A Current Insight into Aurora Kinase Inhibitors and their Potential as Anticancer Agents

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

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BRAC University
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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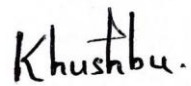

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Approval

The thesis titled “A Current Insights into Aurora Kinase Inhibitors and their Potential as Anticancer Agents” submitted by Samia Sajnin Khushbu (19346013) of Summer, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This project does not involve any clinical trial or human participants and no animals were used or harmed.

Abstract

The Aurora kinases, comprising Aurora A, Aurora B, and Aurora C, constitute a family of serine/threonine protein kinases that plays a fundamental role in ensuring precise mitotic progression, regulating multiple phases of cell division, and maintaining genomic stability. The purpose of this review work is to provide an updated assessment of Aurora Kinase Inhibitors (AKIs) and their role as promising anticancer agents. Additionally, this article compiles the recent advancements in AKI research, encompassing novel compounds, their mechanisms of action, and preclinical/clinical trial outcomes. Aurora A regulates the process of maturation and separation of centrosomes, whereas Aurora B is responsible for ensuring precise chromosomal segregation. The significant correlation between the progression of malignancy and the dysregulation of Aurora kinases highlights their suitability as targets for pharmacological intervention, thereby making them a very promising area of emphasis for the creation of potent and specific anticancer drugs.

Keywords: Aurora kinases, Aurora A, Aurora B, Aurora C, Kinase inhibition (KIs), Anticancer agents, Small-molecule inhibitors, clinical trials, preclinical studies.

Dedication

Dedicated to my father and my teachers

Acknowledgement

To start with, I would want to convey my deepest gratitude to Allah for bestowing upon strength to continue throughout this period with mercy and kindness. I am sincerely grateful to Dr. Nishat Zareen Khair, Asisstant Professor, School of Pharmacy, Brac University, for consistently directing me and assisting during my project. Her patience and guidance have been invaluable throughout this process.

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Table of Contents

List of Tables

List of Figures:

List of Acronyms

AKIs	Aurora Kinase Inhibitors
AKs	Aurora Kinases
ATP	Adenosine Triphosphate
CDK	Cyclin-Dependent Kinase
PI3K	The phosphatidyinositide 3-kinase
pHH3	Phospho-Histone H3
PDB	Protein Data Bank
GD2	Ganglioside D2
VX-680	Tozasertib
IMR-32	A human neuroblastoma cell line
TH-MYCN	Transgenic Mouse Model Overexpressing MYCN
AURKA	Aurora Kinase A
AURKB	Aurora Kinase B
AURKC	Aurora Kinase C
ATP	Adenosine Triphosphate
N-Myc	Neuroblastoma MYC oncogene
P53	Tumor Protein P53
HCT-116	A human colorectal cancer cell line
AKT	Protein Kinase B
MAPK	Mitogen-Activated Peptide Kinases
STAT3	The transcription Mediator and Signalling The transmitter No. 3

Chapter 1: Introduction

1.1: Cancer

Despite significant investments in cancer research in recent decades, cancer remains a prominent contributor to global human mortality, persisting as one of the primary causes of death internationally. Cancer develops through intricate mechanisms that encompass a succession of genetic and epigenetic modifications. Due to the fact that individual cancer patients may contain unique driver mutations and possess diverse genetic backgrounds, it is exceedingly challenging to develop a universal treatment regimen (Mou et al., 2021) Presently employed chemotherapeutic agents for cancer treatment are accompanied by a number of severe adverse effects, lack of selectivity in their mechanism of action, and the development of resistance to clinically relevant anticancer medications Fu et al. (2007). So, novel targets must be identified, and new entities must be designed and developed in order to provide a safer and more effective treatment regimen.

Mitosis and the control of cell cycle checkpoints are facilitated by a multitude of serine/threonine protein kinases called mitotic kinases. Mitosis, an intricately regulated mechanism, guarantees the accurate division of cells within multicellular organisms. It generates two identical copies of daughter cells through the construction of a bipolar mitotic spindle (Cardon et al., 2009). An error in this procedure has the potential to compromise the integrity of the genome, leading to the generation of cells exhibiting genetic instability or aneuploidy (abnormal chromosome content) as shown in figure 1.1, which may promote cellular demise or contribute to the development of tumors (Dalton & Yang, 2009). Moreover, these mitotic abnormalities serve as defining characteristics of the majority of malignancies.

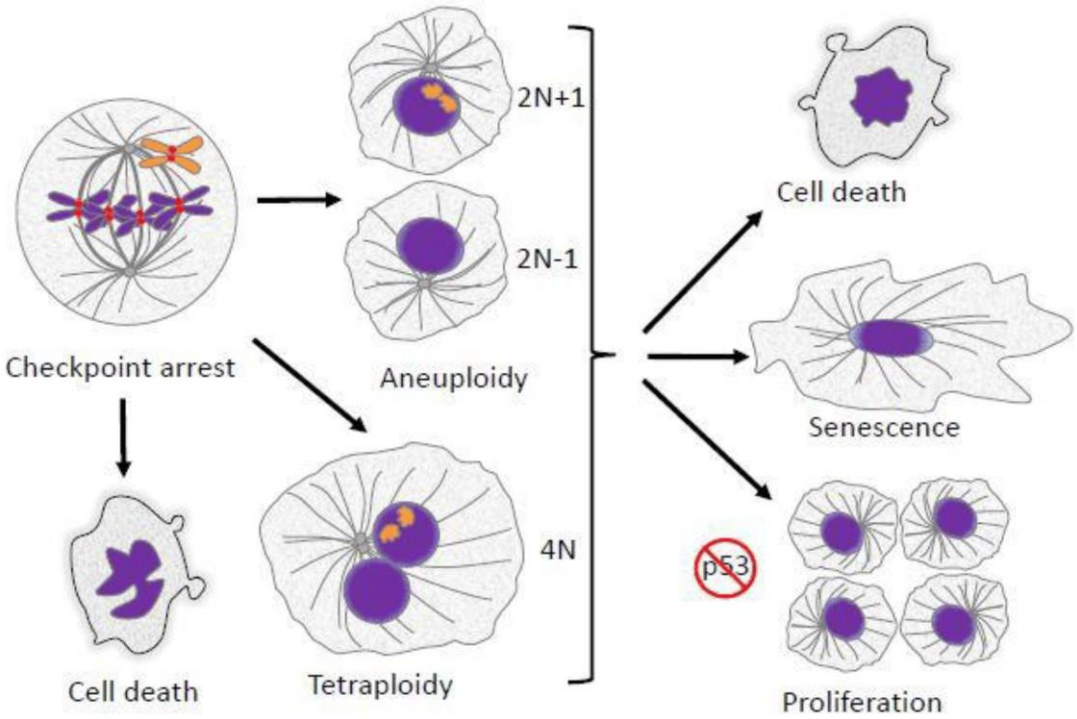


Figure 1: Segregation Errors of Chromosomes During Mitosis (Potapova & Gorbsky, 2017)

Proteolysis, protein localization, and protein phosphorylation are three crucial regulatory mechanisms that effectively propel the cell through the mitotic phase. Targeting these components of mitotic checkpoints is a subject of intensive research due to its potential to regulate tumor progression. The cyclin-dependent kinase-1 family (CDK-1), the polo-like kinase family (Plk), the Aurora family, and the never in mitosis gene a (NIMA)-related family belong to the targets (Ferrari et al., 2005a). Aurora kinases, also known as mitotic kinases, are essential regulatory proteins that govern the process of mitotic cell division (Adams et al., 2010). Aurora kinases constitute a significant class of enzymes that are classified within the Serine/Threonine kinase family (Cheetham et al., 2002).

1.2: Structure of Aurora Kinases

Three distinct subtypes of Aurora kinases are labeled as Aurora A, Aurora B, and Aurora C. Three distinct regions comprise aurora kinases: An N-terminal region (39–139aa), a domain at

the C-terminus (15–20aa) as illustrates in figure 1.2, and an extensively stable kinase domain (250–300aa). A hinge region separates originating from the α-stranded N-terminal region of the α-helical C-terminal section, thereby ensuring the maintenance of the active conformation within the Aurora kinase site (Farrell et al., 2009a). Eleven subdomains that are conserved and are partitioned by insertion sites that are less conserved make up the Aurora kinase domain. Phosphorylation of a surviving portions (Tr288 in AurA) A conformational change occurs in the kinase domain at its C-terminal lobe, which is associated with kinase activity(Ferrari et al., 2005).

