Int J Pharm Bio Sci 2014 Jan; 5(1): (P) 714 - 725

**Review Article** **Bioinformatics**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# DRUG DESIGN EFFORTS TOWARD AURORA KINASE INHIBITORS (REVIEW)

**C.N. DIPKE*[1] AND RENU VYAS[2]**

*[1]Department of Bioinformatics and Computer Science, Dr.D.Y.Patil Biotechnology and Bioinformatics Institute, Tathawade, Pune-411033.*
*[2]Chemical Engineering Division National Chemical Laboratory, Pune -411008*

## ABSTRACT

In the recent years Aurora kinases have attracted increasing attention as serine/threonine kinases with various roles in cell division, including chromosomal agglutination and segration functions of centromeres, centrosomal maturation, spindle formation and cytokinesis. Overexpressed aurora kinases are recently studied and have shown their involvement in oncogenesis and aberrant increase in centrosome number emergence of polykaryocytes and failures of cancer inhibition mechanisms. Several aurora kinase inhibitions have been studied in vitro and in vivo. A number of new aurora kinase inhibitors are being development of numerous aurora kinase inhibitors is likely to increase the number of selectable drugs during treatment. This review showed that different methodology toward the development of aurora kinase inhibitors.

**KEYWORDS: Serine/threonine, Spindle defects, INCENP and Virtual screening.**

**C.N. DIPKE**
**Department of Bioinformatics and Computer Science,**
**Dr.D.Y.Patil Biotechnology and Bioinformatics Institute, Tathawade, Pune-411033.**

*corresponding author

# INTRODUCTION

Aurora kinases are of great interest for cancer researchers. Aurora kinases are a recently discovered family of serine/threonine protein kinases that regulate many processes during cell division. Aurora family kinases play roles in several mitotic processes during cell division. Aurora kinases are involved in the control of the centrosome and nuclear cycles and playing essential functions in mitotic processes such as chromosome condensation, mitotic spindle organization and cytokinasis[1]. The aurora kinases are importantly involved in cell cycle and they exhibit their functions in different stages of cell division. They are involved in some checkpoint regulation pathways including spindle assembly checkpoint, alignment of metaphase chromosome and chromosomal biorientation[2]. The structure of these enzymes has been well conserved throughout evaluation. The first aurora kinase was discovered in *Saccharomyces cerevisiae*, *Drosophila, Caenorhabditis elegans and Xenopus*[3-8]. In human three aurora kinases have been identified: Aurora-A, Aurora-B and Aurora-C. Three mammalian Aurora paralogues are very similar in sequence, in particular within the carboxy terminal catalytic domain, in which human Aurora-A and Aurora - B share 71% identity. Aurora kinases were recently identified as a potential target in anticancer therapy and various Aurora – A and Aurora - B kinase inhibitors are in development. Aurora-A and Aurora-B are expressed in most normal cells, although their localization and the timing of activation during the cell cycle differ. Any abnormal change in the genetic pathways leads to cell transformation and tumorigenesis.

### *Aurora-A*

The Aurora-A also known as AURKA; ALK; AURA; BTAK; STK6; STK15; AURORA2; PPP1R47 found within the region of chromosome 20q13. The protein encoded by this gene is a cell cycle-regulated kinase that appears to be involved in microtubule formation and /or stabilization at the spindle pole during the chromosome segregation. The encoded protein is found at the spindle poles in mitosis.

**Table 1**
***Gene symbol report AURKA.***

| | |
|---|---|
| **Approved Symbol** | AURKA |
| **Approved Name** | aurora kinase A |
| **HGNC ID** | HGNC:11393 |
| **Previous Symbols & Names** | " serine/threonine kinase 6", "serine/threonine kinase 15", STK6, STK15 |
| **Synonyms** | ARK1, AurA, BTAK, PPP1R47, "protein phosphatase 1, regulatory subunit 47", STK7 |
| **Locus Type** | gene with protein product |
| **Chromosomal Location** | 20q13 |

***Note: data source (www.genename.org).***

The aurora kinases were first identified in 1990 during a cDNA screen of Xenopus. The kinase activity is involved in centrosome separation and maturation, as well bipolar spindle assembly and stability. Thus it's over expression leads to several spindle defects [9].

