Aurora kinase inhibitor patents and agents in clinical testing: an update (2009 – 10)

This article is an update to aurora kinase inhibitors review, which appeared in: Expert Opin. Ther. Patents 2009, 19, 1-36 and Expert Opin. Investig. Drugs 2009, 18, 1-20.

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Introduction: Mitosis is a key step in the cell cycle and is controlled by several cell cycle regulators, including aurora kinases. Aurora family members A, B and C are essential for spindle assembly, centrosome maturation, chromosomal segregation and cytokinesis. Overexpression/amplification of aurora kinases has been implicated in oncogenic transformation, including the development of chromosomal instability in cancer cells. Hence, the use of aurora kinase small molecule inhibitors as a potential molecular-targeted therapeutic intervention for cancer is being pursued by various researchers.

Area covered: This review provides an update on aurora kinase inhibitors based on developments from 2009 to 2010. The medicinal chemistry aspects of aurora kinase inhibitors, with a particular emphasis on the patent literature, are reviewed. Databases such as PubMed, SCOPUS®, Scifinder® and www.clinicaltrials.gov database were used to search for literature in the preparation of this review.

Expert opinion: Around a dozen aurora kinase inhibitors are currently undergoing various Phase I – II evaluations for different human cancers. Instead of being applied as a monotherapy, combinations of aurora kinase inhibitors and existing chemotherapeutic compounds seem to give better therapeutic outcomes and are, therefore, a promising future cancer therapy.

Keywords: AMG 900, AT-9283, aurora kinase inhibitors, AZD1152, BI811283, CYC116, ENMD-2076, GSK1070916, MLN8054, MLN8237, PF-3814735, PHA739358, R763, SNS-314, VX-680/MK0457, VX-689/MK-5108, XL228

1. Introduction

Mitosis is a key step in cell cycle that is tightly regulated by many proteins. Abnormal expression or activation of these regulatory proteins could result in aberrant mitosis leading to the development of cancer. At the molecular level, aurora kinases (Aurora-A, -B and -C) are serine/threonine kinases that function as key regulators of mitosis. They are essential for spindle assembly, centrosome maturation, chromosomal segregation and cytokinesis [1,2]. Within the aurora kinase family, Aurora-A and -B are expressed, distributed and function differently during different stages.
of the cell cycle. By contrast, expression, distribution and function of Aurora-C relates closely to Aurora-B kinase. During the cell cycle, Aurora-A colocalizes with centrosome throughout the process where Aurora-B localizes to the centromere in pro-metaphase and metaphase, following by transportation to the spindle midzone in anaphase.

It has been widely demonstrated that aurora kinases are overexpressed in various cancers and also play important roles in the process of tumorigenesis [3-6]. Based on the above findings, the first proof-of-concept pan-aurora kinase inhibitor, VX-680 (MK-0457, tozasertib), was developed in 2004 by Vertex Pharmaceuticals (in collaboration with Merck) with an aim of targeting cancer cells. This aurora kinase inhibitor has been shown to be effective in targeting cancer cells both in vitro and in vivo and was subsequently approved by the FDA for clinical trials [7-9].

In 2007, Merck had suspended enrollment in Phase I – II clinical trials of VX-680 due to safety concerns; QTc prolongation was observed in one patient. Since then, continuous efforts have been made by different pharmaceutical companies in searching for potential aurora kinases inhibitors that exhibit better therapeutic profile and specificity as compared to the first generation inhibitor, VX680 [10-14]. For example, Millennium Pharmaceuticals revealed that their orally active Aurora-A-specific inhibitor, MLN8054, was effective in treating various cancers both in vitro and in vivo [11,14]. Sunesis Pharmaceuticals also revealed the successful use of a pan-aurora kinases inhibitor, SNS-314, to treat cancers in vivo [15,16]. AstraZeneca and GlaxoSmithKline (GSK) have also joined in the discovery and development of aurora kinase-specific inhibitors (AZD1152 and GSK1070916) for the treatment of cancers [10,12,13,17,18].

The mechanisms of actions of aurora kinase inhibitors, agents in clinical trials and patent literature prior to the year 2009 had been fully reviewed in our articles previously published in the Expert Opinion on Therapeutic Patents and Expert Opinion on Investigational Drug [19,20]. The current review focuses on the new developments in aurora kinase inhibitors in clinical trials and new aurora patents filed between 2009 and 2010.

2. Aurora kinase inhibitors in clinical trials

2.1 Clinical trial agents introduced till 2008 (updated information)

In this section, we update 2009 – 10 information for those clinical trial agents introduced before 2009. Please refer to our previous publication for information published till 2008 for these agents [19,20]; refer to Table 1 for structure and summary information for these agents.

2.1.1 VX680/MK-0457 (tozasertib)

VX680 was identified by Vertex scientists by replacing the quinazoline ring of their initial screening hit with a pyrimidine ring followed by side chain modifications [21]. Development of VX-680 was carried out by collaboration with Merck and initial preclinical results were published in 2004. This compound is the first generation pan-aurora kinase inhibitor that inhibits Aurora-A, -B and -C kinases with apparent inhibition constant ($K_{i(app)}$) values of 0.6, 18 and 4.6 nM, respectively, in vitro [7]. In various human tumor cell lines, VX-680 reduced cell proliferation with IC$_{50}$ values ranging from 15 to 113 nM. However, Merck had suspended enrollment in Phase I – II clinical trials of VX-680 in 2007, pending a full analysis of efficacy and safety data after preliminary data showed that one patient experienced QTc prolongation. Assessment of efficacy and safety of this compound has now been completed in patients with advanced solid tumors and results have been revealed in a peer-reviewed journal in 2010. The pan-aurora kinases inhibitor VX-680 was administrated as a 24 h continuous intravenous (i.v.) infusion. The most common adverse effects reported in this study is grade 3 vomiting. In addition, dose-limiting toxicity at 96 mg/m$^2$/h includes grade 4 neutropenia and grade 3 herpes zoster. The maximum-tolerated dose (MTD) was determined as 64 mg/m$^2$/h [22].

Although the efficacy and safety of VX680 monotherapy still require further clinical evaluations, the efficacy of VX680 and in combination with other chemotherapeutic compounds has already been evaluated in various preclinical studies. First, treatment with VX680 and vorinostat (HDAC inhibitor) synergistically increases the inhibition of acute lymphoblastic leukemia cell growth as compared to either VX680 or vorinostat monotherapy [23]. In addition, treatment with VX680 and wortmannin (PI3K inhibitor) showed a synergistic induction of tongue squamous carcinoma cells apoptosis and also suppression of their migration [24]. Interestingly, a recent study reveals that combination of nutlin-3 (p53 activator) and VX-680 selectively targets p53 mutant cancer cells with minor/no effect on p53 wild-type cells [25].