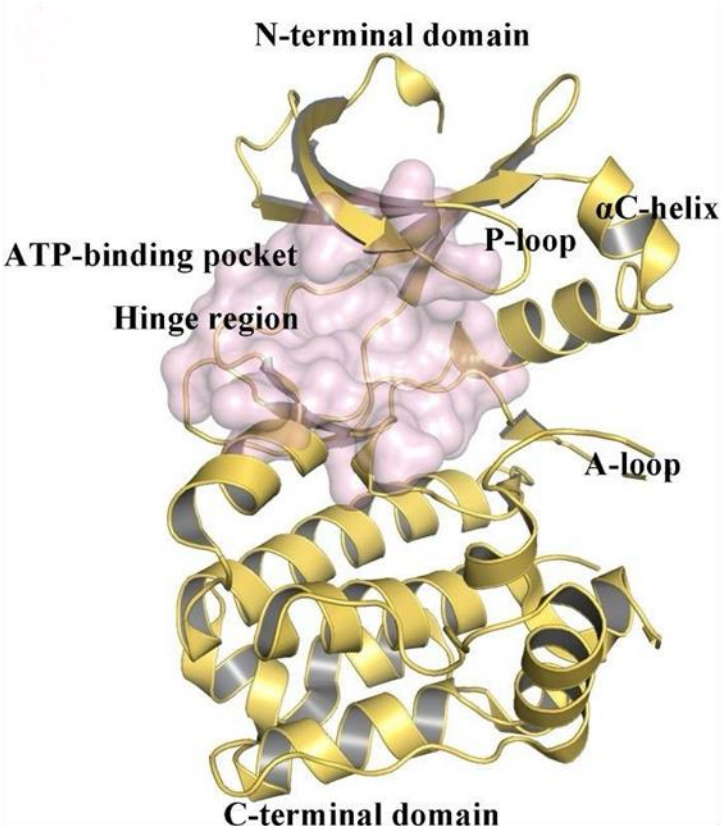


Figure 2: Structure of Aurora Kinase (Zhang et al., 2020)

The Aurora kinase domains consists of linear, concise motifs (SLiMs) that act as degrons which leading to degradation through the proteasome system (Bayliss et al., 2004). There are resemblances between the ATP-binding region found within the Aurora kinase section and those present in SRC kinase and GSK-3β. Following the bending of the domains at the N and

C junctions, an alternative conformation is attained in the site of activity of Aurora kinases. The hydrogen bonds that connect the adenosine purine ring in relation to the kinase domain's junction of AurA are specific(de Souza & Kawano, 2020). AurA-specific inhibitor development is facilitated by the presence of three distinct forms of the ATP-binding domain (T217, L215, R220) in human Aurora proteins that are unique to AurA. Notably, the antagonist VX-680 demonstrates a preference for AurA (0.6 nM) in comparison to AurB (18 nM) and AurC (4.6 nM), signifying the existence of distinct structural attributes shared by the components of Aurora A, B and C.

The size and sequence of the non-catalytic domains at the N and C junctions of Aurora Kinases differ between human and homologous counterparts. After mitosis, the Cdh1 protein in humans recognises degrons in these domains in order to degrade Aurora protein. Cdh1, serving as a substrate-specific adaptor, enhances the activity of the Ube2S E2 enzyme coordinates the degradation of APC/C by E3 ligase. Aurora kinases encompass degrons, including D-boxes, KEN motifs, and DAD/A boxes(Ouchi et al., 2015). In the kinase domain all components of aurora, D-boxes are well-established degrons. Although both AurA and AurB feature KEN motifs, their role in the proteasome-mediated degradation of AurA remains unclear. Both AurA and AurB possess DAD/A boxes, unique degrons that facilitate degradation through APC/C(Ouchi et al., 2015). The C-terminal D-boxes may contribute to intra-molecular interactions, while the N-terminal ones may expose Short, Linear Motifs (SLiMs) declines before degradation controlled by APC/C (Stewart & Fang, 2005).

Additionally, Co-factor interactions that are essential for folding of proteins and Aurora kinase activity are facilitated by the C-terminal domain. Affecting cellular localization, Each Aurora individual's exceedingly varying N-terminal domain can regulate kinase interaction with protein partners(Sugimoto et al., 2002). The subcellular localization of AurA and AurB enables them to reciprocally rescue one another's mitotic functions. Primarily expressed in germ cells,

AurC can function as the somatic cell chromosomal passenger complex (CPC) enzyme in lieu of AurB (Yan et al., 2005). Aurora kinases' distinctive effects in mammalian cells may be largely mediated by their subcellular or cellular localization, according to accumulating evidence. (Borisa & Bhatt, 2017a)

Classification of Aurora kinase: Aurora A, B, and C are the identifiers of three different subtypes that constitute the Aurora kinase family. Despite the fact that they both possess a C-terminal and an N-terminal domain, their functional characteristics exhibit differences.

The expression, localization, and activities of Aurora A during cell entrance and escape from mitosis are depicted in figure 1.3.

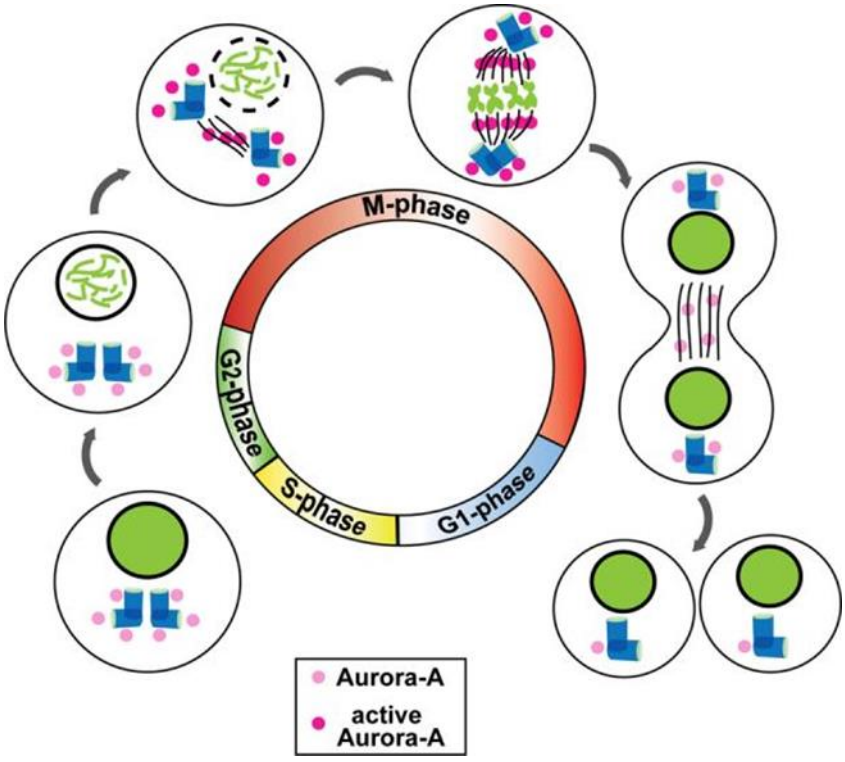


Figure 3: Aurora-A's impact on the cell cycle (Nikonova et al., 2012)

Initial genetic studies on Aurora A variations frequently revealed abnormalities in the formation and control of the bipolar spindle during mitosis. An in-depth examination of Aurora A's expression, activation, and specific phosphorylation targets reveals its role in a sequence of events that occur before mitosis, facilitating the proper advancement through this stage of

the cell cycle. Also, Aurora-A regulates various processes including centrosome maturation and segregation, construction of a bipolar spindle, commencement of mitotic entry, alignment of chromosomes in the metaphase, and the final steps of cytokinesis and abscission. The proteolytic degradation of Aurora A is crucial for cells to transition to the G1 phase. Aurora A protein begins to accumulate significantly at centrosomes during the S phase of the cell cycle and becomes activated at the interface between the G2 and M phases. After moving over the mitotic spindle to the midzone while being active, a significant percentage of the protein undergoes break down and becomes inactive before cytokinesis. Early G1 cells show low levels of Aurora A staining. Inhibiting the function of Aurora A would result in an imbalanced distribution of chromosomes among the daughter cells and the development of abnormal spindle structures. (Nikonova et al., 2013).

In case of Aurora B, during prophase and metaphase it exhibits centromere localization as shown in figure 1.4.

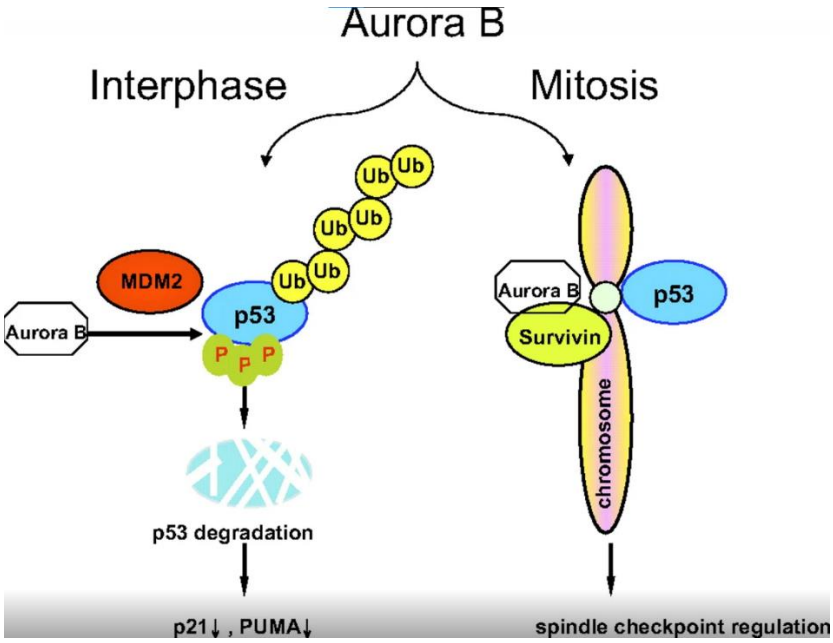


Figure 4: Activity of Aurora B across the cell cycle (Gully et al., 2012)

While during mitosis, Histone H3 is activated at the Serine 10 position. Aurora B kinase enhances cellular viability and facilitates the progression of the cell cycle by synthesizing the tumor suppressor protein p53, hence accelerating its breakdown through the proteasome pathway. Inhibiting Aurora B could bypass the spindle checkpoint and abruptly stop cell mitosis, resulting in the development of polyploidy. In addition to impeding cytokinesis and chromosome segregation, its inhibition may also disrupt microtubule interactions(Hirota et al., 2005).