### *Aurora B*

The Aurora-B is also known as AURKB, AIM-1, ARK2, “Aurora-B”, “Aurora-1”, STK5. The Aurora kinase-B gene is located at the chromosome 17p13.1. Human Aurora-B was first identified in a polymerase chain reaction screens for kineses that were overexpressed in cancer[10]. Aurora-B is a chromosomal passenger protein that is enriched on the chromosome kinetochores from prophase to metaphase, in the midzone during anaphase and in post-mitotic bridge during telophase[11].

**Table 2**
***Gene symbol report AURKB.***

| | |
|---|---|
| **Approved Symbol** | AURKB |
| **Approved Name** | aurora kinase B |
| **HGNC ID** | HGNC:11390 |
| **Previous Symbols & Names** | "serine/threonine kinase 12", STK12 |
| **Synonyms** | Aik2, AIM-1, ARK2, AurB, "aurora-1", "aurora-B", IPL1, PPP1R48, "protein phosphatase 1, regulatory subunit 48", STK5 |
| **Locus Type** | gene with protein product |
| **Chromosomal Location** | 17p13.1 |

***Note: data source (www.genename.org).***

During mitosis, Aurora b plays a critical role in chromosome attachment and biorientation, indicated by the fact that hesperadin, a known inhibitor of Aurora-B, and increased the incidence of mal-oriented chromosome[12]. Aurora-B has three distinct but related functions; it is a histone kinase involved in phosphorylation of chromatin proteins i.e. histone H3, a spindle checkpoint kinase and a cytokinase[13]. In mammalian cells Aurora B is part of a chromosome passenger complex also containing inner centromere protein (INCENP), Borealin and Survivin.

### *Aurora C*

The aurora kinase-c is also known as AIE2; AIK3; AurC; aurora-C; STK13. The Aurora-C gene lies within a region of chromosome 19q13.43 and was only dedicated in testis and not in somatic cell[14-15]. The Aurora kinase C was first through to be involved in meiotic spindle formation[16]. The encoded protein is a chromosomal passenger protein that forms complexes with Aurora-B and inner centromere protein and may play a role in organizing microtubules in relation to centrosome/spindle function during mitosis. This gene is overexpressed in several cancer cell lines suggesting an involvement in oncogenic signal transduction. However, it is reported to highly expressed in cancer cell such as HepG2, HuH7, MAD-MD-453 and HeLa cells[17].

**Table 3**
***Gene symbol report AURKC.***

| | |
|---|---|
| **Approved Symbol** | AURKC |
| **Approved Name** | aurora kinase C |
| **HGNC ID** | HGNC:11391 |
| **Previous Symbols & Names** | "serine/threonine kinase 13 (aurora/IPL1-like)", STK13 |
| **Synonyms** | ARK3, AurC |
| **Locus Type** | gene with protein product |
| **Chromosomal Location** | 19q13.3-qter |

***Note: data source (www.genename.org).***

### *Aurora Kinase and cancer*

The aurora kinases play a critical role in tumorigensis it might be of great importance to evaluate aurora kinases in cancer detection. Aurora kinase family have performed important functions in cycle of mitosis and hence any disorder happened in expression can lead to the cell transformation into cancer. In many tissues like, Breast cancer, Human Gliomas, ovarian, lung cancer, thyroid cancer[18-21]. Aurora kinase over-expression leads to genetic instability (aneuploidy), which may causes cancer. Aneuploidy is a condition which the cells are altered. DNA content may arise from mitotic defects including centrosome duplication, cytokinesis and chromosomal bi-orientation errors. In all these processes aurora kinases are involved. Several aurora kinase inhibitors have been studied *in vitro* and *in vivo*.

**Table 4**

***shows the $IC_{50}$ values for several Aurora kinase inhibitors (different small molecules)***

| Inhibitors | $IC_{50}$ Values in nM | | | |
|---|---|---|---|---|
| | Aurora A Kinase | Aurora B Kinase | Aurora C Kinase | Ref. |
| MLN8054 | 4 | 172 | NA | Manfredi MG, Ecsedy JA, Meetze KA, et al.(22) |
| MLN8237 | 1 | >200 | NA | Gorgun G, Calabrese E, Hideshima T, et al.(23) |
| HESPERADIN | NA | 50 | NA | Carvajal RD, Tse A, Schwartz GK.et al. (24) |
| AZD-1152 | 1369 | 0.36 | 17 | Mountzios G, Terpos E, Dimopoulos M-A. (25) |
| ZM-447439 | 100 | 100 | NA | Walsby E, Walsh V, Pepper C, et al. (26) |
| JNJ-7706621 | 11 | 15 | NA | Emanuel S, Rugg CA, Gruninger RH, et al. (27) |