2.1.2 AZD1152 (barasertib)

AZD1152 is a selective inhibitor of Aurora-B kinase developed by AstraZeneca in 2004 [10,18]. AZD1152 is a dihydrogen phosphate prodrug, which will be converted to the active moiety AZD1152-HQPA in plasma [26]. Recent
<table>
<thead>
<tr>
<th>Structure</th>
<th>Code/name</th>
<th>Company</th>
<th>Target</th>
<th>Route of administration</th>
<th>Indication</th>
<th>Phase</th>
<th>Comment/side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>VX-680/MK-0457 (tozasertib)</td>
<td>Vertex/Merck</td>
<td>Pan-aurora selective</td>
<td>i.v.</td>
<td>CML and ALL</td>
<td>Phase II</td>
<td>Caused QT prolongation in 1/100 patients; monotherapy and in combination with dasatinib</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>AZD1152 (barasertib)</td>
<td>AstraZeneca</td>
<td>Aurora-B and -C selective</td>
<td>i.v.</td>
<td>AML and advanced solid tumors</td>
<td>Phase II</td>
<td>Neutropenia; monotherapy and in combination with low dose cytosine arabinoside</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>PHA-739358 (danusertib)</td>
<td>Nerviano Medical Sciences</td>
<td>Pan-aurora selective</td>
<td>i.v.</td>
<td>B-Cell, Abl, Ret</td>
<td>Advanced solid tumors and CML</td>
<td>Neutropenia, mucositis, and infection due to neutropenia</td>
</tr>
<tr>
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<td>MLN8054</td>
<td>Millennium</td>
<td>Aurora-A selective</td>
<td>Oral</td>
<td>Advanced solid tumors</td>
<td>Phase I (discontinued)</td>
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Cheung, Coumar, Chang & Hsieh
Expert Opin. Ther. Patents (2011) 21(6)
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<th>Target</th>
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<th>Route of</th>
<th>Indication</th>
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<td>Millennium</td>
<td>Aurora-A</td>
<td>selective</td>
<td>-</td>
<td>Oral</td>
<td>Advanced solid tumors and leukemias</td>
<td>Phase I – II</td>
</tr>
<tr>
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<td>SNS-314</td>
<td>Sunesis</td>
<td>Pan-aurora</td>
<td>selective</td>
<td>-</td>
<td>i.v.</td>
<td>Advanced solid tumors and leukemia</td>
<td>Phase I (completed)</td>
</tr>
<tr>
<td><img src="image" alt="CYC116" /></td>
<td>CYC116</td>
<td>Cyclacel</td>
<td>Pan-aurora</td>
<td>selective</td>
<td>-</td>
<td>Oral</td>
<td>FLT3 and VEGFR-2</td>
<td>Advanced solid tumors</td>
</tr>
<tr>
<td><img src="image" alt="PF-3814735" /></td>
<td>PF-3814735</td>
<td>Pfizer</td>
<td>Aurora-A and -B</td>
<td>selective</td>
<td>-</td>
<td>Oral</td>
<td>Advanced solid tumors</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

* No specific information; ALL: Acute lymphocytic leukemia; AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; DLT: Dose-limiting toxicity; i.v.: Intravenous; NA: Information not available.
Table 1. Aurora kinase inhibitors in clinical testing (continued).

<table>
<thead>
<tr>
<th>Structure</th>
<th>Code/name</th>
<th>Company</th>
<th>Target Activity</th>
<th>Route of Administration</th>
<th>Indication</th>
<th>Phase</th>
<th>Comment/side effects</th>
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<td>GSK1070916</td>
<td>GlaxoSmithKline</td>
<td>Aurora-B/-C selectivity</td>
<td>i.v.</td>
<td>Advanced solid tumors</td>
<td>Phase I</td>
<td>-</td>
</tr>
<tr>
<td><img src="image2" alt="Structure 2" /></td>
<td>ENMD 2076 (ENMD-981693: free base)</td>
<td>EntreMed</td>
<td>Aurora-A and multiple tyrosine kinases</td>
<td>Oral</td>
<td>Advanced solid tumors, hematological malignancies and multiple myeloma</td>
<td>Phase I</td>
<td>-</td>
</tr>
<tr>
<td><img src="image3" alt="Structure 3" /></td>
<td>L (+) Tartaric acid</td>
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<td></td>
</tr>
<tr>
<td><img src="image4" alt="Structure 4" /></td>
<td>AF-9283</td>
<td>Astex</td>
<td>Aurora-A</td>
<td>i.v.</td>
<td>Advanced solid tumors, AML and CML</td>
<td>Phase I-lla</td>
<td>-</td>
</tr>
</tbody>
</table>

- No specific information; ALL: Acute lymphocytic leukemia; AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; DLT: Dose-limiting toxicity; i.v.: Intravenous; NA: Information not available.
Table 1. Aurora kinase inhibitors in clinical testing (continued).

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<th>Structure</th>
<th>Code/name</th>
<th>Company</th>
<th>Target</th>
<th>Off-target activity</th>
<th>Route of administration</th>
<th>Indication</th>
<th>Phase</th>
<th>Comment/side effects</th>
</tr>
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<tr>
<td>R-763/AS-703569</td>
<td>Rigel/Merck Serono</td>
<td>Aurora-A and -B/FLT-3</td>
<td>-</td>
<td>Oral</td>
<td>Advanced solid tumors; pancreatic cancer; hematological malignancies</td>
<td>Phase I</td>
<td>Monotherapy and in combination with gemcitabine</td>
<td></td>
</tr>
<tr>
<td>VX-689/MK-5108</td>
<td>Vertex/Merck</td>
<td>Aurora-A selective</td>
<td>-</td>
<td>Oral</td>
<td>Advanced and/or refractory solid tumors</td>
<td>Phase I</td>
<td>Monotherapy and in combination with docetaxel</td>
<td></td>
</tr>
<tr>
<td>AMG 900</td>
<td>Amgen</td>
<td>Pan-aurora selective</td>
<td>-</td>
<td>Oral</td>
<td>Advanced solid tumors</td>
<td>Phase I</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>Boehringer Ingelheim</td>
<td>Aurora-B selective</td>
<td>-</td>
<td>i.v.</td>
<td>Advanced solid tumors; AML</td>
<td>Phase I – IIa</td>
<td>Monotherapy and in combination with oxaliplatin</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>Exelixis, Inc.</td>
<td>Pan-aurora IGFR1, FGFR, Src, ABL</td>
<td>i.v.</td>
<td>Advanced malignancies, CML or Philadelphia chromosome-positive ALL</td>
<td>Phase I</td>
<td>DLT: neutropenia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: No specific information; ALL: Acute lymphocytic leukemia; AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; DLT: Dose-limiting toxicity; i.v.: Intravenous; NA: Information not available.
in vitro and in vivo studies reveal that this compound is effective in targeting the adult T-cell leukemia, hepatocellular carcinoma and breast cancers [27-29]. In addition, AZD1152 sensitizes cancer cells to fractionated irradiation in vivo. Decreases in tumor growth rate were shown in mice implanted with human HCT116 colon cancer cells by AZD1152 (35 mg/kg/day) plus fractionated irradiation (3 Gy for 4 days at the same time as the drug), as compared to the mice treated with fractionated irradiation alone [17]. Besides in combination with fractionated irradiation, combination of AZD1152 and the topoisomerase I poison CPT-11 also shows synergistic antitumor effect in vitro and in vivo [30]. Furthermore, inhibition of Aurora-B kinase by AZD1152 is able to sensitize a subset of human glioma cells to TRAIL treatment through upregulation of the TRAIL receptor, TRAIL-R2 [31]. AstraZeneca published the result of a Phase I clinical trial of AZD1152 in patients with solid malignant tumors in 2010. In this study, AZD1152 was being administered either weekly or biweekly as a 2 h i.v. infusion in a dose-escalating manner (100 – 650 mg). The MTDs of AZD1152 were found to be 200 mg (weekly schedule) and 450 mg (biweekly schedule). Neutropenia and leukopenia are the most frequent adverse events (AEs) reported in that clinical study. No object tumor responses were observed in patients (both weekly treatment and biweekly treatment) as defined by RECIST (response evaluation criteria in solid tumors) [32].