Aurora C is an intermediate protein for the division of chromosomes that is exclusive to the testis and performs an extremely similar function to Aurora B(Sasai et al., 2004). In addition to promoting cancer cell survival, it may induce centrosome amplification and frequently lead to chromosome segregation defects. (Jing & Chen, 2021)

1.3: Association of Aurora Kinase with cancer

Aurora kinases serve crucial roles throughout the process of mitosis; therefore, their aberrant expression may contribute to the cellular alterations that underlie cancer. Upregulation of Aurora kinase in numerous tissues results in genetic instability, or aneuploidy, which has the potential to induce cancer. Aneuploidy, a pathological condition marked by irregular DNA content, can be triggered by mitotic errors like centrosome duplication, separation, cytokinesis, or mistakes in chromosomal bi-orientation. All these mechanisms involve the activity of Aurora kinases. Consequently, it is fascinating to consider that aneuploidy may stem from the abnormal expression of Aurora kinases. As a result, The Aurora genes have received the designation of authentic oncogenes(Keen & Taylor, 2004).

Initially, Aurora A was designated as Breast Tumor Activated Kinase (BTAK) because of its significant increase in mRNA expression in breast cancers and its crucial function in promoting the cellular transformation of breast tumors (BTAK40). The existence of the 20q13 gene in a

breast tumor cannot be relied upon as a prognostic indication(Keen & Taylor, 2004). Reportedly, it has been stated that excessive expression of Aurora A in a location outside of its normal position has the ability to induce tumors in rodents through the transformation of NIHT3 and rat1 cells(Gritsko et al., 2003). The disclosure of these revolutionary findings has heightened interest in the Aurora kinases to an unprecedented degree. By employing both northern and southern blot methodologies, Zhou *et al.* examined an assortment of cellular tumors. Amplifications of Aurora A were identified, ranging from 2.5 to 8 folds, in various cancers including colon cancer cell lines, prostate cancer, leukemia (HL60, K562), neural cell line, and cervical cancer cell line. It has been noted that these alterations lead to a functional polymorphism (Phe31Ile), It is highly linked to the formation of human colon cancer(Sepulveda et al., 2017). Recent reports have identified comparable polymorphisms in lung cancer that is more prevalent among Caucasians.

In numerous other types of tumors, overexpression of Aurora A has been documented. On the basis of the collective findings, it is possible to conclude that Aurora A is a valid oncogene. Tumor formation can also be induced by aberrant Aurora B expression, which is not unexpected given the kinase's involvement in numerous mitotic functions. As a result, Aurora B deserves to be designated as an oncogene. Katayama *et al*. executed western and northern antibody analysis in addition to in situ hybridization on medically extracted colon cancer specimens. The authors documented the upregulation of Aurora B and the progression of the tumor. This noteworthy study sparked enthusiasm regarding the advancement of novel pharmaceuticals targeting Aurora B. The proliferation of invasive thyroid anaplastic carcinoma cells is linked to the suppression of Aurora B45. Aurora B has been found to be over expressed in 46 cases of oral cancer and 47 cases of primary non-small cell lung cancer. Research has investigated the occurrence of Aurora B overexpression in additional tumor classifications. The upregulation of Aurora C has been detected in multiple cancer cell lines.

However, its associated with the advancement of cancer is still unknown. Therefore, current research efforts are focused on identifying Aurora C functions as oncogene. On the basis of these reports, the classification of Aurora kinases as oncogenes is plausible. In contrast to specific oncogenic kinases, their expression is not aberrantly restricted to a single form of tumor. Therefore, the expansion of potential inhibitors of Aurora kinase can target a vast array of tumor types. The potential therapeutic applications of pharmaceuticals that selectively target Aurora kinases are extensive and encompass a broad spectrum of malignancies (Kollareddy et al., 2008). Further details by which Aurora kinases exert their effects in specific forms of cancer is elucidated as follows:

- **Breast Cancer:** An often-observed phenomenon in breast cancer is the increased expression of Aurora A and B, which results in chromosomal instability and aberrant cell division (Thrane et al., 2015). Managing breast cancer becomes notably challenging due to resistance to antiestrogen treatment. Eighteen inhibitors, identified in fulvestrant-resistant and tamoxifen-resistant cell lines, complicate the situation. In resistant cells, WP1130, a deubiquitinase inhibitor, induces caspase-mediated cell death by disrupting Mcl-1. Decreased Mcl-1 levels are linked to increased upregulation. A combination of Aurora kinase and cyclin-dependent kinase inhibitor effectively hinders the proliferation of tamoxifen-resistant cells and induces G2 phase cell cycle arrest. Aurora kinase A significantly influences cell proliferation, demonstrated by knockout studies where its inhibition renders cells responsive to tamoxifen. Notably, JNJ-7706621 and WP1130 which are a dual inhibitor of cyclin-dependent kinases and aurora kinases, exhibit preferential growth inhibition in T47D-derived tamoxifen-resistant cell lines, suggesting Mcl-1 and Aurora kinase A as potential therapeutic targets. In estrogen receptor-positive breast cancer patients undergoing adjuvant tamoxifen treatment, higher Aurora A expression is significantly associated with

shorter disease-free and overall survival, implying Aurora kinase A as a potential biomarker for tamoxifen resistance in breast cancer (Thrane et al., 2015).

- **Ovarian cancer:** Ovarian malignancies often display elevated levels of Aurora A and B, which leads to the aggressive characteristics of the disease. Aurora kinase inhibitors in ovarian cancer specifically target these kinases to hinder cell proliferation, trigger apoptosis, and limit tumor growth. The inhibitors can be utilized in combination therapy to augment the effectiveness of treatment(Yang et al., 2010)

- **Leukemia, including AML and CML:** The use of Aurora kinase inhibitors, such as VX-680/MK-0457, has demonstrated effectiveness in the treatment of both acute and chronic myeloid leukemia. By utilizing mutant forms of tyrosine kinases to target leukemic cells, they induce apoptosis and impede colony formation. These inhibitors offer prospective therapeutic alternatives, particularly in instances where conventional tyrosine kinase inhibitors prove ineffective(Melichar et al., 2015).

- **HCC: Hepatocellular Carcinoma:** VE-465 and other Inhibitors of aurora kinase have demonstrated effectiveness in the management of hepatocellular carcinoma. HCC cells are induced to undergo apoptosis, their proliferation is impeded, and aberrant mitotic spindles are disrupted by these inhibitors. Their potential application is especially auspicious in light of the prevalent upregulation of Aurora-A and -B kinases in HCC(Z.-Z. Lin et al., 2009).

- **Colorectal malignancy:** Frequently, cells derived from colorectal cancer display chromosomal instability and aberrant cell division. Aurora kinase inhibitors cause cell cycle arrest and apoptosis in colorectal cancer cells by targeting these aberrations. The inhibitors have the potential to augment the efficacy of conventional chemotherapy regimens.(X. Lin et al., 2020)

- **Lung malignancy:** The inhibitors of Aurora kinase have been extensively studied as promising efficient treatment for lung cancer. The potential of the inhibitors in particular subtypes of lung cancer could facilitate the development of more targeted treatment strategies(Galetta & Cortes-Dericks, 2020).

1.4: Targeting Aurora Kinase Inhibitors for anticancer drug development

Developing cancer drugs by targeting Aurora kinase inhibitors requires developing chemicals that specifically inhibit the function of Aurora kinases, which are crucial regulators of the cell cycle. The aim of this procedure is to induce a disruption in the cell division, cause mitotic errors, and ultimately induce apoptosis in cancer cells by blocking these kinases.

The challenge in achieving sufficient selectivity for drug development targeting protein kinases like Aurora-A and -B stems from the common ligand design approach. To design individual ligands effectively, it is crucial to possess a comprehensive understanding of the structural conformation of the targeted ATP binding site. Protein kinases, including Aurora-A and -B, are capable of exhibiting two distinct conformations: "closed" (inactive) and "open" (active). The activation process involves essential steps such as repositioning catalytic residues and organizing the regions comprising the substrate-binding pocket. A key residue on the "activation loop" is phosphorylated, which is the driving force behind these alterations. In order for Aurora-A to be fully activated, it must first undergo autophosphorylation and then attach to TPX-2(Bayliss et al., 2003).

Crystal structures provide insights into the underlying mechanisms of cellular processes. Aurora-B activation involves autophosphorylation at Thr248 and binding to INCENP, leading to conformational changes activating the kinase. Aurora-A, when bound to TPX-2, adopts an active conformation aligning catalytic residues for catalysis. In the absence of TPX-2, disorder occurs, creating an incompatible ATP binding pocket. A unique closed conformation, observed

in a pan-Aurora inhibitor complex, offers a potential selective target. The activation of Aurora-B entails self-phosphorylation and binding with INCENP, ultimately resulting in enhanced activity. The lack of comprehensive Aurora-B structural characterization hinders understanding and selective targeting. Chemical 3 inhibits AURKA and AURKB in a closed conformation, suggesting similarity between inactive forms. While Aurora-C lacks documented structures, exploring both Aurora-A and Aurora-B structures unveils strategies for specific inhibitors. The unique configuration of inactive Aurora-A, with a confined hydrophobic pocket near the ATP-binding site, provides specificity. Aurora kinases' flexibility, seen in structural changes during activation, offers opportunities for inhibitors inducing conformational alterations in both active and inactive states. Ligands can immobilize specific conformations or induce localized changes, advantageous in targeting flexible Aurora kinases (Sessa et al., 2005).

1.5: Objectives of the review

- The objective of this review paper is to provide a concise summary of the existing inhibitory compounds of Aurora kinases, encompassing both Aurora-A and B. To accomplish this, it is necessary to provide a detailed account of the inhibitory categories, compositions, and associations of the inhibitors with the kinase targets.
- To summarize the information about the inhibitors function, their mechanism of action, including their effect on the regulation of the cell cycle, specifically in mitosis.
- This essay will also provide an update on the progress of Aurora kinase inhibitor for the development of anticancer agent by analyzing ongoing clinical and preclinical trials. The article also discusses issues including drug resistance and toxicity in clinical environments and investigates possible solutions.