### *Drug Design Methods*

Computers are routinely used in the drug discovery process. Virtual screening is a techniques where evaluating compounds desirability in computational model. Usually the predicted property is the bioactivity of a compound in an in-vitro assay. The virtual screening is either ligand-or structure-based. In 3D virtual screening, models of ligand and target protein are used. In the ligand-based approach, the similarity of known chemical structure (ligands) is used. In the search for novel structure is used. On the other hand structure-based virtual screening, compounds are docked into a protein model of the drug target. The virtual screening techniques is used for fast prediction of biological activity for million of compounds. As virtual screening is a relatively new field of science, there is a need for novel methods and for the improvement of existing virtual screening protocols. Discovery of new therapeutic solution is an expensive and time consuming process. It is estimated that a typical drug discovery cycle (fig.1) from lead identification through to clinical trials can take more than 14 years[28]. An emerging technology, computer aided drug design accumulated information of existing drug and diseases, during the early 1980s, structural biologists began to design rational drug based on protein structure. There are two major approaches in drug design. The first is referred to as ligand based design and the structure based drug design.

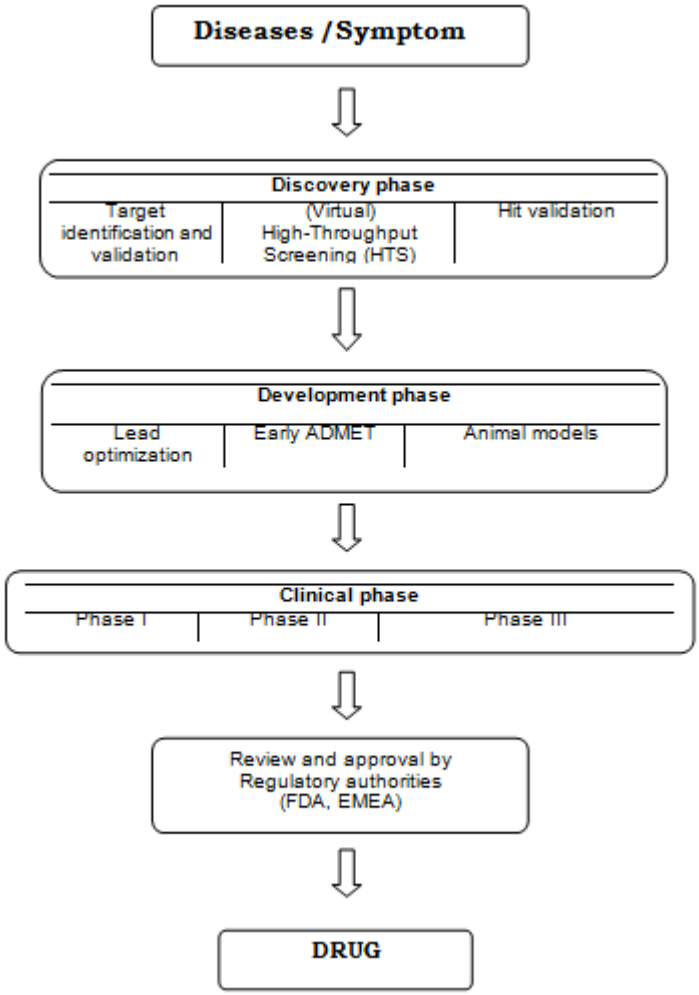


**Figure 1**
***typical drug discovery cycle.***

### *Structure –Based Drug Design*

'Structure-based' computational approaches require the 3D structure of the target. Typically a high- resolution (<2.5A$^0$) structure from X-ray crystallography or a suitable well-defined structure from NMR spectroscopy is used. It is also possible to design ligands for and screen against a homology model for which there is a high degree of confidence. The structure based drug design is beginning to play an important role in the discovery of new therapeutic molecules. Particularly when a lead compound is already known, and the three dimensional structure of the protein ligand complex is determined, computer modeling provide an opportunity for assessment of the feasibility of related compounds as ligands. Using the structures of biological target, candidate drug that are predicted to bind with high affinity and selectivity of the target may be designed using interactive and the intuition of a medicinal chemist. Alternate various automated computational procedure may be used to suggest new drug candidates. The program like Glide, LigandFit, and GOLD are useful for performing docking studies.

