Aurora-B Kinase Inhibitors for Cancer Chemotherapy

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Abstract: Cancer cells undergo mitosis frequently, and many mitotic regulators are aberrantly expressed in these cells. Members of the Aurora family of serine/threonine kinases are expressed during mitosis and carry out vital functions in chromosome alignment, segregation and cytokinesis. Here we review the functions of Aurora-B kinases in mitosis and summarize the current literature on Aurora-B kinase inhibitors. In the process of developing these inhibitors as anticancer drugs, the Aurora kinase inhibitors have also helped to advance our understanding of the role of Aurora kinases in mitosis. The mechanism of action and structure-activity relationship of a selective Aurora-B inhibitor are also discussed. The future may see mechanism guided design of chemotherapy combinations that include these cell-cycle phase-specific drugs. The therapeutic potential of Aurora-B inhibitors is promising.

Key Words: Oncogenes, Aurora-B kinase, mitosis, cytokinesis, cancer, drug target.

INTRODUCTION

When compared with normal cells, a major distinguishing characteristic of cancer cells is frequent mitosis, which creates a therapeutic window for most of the antineoplastic drugs. Mitosis is a complex biological process. Aurora family kinases regulate many steps in mitosis including centrosome maturation and separation, mitotic spindle assembly, chromosome segregation and cytokinesis. The survival of daughter cells depends on the accurate handling of the genetic material, and multiple checkpoints have evolved to ensure accurate copying and equal segregation of the copied genome in a temporally and spatially coordinated manner. Dysregulation of Aurora kinases due to gene amplification and protein overexpression results in aneuploidy and may contribute to tumorigenesis.

The Aurora kinases are conserved during eukaryotic evolution. While the genomes of yeast encode only one Aurora kinase (Ipl1 in budding yeast and Ark1 in fission yeast), higher eukaryotes express two or more members in the Aurora kinase family. In mammals, there are 3 members (Aurora-A, -B, and -C). Aurora-A (also known as Aurora 2, ARK1, and BTAK) is present among all vertebrates. Aurora-B (also known as Aurora 1, ARK2, and AIM1) and Aurora-C (also known as Aurora 3 and AIK3) came into existence from gene duplication events during the evolution of mammals [1].

A high level of interest in Aurora kinases as drug targets for cancer chemotherapy has generated many small molecule inhibitors of Aurora kinases, which are under investigation in translational studies and early phase clinical trials. The amount of information about aurora kinases and their inhibitors has exploded in recent years. An ongoing debate is which Aurora kinase is the best drug target, and so far Aurora-B seems to be the leading drug target [2]. In this short review, we shall focus on Aurora-B inhibitors and their potential as novel entries in the antineoplastic armamentarium.

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AURORA-B KINASE BIOLOGY

The biology of Aurora-A, B and C has been reviewed extensively [3-5]. The expression of Aurora kinases depends on the phase of the cell cycle. The levels of Aurora-A and Aurora-B are very low in the non-M phases. The subcellular locations of the Aurora kinases also change in different phases of mitosis. During prophase, Aurora-A is concentrated at the centrosomes while Aurora-B starts to appear in the nucleus [6]. During metaphase, Aurora-A is near the spindle poles on the microtubules while Aurora-B is at the centromeric regions of chromosomes as a chromosomal passenger protein. During anaphase, Aurora-A is predominantly at the polar microtubules while Aurora-B relocates from the centromeres to the spindle mid-zone (spindle equator) where the microtubules from the opposite poles interdigitate [7]. During cytokinesis, Aurora-B accumulates in the cell cortex at the cytoplasmic cleavage furrow before finally concentrating at the midbody. The temporo-spatial dynamics of Aurora-B is determined by its binding to tubulin [8], regulation by other kinases and proteasomal degradation after ubiquitination by the Cul3-containing [9] and/or APC-cdh1 E3 ligases [10].

Molecular details about the function of Aurora-B have emerged. Regarding mitotic chromosome condensation, Aurora-B directly phosphorylates histone H3, not only at Ser10 but also at Ser28. The level of Ser28 phosphorylation is rendered undetectable by protein phosphatase 1 (PP1) just prior to entry into mitosis [11]. A complex of histone deacetylase 3 (HDAC3) with A-Kinase-Anchoring Proteins (AKAP95) and a nuclear protein homologous to AKAP95 (HA95) acts on mitotic chromosomes to provide a hypoacetylated H3 tail that is the preferred substrate of Aurora-B kinase. Phosphorylation of H3 at Ser10 by Aurora-B leads to

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dissociation of heterochromatin protein 1 (HP1) from methylated histone H3 lysine 9 residues on mitotic heterochromatin. This transcription-independent pathway is required for normal progression through mitosis and is another mechanism of antineoplastic action of HDAC inhibitors [12].