Chapter 2: Methodology

In order to compose this review article, a systematic and methodical synthesis of the extant literature on the topic is required. The objective and scope of this review paper is clearly defined by describing the classifications, mechanisms of action, preclinical and clinical investigations, and potential anticancer properties of Aurora kinase inhibitors. To write this review paper, firstly related keywords are searched in authentic sources to identify pertinent papers, forming the basis for composing this review article. Then a comprehensive literature search is performed by utilizing trustworthy sources, academic papers, and other scholarly databases. For instance, PubMed, ScienceDirect, SpringerLink, Nature is used to gather all the data necessary to compose this review article. Also, to capture the current findings in clinical and preclinical development, Lancet, Peer-reviewed research, MDPI open-access publication, Elsevier are used correctly and referenced. These authentic sources corroborate my content's legitimacy and correctness.

Chapter 3: Inhibitors of Aurora Kinases

3.1: Overview of the inhibitory compounds:

As of now, there is only one FDA-approved medication, alisertib, which has been identified through the efforts of a biopharmaceutical company named "Puma Biotechnology." Functioning as an inhibitor of Aurora kinase A, the U.S. Food and Drug Administration (FDA) has given Orphan Drug Identification to alisertib particularly for the treatment of small cell lung cancer (SCLC). Multiple Aurora inhibitors, after completing their phase 3 clinical trials, have demonstrated anti-cancer capabilities. Nevertheless, current research still remains ongoing for numerous additional inhibitors in the field. (Puma Biotechnology, n.d.).

The expression of all three Aurora kinases (AURKs) is notably observed in many solid and hematological cancers, rendering them important targets for cancer treatment. Various small compounds, such as ZM447439, VX680, AZD1152, MLN8054, and hesperidin, have been identified as specific inhibitors of Aurora-A that directly compete with ATP (Bebbington et al., 2009; Borisa & Bhatt, 2017b; Georgieva et al., 2010). In this chapter, different classes of Aurora kinase inhibitors and their selectivity for AURKs will be discussed. Also, the small molecule inhibitors which demonstrate similar kind of characteristics will be also discussed.

ZM447439, Hesperadin8, and VX-680, three inhibitors of Aurora kinase which has been found initially, exhibit promisingly similar characteristics in cellular experiments. In particular, all three substances hinder both cell division and serine 10 phosphorylation of histone H3. Despite this effect, they do not hinder the advancement of the cell cycle(Hauf et al., 2003). Instead, tumor cells treated with these drugs undergo mitosis and replicate their genomes again at a pace similar to untreated cells, leading to the development of tetraploidy. Their conduct exhibits a departure from the conventional 'antimitotic' agents. It appears that the effects of protracted exposure to these medications differ between cell lines(Ditchfield et al., 2003). In

some cells, there is progression through additional cell cycles, leading to extensive polyploidity due to unsuccessful cell division. On the contrary, alternative cell types enter a pseudo-G1 state or endure apoptosis. The observed variations in findings are certainly not caused by the distinctive function of aurora kinase, but rather by the requirements of various cell lines, specifically related to the structure of the post-mitotic checkpoint that relies on p53. (Harrington et al., 2004).

In summary, regulating the process of repair (or apoptosis) by inducing a G1 or S/G2 phase halt in a reaction to DNA damage, p53 appears to regulate an aberrant mitosis-induced split of cells by inducing a G1-like arrest. P53 may serve as a substitute mechanism for the spindle-checkpoint system, thereby obstructing the growth of cells that contain an aberrant genome. After treatment with the Aurora inhibitor ZM447439, endoreduplication in U2OS cells is unfailingly inhibited by P53(Ditchfield et al., 2003). Similar effects are observed in mitosis with both ZM447439 and Hesperadin. Surprisingly, despite the suppression of serine 10 modification of histone H3 Consolidation of chromosomes persists in cells that have been treated with these compounds.

The impacts of VX-680 on mitosis lack elaboration upon in the passage. In contrast, Tumour cells in vivo exhibit a significant decrease in histone H3 phosphorylation at serine 10, suggesting that the inhibitor effectively suppresses Aurora-B kinase activity The passage concludes by highlighting the promising antitumor characteristics of VX-680, which were observed in xenograft experiments using mice. (Pradhan et al., 2021)

3.2: Different chemical classes of AKIs

3.2.1: Pyrimidines as inhibitors of Aurora Kinase

The last couple of years have been emphasized by prominent developments that has been achieved in exploring substituted pyrimidines to identify potential inhibitors of Aurora kinase. In their comprehensive study, Qin *et al.* provided a clear description of the process and assessment of 2,4-diaminopyrimidines, showing them to be potent inhibitors of Aurora A kinase. Various compounds were synthesized and assessed for their cytotoxic characteristics in relation to VX-680. Notably, the IC_{50} values for compounds 1a and 1b were found to be the highest (ranging from 0.5 to 3.6 mM and 2.1 to 4.5 mM) compounds that exhibited activity in opposition to HCT-8, A-549, HeLa, and Hep-G2 tumour cell lines were designed(Qin et al., 2015a). Further verification of the findings was achieved via docking simulations, which revealed that the compound conformed more closely to the binding pocket of AURKA (PDB code 3D14) in comparison to AURKB (PDB code 4C2V), with Aurora A possessing a binding energy of 9.05 kcal/mol and Aurora B 10.02 kcal/mol(Xu et al., 2020).

Furthermore, the substance under investigation created hydrogen bonds with key residues in the enzyme's active site through hydrophobic interactions. Several compounds were conceived and synthesized with the aim of inhibiting Aurora kinase and demonstrating promising anticancer properties(Lee et al., 2019).

Potent AKIs were produced by Liang Long *et al.* using an N-trisubstituted pyrimidine scaffold. Twelve N-trisubstituted pyrimidine derivatives were synthesized, with IC_{50} values of 0.0071 and 0.0257 μM for AURKA and AURKB inhibition. Furthermore, leukemia cells were polyploidized. The findings demonstrated that these compounds exhibited a higher degree of inhibition towards AURKA compared to AURKB. Additionally, they displayed

antiproliferative effects against the U937 human leukemia cell line (IC_{50} value of 0.012M) (Long et al., 2018).

The cumulative findings of these investigations underscore the encouraging prospects of these compounds as feasible candidates for further investigation and progression as targeted anticancer agents. Informed by their prior investigations, Long *et al*. evaluated the cytotoxic properties of an extensive variety of human cancer, including epidermoid carcinoma (A431), cervical cancer (Hela), leukaemia tumour (U937 and K562), and Breast tumours among humans (MCF-7 as well as MDA-MB-231). As a standard for validating these pyrimidines, the authors employed VX-680. Compound 8, which was produced in a series, demonstrated significant antiproliferative characteristics. The compound demonstrated a low micromolar inhibitory effect on diverse cellular strains, such as K562, Hela, MDA-MB-231, MCF-7, HCT116, and A431(Luo et al., 2014). Specifically, it exhibited an IC_{50} value of 12.2 nM against U937. Subsequent investigations utilizing flow cytometry and immunofluorescence techniques revealed that compound 8 exerts its effects via the promotion of spindle formation defects, chromosome alignment, and polyploidy. In addition, compound 8 exhibited advantageous chemical, thermal, and physical stabilities Compound eight has the potential to be used as a precursor in the development of a medication that particularly targets tumors.(Qin et al., 2015b)

Through inside screening, the bisanilinopyrimidine 1 substituted with o-carboxylic acid was identified as a potent finding (Aurora A $IC_{50} = 6.1 \pm 1.0$ nM) as shown in figure 3.1. According to comprehensive structure–activity relationship (SAR) investigations, polar substituents situated at the para position of the B-ring play a crucial role in generating potent activity. B-Ring modifications were implemented in an effort to increase the permeability and solubility of the substance. It was discovered that 9m and other compounds possessing water dissolving groups at the opposite end of the B-ring inhibited Aurora A activation in breast cancer cells. (Lawrence et al., 2012).

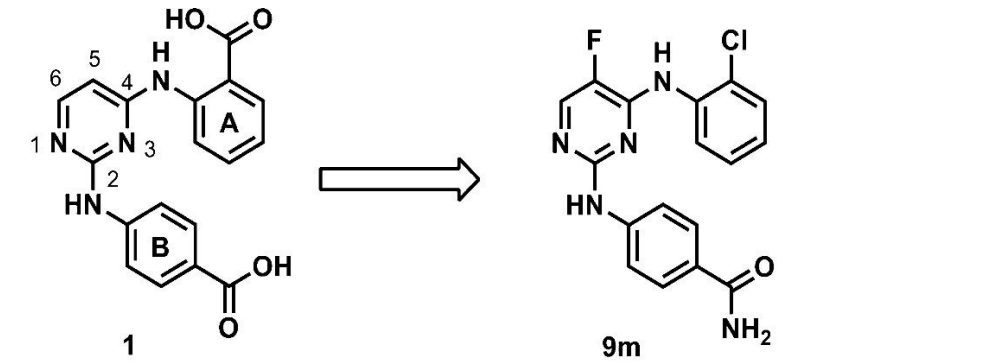


Figure 5: Highly potent o-Chlorophenyl Substituted Pyrimidines (Lawrence et al., 2012b; Xu et al., 2020b)

3.2.2: Quinazolines and quinolines as inhibitors of Aurora Kinase

Yung Chang Hsu *et al*. identified a quinazoline-based multi kinase inhibitor, BPR1K871 which exhibits dual-targeting activity against acute myeloid leukaemia (AML) and solid tumours via FMS-like receptor AURKA. Fourteen analogs from the quinazoline series were synthesized. BPR1K871 was identified as the most effective quinoline-based kinase inhibitor (Chang Hsu et al., 2016).