### *Homology modeling/Comparative Modeling*

A major goal of structural biology is to predict the three dimensional structure from the sequence, pursuit that has not yet been realized. Thus, alternative strategies are being applied to develop models of protein structure when the constraints from X-ray diffraction or NMR are not yet available[29]. One method that can applied to generate reasonable models of

protein structures is homology modeling. This procedure, also termed comparative modeling or knowledge based modeling, develops a three-dimensional model from a protein sequence based on the structures of homologous proteins.

**Table 5**
***Database of Automated comparative protein Models.***

| | Model Database Resource | Ref. |
|---|---|---|
| MODBASE | http://www.salilab.org/modbase/ | 30, 31 |
| SWISS_MODEL Repository | http://swissmodel.expasy.org/ | 32, 33, 34 |
| Protein Model Portal | http://www.proteinmodelportal.org | |

***List of Protein modeling Software's:***

---

| | |
|---|---|
| HHPred [35] | http://toolkit.tuebingen.mpg.de/hhpred |
| Modeller [36, 37] | http://salilab.org/modeller/ |
| SCWRL [38] | http://dunbrack.fccc.edu/SCWRL3.php |
| WhatIf [39] | http://swift.cmbi.ru.nl/whatif/ |
| Rosetta [40] | http://www.rosettacommons.org/ |

---

Homology modeling is one of the most accurate computational methods to generate reliable tertiary protein structure from its sequence and is routinely used in many biological applications. Recently, there are four crystal structures of Xenopus laevis Aurora kinase-B were deposited in Protein Data Bank (PDB, www.rcsb.org) which shows high similarity and identity with human Aurora kinase-B, but till date, there is not any X-ray crystal structure (3D-structure) of human Aurora kinase-B. Hence, the homology model for human Aurora kinase-B was carried out to find its tertiary structure. S. Sakkiah et al has reported, Human Aurora kinase-B primary sequence was retrieved from Swiss-Prot Protein Database (Accession ID: Q96GD4) which has 344 amino acids. To find a suitable template for human Aurora kinase-B a similarity search against PDB was performed using BLAST (http://www.ncbi.nlm.nih.gov) server Aurora kinase-B from Xenopus laevis (PDB ID: 2VRX, Resolution: 1.86 $A^0$) was selected as a best template to construct human Aurora kinase-B using MODELLER algorithm in DS. The final model was checked using the PROCHECK program to search for deviations from normal protein conformational parameters. Average structure with low RMSD value was selected as the best model for molecular docking studies.

***Validation of homology model using molecular dynamics simulation***

Molecular dynamics (MD) simulation was performed to refine the side chain orientations and also to gain a better relaxation as well as more correct arrangement of the atoms in Aurora kinase- B model. The GROningen MAchine for Chemical Simulations (GROMACS)[41-42].

***Docking***

The quality of any docking result depends on reasonable starting structures for both the protein and the ligand. It is strongly recommended that you process protein and ligand structure with these facilities in order to achieve the beast result. Molecular docking is used to predict the structure of the intermolecular complex formed between two or more molecules. The most interesting case is the protein ligand interaction, because of its applications in medicine. Ligand is a small molecule, which interacts with protein's binding sites. Binding sites are areas of protein known to be active in forming of compounds. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. Molecular docking is a computational technique that sample

conformations of small compound in protein binding site; scoring functions are used to assess which of these conformations were best complements to the protein binding site. Molecular docking programs consists of two essential parts: an algorithm that searches the conformational, rotational and translational space available to candidate molecules within binding site and an objective function to be minimized during the process.

***Some common searching algorithms and tools***

***Monte Carlo methods***

Programs using MC methods include AutoDock, ProDock, ICM, MCDOCK, DockVision, QXP and A_nity

***Genetic algorithms***

Programs using GAs are GOLD, AutoDock, DIVALI and DARWIN.

***Fragment-based methods***

Programs using fragment based methods are FlexX and DOCK

***Point complementary methods***

Programs using point complementary methods are FTDOCK, SANDOCK, FLOG and the Soft Docking algorithm.

***Ligand Based Drug Design and Screening:***

The virtual screeening can be divided into two categories structure based techniques and ligand based techniques. The first structure based techniques is widely used in drug design, requires a protein structure or homology model as a starting point. On the other hand ligand-based approches require knowledge of only one active molecule.

The virtual screen is then conducted by identifying molecules that share common prosperities with that single active molecule given that molecules bind to their active site of receptor and shows some effect on that receptor in a three dimensional manner.