Aurora-B binds three other chromosome passenger proteins — inner centromere protein (INCENP), survivin and borealin. This quaternary complex may be required to target the Aurora-B kinase to both centromeres and the spindle. Survivin stimulates Aurora-B kinase activity and helps correctly target Aurora-B to its substrates during the cell cycle [13]. The phase of the cell cycle, microtubule attachment, and Aurora-B kinase activity regulate the rate of survivin turnover at the centromeres [14]. The assembly of the outer kinetochore, which binds microtubules to control chromosome movement, is restricted to mitosis, whereas the inner kinetochore remains tethered to the centromeres throughout the cell cycle. Aurora-B and PP1 coordinate and mediate cell cycle-dependent changes in kinetochore assembly by regulating the phosphorylation status of kinetochore substrates [15]. The affinity of mitotic checkpoint kinase Budding Uninhibited by Benzimidazoles1 beta (BubR1) for kinetochores is regulated by the survivin/Aurora-B complex. These functions of Aurora-B are essential for chromosome biorientation, a requirement for proper chromosome segregation [16]. Checkpoint kinase 1 (Chk1) is a component of the DNA damage checkpoint, and it augments spindle checkpoint signaling by regulating Aurora-B and BubR1 [17]. Aurora-B is also required for the tension-sensitive mitotic-checkpoint com-ponents to maintain mitotic-checkpoint arrest [18]. Aurora-B phosphorylates mitotic centromere-associated kinesin (MCAK) at merotelic attachments and regulates the microtubule depolymerase activity of MCAK [19]. Aurora-B regulates the cleavage furrow-specific vimentin by phosphorylation and controls vimentin filament segregation in the cytokinetic process [20].

ASSOCIATION OF AURORA-B KINASE WITH CANCER

Aurora-B has not been shown to behave like an oncogene. However, it is a protein required for mitosis and is expressed during mitosis. There is some circumstantial evidence to suggest that Aurora-B may be involved in carcinogenesis. Aberrant expression of Aurora-B together with alternative splicing may play a role in hepatocarcinogenesis. Cell cycle phase-inappropriate phosphorylation of histone H3 in the entire cell cycle may enhance proliferation of liver cancer cells [21]. Transcription of AURKB gene and Aurora-B protein level are frequently increased in lung cancer samples compared with matched normal tissue [22, 23]. In lung cancer samples, Aurora-B expression correlates with expression of survivin in the nucleus, but not with expression of p16 [23]. Progressive increase in nuclear expression of Aurora-B is observed when normal prostate glands are compared to hyperplastic glands and to prostate cancers with increasing Gleason grades [24]. Immunohistochemical analysis of archive samples showed high expression of Aurora-B in anaplastic thyroid carcinomas while Aurora-B expression is not detectable in normal thyroid tissue [25]. These circumstantial associations may simply reflect the high mitotic rate in aggressive cancers.

Aurora-B may be a prognostic factor predicting cancer survival. High Aurora-B expression correlates well with cell proliferation, histological dedifferentiation, and metastasis in oral cancer [26] and in the subset of radically resected lung cancer patients [23]. High Aurora-B expression predicts short survival for patients with lung adenocarcinoma [23] and patients with glioblastoma multiforme [27], and aggressive hepatocellular carcinoma recurrence [28].

WHAT ARE THE BIOLOGICAL CONSEQUENCES OF LOSS OF AURORA-B FUNCTION?

Genetic disruption of Aurora-B expression, inhibition of Aurora-B function by dominant negative mutants and small molecule Aurora-B inhibitors have yielded important information about the biologic consequences of loss of Aurora-B function. In contrast to inhibition of Aurora-A which leads to centrosome separation abnormalities and formation of monopolar spindles resulting in arrest of the cell cycle in mitosis, inhibition of Aurora-B will force tumor cells through a catastrophic mitotic exit leading to polyploid cells that rapidly lose viability [29]. Inactivation of Aurora-A activates checkpoint kinase BubR1 in an Aurora-B-dependent manner, and the mitotic requirement for Aurora-A kinase is bypassed when Aurora-B kinase expression is inhibited using RNAi [30]. Therefore, Aurora-B mediates the mitotic arrest in the absence of Aurora-A, and the mitotic requirement for Aurora-A is bypassed in the absence of Aurora-B [30].

Following inhibition of expression of Aurora-B by RNAi or antagonization of Aurora-B function by overexpressing a dominant-negative kinase mutant, Aurora-B is no longer detectable at centromeres. Using a dominant-negative Aurora-B kinase mutant, Aurora-B is shown to be required for kinetochore function [7] and to regulate the association of motor proteins with kinetochores to control microtubule organization, chromosome movement and the release of the spindle checkpoint [31]. The phenotype of loss of Aurora-B function is consistent with a major kinetochore defect. By preventing kinetochores from resolving inappropriate microtubule interactions, loss of Aurora-B function prevents chromosome alignment and segregation (Fig. **1**).

The functional status of the post-mitotic checkpoint determines whether cells with loss of Aurora-B function continue to divide (become massively polyploid), arrest in a pseudo-G1 state or die from apoptosis. The post-mitotic checkpoint is a 'back-up' to the spindle-checkpoint system to prevent further proliferation of cells that have undergone massive spindle damage or cells that have abnormal genomes [32, 33]. This post-mitotic checkpoint is p53-dependent. p53 normally associates with centrosomes in mitotic cells, and abnormality in the mitotic spindles induces changes in the subcellular localization of p53 to induce cell cycle arrest in the offspring of such defective mitoses [34]. Loss of Aurora-B function increases in multinucleated cells in cells lacking p53 function [35]. In cells with defective post-mitotic checkpoint, these highly abnormal aneuploid cells continue proliferating in the presence of massive genomic instability, leading to cell death.

