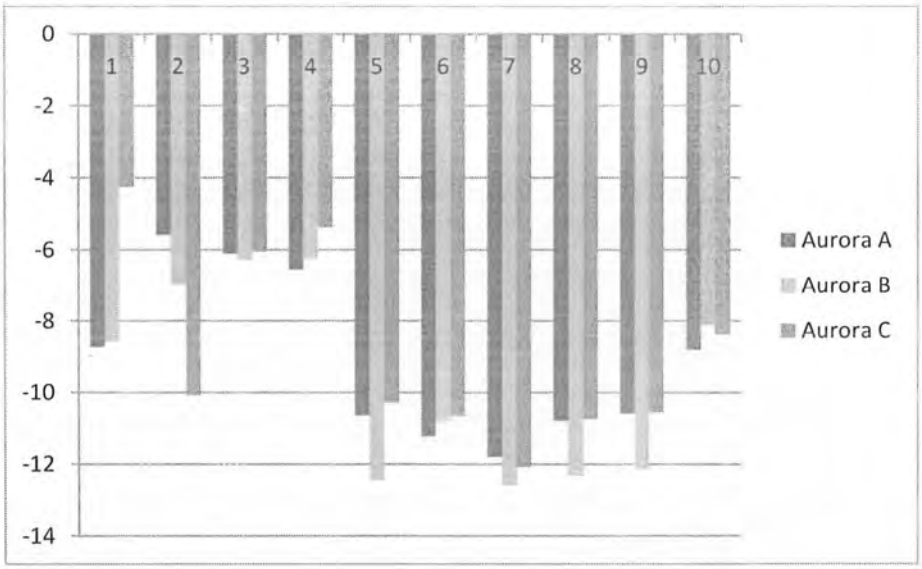


Figure 3.38: Binding Energies for Uorsy Extracted compounds.

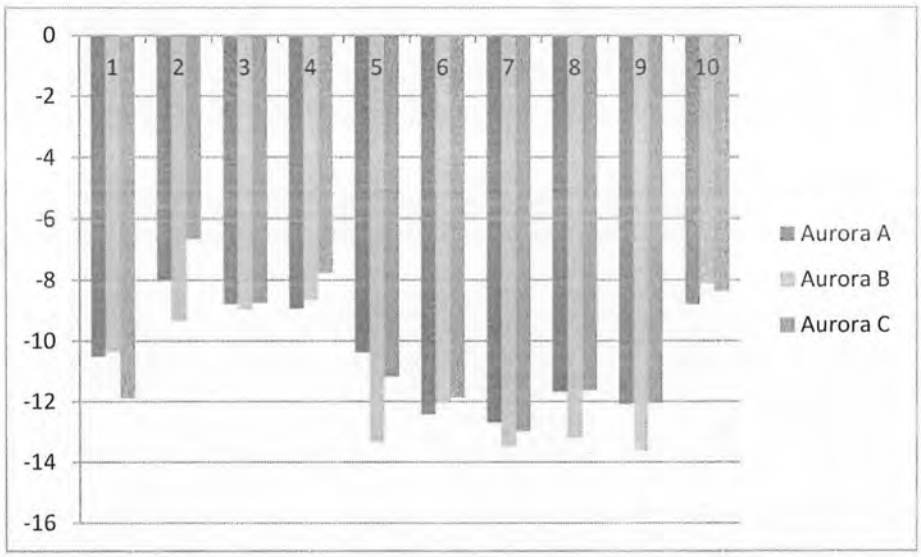


Figure 3.39: Docking Energies for Uorsy extracted compounds.

3.3.8 Binding Mode of Uorsy Extracted Compounds

Binding energy values for Uorsy screened compounds involved in Aurora A binding ranged from -5.59 to -11.8 Kcal/mol, while docking energies ranged from -7.98 to -12.7 Kcal/mol. In case of Aurora B binding compounds, binding energies ranged from -6.25 to -12.59 Kcal/mol, docking energies ranged from -8.64 to -13.49 Kcal/mol. For compounds interacting with Aurora C, binding energies ranged from -4.25 to -12.09

Kcal/mol, docking energies ranged from -11.88 to -12.98 Kcal/mol. Compounds 1-9 belong to carboxamide family and 10th compound belongs to acetamide family.

DISCUSSION

4 Discussion

Recently, structures of known ligands in complex with their receptors have been correctly predicted computationally using the structures of the independent receptor and ligand molecules (Udier-Blagovic *et al.*, 2003). From the standpoint of exploring chemical space, computational screens of chemical databases have identified new ligands for over 50 receptors of known or even, in some cases, computer-modelled structures (Schapira *et al.*, 2003). The use of virtual screening to discover new inhibitors is becoming a common practice in modern drug discovery (Shoichet, 2004). Until now, development of a large number of drugs was based on structure-based design and screening strategies (Kitchen *et al.*, 2004). In this context, recent identification of novel binding partners and key downstream effectors, together with small molecule inhibitors that display efficacy against tumours, heralds an upsurge of interest in Aurora kinases (Weaver and Cleveland, 2005). Aurora kinases are important regulators of diverse cell cycle events, ranging from entry into mitosis, centrosome function, mitotic spindle formation, chromosome biorientation and segregation and cytokinesis.