Now there has been a continued interest in 3D techniques in ligand-based virtual screening. Among the best known techniqes pharmacophore approach, Which attempt to abstract features of an active molecule that are likely to be important in binding to the forget receptor.

The virtual screen is than conducted by identifying molecules that should potentially match this set of features. Other 3D ligand-based approaches include shape similarity, which attempts to score database molecules based on their overall shape similarity to a query molecule, rather than just on the molecule's ability to match an abstracted set of features, as pharmacophore tools do. If for a given therapeutic project, a set of active ligand molecules is known for the macromolecular target, but little or no structural information exists for the target, ligand-based computational methods can be employed. More specifically quantitative structural activity relationship (QSAR) method can be used, pharmacophore models developed and shape searches performed based on the set of ligands. QSAR approaches involved the statistical analysis of a set of properties or descriptors for series of biological active molecules; the statistical model that is developed is then used to predict the activity of additional compounds against the target.

***QSAR and Pharmacophore***

Currently, three-dimensional quantitative structure–activity relationship (3D-QSAR) methods have been successfully employed to assist the design of novel small molecule drug candidates[43]. Comparative molecular field analysis (CoMFA)[44], one of the most popular 3D-QSAR methods that use statistical correlation techniques for the analysis of the quantitative relationship between the biological activity of a set of compounds and their three-dimensional electronic and steric properties, is widely used to optimize binding affinity and specificity of series of compounds acting on the same target and through the same mechanism of action[44]. In the 3D-QSAR studies, molecular alignment and 'active' conformation

determination are the most important steps that affect the model reliability. The selection of the active conformer for each compound is the key step in the 3D-QSAR analysis, followed by the molecular Alignment rule. However, generating 3D conformations and the alignment of the compounds is a difficult and time-consuming process, especially when the compounds are large in size and contain several rotatable bonds[45]. Usually, a bioactive conformation of the ligand can be obtained from a structural determination of the ligand–receptor complex by X-ray crystallography. In computer aided drug design process, pharmacophore based molecular docking was one of the most reputable method which was used to find the accuracy of binding orientation (poses) of the ligands into the protein active site[46].

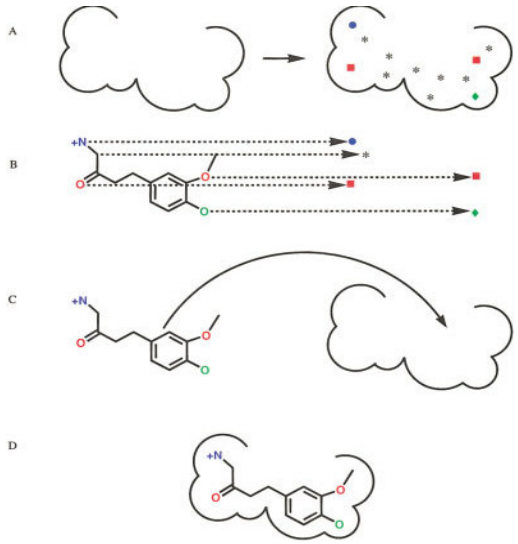


**Figure 2**
***Schematic of DOCK methodology.***

In the first step (**A**), the target binding site is filled with site points that may be colored. Then (**B**), distances between pairs of atoms in a database molecule are matched to distances between pairs of site points. A transformation matrix is calculated for the orientation (**C**), the molecule is docked into the binding site (**D**), and the fit of that conformer of that molecule is scored.

### *Bioassay*

Bioassays are typically conducted to measure the effects of a substance on a living organism and are essential in the development of new drugs and in monitoring environmental pollutants. Both are procedures by which the potency or the nature of a substance is estimated by studying its effects on living matter. Determination of the relative purity of a substance, such as a drug or hormone, by comparing its effects with those of a standard preparation on a culture of living cells or a test organism.

### *USES OF BIOASSAY*

To measure the pharmacological activity of new or chemically undefined substances, to investigate the function of endogenous mediators and to measure drug toxicity and unwanted effects.