Cytokinesis is regulated by a complex network involving many components. Bits and pieces of mechanistic details

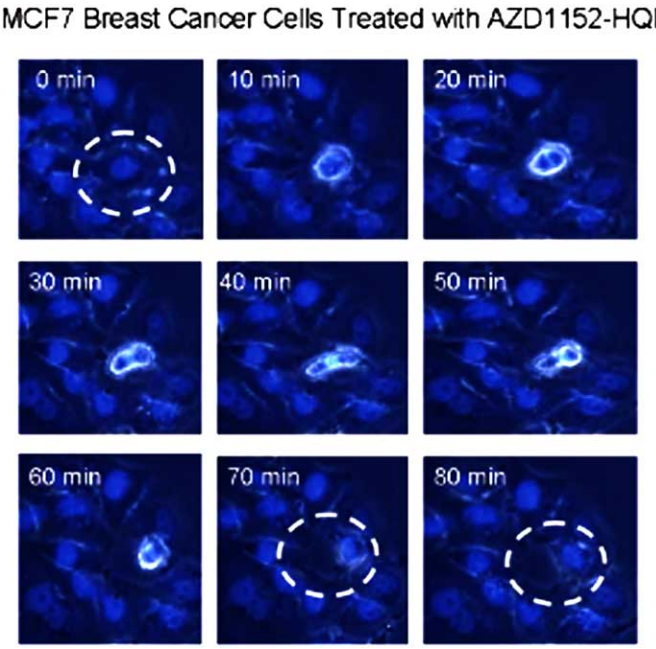


Fig. (1). Mitotic catastrophe induced by a specific Aurora-B kinase inhibitor.

Human breast cancer cells (MCF7) were incubated with AZD1152 hydroxyquinazoline pyrazol anilide [AZD1152- HQPA] 40nM for 48 hours. The DNA was stained with Hoechst-33258 dye and photographed in fluorescent microscopy and phase-contrast microscopy in time lapse at 10 minute intervals. The combined images (fluorescence + phase-contrast) are shown with the time of observation labeled in the left upper corners. The parent cell and daughter cells are indicated by the dashed ovals. The chromatin failed to separate and the mitotic cell division ended up with one anucleate daughter cell and one mononuclear cell with polyploidy.

have begun to emerge only recently. Aurora-B kinase activity is required for regulation of microtubule dynamics in order for cytokinesis to occur properly at the cell equator [36]. The central spindle, or midzone, consists of antiparallel, overlapping microtubules that form during anaphase and persist after chromosomal segregation at the midbody. Central spindle microtubules are directly in contact with and directly stimulate Aurora B kinase activity [37]. MKlp2 is involved in the localization of Aurora-B to the central spindle [38]. MgcRacGAP, a GTPase-activating protein for RhoA, controls the assembly of the contractile ring and the initiation of cytokinesis [39], and it is phosphorylated by Aurora B as well as Cdk1 but dephosphorylated by PP2A [40]. Aurora-B as well as other mitotic kinases indirectly modulates localized RhoA activation during cytokinesis through GEF-H1 [41]. Nevertheless, inhibition of Aurora-B inhibits cytokinesis resulting in multinucleate giant cells.

PHARMACOLOGIC DISRUPTION OF AURORA-B KINASE

Targeting the kinase activity with small molecules that bind and occupy the catalytic ATP binding site or substrate binding site has resulted in a list of Aurora-B kinase inhibitors with various degrees of selectivity (Table **1**). A number of Aurora kinase inhibitors have demonstrated anti-tumor activity and have subsequently entered clinical evaluation. Some that are in clinical trials (Table **2**) (AT9283, CYC116, SNS-314, PF-03814735) have few or no published studies so far, and will not be discussed. Because of the high potential of Aurora-B kinase as a drug target, the search for novel inhibitors continues [42-46]. The inhibitors that have activity against Aurora-B and have at least two published reports are discussed below.

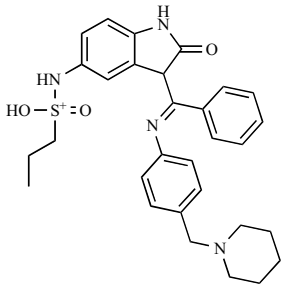
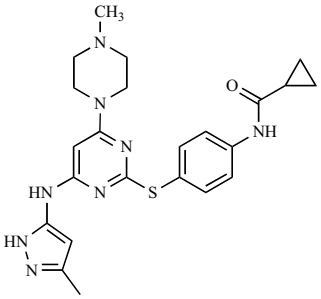
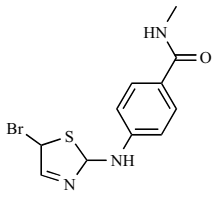
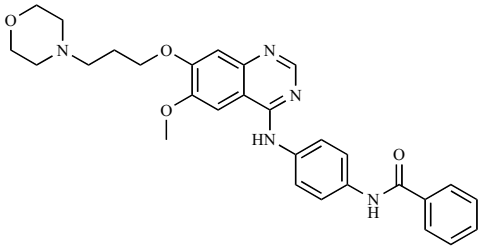
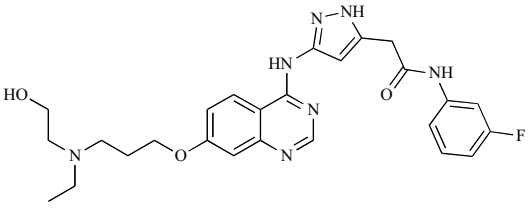
JNJ-7706621

JNJ-7706621 is a small molecule kinase inhibitor that showed potent inhibition of Aurora kinases and several cyclin-dependent kinases (CDK). It selectively inhibits proliferation of tumor cells of various tissue origins over normal human cells *in vitro* [47]. However, the multi-drug resistance protein ATP-binding cassette, sub-family G, member 2 (ABCG2) mediates acquired resistance to JNJ-7706621 and alters the absorption of the compound following administration [48].