Six cancer cell lines were subjected to screening of the newly synthesized analogues: K562, U937, A549, NCI-H661, HT29, and LoVo. While a considerable proportion of the compounds demonstrated substantial inhibitory effects against four evaluate tumour cell lines, their activity against two leukaemia cell lines was significantly reduced. Compounds 20a–d demonstrated a high extent of inhibition, as evidenced by their IC_{50} values ranging from 0.9 to 3.4 mM, which surpassed those of the standard ZM447439(Cai et al., 2014).

In addition, an investigation was conducted to find out the inhibitory effects of these substances on Aurora kinase and the majority of these compounds demonstrated a severe degree of sensitivity towards Aurora A in contrast to Aurora B. Coupling simulations confirmed that 20b

and Aurora A's active ATP binding site sustained interaction through numerous hydrogen bonds with the active site's critical areas (Fan et al., 2020).In their research, Fan *et al.* provided a comprehensive concept, synthesis, and biological evaluation of imidazole-coupled analogues of this molecule. They identified these derivatives as unique anticancer medicines with the ability to inhibit Aurora kinase.

Furthermore, docking experiments were performed in order to determine the justification behind the compound's selective inhibitory tendency towards AURKA (PDB ID: 3P9J)(Al-Sanea et al., 2020).

3.2.3: Pyridine as an inhibitor of Aurora Kinase

To explore innovative anticancer agents, three separate investigations demonstrate the synthesis and assessment of compounds that specifically target Aurora kinases. Song *et al.* conducted a study wherein they synthesized pyrrolopyridinyl linked indazole derivatives (Compound 26) and assessed their ability to prevent the proliferation of different types of malignant cells. Compound 26a demonstrated considerable cytotoxicity towards HCT116 cells, outperforming Taxol. Apoptosis followed by cell cycle stoppage in the G2/M phase was determined through mechanistic investigations. The 5-amide linkage increased the affinity of binding to the ATP site of AURKA, as determined by molecular docking(Song et al., 2015).

To develop inhibitors of Aurora kinase A, Lomov *et al.* utilized a computational methodology to design imidazolyl pyridine derivatives. The compounds that were synthesized exhibited robust interactions requiring binding within the active region of AURKA.

Rashdan *et al.* generated 1,2,3-triazolyl-pyridine hybrids via molecular hybridization and assessed the antiproliferative function against HepG2 cells(Rashdan et al., 2021). Significantly, it exhibited remarkable efficacy, surpassing that of doxorubicin, while causing minimal harm

to healthy cells. In silico investigations and docking simulations both demonstrated efficacious binding to AURKB.

Furthermore, in order to overcome the oral bioavailability constraints of pyrazoloamino-pyridines inspired by VX-680, Morioko investigated a novel category of pyrazoloamino-pyridines and it has been recognised as Aurora A and B kinase dual inhibitors, demonstrating significant GI_{50} values in HCT116 cells. (Morioka, 2016).

3.2.4: Benzimidazole as an AURKA blocker

Amira *et al.* made a new group of benzimidazoles as shown in figure 3.2 with a pyrimidine group attached to them as possible Aurora kinase inhibitors. These chemicals were very effective against several types of tumor cells, such as HCT116, HepG2, and A2780. chemicals 33a and 33b were the most effective. Their ability to fight cancer was proven by finding a link between Aurora kinase A and KSP reduction, which stopped the growth of cells. The chemicals that were made were very good at stopping KSP at a single-digit nanomolar level and very bad at stopping AURKA at a low micromolar level(Abd El-All et al., 2015).

The structural traits of these compounds were confirmed even more by spectral characterization. Compound 34, which is made up of benzimidazole joined quinazolines, was studied by Sharma *et al.* as an Aurora kinase inhibitor. The in vitro test showed that compound 34a had the most powerful effect against Aurora A, beating out the standard drug ZM447439. Molecular linking investigations demonstrated that strong interactions and high energy binding are exhibited by these compounds with vital residues located in the active pocket of AURKA(Sharma et al., 2016).

This makes them a possible lead for developing drugs that can fight cancer. Magd-El-Din and his colleagues made benzimidazole-based Schiff bases and their metal complexes, then tested how well they worked against tumors. It showed the strongest activity against cancer cell lines

of the breast (MCF-7), hepatic (HepG2), and respiratory (A549). On Ehrlich's ascites malignancy (EAC) cell lines, in vivo cytotoxicity assays indicated that all formed complexes were extremely toxic, suggesting that they may have therapeutic potential for EAC. Overall, these results are very helpful for understanding how to make benzimidazole derivatives that might work against cancer by hitting Aurora kinases. (Fancelli et al., 2005).

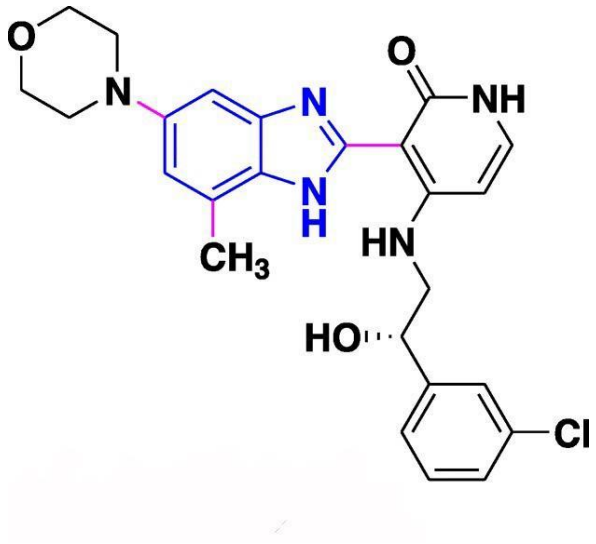


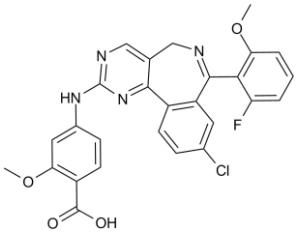
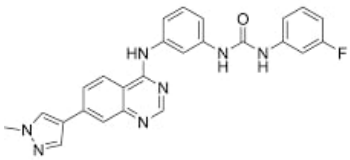
Figure 6: Chemical structure of benzimidazole (Ibrahim & Refaat, 2020)

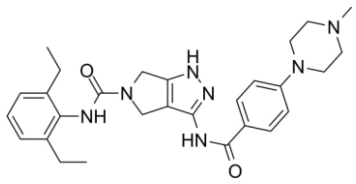
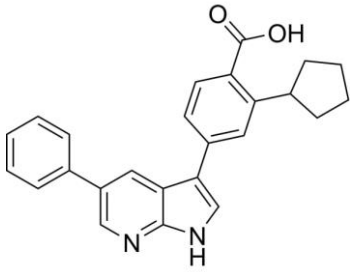
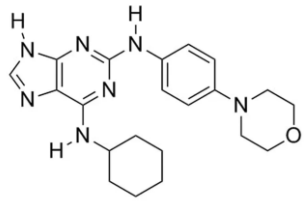
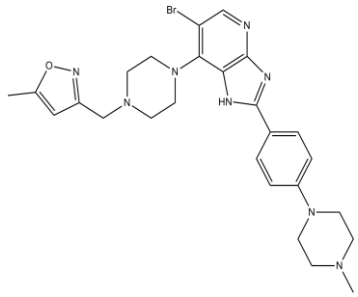
3.3: Preclinical Studies: In vitro and in vivo findings

There have been reports of over thirty AKIs that are undergoing preclinical testing; numerous articles have been devoted to their synthesis, development, and biological activity. On the basis of their origin, AKIs have been categorized as either naturally occurring compounds or synthetic compounds. Preclinical studies have provided evidence that Aurora kinase inhibitors, at varying concentrations, can selectively inhibit the activity of Aurora-A, -B, and -C kinases. The specific inhibition of these kinases is intended to interfere with their vital functions, which include mitotic progression and cell division. Additionally, there is a comprehensive overview of the outcomes is provided which are derived from in vitro and in vivo investigations conducted on these compounds. The investigation of the molecular mechanisms that govern the activity of Aurora kinase inhibitors is a fundamental objective of preclinical research.

Frequently, research examines the effects of these inhibitors on mitotic events, cell cycle regulation, and downstream signaling pathways. This information aids in clarifying the therapeutic significance of these inhibitors (Sankhe et al., 2021a). A comprehensive list of Aurora kinase inhibitors in preclinical development is shown in table 3.1.

Table 1: AKIs in Preclinical investigations

Compound Names	**IC_{50} values**	**Preclinical activity observed both in vivo and in vitro settings**
CCT137690 	A: 42 nM B: 198 nM C: 227 nM	• Increased p53 stability and reduces histone H3 activation in HCT116 cells • Activates p21 in several cell lines through both p53-dependent and p53-independent mechanisms. • Histone H3 phosphorylation decreased by fifty percent, and tumor growth was inhibited by 57.7 percent, in experimental rodents infected with HCT116 xenografts.
SP-96 	A: n/a B: 0.316 nM C: n/a	• Selective reduction of growth in NCI60 screening • GI_{50} < 1000 nM for the majority of the cell lines • Inhibits Aurora B that operates via ATP-non-competitive mechanisms.
PHA-680632	A: 27 nM B: 135 nM C: 120 nM	• Polyploidy arises in tumor cells • By enhancing cellular apoptosis and decreasing cell proliferation in tumors, it has been observed

		to inhibit the growth of various types of malignant cells in mice xenografts.
GSK650394 	A: n/a B: 5.28 nM C: n/a	• Induces cell cycle arrest specifically in the G2/M phase. • Inhibits the growth of cancer cells and Aspergillus fumigatus.
Reversine 	A: 400 nM B: 500 nM C: 400 nM	• Suppresses the growth of cells and triggers targeted cell death by altering the activity of caspase 3 and Bax/Bcl-2 proteins • Demonstrates an IC_{50} value of 660 nM for the A3 adenosine receptor in humans • Blocks the process of histone H3 phosphorylation in HCT116 cells.
CCT137690 	A: 15 nM B: 25 nM C: 19 nM	• Exhibits antiproliferative effects in several malignant cell lines • Promotes cell death, multiplication, and mitotic a bnormalities in cancer cells.