A study published in 2009 has revealed a potential weakness of using AZD1152 in clinical setting in which AZD1152 was shown to be a substrate of the well-known multidrug resistant protein MDR1 (P-gp). The overexpression of the ABC transporter, MDR1, has been shown related to the causation of drug resistance in patient with prolonged chemotherapeutic therapy. Substrates of MDR1 include various chemotherapeutic compounds such as vincristine and etoposide (VP16) [33]. Interestingly, Guo et al. revealed that overexpression of both MDR1 and the breast cancer resistant protein can interfere with the sensitivity of AZD1152 and the above mentioned aurora kinase inhibitor, VX680, in cancer cells [34].

2.1.3 PHA-739358 (danusertib)

In 2003, Nerviano Medical Sciences filed a patent claiming the use of pyrrolo[3,4-c]pyrazole derivatives as aurora kinase inhibitors. PHA-739358 is an Aurora kinase inhibitor based on the 3-aminopyrazole moiety that had been shown to inhibit cancer cell growth in various in vitro and in vivo models previously [35,36]. Benten et al. recently revealed on a peer-reviewed journal that this compound is also effective in inhibiting the growth of hepatocellular carcinoma both in vitro and in vivo [37]. At cellular and molecular levels, PHA-739358 induces endoreduplication of tumor cells and inhibits phosphorylation of histone H3. Animal study reveals that dosage of PHA-739358 at 15 mg/kg, twice a day, successfully delayed tumor growth in Huh-7 xenograft model [37]. Based on these excellent results in preclinical testing, PHA-739358 was progressed to Phase I clinical investigation. In one clinical study, 56 patients with advanced solid tumor were treated with PHA-739358 in various dosages (45, 90, 180, 360, 500, 580, 650 mg/m²) with/without G-CSF [38]. Out of 40 patients (without G-CSF), dose-limiting toxicity such as grade 4 mucositis, febrile neutropenia and neutropenic infection were observed in 6 patients. No dose-limiting toxicity has been reported in the first five patients co-treated with 750 mg/m² of PHA-739358 and G-CSF. Without G-CSF, 39% of patients had stable disease after the treatments and 500 mg/m² was the recommended dose for the Phase II trial [38]. The pharmacokinetic and pharmacodynamics of PHA-739358 are also evaluated in another Phase I clinical study with patients with advanced or metastatic solid tumors [39]. Fifty patients were treated with PHA-739358 in a schedule of either 6 or 3 h i.v. on days 1, 8 and 15 every 28 days. Disease stabilization was shown in 23.7% of the treated patients. The recommended dose of PHA-739358 for Phase II study was 330 mg/m², infused over 6 h on days 1, 8 and 15 every 28 days [39].

In terms of patent, Nerviano further filed three patent applications in 2009 claiming the combinational use of PHA-739358 with either imatinib (BCR/ABL kinase inhibitor), bevacizumab (VEGF inhibitor), gemcitabine (anti-metabolite), cisplatin (DNA damaging agent) or irinotecan (topoisomerase I inhibitor) in treating cancers [40-42]. Leukemia cells treated with PHA-739358 in combination with imatinib have been shown to induce synergistic growth inhibition in vitro [41]. Moreover, intraperitoneal administration of PHA-739358 at a dosage of 15 mg/kg (twice daily and continuously for 9 days) combined with imatinib at a dosage of 100 mg/kg clearly demonstrated the synergistic effect in tumor growth inhibition as compared to the monotherapy in mice implanted with K562 cancer cells [41]. On the other hand, combinational treatment using PHA-739358 at a dosage of 15 mg/kg (twice a day, continuously for 10 days) together with bevacizumab at a dosage of 20 mg/kg (once per day for 3 days) has also been shown effective in inducing synergistic inhibition of tumor growth in mice implanted with human DU145 prostate cancer cells.

2.1.4 MLN8054

In 2004, Millennium Pharmaceuticals filed a PCT (patent cooperation treaty) application that described the synthesis and development of benzazepine-fused pyrimidine ring compounds that inhibit aurora kinase. One of the included compounds, MLN8054, functions as an ATP-competitive and reversible inhibitor of Aurora-A kinase [11,14]. It has been demonstrated that MLN8054 suppresses cell viability and promotes apoptosis in p53-deficient cancer cells [43]. In 2010, Huck et al. revealed that MLN8054 also induces senescence in human tumor cells [44]. Despite early successes of the development of MLN8054, a recent study reveals that the anticancer effect of this compound can be hampered in cells that carry the Aurora-A T217D mutation [45]. The pharmacokinetic and pharmacodynamics of MLN8054 have been evaluated in two Phase I clinical studies in patients with advanced solid tumors. In one study, MLN8054
The discovery of CYC116 by Cyclacel was reported in the 2.1.6 CYC116 A urora-A kinase with an IC50 value of 1 nM [48]. It inhibited studies revealed that MLN8237 inhibited the recombinant Aurora-A kinase inhibitor entitled MLN8237. Previous Millennium Pharmaceuticals has also developed another Aurora kinase inhibitor patents and agents in clinical testing: an update (2009 administration of CYC116 at 45 and 67 mg/kg twice daily ity evaluation of CYC116 was carried out in an intraperitoneally showed nanomolar level anti-proliferative activity. with several human derived cancer cell lines of different origins, was observed in patients as defined by RECIST. Consistent with the results of the first clinical study, patients in the 60 mg cohort were shown not able to maintain a steady-state plasma concentration of 2 µM [47].

2.1.5 MLN8237 Millennium Pharmaceuticals has also developed another Aurora-A kinase inhibitor entitled MLN8237. Previous studies revealed that MLN8237 inhibited the recombination Aurora-A kinase with an IC50 value of 1 nM [48]. It inhibited growth of various cancer cell lines such as HCT-116, PC3, SK-OV-3 and LY-3 with growth inhibition (GI50) values ranging from 16 to 469 nM in vitro [48]. In 2010, Gorgun et al. revealed that this compound is potent in targeting multiple myeloma cells. The same study further showed that combination of MLN8238 with either doxorubicin (intercalating agent) or bortezomib (proteasome inhibitor) induces synergistic anticancer activity in vitro [49]. Interestingly, the anticancer activity of MLN8237 has also been evaluated by the pediatric preclinical testing program. It has been demonstrated that this compound is effective in targeting acute lymphoblastic leukemia cells both in vitro and in vivo. Moreover, the same study revealed that ~ 50% of the MLN8237-administrated mice with neuroblastoma xenograft maintained complete responses [50].