In this study, we explored novel inhibitors for Aurora Kinases using computational approaches. In total, two datasets were used in this study. The first dataset was prepared from Aurora kinase focused library taken from Enamine database and 2D profiles were generated. Compounds that did not fulfill Lipinski rule of five criteria had been eliminated whereas remaining compounds were screened against Aurora A, B and C. Lipinski's Rule of five is drawn from the statistical study of existing drugs and focuses on the "drug-likeness" of small molecules (Lipinski *et al.*, 2001). Subsequently, each compound was docked into the defined grid representation. Binding and docking energies for these compounds were computed and monitered. The compounds with positive binding and docking energies were eliminated as more negative energy score (kcal/mol) corresponds to increased binding affinity (Gilson and Zhou, 2007). Later, binding site interactions were mapped for all Aurora kinases. The compounds showing frequent interactions with binding site were further selected and classified. These compounds belonged to different families including quinozaline, benzoic acid, carboximide and benzimide. Binding modes of these compounds were studied in detail which indicated that four compounds fulfilled the criteria of binding,

docking and inhibition constant. These four compounds were then subjected to pharmacophore modeling using Ligandscout tool (Wolber and Langer, 2005). Altogether, ten pharmacophore models were generated. Among them, the first pharmacophore model showed the highest score. Its features included one hydrogen acceptor, one hydrogen donor, one hydrophobic region and two aromatic rings and this model was used as a reference to screen libraries isolated from Princeton and Uorsy databases. Virtual screening of these libraries was performed and compounds showing pharmacophore like features were selected and subjected to docking studies against Aurora kinase proteins. Finally, representative molecules on the top hits list were selected and further analyzed for their interactions with target proteins. The binding analysis indicated that these molecules could be potential drugs for Aurora kinases.

Family analysis for selected compounds resulted in presence of multiple families including phenol, benzoate, ethanone, chromen-2-one, carboxamide, sulfonamide, benzamide, propanoate, acetamide, acetic acid, quinazoline and phosphane families respectively. Table 4.1 shows reported inhibitors for Aurora kinases along with their family origin.

Table 4.1: Reported Aurora kinase inhibitors.

Compounds	Name	Origin	Reference
1	AZD1152	Acetamide	(Tsuboi *et al.*, 2011)
2	VX680	Carboxamide	(Fiskus *et al.*, 2010)
3	MLN8237	Benzoic acid	(Maris *et al.*, 2010)
4	PHA680632	Carboxamide	(Soncini *et al.*, 2006)
5	Hesperadin	Sulfonamide	(Jetton *et al.*, 2009)
6	ZM447439	Benzimide	(Girdler *et al.*, 2008)
7	CCT129202	Acetamide	(Chan *et al.*, 2007)
8	AT9283	Urea	(Dawson *et al.*, 2010)
9	SNS314	Urea	(Arbitrario *et al.*, 2010)
10	ENMD2076	Amine	(Diamond *et al.*, 2011)
11	PF03814735	Acetamide	(Jani *et al.*, 2010)
12	CYC116	Amine	(Wang *et al.*, 2010)
13	K00590	Quinazoline	(*www.pubchem.com*)

To our knowledge, this study is the first one reporting compounds belonging to phenol, benzoate, ethanone, chromen-2-one, acetic acid, propanoate and phosphane families could contribute as inhibitors for Aurora kinases. Involvement of carboxamide, sulfonamide, benzamide, acetamide and quinazoline families as Aurora kinase inhibitors is already known (Table 4.1).

Next, second dataset was prepared from known inhibitors of Aurora kinases which included MLN8237; a reported inhibitor for Aurora kinase A, Hesperadin a reported inhibitor for Aurora kinase B and 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline which is a reported inhibitor for both Aurora kinase A and B. Docking analysis of these compounds was carried out to validate the bindings of these compounds with all three members of Aurora proteins. Docking studies showed that MLN8237, hesperadin and 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline bounds at specific binding sites of all three Aurora proteins. We propose that MLN8237 could be used as an inhibitor against Aurora B and Aurora C, hesperadin could be used as inhibitor for Aurora A and C while 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline could be used as inhibitor for Aurora C.

Derivatives for these known inhibitors were also generated and filterted using specific criteria and selected derivatives were docked for monitoring their interactions. The binding and docking energy values of the ligand molecules were observed and compared to pre-existing inhibitor molecules MLN8237, Hesperadin and 4-(4'-Benzamidoanilino)-6,7-dimethoxyquinazoline. These comparisons indicated that higher binding affinity of designed analogues to the receptor is present than the original. The derivatives showing noticable interactions with predicted binding site were picked. The 3D pharmacophore model was generated using known inhibitors dataset. Ten pharmacophore models were generated for this dataset also and among them the first one showed highest score and included features such as seven hydrogen acceptors, two hydrogen donors and one aromatic ring. A library from Uorsy database was taken and screened using this pharmacophore model. Virtual screening of library was carried out and compounds showing pharmacophore like features were short listed which were further used for docking in order to check their binding affinities with Aurora proteins. The compounds having interactions with binding site residues were selected and analyzed.

In this study, a two-round screening schema was adopted in order to get a balance between time, cost and accuracy. In the first round, ligand based drug designing approach was used to shortlist the compounds according to pharmacophore like features and during the second phase, structure based drug designing approach was used to identify the compounds that bind to the drug targets efficiently. Currently, computer aided drug design approaches mainly focus on structure based properties of a drug target and its possible binder to design a chemical that could bind the target efficiently. Essentially, they are based on the "lock and key" principle proposed by Fisher more than 100 years ago (Pyrkov *et al.*, 2010).

Conclusively, our results proved the importance of *in silico* strategies for the screening of ligands that can bind to macromolecules with both available and unavailable crystallographic structure. Several novel lead compounds were identified using virtual screening and molecular docking against Aurora kinase proteins. The principal goal in the development of Aurora kinase inhibitors is to assess whether the administration of these small molecules to patients will yield a clinical benefit. We want to propose inhibitors that have minimum toxicity, fewer side effects and are highly specific. In future, subjecting these molecules into *in vitro* studies may lead to develop a potent lead for the inhibition of Aurora kinases.

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