Note: n/a; Data is not available

3.4: Aurora Kinase inhibitors in clinical trials according to recent progress

Clinical trials involving Aurora kinase inhibitors are establishing themselves as a promising area of investigation in cancer research. As their involvement in the development of cancer has been established, kinases represent an appealing therapeutic target. The main purpose of the clinical trials is to assess the safety, efficacy, and tolerability of Aurora kinase-targeting small-molecule inhibitors. Certain inhibitors have already exhibited promising results in preclinical studies, demonstrating the ability to inhibit the growth of cancer cells and ultimately induce apoptosis. The variety of cancer types involved in the trials is representative of the extensive spectrum of malignancies in which Aurora kinases are implicated. Scholars and practitioners are extremely motivated to comprehend the therapeutic effects of these inhibitors, both when used in isolation and in conjunction with established treatments. At least seventy clinical trials evaluating the efficacy of Aurora kinase inhibitors have been initiated since the initial trials utilizing these compounds commenced in 2005 (Martin et al., 2017). Table 3.2 displays the current state of certain compounds in clinical phase that act as inhibitors of Aurora kinase.

Table 2: Clinical investigations of AKIs

AKIs in clinical phase III

Drugs	Clinical Phase	indication	Current status	IC_{50} values (µm)		Reference
				AURKA	AURKB	
Barasertib (AZD1152) AstraZeneca	Phase III	Acute myeloid leukaemia, Advanced solid malignancies	Completed	1.369	0.00037	(Kovacs et al., 2023a)
CS2164 Cancer research uk	Phase III	Advanced solid tumors	Completed	-	0.009	(Kovacs et al., 2023a)

AKIs in clinical phase II

Drugs	Clinical phase	indication	Current status	IC_{50} values(µm)		Reference
				AURKA	AURKB	
Danusertib Nerviano Pharmaceuticals	Phase II	Leukemia, solid tumors	On going	0.013	0.079	(Kovacs et al., 2023a)
ENMD−2076 University health	Phase II	Leukemia, solid tumors	On going	0.014	0.35	(Sankhe et al., 2021b)

network, Toronto						
AT92 Astex Pharmaceuticals	Phase II	Leukemia, solid tumors	Terminated	0.003	-	(Borah & Reddy, 2021)

AKIs in clinical phase I

Drugs	Clinical phase	Indication	Current status	IC_{50} values(μm) AURKA	AURKB	Reference
AZD2811 AstraZeneca	Phase I	Advanced solid tumors, NSCLC	On going	-	0.37	(Johnson et al., 2023)
AMG 900 Amgen	Phase I	Solid tumors, acute myeloid leukemia	On going	0.005	0.004	(Kovacs et al., 2023a)
PF− 03814735 Pfizer	Phase I	Solid tumors	On going	0.0008	0.005	(Sankhe et al., 2021c)

3.4.1: TAK-901

The IC_{50} values for its activity against AURKA and AURKB are 0.021 and 0.015 µM. In vitro investigations into enzyme inhibition demonstrated that TAK-901 inhibited AURKA and AURKB in a time-dependent manner. Using an affinity constant of 0.00002 M, the interaction between TAK901 and AURKB was determined (Farrell et al., 2009b). To figure out the maximal tolerated dose in patients with advanced solid tumours or lymphoma, a Phase 1 study was undertaken. The aforementioned trial contributed to the determination of the optimal phase 2 dose, infusion duration, and predictive pharmacokinetics of TAK-901. The results have not yet been disclosed(Kovacs et al., 2023a).

3.4.2: Barasertib (AZD1152)

Barasertib, as shown in figure 3.3, is an ATP-competitive inhibitor of Aurora B, belonging to the pyrazoloquinazoline derivative class. IC_{50} = 0.37 nM Aurora-B is significantly and selectively inhibited by barasertib. Extensive investigations have been conducted in clinical trials involving phases I, II, and III. Fifteen clinical trials have been undertaken in all. The malignancies being investigated are acute myeloid leukemia (in phases I, II, and III, ongoing), refractory diffuse B-cell lymphoma (in phase II, with limited anti-tumor response and administration issues), small-cell lung cancer (in phase II, currently recruiting participants), and advanced solid tumors (in phase I, with manageable effects). In general, Barasertib shows potential as an effective inhibitor of Aurora B for treating cancer. However, further clinical trials are necessary to evaluate its effectiveness in other types of cancer (Kovacs et al., 2023b).

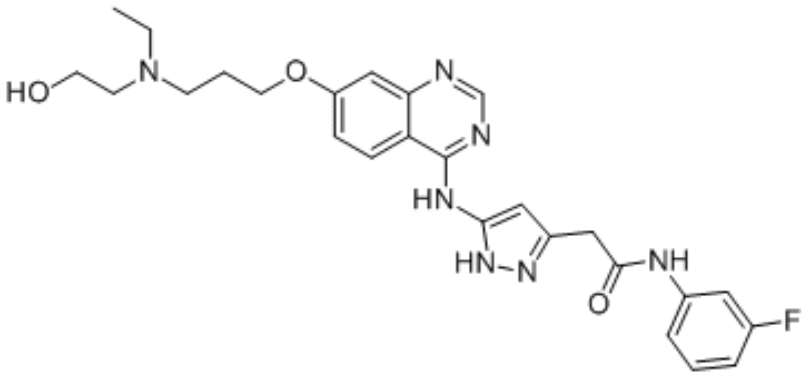


Figure 7: Structure of Barasertib (AZD1152)

3.4.3: MLN8054

MLN8054 is a potent inhibitor of Aurora-A, a kinase that competes with ATP. Figure 3.4 illustrates the molecular structure of this molecule. This chemical exhibited a selectivity that was 40 times higher for Aurora-A compared to Aurora-B. The concentration at which AURKA is inhibited is 4 nM(IC_{50}). The suppression of growth of numerous cell lines in a laboratory setting was observed with compound 61, as indicated by IC_{50} values varying between 0.11 µM and 1.43 µM. Compound 61 caused a disruption in the spindle formation, which is the fundamental work flow of Aurora-A, within the cells. As a result, the inhibitory impact of Aurora-A on cultured cells was more pronounced than that of Aurora-B. These defects impede the process of mitosis by facilitating the accumulation of G2/M. The experimental results indicate that compound 61 exhibited the ability to disrupt DNA double strands in androgen-resistant prostate cancer. Treatment of the tumour tissue with this compound increased both tubulin staining and beta-galactosidase activity, according to research conducted on an HCT-116 xenograft model(Manfredi et al., 2007; Moretti et al., 2011). Two phase I clinical trials targeting advanced solid tumours have been started.(Kovacs et al., 2023b)

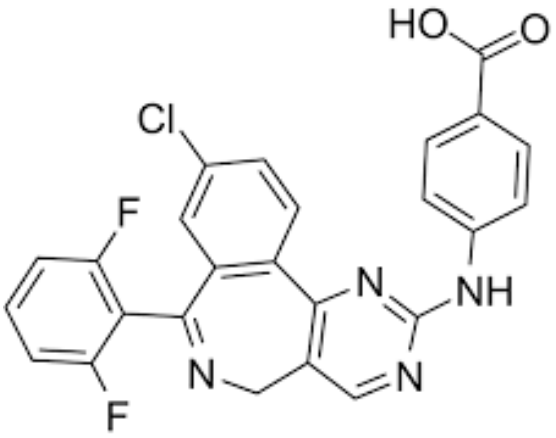


Figure 8: Structure of MLN8054

3.4.4: Danusertib

Danusertib as shown in figure 3.5, is an Aurora B inhibitor that competes with ATP and is derived from 3-aminopyrazole. There are currently six clinical studies underway. These trials are specifically studying multiple myeloma (phase II terminated), chronic myeloid leukemia (phase II ongoing), accelerated or blast phase Philadelphia chromosome-positive acute lymphoblastic leukemia (phase I ongoing), prostate cancer (phase II ongoing), and advanced solid tumor malignancies (phase II ongoing)(Kovacs et al., 2023b).

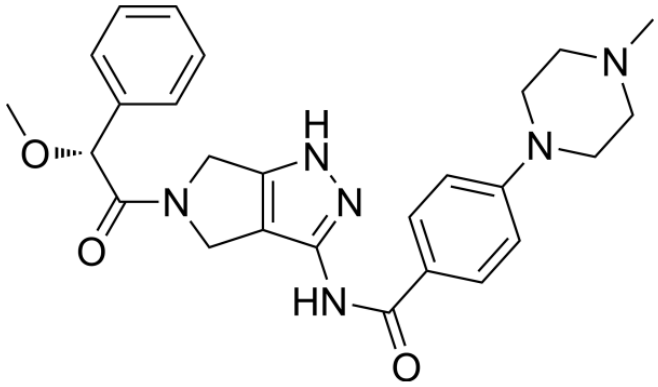


Figure 9: Chemical structure of Danusertib

3.4.5: AMG-900

AMG 900 is a compound that is derived from phtalazinamine. The compound demonstrates potent inhibitory effects on three kinases, namely AURKA, AURKB, and AURKC, as indicated by its IC50 values of 0.005, 0.004, and 0.001 µM, correspondingly. According to in vitro research, it hindered the autophosphorylation of AURKA and histone H3 on Ser10 of AURKB in HeLa cells. According to in vivo tests, it was found that the proliferation of HCT-116 cells was suppressed and the activity of histone H3 was stopped in a manner that depended on the dosage (Carducci et al., 2018).