2.1.6 CYC116 The discovery of CYC116 by Cyclacel was reported in the Journal of Medicinal Chemistry in 2010 [51]. The initial lead compound was identified through a cell-based screening, which was followed by lead optimization to get CYC116. It potently inhibits both Aurora-A and -B kinase with Ki values of 8 and 9.2 nM, respectively. In addition, it also inhibits FLT3 and VEGFR2 potently with a Ki value of 44 nM. When evaluated with several human derived cancer cell lines of different origins, such as MCF7 (breast), HCT-116 colon), HL60 (leukemia), it showed nanomolar level anti-proliferative activity. In vivo activity evaluation of CYC116 was carried out in an intraperitoneally implanted P388/0 murine leukemia mouse model. Oral administration of CYC116 at 45 and 67 mg/kg twice daily on days 1 – 5 and 7 – 9 resulted in a significant increase in lifespan of 172 and 183%, respectively, compared to control. CYC116 was tested orally in humans in Phase I clinical trials for advanced solid tumors first in 2007. However, the testing was discontinued and the reason is not yet disclosed by Cyclacel.

2.1.7 SNS-314 SNS-314 developed by Sunesis Pharmaceuticals is a pan-selective aurora kinase inhibitor, which has now completed a Phase I clinical trial for safety and tolerability in patients with advanced solid tumors. Anti-proliferative effects of SNS-314 in combination with common chemotherapeutics in cell culture were tested and revealed that sequential administration of SNS-314 followed by docetaxel or vincristine resulted in profound anti-proliferative activity in HCT-116 cell lines. Moreover, SNS-314 also potentiated in vivo efficacy of docetaxel in xenograft models, providing rationale for future combination therapy in clinical testing [16]. In addition, when tested in the HCT116 human colon cancer xenograft model, SNS-314 significantly inhibited tumor growth in a dose-dependent manner under a variety of dosing schedules including weekly, bi-weekly and 5 days on/9 days off cycles, suggesting flexible dosing schedules [15]. Recently, Sunesis has disclosed prodrugs and benzimidazole bioisosters of SNS-314 [52,53].

2.1.8 GSK1070916 GSK was granted a US patent in 2007 which describes the process of synthesizing 7-azaindole-based compounds including GSK1070916 as an aurora kinase inhibitor. However, detailed information of this compound has not been revealed in a peer-reviewed journal until 2009. GSK1070916 is a reversible and ATP competitive inhibitor of the Aurora-B–INCENP and Aurora-C–INCENP complexes with Ki values of 0.38 and 1.5 nM, respectively [13]. The inhibition IC50 values of GSK1070916 to Aurora-B–INCENP and Aurora-C–INCENP complexes are 5 and 6.5 nM, respectively, whereas the dissociation half-life is > 480 min for Aurora-B–INCENP and 270 min for Aurora-C–INCENP [13,54]. The same study also reveals that this compound inhibited the phosphorylation of histone H3 in Colo205 cancer cells in vivo [54]. Another study published in 2009 revealed that Colo205 cells treated with GSK1070916 induced the activation of caspase-3 and increased cleavage of PARP, indicating that the drug-treated cells had undergone apoptosis. Moreover, GSK1070916 showed potency in inhibiting tumor growth in various human tumor xenograft models including A549 (lung), HCT116 (colon), SW620 (colon) and MCF-7 (breast) [12]. This compound is being progressed to a Phase I clinical trial.

2.1.9 PF-03814735 PF-03814735 is an ATP-competitive, orally bioavailable, reversible inhibitor of Aurora-A and -B kinases. PF-03814735 inhibits Aurora-A and -B kinases with an IC50 value of 5 and 0.8 nM, respectively. The latest study
reveals that once-daily oral administration of PF-03814735 to mice bearing human xenograft tumors produces a reduction in the phosphorylation of histone H3 at Ser10 in tumors at doses that are tolerable and that result in significant inhibition of tumor growth. In addition, the combination of PF-03814735 and docetaxel in xenograft mouse tumor models shows additive tumor growth inhibition [55].

2.1.10 ENMD-2076
ENMD-2076 (L-(-)-lactic acid salt of ENMD-981693) developed by EntreMed is an orally active, vinyl-pyrimidine-based compound and selectively inhibits Aurora-A with an IC$_{50}$ value of 14 nM, instead of Aurora-B kinase (290 nM) [56]. Interestingly, a recent study reveals that this compound rapidly inhibited the PI3K/AKT pathway and downregulates both survivin and XIAP (members of the inhibitor of apoptosis family) in multiple myeloma cells after 6 h of post-treatment, whereas the inhibition of Aurora-A and -B kinases happened between 24 and 48 h [57]. In addition, mice orally treated with ENMD-2076 (50, 100, 200 mg/kg/day) have shown a dose-dependent reduction in the rate of H929 tumor growth [57]. The efficacy of ENMD-2076 has also been evaluated in another preclinical study. Oral administration of ENMD-2076 (100 or 200 mg/kg/day) for 28 days was shown inhibiting tumor growth and subsequently induce tumor regression in HT-29 human colorectal cancer xenografts [58].

A Phase I clinical study has been conducted to determine the MTD and toxicities of ENMD-2076 in patients with relapsed or refractory leukemia. In the study, 27 patients received escalating doses (225, 375, 325 or 275 mg/day) of ENMD-2076 by oral administration. The most common non-hematological toxicities of any grade observed in patients were shown to be fatigue, diarrhea, dysphonia, dyspnea, hypertension, constipation and abdominal pain. No patient experienced grade 4 toxicities or death from ENMD-2076 at all doses. The Phase II dose (RPTD) of this compound is recommended as 225 mg/day [59]. In 2010, EntreMed announced that the FDA has granted orphan drug designation for ENMD-2076 for the treatment of acute myeloid leukemia (AML).

2.1.11 AT9283
The pyrazol-4-yl urea AT9283 developed by Astex is a multi-targeted kinase inhibitor that inhibits tyrosine and serine/threonine kinases such as Aurora-A and -B, JAK-2 and -3, Tyk2, RSK2 with IC$_{50}$ values < 10 nM in vitro [60]. It has been demonstrated that this compound is effective in targeting a variety of different imatinib-resistant BCR-ABL+ cancer cells [61]. In 2009, Astex reported results from its Phase I clinical study of AT9283 in patients with refractory solid tumors at the ASCO annual meeting. AT9238 was being administered as a 72 h continuous i.v. infusion schedule repeated three weekly according to a standard ‘3 + 3’ design. The MTD was suggested to be 9 mg/m$^2$/day [62]. In the same year, Astex Therapeutics announced that it has been granted orphan drug designation by the FDA for AT9283 for the treatment of patients with AML. In addition, AT9283 was also granted an orphan medicinal product designation by the European Commission for treating the same type of cancer.