Hesperadin

Hesperadin was identified as an inhibitor of chromosome alignment and segregation, and was found to be an inhibitor of Aurora-B function (IC_{50} of 250 nM) [49]. In addition to Aurora-B, hesperadin at 1 μM also significantly inhibits at least six other kinases, including CHK1, LCK, MAPKAP-K1, MKK1, PHK and AMPK [6]. This drug has been used to investigate the biological functions of Aurora kinases. It causes cells arrested by taxol or monastrol to enter anaphase within <1 h, whereas cells in nocodazole stay arrested for 3-5 h [49]. It causes cytokinesis failure; actin filaments, myosin II, and RhoA are distributed along the lateral and/or polar cortex in the drug-treated cells instead of localizing at the equator as in normal cells, and astral microtubules are abnormally bundled [36].

Table 1. Structures and Selectivities of Small Molecule Aurora-B Kinase Inhibitors

Compound	Structure	Aurora-A Kinase Assay	Aurora-B Kinase Assay	Selectivity Index*	Reference
Hesperadin		n. d.	IC50 = 250 nM	-	[45]
		n. d.	IC50 = 50 nM in cells	-	[77]
VX-680		*Ki = 0.6 nM*	*Ki = 18 nM*	0.033	[58]
PHA-680632		IC50 = 27 nM	IC50 = 135 nM	*0.2*	[78]
ZM447439		IC50 = 110 nM	IC50 = 130 nM	*0.846*	[79]
		IC50 = 1000 nM	IC50 = 50 nM	*20*	[29]
AZD1152-HQPA		IC50 = 687 nM	IC50 = 3.7 nM	*185.7*	[77]
		Ki = 1368 nM	*Ki = 0.37 nM*	3697	[65]

n.d. – not done.

* Selectivity Index defined as the inhibition constant (Ki) for Aurora-A divided by Ki for Aurora-B.

Ki can be calculated from the IC50 of an inhibitor in the presence of a specific substrate using the Cheng-Prusoff equation: Ki=IC50*Km/(S+Km), where S is the concentration of substrate, and Km is the substrate concentration (in the absence of inhibitor) at which the velocity of the reaction is half-maximal. When Ki data are not available, the selectivity index is estimated by dividing the IC50 for Aurora-A with the IC50 for Aurora-B and is indicated by italics.

MK-0457 (Formerly VX-680, also known as VE-465)

MK-0457 is a potent inhibitor of all 3 Aurora kinases. Although it shows selectivity against Aurora-A and Aurora-B as dual intracellular targets [50], it also inhibits both Flt-3 and Abl kinases at low nanomolar concentrations. The response of cell lines treated with this drug most closely resembles that described for inhibition of Aurora-B. MK-0457 inhibits anaplastic thyroid cancer cell lines [51], myeloma

Table 2. Current Clinical Trials with Inhibitors that also Inhibit Aurora-B Kinase

Clinical Trials. Gov Identifier	Agent	Patient Population	Status	Sponsor
NCT00443976	AT9283	Lymphoma; Unspecified Adult Solid Tumor, Protocol Specific	recruiting	National Cancer Institute of Canada
NCT00522990	AT9283	Leukemias	recruiting	Astex Therapeutics
NCT00530465	CYC116	Advanced Solid Tumors	recruiting	Cyclacel Pharmaceuticals, Inc.
NCT00560716	CYC116	Advanced solid tumors	recruiting	Cyclacel Pharmaceuticals, Inc.
NCT00424632	PF-03814735	Advanced solid tumors	recruiting	Pfizer
NCT00519662	SNS-314	Advanced solid tumors	recruiting	Sunesis Pharmaceuticals
NCT00099346	VX-680	Advanced Colorectal Cancer and Other Advanced Solid Tumors	terminated	Merck
NCT00104351	VX-680	Advanced cancer	terminated	Merck

cell lines and primary myeloma plasma cells [52] and oral squamous cancer cells [53]. MK-0457 induces endoreduplication and apoptosis preferentially in cells with compromised p53-dependent postmitotic checkpoint function [54]. It increases Bax/Bcl-2 ratio, a predictor for drug response and survival in acute myelocytic leukemia (AML) [55]. It enhances the cytotoxic effect of etoposide (VP16) on AML cells [55] and that of imatinib in K562 cells [56]. Its combination with dexamethasone improves cytotoxicity over either drug alone [52]. It also potently inhibits Abl and the imatinib resistant Abl mutant (T315I). Its effectiveness against mutant forms of Bcr-Abl makes it a potential rescue for patients with Bcr-Abl-positive leukemias resistant to imatinib and dasatinib [57]. It inhibits tumor growth in xenograft models, leading to regression of human colon cancer, pancreatic cancer and leukemia cell lines at well-tolerated doses [58]. Reversible bone marrow suppression was observed as toxicity of this drug. MK-0457 was in phase I and II trials in patients with advanced solid tumors and leukemia. A clinical safety finding of QTc prolongation was observed in one patient in a phase I trial, which has led to suspension of enrollment in clinical trials.