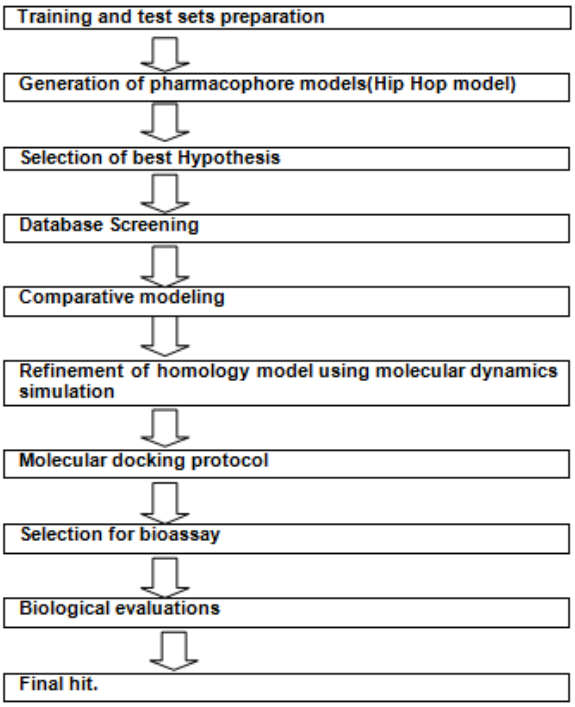


**Figure 3**
***Flowchart of virtual screening Process.***

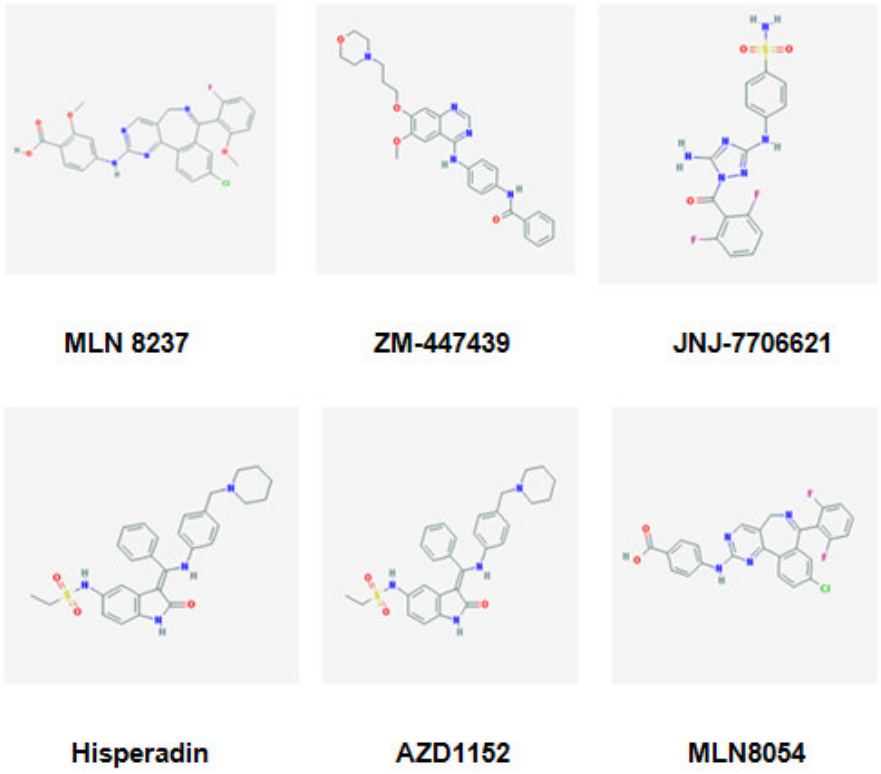


**Figure 4**
***The chemical structures of different Aurora kinase inhibitors.***

## CONCLUSION

This review has emphasized a view on aurora kinase inhibitors designing functions to be the candidate targets to inhibit tumor cell growth. We believe that uncovering the molecular functions of aurora kinases will bring great rewards in understanding cell cycle control and provide new strategies for drug design in cancer therapy. The aurora kinase family provides a new concept to understand the mitosis process, tumorigenesis and the possible relationship between them.

## ACKNOWLEDGMENT

We thank all the other members in our laboratory for valuable comments and discussions.