2.1.12 R763/AS703569
R763 is an orally available inhibitor of Aurora-A, Aurora-B and several other kinases such as FLT3 and VEGFR2 [63]. R763 induces anti-proliferative effects in various cancer cell lines including A549, DU145, HeLa, Colo205 and U2OS with IC$_{50}$ values < 0.5 µM in vitro [63]. The antitumor activity of this compound has been examined in human MiaPaCa-2 pancreatic tumor xenograft, MV4-11 and MOLT-4 leukemia models, and oral administration of R763 demonstrated significant antitumor activity in vivo [63].

2.1.13 VX-689/MK-5108
Besides VX680/MK-0457, Vertex and Merck have also developed another Aurora-A kinase inhibitor entitled VX689/MK-5108 and the results of its preclinical evaluation have been revealed in 2010. A recent study reveals that VX689 specifically inhibits the activity of aurora kinase A with an IC$_{50}$ value of 0.064 nM. It exhibits potent growth inhibitory activity (IC$_{50}$ < 2 µM) in various cancer cell lines including MCF-7, AU565, SW48, Colo205 and SKOV-3. Surprisingly, this compound seems less potent (IC$_{50}$ > 30 µM) in targeting some of the breast cancer cell lines such as T47D, HCC1419 and BT474. In animal models, it has been demonstrated that combination of VX689 with docetaxel induces synergistic antitumor activity [64].

2.2 Clinical trial agents introduced from 2009

2.2.1 AMG-900
AMG-900 is a pan-aurora kinase inhibitor developed by Amgen, Inc. It inhibits the activity of Aurora-A, -B and -C kinases with IC$_{50}$ values of 5, 4 and 1 nM, respectively. It also inhibits the autophosphorylation of Aurora-A and -B and the phosphorylation of histone H3 (substrate of Aurora-B) at Ser10 in cells [65]. A recent study reveals that AMG 900 exhibits potent anti-proliferative activity against various types of cancer cells in vitro. Interestingly, AMG 900 is effective in targeting cancer cells that overexpress the multiple drug resistance protein (MDR1) and also have previously shown resistance to other aurora kinase inhibitors such as AZD1152 and MK-0457. Oral administration of AMG 900 was shown effective in inhibiting tumor growth in both human HCT116 tumor and NCI-H460-PTX (MDR overexpressing cells) tumor xenograft models [65].

2.2.2 BI 811283
Boehringer Ingelheim in 2010 disclosed the development of BI 811283 at the ASCO annual meeting. BI 811283 is an Aurora-B selective inhibitor, which is currently in Phase I
Aurora kinase inhibitor patents and agents in clinical testing: an update (2009 – 10)

clinical testing for advanced solid tumors [66,67], BI 811283 potently inhibited Aurora-B kinase (IC_{50} = 9 nM) and blocked several cancer cell line proliferation (EC_{50} < 14 nM). In nude mouse xenograft models of human NSCLC, BI 811283 dose-dependently inhibited tumor growth. A Phase I dose escalation study was carried out to determine the MTD in humans. In an initial 4-week study, BI 811283 as a 24 h i.v. infusion on days 1 and 15 of each treatment cycle (q2w) showed that MTD was exceeded at 140 mg. Reversible hematotoxicity was the main AE; dose-limiting toxicity at 140 mg was neutropenia. Further testing is going on to determine the MTD.

2.2.3 XL228
Exelixis, Inc. in 2010 disclosed the development of XL228 at the ASCO annual meeting [68]. XL228 is a multi-targeted protein kinase inhibitor targeting IGF1R, the aurora kinases, FGFR1-3, ABL, ALK and SRC family kinases. In Phase I clinical testing, XL228 is administered as weekly 1 h i.v. infusions for evaluation for safety, preliminary efficacy, pharmacokinetics and pharmacodynamic effects. The dose-limiting toxicity was grade 3 and 4 neutropenia occurring at the 8 mg/kg dose level. Pharmacodynamic assessment of tumor biopsies has demonstrated decreases in phosphorylation of IGF1R, FGFR and AKT for up to 24 h, post dosing. In a patient with melanoma, enlargement of tumor nuclei suggests Aurora-B inhibition and endoreduplication.

3. Aurora kinase inhibitor patents filed in 2009 – 10

3.1 Abbott Laboratories
Abbott in 2009 disclosed two patents WO2009012312A1 [100] and WO2009136966A1 [101] describing compounds with general formula 1(G) and 2(G), respectively. Both patents claimed aurora kinase inhibitors with a similar side chain, as exemplified by compounds 1 and 2, the only difference being the core structures claimed in the patent. In 2010, Abbott disclosed a series of thienopyridine core compounds in WO2010065825A2 [69] with the general formula 3(G) as aurora kinase inhibitors with VEGFR and PDGFR kinase inhibitory activity (Table 2). They have claimed these compounds have advantage over their previous class of compounds published in 2005 WO2005/010009, due to their lower ability to inhibit cytotoxicity on P450 enzymes CYP3A4 and hence to possess less drug-drug interaction. Exemplary compound 3 inhibited Aurora-A (IC_{50} = 675 nM) and Aurora-B (IC_{50} = 7 nM); it also inhibited VEGFR family (KDR, FLT1) and PDGFR family (FLT3, cKIT) members with IC_{50} values ranging from 1 to 20 nM. Compound 3 showed a very low potential for inhibiting CYP3A4 with an IC_{50} > 10 µM while ketoconazole, a known inhibitor of CYP3A4, showed 70 – 80% inhibition of the enzyme at 100 nM concentration.

3.2 Banyu/Vertex
Banyu Pharmaceuticals in 2009 filed a PCT application describing novel aminopyridine derivatives with general formula 4(G) as Aurora-A selective inhibitors (Table 3) [70]. They have reported several compounds in this series to show selective Aurora-A inhibition with cellular anti-proliferative activity in HeLaS3 human derived cancer cell line. One such selective compound 4 shows an Aurora-A inhibition IC_{50} value of 1.14 nM and an Aurora-B IC_{50} value > 1000 nM. Structurally these aminopyridine compounds are similar to Merck’s Phase I clinical trial compound VX-689/MK-5108 [7], which is claimed in the PCT application WO2008026768A1 [71]. In 2010, Banyu and Vertex Pharmaceuticals filed three patents WO2010111050A1 [72], WO2010111056A1 [73] and WO2010111057A1 [74], claiming closely related Aurora-A selective pyrazolylaminoprimidine inhibitors. Representative compounds 5 (Aurora-A IC_{50} 37 nM and Aurora-B IC_{50} > 10 µM), 6 (Aurora-A IC_{50} 9.1 nM and Aurora-B IC_{50} 7.394 µM) and 7 (Aurora-A IC_{50} 2.3 nM and Aurora-B IC_{50} 730 nM) from these series are shown. These compounds are claimed to show potent anticancer activity in combination with known antitumor agents.

3.3 Merck Serono
Merck Serono has disclosed in 2009 – 10 three PCT application WO2009108670A1 [75], WO2010002779A2 [76] and WO2010077530A1 [77] claiming naphthridinone compounds of general formula 8(G) – 10(G) as multi-kinase inhibitors useful in the treatment of various kinase-related disorders including cancer, arteriosclerosis, ocular disease, diabetes, inflammatory and immune diseases (Table 4). Representative compounds 8 showed aurora kinase A inhibition with an IC_{50} < 10 nM and 10 showed inhibition in the range of 1 – 10 nM.