PHA-739358

PHA-739358 is a small-molecule 3-aminopyrazole derivative with strong activity against Aurora kinases (A and B, being more potent inhibitors of Aurora-A) and cross-reactivities against some receptor tyrosine kinases relevant for cancer such as Bcr-Abl. The phenotype of cells treated with PHA-739358 resembles that of Aurora-B kinase inhibition *in vitro* and *in vivo* [59]. This multi-target inhibitor is effective against imatinib-resistant Bcr-Abl mutations including T315I. Thus, PHA-739358 also represents a promising rescue strategy for Bcr-Abl-positive leukemias that are resistant to imatinib and dasatinib [60].

ZM447439

ZM447439 inhibits Aurora-A and -B, and interferes with the spindle integrity checkpoint and chromosome segregation. ZM447439 reduces histone H3 phosphorylation at Ser10 in Hep2 cells. It induces multipolar spindles and polyploidy, similar to the phenotype of Aurora-B inhibition [61]. It induces apoptosis in AML cell lines and primary cultures [62].

Reversine

Reversine inhibits by >33% in 16 out of 56 of kinases tested (29%), including Aurora-A, B and C, JAK2, Syk, PTK2 (FAK), TRKA, TRKB, c-kit, and c-met [63]. It causes a failure in cytokinesis and induces polyploidy, and inhibits the phosphorylation of histone H3 [64]. Therefore, the phenotype of cells treated with reversine primarily resembles that of inhibition of Aurora-B.

AZD1152

AZD1152 is a small molecule ATP binding pocket competitor and is a dihydrogen phosphate prodrug which is converted in the plasma to AZD1152-HQPA [hydroxyquinazoline pyrazol anilide]. It is derived from 5-acetanilide-3-aminopyrazole (3-pyrazole)-substituted quinazolines to achieve selectivity for Aurora-B and high solubility. This drug selectively inhibit Aurora-B [inhibition constant (Ki) = 0.36 nM] compared with Aurora-A [Ki = 1,369 nM] and a panel of 50 other kinases [65]. AZD1152 is the first Aurora-B selective inhibitor to enter clinical evaluation.

Antineoplastic effect of this drug has been demonstrated in human cancer cell lines, including colon, lung, breast, and cervix, as well as leukemia cell lines and primary acute myeloid leukemia cultures [62]. Antitumor activity (enhanced apoptosis and tumor growth inhibition) can be achieved with

either high doses of AZD1152 for short durations or lower doses for longer durations. AZD1152 potently inhibited the growth of human colon, lung, and hematologic tumor xenografts in immunodeficient mice [65]. In SW620 (a colorectal cancer cell line) xenograft-bearing athymic rats, AZD1152 induces transient suppression of histone H3 phosphorylation followed by accumulation of polyploid cells in the xenografts. Aberrant cell division concurs with an increase in apoptosis in AZD1152-treated xenografts [65]. The combination of ionizing radiation plus AZD1152 enhances tumor growth delay in xenograft mouse model as compared to radiation alone. This effect is more pronounced with p53-/- HCT116 and p53-mutant xenografts. CHK2 and 14-3-3 sigma may be required for this radiosensitizing effect of AZD1152 [66]. The fact that the radiosensitizing effects are enhanced in p53-deficient cancer cells is of great interest for further clinical investigation [66]. In rodent xenograft models, at doses that inhibited tumour growth, AZD1152 is generally well-tolerated with mild, transient myelosuppression [65, 67].

A phase I study in patients with advanced solid tumors, 2-hour infusion of AZD1152 at doses ranging from 100 to 450 mg weekly or every 2 weeks caused neutropenia in 5 of 19 patients, and was the predominant dose-limiting toxicity [68]. Three patients had disease stabilization and remained on therapy for ≥12 weeks. To establish that Aurora-B can be inhibited in humans at a safe dose, the phosphorylation status of histone H3 at serine 10 is a useful biomarker of inhibition of Aurora-B. Preclinical validation of this biomarker for Aurora kinase inhibition has led to its application in clinical trials [69].

Toxicity

As the Aurora kinases are only expressed during mitosis, non-proliferating cells would not be adversely affected by these drugs. Given that most normal cells in the body do not proliferate rapidly, Aurora-kinase inhibitors could have a broader therapeutic index than non-specific cytotoxic agents, such as alkylating agents (e.g., cisplatin, cyclophosphamide) that act in a non-cell-cycle-specific manner. There was some evidence of myelosuppression in treated mice; however, full recovery of bone marrow followed the discontinuation of treatment [65, 67]. Neutropenia was observed in a Phase I trial of Aurora-B inhibitors [68].

Summary

The majority of the multi-target inhibitors all induce similar phenotypes in cell-based assays. The phenotype of Aurora-B inhibition seems to be dominant. However, they do not inhibit cell-cycle progression, except perhaps JNJ-7706621 due to its inhibition of CDKs. Rather, drug-treated tumor cells enter and exit mitosis with normal kinetics and then go on to re-replicate their genomes again, resulting in polyploidy. This effect distinguishes these inhibitors from the classic "antimitotic" agents that interfere with microtubule function.