Two clinical trials have been conducted on malignancy targets, comprising acute myeloid leukaemia (phase I, tolerable results) and advanced solid tumour (phase I, tolerated) (Kovacs et al., 2023b). Figure 3.6 below illustrates the chemical structure of this compound.

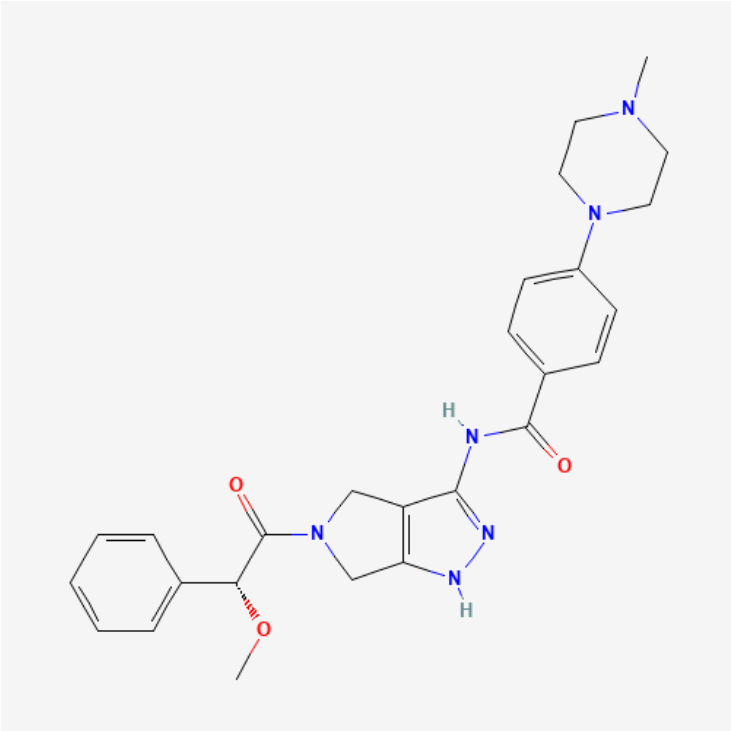


Figure 10: Chemical Structure of AMG-900

3.4.6: GSK1070916

GSK1070916, an azaindole-based inhibitor, is recognized as a reversible, ATP-competitive antagonist targeting Aurora B. Demonstrating significant efficacy, it exhibits IC_{50} values of 0.38 and 1.5 nM for Aurora B and C, respectively, with a notable >250-fold preference for Aurora B over Aurora A(Adams et al., 2010). In in vivo experiments involving human lung cell carcinoma lines A549, an IC_{50} of 7 nM was observed. Across more than 100 diverse human cancer cell lines, the compound displayed an IC_{50} below 10 nM, accompanied by a promising reduction in tumor growth. Mouse xenograft studies revealed its anticancer effectiveness against human colon, lung, and breast malignancies(Hardwicke et al., 2009). The structural composition of this compound is visually represented in figure 3.7 below.

Ongoing phase I clinical trials aim to unveil further insights into its potential as a treatment for advanced solid malignancies(Kovacs et al., 2023a).

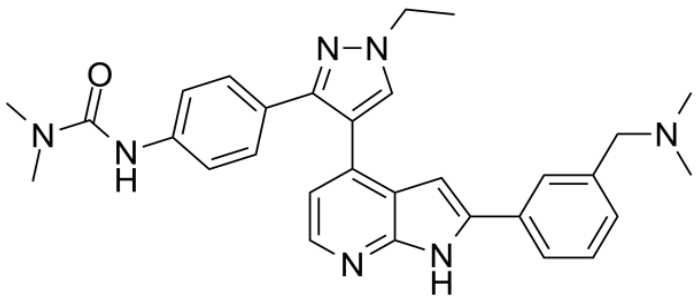


Figure 11: Chemical Structure of GSK1070916

3.5: FDA approved Aurora Kinase Inhibitors:

In 2023, a biopharmaceutical company named “Puma Biotechnology” announced that alisertib, a drug that inhibits the activity of Aurora A and is taken orally, has been granted Orphan Drug Designation by the U.S. Food and Drug Administration (FDA) for the treatment of small cell lung cancer (SCLC). Small cell lung cancer (SCLC) is an extremely deadly kind of lung cancer that offers limited therapeutic choices for patients who have advanced beyond platinum-based

chemotherapy. Alan H. Auerbach, the CEO, President, and Founder of Puma, expressed enthusiasm about the FDA's recognition, highlighting the company's dedication to advancing alisertib for SCLC treatment. Given the urgent need for new therapies, Puma anticipates commencing the Phase II trial (Study PUMA-ALI-4201) for alisertib in small cell lung cancer.

Alisertib (MLN8237), as shown in figure 3.8 is a highly selective, reversible ATP competitive inhibitor with an IC_{50}<10 nM for AURKA. Millennium has developed MLN8237, an orally administered AKI with high selectivity, which functions by selectively targeting the ATP-binding site of AK. With an IC_{50} of 0.0012 M, in vitro investigations demonstrated that AURKA exhibited a selectivity that was 200 times greater than that of AURKB. The compound effectively suppressed the phosphorylation of AURKA and caused a substantial reduction in the number of cells in the M phase of multiple myeloma (MM) cell lines (IC_{50} = 0.003–1.71 μM). Tumor growth suppression was documented through in vivo investigations, which revealed a reduction in both size of tumors and growth at concentrations of 15mg/kg and 30mg/kg. In the clinic, Alisertib was evaluated in patients with peripheral T-cell lymphoma (PTCL) that had relapsed or become resistant. The drug was administered orally for seven consecutive days (Cycle Days 1–7) for a total of twenty-one days (up to 148 weeks) at a dose of 50 mg enteric-coated tablet formulation, twice daily, with positive results.

The side effects that were reported most frequently were neutropenia (47 percent of patients treated with Alisertib) and anemia (53 percent of patients treated with Alisertib) (O'Connor et al., 2019).

Orphan Drug Designation, granted by the FDA, is reserved for investigational therapies addressing rare diseases affecting fewer than 200,000 people in the United States. This designation offers various advantages for drug developers. In August 2023, Puma received FDA clearance for its Investigational New Drug application, allowing clinical development of

alisertib monotherapy for extensive stage SCLC. The company expects to initiate the Phase II and they anticipate that the research will offer essential understanding regarding the clinical effectiveness of alisertib in small cell lung cancer. Specifically, the focus is on patients with molecularly defined tumors that could potentially be addressed using an aurora kinase A inhibitor such as alisertib (Puma Biotechnology, n.d.).

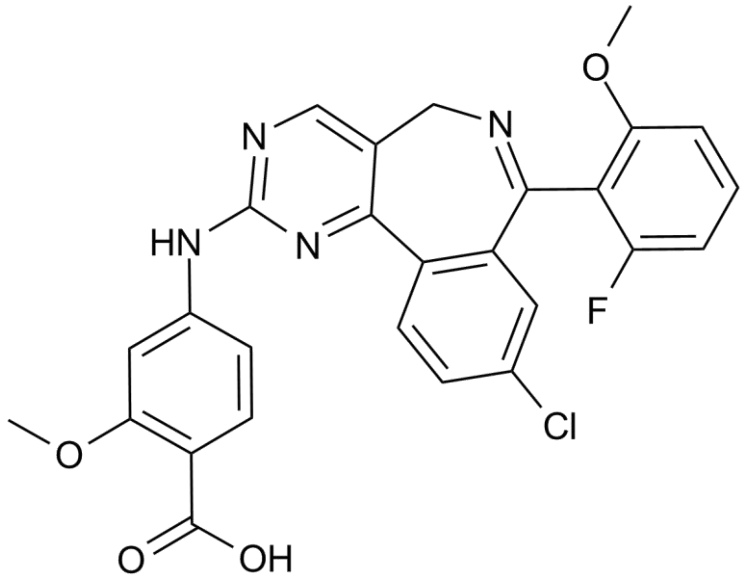


Figure 12: Chemical structure of Alisertib

3.6: Combination therapy with AKIs

The integration of targeted therapeutics with AKIs: The concurrent targeting of several oncogenes may increase the efficacy of AKIs. The ability of HDAC inhibitors to inhibit the expression of AURKA in diverse array of cancer cells has been demonstrated in studies. Moreover, AKIs are capable of inhibiting the functionality of HDAC proteins. This implies that the concurrent use of AKIs and HDAC inhibitors may yield synergistic outcomes. Previous studies have established that the HDAC inhibitor vorinostat augments the cytotoxic activity of MK-0457 against breast cancer and leukaemia cells(Chuang et al., 2013).

In addition, it has been demonstrated that the concurrent administration of vorinostat and MK-0457 or MK-5108, Aurora kinase inhibitor, increases lymphocyte lysis through the inhibition

of c-Myc, hTERT, and microRNA expression. The combination of AURKA inhibitor and the HDAC inhibitor romidepsin has a significant synergistic effect by influencing cytokine regulation, according to a study on T-cell lymphomaAMG900 and vorinostat inhibit cell growth synergistically when administered concurrently, in both laboratory and living organism contexts(Park et al., 2004). A clinical trial examining the combination of AURKA inhibitor and vorinostat for the treatment of relapsed/refractory lymphoid malignancies in patients has shown encouraging results in terms of clinical efficacy and safety. The application of EGFR inhibitors has transformed the treatment of NSCLC(Shah et al., 2019).