They also filed a PCT application in 2010, claiming macrocyclic pyrimidine compounds of general formula 11(G) as aurora kinase inhibitors with multiple kinase inhibition profile useful for aurora kinase-related disorders, including cancer. A representative compound 11 was shown to inhibit aurora kinases A and B with an IC_{50} < 100 nM. Moreover, 11 exhibited anti-proliferative activity in A549 and MiaPac2 cancer cell line with an IC_{50} < 100 nM and also inhibited 121 out of 267 kinases tested with > 79% inhibition at concentration of 1 µM [78].

3.4 National Health Research Institutes
We at the National Health Research Institutes in Taiwan filed two patent applications in 2009 claiming the use of urea compounds as aurora kinase inhibitors (Table 5) [79,80]. Several heterocyclic core structures including furanopyrimidines, imidazopyrimidines and quinazolines bearing a urea containing side chain with general structural formula 12(G) and 13(G) were claimed for aurora kinase inhibition. Two of the representative compounds 12 and 13 from these patents are shown.
Table 2. Aurora kinase patents filed by Abbott and their features.

<table>
<thead>
<tr>
<th>Patent no</th>
<th>General structure</th>
<th>Representative examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>WO2009012312A1 [100]</td>
<td><img src="1G" alt="Image" /></td>
<td><img src="1" alt="Image" /></td>
<td>Aurora kinase inhibitors</td>
</tr>
<tr>
<td>WO2009136966A1 [101]</td>
<td><img src="2G" alt="Image" /></td>
<td><img src="2" alt="Image" /></td>
<td>Aurora kinase inhibitors</td>
</tr>
<tr>
<td>WO2010065825A2 [69]</td>
<td><img src="3G" alt="Image" /></td>
<td><img src="3" alt="Image" /></td>
<td>Aurora, VEGFR and PDGFR kinase inhibitors</td>
</tr>
</tbody>
</table>

(G): General structure.
Table 3. Aurora kinase patents filed by Banyu/Vertex and their features.

<table>
<thead>
<tr>
<th>Patent no</th>
<th>General structure</th>
<th>Representative examples</th>
<th>Comments</th>
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<td>WO2009104802A1 [70]</td>
<td><img src="image1" alt="Image of molecule" /></td>
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<td>Aurora kinase A selective inhibitors</td>
</tr>
<tr>
<td>WO2010111050A1 [72]</td>
<td><img src="image3" alt="Image of molecule" /></td>
<td><img src="image4" alt="Image of molecule" /></td>
<td>Aurora kinase A selective inhibitor</td>
</tr>
<tr>
<td>WO2010111056A1 [73]</td>
<td><img src="image5" alt="Image of molecule" /></td>
<td><img src="image6" alt="Image of molecule" /></td>
<td>Aurora kinase A selective inhibitor</td>
</tr>
</tbody>
</table>

(G): General structure.
Compound 12 was reported as a potent aurora kinase inhibitor identified through structure-based drug design from a furanopyrimidine lead identified by screening internal compound library [81,82]. Compound 12 possess Aurora-A-specific inhibitory activity with an IC$_{50}$ of 43 nM and also showed anti-proliferative activity in HCT-116 with an IC$_{50}$ of 400 nM. Moreover X-ray co-crystal structure of 12 in complex with Aurora-A protein revealed that the furanopyrimidine ring forms interaction with the hinge region and the urea carbonyl group interacts with the Lys272 residue through hydrogen bonds [82].

Compound 13 claimed in WO2010036629A2 revealed that this compound inhibits aurora kinase A and the proliferation of HCT-116 cancer cells with an IC$_{50} < 1$ µM. Moreover, 13 showed superior in vivo efficacy when administered intravenously at a dosage of 25 and 50 mg/kg (5 days per week, continuously for 2 weeks), as compared to the first generation aurora kinase inhibitor, VX680 (50 mg/kg, same schedule), in mice implanted with HCT-116 cancer cells.

3.5 Schering and Albany Molecular Research

Schering and Albany Molecular Research in 2009 claimed a series of imidazopyrazine compounds as aurora kinase inhibitors, with general formula 14(G) [83]. An exemplary compound 14 is shown, which shows both Aurora-A and -B inhibitory activity. Also in 2009, Schering Co. filed another PCT application WO2009017701A1 [84] for related imidazopyrazine compounds 15(G) as aurora kinase inhibitors, which were reported as useful for combination therapy with other anti-mitotic agents (Table 6).

3.6 University Health Network

University health network of Canada in 2009 claimed substituted indolinone compounds of general formula 16(G) as aurora kinase B and/or PLK4 inhibitors (Table 7) [85]. Exemplary compound 16 shows PLK4, aurora kinases A and B inhibitory activity with an IC$_{50} < 5$ µM, and also shows inhibition activity towards other oncology kinase such as Abl, FLT-1, KDR, PDGFR-β and Ret with an IC$_{50} < 5$ µM. Moreover, 16 inhibited the proliferation of several cancer cell lines, including MCF-7, MDA-MB-468, HCC-1954, A172, Colo205 and HCT-15 with an IC$_{50} < 5$ µM.

Further in 2010, they claimed another series of indolinone compounds of general formula 17(G) as aurora kinase B and/or PLK4 inhibitors [86]. Exemplary compound 17 shows PLK4, aurora kinases A and B inhibitory activity with an IC$_{50} < 100$ nM, and also shows inhibition activity towards other oncology kinases. Moreover, 17 inhibited the proliferation of several cancer cell lines, including MCF-7, MDA-MB-468, A-549, SW-620, A172 and Colo205 with an IC$_{50} < 100$ nM.

3.7 Patents from Arrow Therapeutics, Bayer and Curis

Arrow therapeutics in 2009 disclosed a PCT application claiming 2,4-diaminopyrimidine derivatives of general
### Table 4. Aurora kinase patents filed by Merck group and their features.

<table>
<thead>
<tr>
<th>Patent no</th>
<th>General structure</th>
<th>Representative examples</th>
<th>Comments</th>
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<td>WO2009108670A1 [75]</td>
<td><img src="image" alt="8(G)" /></td>
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<td>Multi-kinase inhibitors, including aurora, Lck, Src, KDR, FLT-3 and FGRF3 kinase</td>
</tr>
<tr>
<td>WO2010002779A2 [76]</td>
<td><img src="image" alt="9(G)" /></td>
<td><img src="image" alt="9" /></td>
<td>Multi-kinase inhibitors, including aurora, Lck, Src, KDR, FLT-3 and FGRF3 kinase</td>
</tr>
<tr>
<td>WO2010077530A1 [77]</td>
<td><img src="image" alt="10(G)" /></td>
<td><img src="image" alt="10" /></td>
<td>Multi-kinase inhibitors, including aurora, Lck, Src, KDR, FLT-3 and FGRF3 kinase</td>
</tr>
</tbody>
</table>

(G): General structure.
formula $18(G)$ as inhibitors of aurora kinase (Table 8) \[87\]. Representative compound 18 was shown to inhibit aurora kinase in vitro with an IC$_{50}$ of 3 nM.