## REFERENCES

1. Bedrick B. gadea and Joan V. Ruderman Aurora kinase Inhibitor ZM447439 Blocks Chromosome- induced spindle Assembly, the Completion of chromosome Condensation, and the Establishment of the Spindle Integrity Checkpoint in Xenopus Egg Extracts Molecular Biology of the Cell Vol. 16, 1305-1318, March 2005.
2. M. Kollareddy, P. Dzubak, D. Zheleva, M. hajduch Aurora Kinases: Structure, Functions and Their Association with Cancer. Biome Pap Med Fac Univ Olomouc Czech Repub.2008, 152 (1):27-33.
3. Glover DM, Leibowitz MH, McLean DA, Parry H. Mutations in aurora prevents centrosome separation leading to the formation of monopolar spindles. Cell 1995; 81:95 – 105.
4. Schumacher JM, Ashcroft N, Donovan PJ, Golden A. A highly conserved centrosomal kinase, AIR-1, is required for accurate cell cycle progression and segregation of developmental factors in Caenorhabditis elegans embryos. Development 1998;125:4391 – 402.
5. Schumacher JM, Golden A, Donovan PJ. AIR-2: An Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in Caenorhabditis elegans embryos. J Cell Biol 1998; 143:1635 – 46.
6. Mesilaty-Gross S, Reich A, Motro B, Wides R. The Drosophila STAM gene homolog is in a tight gene cluster, and its expression correlates to that of the adjacent gene ial. Gene 1999; 231:173 – 86.
7. Roghi C, Giet R, Uzbekov R, et al. The Xenopus protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. J Cell Sci 1998; 111:557 – 72.
8. Adams RR, Wheatley SP, Gouldsworthy AM, et al. INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. Curr Biol 2000; 10:1075 – 8.
9. Glover, D.M. et al Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles cell 1995, 81(1): 95-105.
10. Bischoff, J.R. et al A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancers. EMBOJ.17-3052-3066(1998).
11. Carmena M, Earnshaw WC. The cellular geography of aurora kinases. Nat Rev Molcell Biol 2003;4(11):842-5.
12. Haufs, cole RW, laTerra S, et al. the small molecule Hesperadin reveals arole for aurora B in correcting Kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. J Cell Biol 2003; 161(2):281.94

13. Giet R, petrettic, Pringent C. Aurora kinases, aneuploidy and cancer a coincidence or a real link? Trends cell Biol 2005; 15(5):241-50.
14. Jiang Y, Z hang Y, Lees E, Seghezzi W. Aurora A overexpress overrides in mitotic spindle checkpoint tiriggred by nocoelazole, a microtubule destabilize. Oncogene 2003; 22:8293-301.
15. Katayama H, sasai k, Kawai H, et al. Phosporylation by aurora kinase A induces Mdm2-Mediated destabilization and inhibitaion of p53. Net Genet 2004;36:55-62.
16. Liu. Q Kaneko s, yang L, et al. Aurora-A abrogation of p53 DNA binding and transactivaion activity by phosphorylation of Serine 215. J Biol Chem 2004;279:52175-82.
17. Kimura M. mastsuda Y, Yoshioka T. okano Y. cell cycle dependent expression and centrosome localization of a third human aurora/lpl1-related protein kinase, ALK3. J Biol chem. 1999; 274:7334-40.
18. Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR, Sen S. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat. Genet. 1998; 20:189-93.
19. Gu J, Gong Y, Huang M, Lu C, Spitz MR, Wu X. Polymorphisms Of STK15 (Aurora-A) gene and lung cancer risk in Caucasians. Carcinogenesis 2007; 28:350-5.
20. Sorrentino R, Libertini S, Pallante PL, Troncone G, Palombini L, Bavetsias V, Spalletti-cernia D, Laccetti P, Linardopoulos S, Chieffi P, Fusco A, Portell G. Aurora B overexpression associates with the thyroid carcinoma undiff rentiated phenotype and is required for thyroid carcinoma cell proliferation. J. Clin. Endocrinol. Metab. 2004; 90:928–35.
21. Reichardt W, Jung V, Brunner C, Klein A, Wemmert S, Romeike BFM, Zang KD, Urbschat S. The putative serine/threonine kinase gene STK15 on chromosome 20q13.2 is amplifi ed in human gliomas. Oncol. Rep. 2003; 10:1275-9.
22. Manfredi MG, Ecsedy JA, Meetze KA, et al. Antitumor activity of MLN8054, an orally active small-molecule inhibitor of aurora A kinase. Proc Natl Acad Sci. 2007; 104:4106–4111. [PubMed: 17360485]
23. Gorgun G, Calabrese E, Hideshima T, et al. A novel aurora-A kinase inhibitor MLN-8237 induces cytotoxicity and cell-cycle arrest in multiple myeloma. Blood. 2010; 115(25):5202–13. [PubMed: 20382844] 45. Kelly KR, Swords
24. Carvajal RD, Tse A, Schwartz GK. Aurora kinases: new targets for cancer therapy. Clin Cancer Res. 2006; 12(23):6869–75. [PubMed: 17145803]
25. Mountzios G, Terpos E, Dimopoulos M-A. Aurora kinases as targets for cancer therapy. Cancer Treat Rev. 2008; 34:175–82. [PubMed: 18023292]
26. Walsby E, Walsh V, Pepper C, et al. Effects of the aurora kinase inhibitors AZD1152-HQPA and ZM447439 on growth arrest and polyploidy in acute myeloid leukemia cell lines and primary blasts. Haematologica. 2008; 93(5):662–9. [PubMed: 18367484]
27. Emanuel S, Rugg CA, Gruninger RH, et al. The in vitro and in vivo effects of JNJ-7706621: A dual inhibitor of cyclin-dependent kinases and aurora kinases. Cancer Res. 2005; 65(19):9038–46. [PubMed: 16204078]
28. Myers S, Baker A. Drug discovery-an operating model for anew era. Nat Biotechnol 2001;19:727-30.
29. Reddy, C.S.; Vijayasarthy, k; Srinivas, E; Sastry, G.M.;Sastry, G.N. Homology modeling of membrane preotins: a critical assessment. Comput.Biol.Chem. 2006,30,120-126
30. Pieper U, Eswar N, Davis FP, *et al*. (2006) MODBASE: A database of annotated comparative protein structure models and associated resources. *Nucl Acids Res* 34(Database Issue): D291–D295.
31. Sanchez R, Sali A. (1999) ModBase: A database of comparative protein structure models. *Bioinformatics* 15(12): 1060– 061.
32. Peitsch MC, Wilkins MR, Tonella L, *et al*. (1997) Large-scale protein modeling and