MECHANISM OF ACTION

The molecular structure of Aurora-B has been determined by crystallography (Fig. **2**) [45, 70, 71]. All these small molecule inhibitors target the ATP-binding pocket of aurora kinases. When the amino acid sequences of the ATP-binding pockets of Aurora-A, B and C are compared, 26 amino acid residues can be identified to line the active site (Fig. **3A**). Three Of the 26 residues lining the active site in Aurora-A, Leu215, Thr217 and R220, are different from the corresponding residues in Aurora-B and C [1]; there is no difference in these 26 residues between Aurora-B and C. The manner in which Aurora A binds to ATP [72] and ADP [73] have been modeled. Because of the high degree of conservation of the active site between Aurora-A and B, Aurora-B is expected to interact with ATP and ADP in the same way as reported for Aurora-A. There are data available from the Protein Data Bank website (http://www.pdg.org) regarding the interaction of Aurora-B with 3 inhibitors [AD6 (4-[(5-bromo-1,3-thiazol-2-yl)amino]-N-methylbenzamide): PDB ID 2vgp [45], ZM447439 (N-(4-([6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin- 4-yl]amino)phenyl)benzamide): PDB ID 2vrx [70], and hesperadin (n-[2-oxo-3-((e)-phenyl[[4-(piperidin-1-ylmethyl)phenyl]imino]methyl)- 2,6-dihydro-

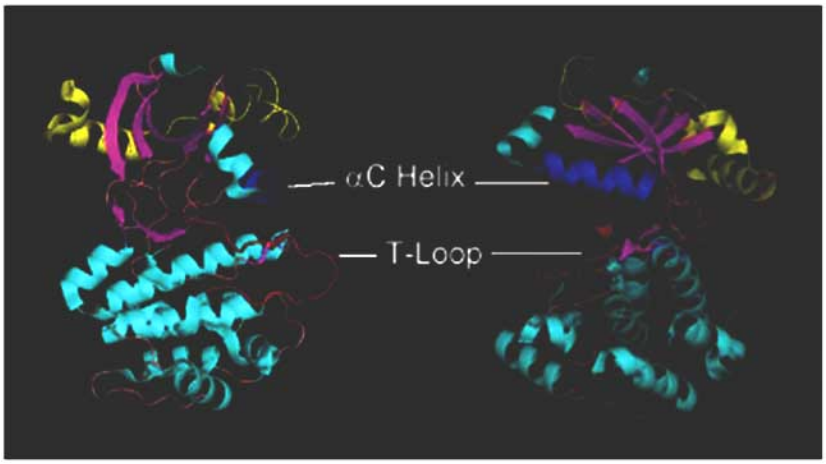


Fig. (2). The structure of Aurora-B. A fragment (amino acids 77 – 361) of Aurora-B (PDB ID 2vrx) in complex with a fragment of INCENP-A (amino acids 798 – 840) is rendered as a cartoon using the program PyMOL. Two views from two different angles are shown. Yellow – INCENP-A; cyan - α-helix; Magenta - β-sheet; beige – loop. The T-loop (red) is important for substrate recognition as well as catalytic activation that involves phosphorylation of a conserved threonine. The αC-helix (blue) is also important for catalytic activity [45].

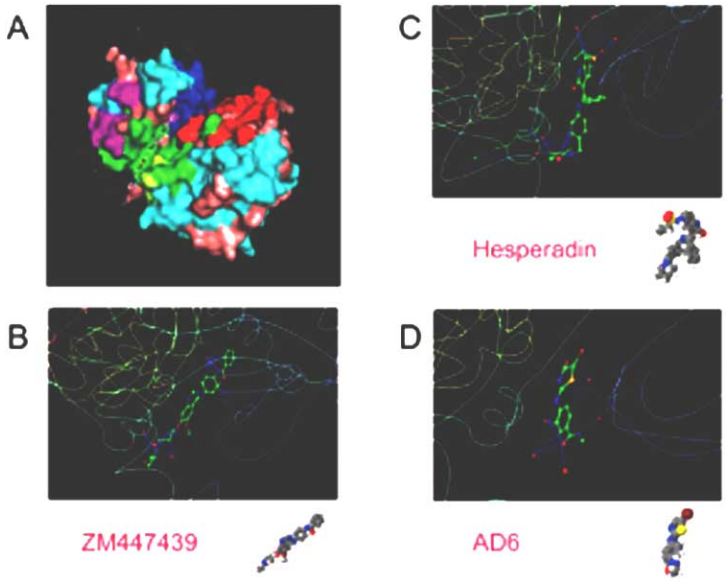


Fig. (3). Small molecule inhibitors of Aurora-B.

A: The surface model of ZM447439 bound to Aurora-B (amino acid 77-361) is rendered using the program PyMOL. ZM447439 (in stick molecular structure) is shown in the active site ATP-binding pocket of Aurora-B (green), and the 3 residues that differ between Aurora-B and A are highlighted in lime yellow. The α-C helix (blue) and the T-loop (red) are also shown. For the remainder part of the molecule, helices are in cyan; beta-sheets in magenta; loops in beige. INCENP-A (amino acid 798-840) in complex with Aurora-B is hidden by coloring it black. B: The hydrogen bond interaction (blue lines) between ZM447439 (green carbon backbone) and the Aurora-B active site (colored line cartoon) is shown adjacent to the molecular model of the inhibitor. C: Hydrogen bond interaction for hesperadin. D: Hydrogen bond interaction for AD6.