Bayer disclosed a PCT application describing pyrrolothiazine derivatives as aurora kinase inhibitors. These compounds are reported to inhibit both aurora kinases A and B with an IC$_{50}$ < 100 nM in a biochemical enzyme assay, as well as in a cell-based autophosphorylation assay in HT29. A representative example of these compounds is shown as 19 \[88\].

Curis, Inc. in 2009 disclosed a PCT application claiming pyrimidine analogs, which are closely related to VX-680, as inhibitors of both aurora kinase and HDAC enzymes \[89\]. The presence of zinc-binding moieties (e.g., hydroxamate group in 20) in the compounds helps to target HDAC. Compound 20 was shown to inhibit both aurora kinase A and HDAC with an IC$_{50}$ < 100 nM.

### 3.8 Patents from Mikana Therapeutics, Shanghai Genomics and Telik

Mikana Therapeutics in 2010 disclosed a PCT application claiming the use of pyrazole derivatives of general formula $21(G)$, which is closely related to VX-680 as aurora kinase inhibitors, for the treatment of cancer (Table 9) \[90\]. Representative compound 21 showed Aurora-A, src, Flt3 and KDR kinase inhibition activity with an IC$_{50}$ < 100 nM, while Aurora-B was inhibited at a lower concentration level (IC$_{50}$ 100 – 1000 nM), suggesting Aurora-A selective inhibitory activity for these compounds. Moreover, 21 inhibited proliferation of HCT-116 with an IC$_{50}$ of 100 – 1000 nM.

Shanghai Genomics had disclosed a PCT application claiming the use of pyrimidine analogs of general formula $22(G)$ as multi-kinase inhibitors for the treatment of various disorders associated with kinase signal disruption, particularly for the treatment of cancer \[91\]. One representative compound 22 is shown to inhibit several kinases including CDK subtypes and both aurora kinases A (IC$_{50}$ 30 nM) and B (IC$_{50}$ 700 nM) with a nanomolar IC$_{50}$ range in vitro. It inhibited several cancer cell lines including HeLa, A549, HepG2 and MCF-7 at low concentration (IC$_{50}$ 680 – 1700 nM) in vitro. Moreover, 22 showed significant in vivo antitumor activity in xenograft mouse model, when administered intraperitoneally at a dose of 12.5 – 100 mg/kg/day for 21 days.

Telik in 2010 disclosed a PCT application claiming benzimidazol- and benzothiazol-ylidene acetamide derivatives as aurora kinase and VEGFR2 kinase inhibitors for the treatment of cancer \[92\]. They have disclosed several compounds with potent in vitro activity along with their in vivo anticancer activity in tumor xenograft models. Representative compound 23 was shown to inhibit Aurora-A (IC$_{50}$ 10 nM) and -B (IC$_{50}$ 5 nM) and VEGFR2 (IC$_{50}$ 7 nM) in vitro. Compound 23 inhibited the proliferation of HCT-116 (IC$_{50}$ 300 nM), HL60 (IC$_{50}$ 500 nM) and HUVEC (IC$_{50}$ 60 nM) cancer cell lines in vitro. Moreover, when
Table 5. Aurora kinase patents filed by National Health Research Institutes and their features.

<table>
<thead>
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<td>WO2009134658A2 [79]</td>
<td><img src="image1" alt="12(G)" /></td>
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<td>WO2010036629A2 [80]</td>
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<td><img src="image4" alt="13" /></td>
<td>Aurora kinase inhibitors</td>
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(G): General structure.
Table 6. Aurora kinase patents filed by Schering Corp. and their features.

<table>
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<td>WO2009097233A1 [83]</td>
<td><img src="G" alt="Structure 1" /></td>
<td><img src="G" alt="Example 1" /></td>
<td>Aurora kinase A and B inhibitors</td>
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<tr>
<td>WO2009017701A1 [84]</td>
<td><img src="G" alt="Structure 2" /></td>
<td><img src="G" alt="Example 2" /></td>
<td>Aurora kinase inhibitors used in combination with anti-mitotic agents</td>
</tr>
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</table>

(G): General structure.
Table 7. Aurora kinase patents filed by University Health Network and their features.

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<tr>
<td>WO2009079767A1 [85]</td>
<td>![Structure 16(G)]</td>
<td><img src="image" alt="Example 16" /></td>
<td>Aurora kinase B and/or PLK4 inhibitors</td>
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<tr>
<td>WO2010115279A1 [86]</td>
<td>![Structure 17(G)]</td>
<td><img src="image" alt="Example 17" /></td>
<td>Aurora kinase B and/or PLK4 inhibitors</td>
</tr>
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</table>

(G): General structure.
Table 8. Aurora kinase patents filed by Arrow Therapeutics, Bayer, Curis and their features.

<table>
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<th>General structure</th>
<th>Representative examples</th>
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<td>WO2009063240A1</td>
<td><img src="image1" alt="Structure 18(G)" /></td>
<td><img src="image2" alt="Example 18" /></td>
<td>Aurora kinase inhibitors</td>
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<tr>
<td>WO2009042543A1</td>
<td><img src="image3" alt="Structure 19(G)" /></td>
<td><img src="image4" alt="Example 19" /></td>
<td>Aurora kinase A and B inhibitors</td>
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<tr>
<td>WO2009086012A1</td>
<td><img src="image5" alt="Structure 20(G)" /></td>
<td><img src="image6" alt="Example 20" /></td>
<td>Aurora kinase A and B inhibitors</td>
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</table>

(G): General structure.
Table 9. Aurora kinase patents filed by Mikana Therapeutics, Shanghai Genomics, Telik and their features.

<table>
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<th>Patent no</th>
<th>General structure</th>
<th>Representative examples</th>
<th>Comments</th>
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<td>WO2010062848A1 [90]</td>
<td><img src="image" alt="Structure 21(G)" /></td>
<td><img src="image" alt="Structure 21" /></td>
<td>Aurora kinase A inhibitors</td>
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<tr>
<td>WO2010051781A1 [91]</td>
<td><img src="image" alt="Structure 22(G)" /></td>
<td><img src="image" alt="Structure 22" /></td>
<td>Protein kinase inhibitors</td>
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<tr>
<td>WO2010036873A1 [92]</td>
<td><img src="image" alt="Structure 23(G)" /></td>
<td><img src="image" alt="Structure 23" /></td>
<td>Aurora kinases A and B and VEGFR2 inhibition</td>
</tr>
</tbody>
</table>

(G): General structure.
tested in vivo after oral administration, it reduced tumor growth of HCT-116 (56% inhibition; 150 mg/kg/day, on days 1 - 5 and 8 - 12) and HL60 (47% inhibition; 150 mg/kg/day, on days 1 - 5 and 8 - 10) in mouse xenograft models.