EGFR inhibitor resistance has been attributed to various mechanisms, one of which involves the activation of supplementary oncogenic proteins. Recent research indicates that EGFR-mutant LUAD cells that are resistant to third-generation EGFR inhibitors are susceptible to AKIs(Shah et al., 2019).

Combining AKIs and EGFR inhibitors further inhibits tumour growth in an EGFR-mutant LUAD PDX model by a substantial margin. There is potential for synergistic anticancer effects when both BRD4 and AURKA, which modulate the MYC gene at translational and posttranslational levels, are targeted. MLN8237 treatment combined with JQ1 treatment to inhibit BRD4 activity increases cell mortality in expressing human glioma cells(Ratushny et al., 2012).

Neuroblastoma is efficiently combated in vitro and in vivo by the combination of I-BET151 which is a BET inhibitor & Aurora A kinase inhibitor, with or without MYCN amplification. Two studies investigated the effectiveness of melanoma treatments that combined p53-activating MDM2 antagonists and senescence-inducing AKIs(Alcaraz-Sanabria et al., 2017). Antagonism of AURKA and MDM2 with MLN8237 and Nutlin-3 halted the growth of melanoma by inducing senescence, growth arrest, and increased immune infiltration and

clearance (Vilgelm et al., 2015). Protlin-3 and MK-0457 administered concurrently induced the activation of postmitotic checkpoints in the pseudo-G1 phase, resulting in proapoptotic signalling and mitochondrial apoptosis in AML, according to one study. In addition to AURKA, preclinical investigations have identified a number of molecules that may serve as targets, including SRC, CHEK1, mTOR, WEE1, PDK1, and MEK. (Maria et al., 2021)

The synergy between AKIs and immunotherapy: The application of targeted monoclonal antibodies and immunotherapy has been extensive in the treatment of cancer. These agents have the potential to augment treatment efficacy when combined with AKIs. For example, MK-5108 improves the inhibitory effect of anti-ganglioside (GD2) antibody 14G2a against human neuroblastoma cells. This enhancement is associated with an increase in PHLDA1 and p53 protein levels and a decrease in N-Myc expression(Horwacik et al., 2013). In IMR-32 neuroblastoma cells, concurrent administration of the anti-GD2 14G2a antibody and MK-5108 also stimulates autophagy. Antibody activating death receptor 5 has been identified as the agent responsible for inducing substantial cellular demise in tumour cells that are undergoing MLN8237-induced therapy-induced senescence(Brodeur & Bagatell, 2014).

The combination group demonstrated a significant decrease in tumour growth in melanoma xenografts derived from patient tissues and cell lines. Alisertib generates an immune microenvironment that combats cancer by increasing activated CD8+ and CD4+ T lymphocytes and decreasing myeloid-derived suppressor cells (Brodeur & Bagatell, 2014). Significantly, For the treatment of breast cancer cells, simultaneous administration of alisertib and a PD-L1 antibody has shown a synergistic effect. As a potential cancer treatment strategy, the combination of anti-PD-1/PD-L1 immune checkpoint therapy and AKI treatment is highly encouraging.(Du et al., 2021)

3.7: The limitations of Aurora Kinase inhibitors

The effectiveness of cancer treatment faces a major obstacle due to the existence of inactive or resistant cells, leading to potential disease recurrence. Increased expression of drug transport pumps often leads to elevated drug expulsion, commonly causing resistance to multiple drugs. Additionally, the activation of DNA repair processes and compromised apoptosis further contributes to the development of drug resistance. Research has emphasized the presence of mutations in the p53 tumor suppressor gene, observed in more than 50% of human malignancies, including colorectal cancer.

Madhu Kollareddy and colleagues have identified CYC116 as an inhibitor of AURKA, AURKB, AURKC, and VEGFR2. However, its efficacy is compromised in tumors overexpressing the antiapoptotic Bcl-xL protein, potentially leading to insensitivity to drugs like AZD1152, VX-680, and MLN8054 due to high cross-resistance. Combining CYC116 with a Bcl-xL inhibitor is suggested as a potential strategy to overcome or prevent resistance to AURK inhibitors. Guo *et al.* observed that the AZD1152 AURK blocker built up resistance in SW620 and MiaPaca cell lines by increasing the expression of BCRP and PgP (Guo et al., 2009).

Furthermore, Seamon et al. identified an increase in BCRP expression in HeLa cell lines resistant to JNJ-7706621 (Seamon et al., 2006), and Girdler *et al.* identified multiple AURKB mutations in HCT116 cell lines that were resistant to ZM447439 (Girdler et al., 2008). By employing 2D electrophoresis and MALDI TOF/TOF, Rita Hrabakova *et al.* analyzed the protein composition of HCT116 colon cancer cells and identified platelet-activating factor acetyl hydrolase and GTP-binding nuclear protein Ran as factors that play a role in the resistance of these cells to ZM447439. In the absence of p53 effect, serine hydroxymethyltransferase was discovered to promote tumor growth in CYC116-resistant cells.

Particularly noteworthy are the proteins serine hydroxymethyltransferase, serpin B5, and calretinin, which have the potential to assist combination therapies in surmounting resistance.

In addition, resistance to AURK inhibitors was observed in CCRF-CEM (a leukemia cell line) and A549 (a lung adenocarcinoma) cell lines, where overexpression of these proteins was detected. This suggests that they may serve as viable targets for overcoming drug resistance in cancer. Hence, it is critical to comprehend the processes that give rise to drug resistance in order to identify promising targets for anti-cancer medications that can specifically eradicate resistant cells at distinct stages of the disease.

Chapter 4: Conclusion

To summarize, this review has presented a thorough analysis of the present status of studies on Aurora kinase inhibitors as potential medications for treating cancer and emphasizes the vital role of Aurora kinases, specifically Aurora A and B, in regulating essential cellular processes related to mitosis and cell division. Targeted inhibition of these kinases has become a strategic method to interrupt abnormal cell cycle progression, trigger mitotic catastrophe, and promote death in cancer cells. Also, Aurora kinase inhibitors have exhibited promise in treating an extensive variety of malignancies, including glioblastoma multiforme, ovarian cancer, leukemia, hepatocellular carcinoma, colorectal cancer, and lung cancer, as well as gastrointestinal stromal tumors. Significantly, these inhibitors have demonstrated effectiveness in preclinical and in vitro models, which has identified the initiation of clinical trials to evaluate their safety and efficacy in human. Moreover, there is a current demand for the creation of anticancer drugs that have reduced side effects and beneficial pharmacological qualities. Targeting Aurora kinases can achieve this potential. The literature reports and clinical trial data have demonstrated that these inhibitors may play a pivotal role in the next generation of anticancer therapies, offering hope for improved outcomes and enhanced patient survival.

Chapter 5: Future perspective and therapeutic potential

The current main research approach of Aurora kinase inhibitors is focused on highly selective Aurora kinase inhibitors or multi-target inhibitors used either alone or in combination therapy. So, the potential of Aurora kinase inhibitors as anticancer agents in the future is extremely encouraging for the advancement of cancer treatment. Aurora kinases serve as vital cellular division regulator and their dysregulation is frequently observed in a variety of malignancies. Therefore, they are desirable drug development targets. Aurora kinase inhibitors possess therapeutic potential due to their capacity to impede the progression of the cell cycle, stimulate apoptosis, and selectively target malignant cells exhibiting aberrant Aurora kinase activity.

- **Integration of Therapies:** By Examining the potential for synergistic effects when Aurora kinase inhibitors are combined with well-established therapeutic techniques such as chemotherapy, immunotherapy, and targeted therapies. Combinatorial strategies have the potential to augment treatment effectiveness, surmount resistance, and mitigate adverse effects.

- **Integration of Precision Medicine:** Create individualized therapeutic approaches by considering the distinct molecular attributes of tumours. Targeting Aurora kinases with precision could result in more efficient and personalized therapy, hence reducing the occurrence of off-target effects.

- **Specific Isoform Targeting:** Develop inhibitors that exhibit improved selectivity towards particular isoforms of Aurora kinase. By mitigating the detrimental consequences linked to non-specific inhibition, this methodology has the potential to elevate the therapeutic index and bolster the safety profile of these agents.

- **Exploring the Mechanisms of Resistance:** Examine in greater detail the molecular processes that contribute to resistance to inhibitors of Aurora kinase. The

comprehension and resolution of resistance mechanisms will be critical in the advancement of therapeutic strategies that are more robust and long-lasting.

- **Innovative Systems for Drug Delivery:** Explore novel approaches to drug delivery in order to enhance the tissue distribution and bioavailability of inhibitors of Aurora kinase. By minimizing systemic adverse effects and optimizing the therapeutic concentration at the target site, enhanced drug delivery strategies are possible.
- **Utilizations in Pediatric Oncology:** Considering the role of Aurora kinases in the process of regular cell division, examine the safety and effectiveness of Aurora kinase inhibitors in pediatric cancer. Customizing these inhibitors for pediatric patients could potentially circumvent the distinct obstacles and needs associated with childhood malignancies.
- **Biomarker Formation:** Determine and authenticate dependable biomarkers for prognosticating the impact of Aurora kinase inhibitors. Patient selection will be facilitated by robust biomarkers, which will enable more precise prognostications of treatment efficacy.
- **Measures to Prevent Metastasis:** Evaluate the capacity of inhibitors of Aurora kinase to impede or restrict the spread of metastases. Aurora kinase inhibition may disrupt processes that are critical for metastasis, thereby providing novel therapeutic approaches to manage advanced stages of cancer.

In the future, the clinical use of inhibitors of Aurora kinase as anticancer drugs is expected to include a holistic approach that incorporates precision medicine, combination therapies, and new ways to boost the therapeutic potential of these medications. In order to fully realise the full range of benefits that these inhibitors can give in the ongoing fight against cancer, it is essential that research and clinical exploration be continue.

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