1h-indol-5-yl]ethanesulfonamide): PDB ID 2bfy] [71]. The surface model of the data for ZM447439 bound to Aurora-B (PDB ID 2vrx) is rendered using the program PyMOL (Windows-XP version, 2006, Delano Scientific, LLC) (Fig. **3A**). The hydrogen bond interaction (blue lines) between ZM447439 (green carbon backbone) and the Aurora-B active site (colored line cartoon) based on PDB ID 2vrx is shown in Fig. (**3B**). Similar hydrogen bond interaction for hesperadin (PDB ID 2bfy) is shown in Fig. (**3C**), and for AD6 (PDB ID 2vgp), Fig. (**3D**). The list of small molecule inhibitors of Aurora-B (Table **1**) is growing, especially with the addition of new inhibitors selective for Aurora-B [45].

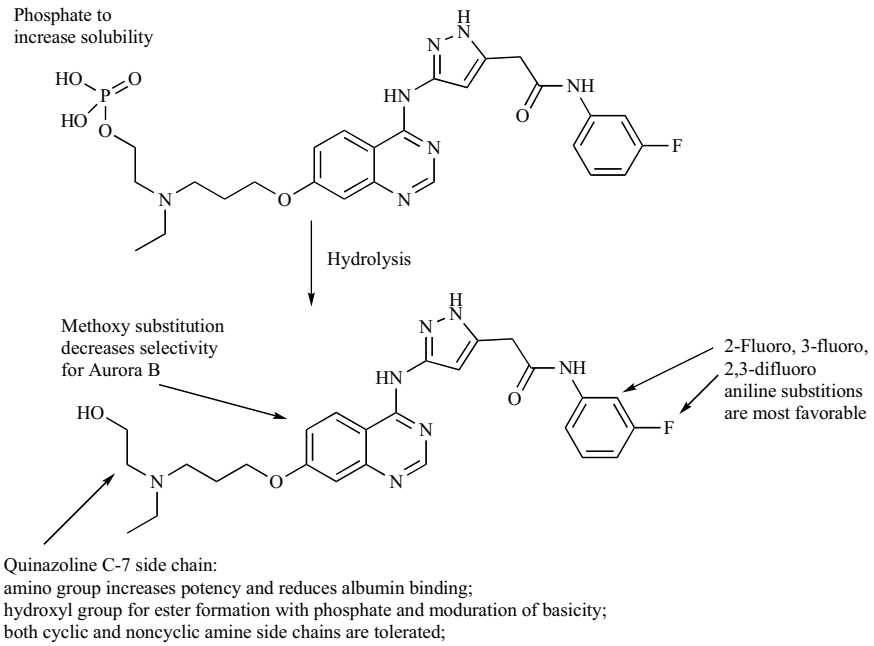
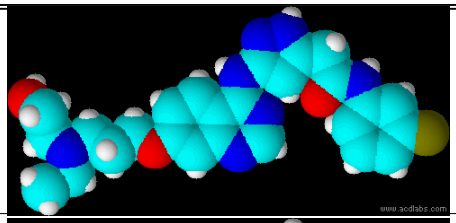
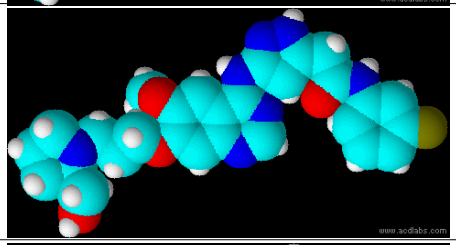
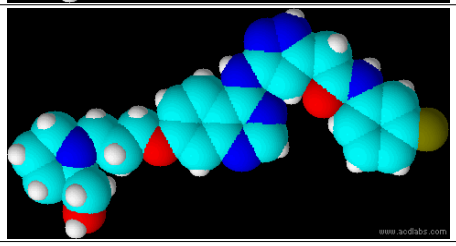
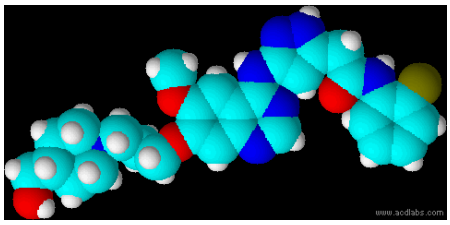
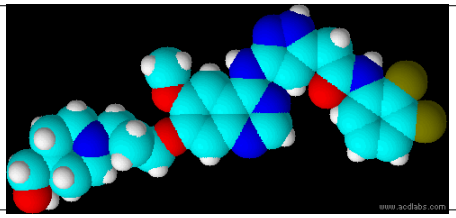
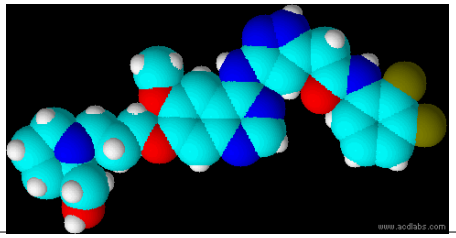
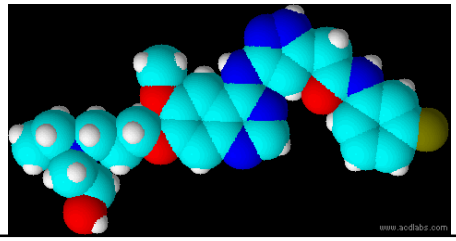


Fig. (4). Structure-Activity Relationship of AZD1152.