integration with the SWISS-PROT and SWISS-2DPAGE databases: the example of *Escherichia coli*. *Electrophoresis* 18(3–4): 498–501.

33. Kopp J, Schwede T. (2006) The SWISS-MODEL Repository: new features and functionalities. *Nucl Acids Res* 34(Database Issue): D315–318.
34. Kopp J, Schwede T. (2004) The SWISS-MODEL Repository of annotated three-dimensional protein structure homology models. *Nucl Acids Res* 32(Database Issue): D230–D234.
35. Soding J, Biegert A, Lupas AN. (2005) The HHpred interactive server for protein homology detection and structure prediction. *Nucl Acids Res* 33 (Web Server Issue): W244–W248.
36. Sali A, Blundell TL. (1993) Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol* 234(3): 779–815.
37. Eswar N, John B, Mirkovic N, *et al*. (2003) Tools for comparative protein structure modeling and analysis. *Nucl Acids Res* 31(13): 3375–3380.
38. Canutescu AA, Shelenkov AA, Dunbrack RL, Jr. (2003) A graph-theory algorithm for rapid protein side-chain prediction. *Protein Sci* 12(9): 2001–2014.
39. Vriend G. (1990) WHAT IF: a molecular modeling and drug design program. *J Mol Graph* 8(1): 52–56, 29.
40. Rohl CA, Strauss CE, Misura KM, Baker D. (2004) Protein structure prediction using Rosetta. *Meth Enzymol* 383: 66–93.
41. GROMACS, v3.1.4, Department of Biophysical Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The etherlands. <http://www.gromacs.org/>.
42. D. Van Der Spoel, E. Lindahl, B. Hess, G. Groenhof, A.E. Mark, H.J. Berendsen, J Comput. Chem. 26 (2005) 1701–1718.)
43. Cramer R.D. III, Patterson D.E., Bunce J.D. (1988) Comparative Molecular Field Analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. J Am Chem Soc;110:5959–5967.
44. Klebe G., Abraham U., Mietzner T. (1994) Molecular Similarity Indices in a Comparative Analysis (CoMSIA) of drug molecules to correlate and predict their biological activity. J Med Chem;37:4130–4146.
45. Green S.M., Marshall G.R. (1995) 3D-QSAR: a current perspective.
46. Trends Pharmacol Sci;16:285–291.
47. Diane Joseph-McCarthy,* Bert E. Thomas IV, Michael Belmarsh, Demetri Moustakas, and Juan C. Alvarez Pharmacophore-Based Molecular Docking to Account for Ligand Flexibility PROTEINS: Structure, Function, and Genetics 51:172–188 (2003)