Table 3. Aurora-B-Specific Kinase Inhibitors

Compound	3-D Structure	Ki for Aurora-A Assay	Ki for Aurora-B Assay	Selectivity Index*
AZD1152-HQPA		1368 nM	0.37 nM	3697
Comp. **5** [44]		1100 nM	1 nM	1100
Comp. **7** [44]		3000 nM	<1 nM	>3000
Comp. **7** [76]		1400 nM	14 nM	100
Comp. **10** [76]		410 nM	2 nM	205
Comp. **18** [76]		94 nM	1 nM	94
Comp. **18** [76]		190 nM	1 nM	190

* Selectivity Index defined as the inhibition constant (Ki) for Aurora-A divided by Ki for Aurora-B.

White – hydrogen; cyan – carbon; blue – nitrogen; red – oxygen; green – fluorine.

STRUCTURE ACTIVITY RELATIONSHIP

Bioinformatics and computational methods have demonstrated their usefulness in assisting chemical and biological experimentation in the drug discovery process. A 3- dimensional structure-based design approach has used structural models of Aurora kinases and molecular docking simulations to design and guide the synthesis of novel compounds, leading to the identification of compounds selective for the Aurora kinases in a panel of diverse kinases [74]. The enzymatic function of kinases entails binding ATP, weakening the covalent bond of the γ-phosphate, and transfer of the phosphate to the substrate. This function dictates the evolutionary conservation of the kinase active-site topology. Therefore, the kinase active sites usually vary in a very small number of residues. Yet, these minor differences form the structural basis for selectivity of small molecular inhibitors that bind to kinase active sites [75]. An integrated computational approach involving molecular dynamics simulations, static docking calculations and free energy perturbation may help explain how changing the bromine substitution from 6- to 7-position converts the glycogen synthase kinase-3α/β selective inhibitor 6-bromoindirubin-3'-oxime to a potent inhibitor of Aurora B and C kinases. The residue (Aurora-A Thr217, Aurora-B Glu161, Aurora-C Glu127) located in the active site is the major contributor to the enhanced affinity of 7 bromoindirubin-3'-oxime for Aurora B and C versus Aurora A [75].

AZD1152 belongs to a series of pyrazoloquinazolines with >1000-fold selectivity for Aurora-B over Aurora-A. As discussed above, AZD1152 possesses good antineoplastic activity in animal models, and is currently in clinical trials [76]. The structure-activity relationship of this series of pyrazoloquinazolines has been reported [44, 76]. Several features of the structure-activity relationship can be noted (Fig. **4**). Some of the compounds related to AZD1152 are listed with structures and selective indices in Table **3**. The phosphate prodrug has good solubility which allows easy formulation of parenteral dosage forms. Fluoro substitutions at 2- and 3- positions of the aniline moiety most favor potency of inhibition of Aurora-B [76]. Selectivity for Aurora-B over Aurora-A is enhanced by replacing the methoxy group at the C-6 position of the quinazoline with a hydrogen [44, 76]. The quinazoline C-7 side chain is important for cellular potency. Amino groups increases potency and the basicity reduces binding to plasma albumin. A hydroxyl group in this C-7 side chain allows the synthesis of phosphate ester and moderates the basicity of the amino group. Both cyclic and acyclic amine side chains will provide excellent cellular potency without significant increase in lipophilicity.

FUTURE DIRECTIONS

Will Aurora-B inhibitors represent an advance over current therapies? Non-specific Aurora kinase inhibition has enhanced the activity of cytotoxic agents in preclinical studies. It will be important to understand the behavior of specific Aurora-B inhibitors when delivered in combination (and in sequence) with other anticancer agents. Mechanism-guided design of chemotherapy combinations taking into consideration of the cell-cycle phase-specific nature of these drugs will have improved chances of success.

What kind of malignancy should we use Aurora-B inhibitors to treat? Factors that determine sensitivity to Aurora-B kinase inhibitors may include the p53 functional status of the tumor. Of course, there are many other factors that also govern drug sensitivity, including the kinase-selectivity profile of the drug, susceptibility of the drug to active transport by drug resistance proteins, the proliferation rate of the tumor (i.e., the fraction of mitotic cells in the whole cell population susceptible to Aurora-B kinase inhibition), and the complexity of the genotype of the tumor. Characterization of cancers susceptible to Aurora-B inhibitors will maximize clinical efficacy for the identified patient population.

In vitro selection of resistant clones to ZM447439 yields mutants that retain wild-type catalytic activity and are cross-resistant to other Aurora inhibitors [70]. Three point mutations are mapped to the ATP binding pocket. Thus, the plasticity of the ATP-binding pocket may render Aurora-B kinase insensitive to multiple inhibitors [70]. Therefore, acquired drug resistance is a potential clinical problem limiting clinical efficacy. The question whether multi-target kinase inhibitors are less likely to encounter acquired drug resistance in the clinical setting than selective Aurora-B inhibitors remains to be answered.

CONCLUSION

Aurora-B kinase represents a novel cell-cycle specific mitotic target for cancer therapy.

Inhibition of Aurora-B kinase has been demonstrated to result in apoptosis of cancer cells and inhibition of tumor growth in xenograft models. A better understanding of Aurora-B kinase biology and activity should lead to improved selection of patients. Aurora-B inhibitors are being evaluated in clinical trials to assess the potential clinical activity. Many questions remain about Aurora-B function and the answers will be of great interest, not only to basic researchers but also to clinicians and patients. Aurora-B inhibitors may soon be important weapons in the fight against cancer.

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