3.9 Patents from University Leipzig and Vitae Pharma

University Leipzig and others in 2010 disclosed a PCT application claiming quinoline derivatiges of general formula 24(G) as aurora kinase inhibitors useful for the treatment of cancer (Table 10) [93]. Representative compound 24 was shown to selectively inhibit Aurora-A (IC50 2.75 nM) over Aurora-B (IC50 3380 nM) in vitro. Moreover, HeLa cells treated with 24 showed a dose-dependent increase in proportion of cells with 4N DNA content. However, treatment did not result in cells with 8N DNA content, further indicating that 24 as an Aurora A selective inhibitor.

Vitae Pharma in 2009 filed a PCT application claiming quinazolinyl aminopyrazolyl acetamides of general formula 25 (G) as aurora kinase inhibitors for the treatment of cancers, particularly colorectal, breast and leukemia (Table 10) [94]. A representative example 25 from this series shows potent aurora kinase A (IC50 1.93 nM) and B inhibition (IC50 0.48 nM) in vitro. Also, 25 showed potent anti-proliferative activity in several cancer cell lines such as Colo205 (IC50 1.82 nM), HCT-116 (IC50 5.32 nM), HL-60 (IC50 1.32 nM) and MCF-7 (IC50 16.91 nM). The compounds claimed in this patent have high structural similarity to AZD1172, except for their phenyl substitution at 6-position of quinoline ring.

4. Conclusion

Mitosis is a key stage of the cell cycle, and tight regulation of the microtubule dynamics is important for the completion of mitosis. Therefore, various anti-tubulin/microtubule compounds have been developed as anticancer agents, including vinca alkaloids (vincristine, vinblastine) and taxanes (docetaxel, paclitaxel) [95]. However, microtubule also plays an important role in different cell cycle unrelated functions, such as vesicle transportation and cell signaling; these anti-microtubule compounds can cause toxicity to non-dividing cells [96]. Therefore, interfering with the process of mitosis by targeting the non-structural components, that is, proteins that regulate the mitotic step of the cell cycle, may gives less adverse effects during cancer treatment.

Aurora kinases are key regulators of mitosis. Under normal physiological conditions, they are essential for spindle assembly, centrosome maturation, chromosomal segregation and cytokinesis. However, under pathological conditions, it has been demonstrated that aurora kinases are overexpressed in various cancers and also played important roles in the process of tumorigenesis. Because overexpression of Aurora-A and -B is frequently associated with tumorigenesis, these molecules are the attractive drug targets for anticancer therapy.

Currently, around a dozen aurora kinase inhibitors are actively pursued in clinical trials for the treatment of various cancers. The most actively pursued compounds in clinical testing are AZD1152, PHA739358 and MLN8237. Particularly, MLN8237 is registered for 13 clinical trials in www.clinicaltrials.gov database. Most of the aurora kinase inhibitors are tested as single agents and a few of them are being tested in combination with other established chemotherapeutic agents, for example, AZD1152 with cytarabine, MLN8237 (with paclitaxel or bortezomib), R-763/ AS-703569 (with gemcitabine), VX-689/MK-5108 (with docetaxel) and BI 811283 (with cytarabine). Both the literature and patent reviews show that the current preclinical testing for these agents is moving towards combination therapy. This suggests that more of these agents could be tested in clinics in combination with other established treatments of cancer. For example, combination of AZD1152 with CPT-11 (irinotecan, a topoisomerase I poison) showed superior in vitro and in vivo anticancer efficacy as compared to both AZD1152 and CPT-11 monotherapies [86]; SN5-314 shows synergistic growth inhibitory effect when used in combination with other antimitotic agents [16]. Moreover, pharmaceutical companies filed patent applications claiming combination therapy of aurora kinase inhibitors with other established treatments of cancer. For example, Neriviano Medical Sciences claimed the use of PHA739358 and related compounds, in combination with BCR-ABL kinase inhibitors (imatinib, dasatinib, nilotinib), in US20100022553A1 [96]. They also claimed the use of PHA739358 and related compounds in combination with anti-neoplastic agents selected from the group consisting of platinum derivative, anti-metabolite agents, topoisomerase I inhibitors and anti-microtubule agents in a PCT application WO20100009967A1 [97]. It is likely that aurora kinase inhibitors could be more beneficial when used in combination with other established anticancer drugs than when used as monotherapy.

Another important aspect of aurora kinase inhibitors is that they may show more promise in the treatment of leukemias than solid tumors. The majority of the aurora kinase inhibitors in clinical trials are being tested for the treatment of leukemia. For example, AZD1152 is tested in patients with AML, PHA739358 in chronic myeloid leukemia (CML), and AT-9283 in AML and CML patients both in Phase I and II testing. Currently, it is not clear if the therapeutic activity of these compounds in leukemia is primarily due to aurora inhibition or due to the multi-kinase targeting of BCR-ABL kinase and/or FLT3 kinase. The most promising application for aurora kinase inhibitors appears to be in FLT3-mutated AML and imatinib-resistant CML/Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia, particularly when caused by the T315I mutation [98].

In summary, aurora kinases have emerged as key regulators of mitosis and abnormalities in their expression/activity are
Table 10. Aurora kinase patents filed by University Leipzig, Vitae Pharma and their features.

<table>
<thead>
<tr>
<th>Patent no</th>
<th>General structure</th>
<th>Representative examples</th>
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<td>WO2009111028A1 [94]</td>
<td><img src="image" alt="25(G)" /></td>
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<td>Aurora kinase A and B inhibitors</td>
</tr>
</tbody>
</table>

(G): General structure.
closely related to the cancer development and progression. Preclinical testing has shown both aurora kinases A and B as suitable drug targets for cancer treatment. These agents both single as well as in combination with existing cancer treatment hold promise for future cancer therapy.

5. Expert opinion

As previously discussed by us [19], analysis of the literature reveals three common strategies utilized by different groups in developing aurora kinase inhibitors, which are:

(i) Through a computer aided design  
(ii) High-throughput screening or  
(iii) Through modification of known kinase inhibitor structures (Figure 1).

Particularly, the strategy of modifying a known kinase inhibitor structure to develop novel patentable series of compound is very attractive both from the design point of view and also for the generation of new intellectual property rights (IPR). Such a strategy was successfully applied by various pharmaceutical companies in an effort to develop new IPRs.

Figure 1. Common strategy used for developing new aurora kinase inhibitors: chemical modification of known inhibitors.
For example, Mikana Therapeutics claimed pyrazole derivative 21 in WO2010062848A1 [90], which was closely related to VX-680 as aurora kinase inhibitors for the treatment of cancer. This new patent seems to be an extension to their previous PCT application WO07041358A2, which claimed the use of ENMD-2076 in cancer treatment. Vitae Pharma filed WO2009111028A1 [94], claiming quinazolinyl aminopyrazolyl acetamide 25, which is structurally related to a Phase II clinical trial compound AZD1152. Such a strategy to modify the existing aurora kinase inhibitors to develop novel patentable molecules through either modification of the heterocyclic core (hinge-binding core) or side chains appended to it could also be utilized by others in the future. Nevertheless, it should be noted that the initial holders of IPR do file a series of patents covering structurally related compounds and competitors need to carefully bypass these patents to develop novel compounds.

As discussed in the previous section, some of the aurora kinase inhibitors are being tested in clinics as combination with other established chemotherapeutic agents, for example, AZD1152 (with cytarabine), MLN8237 (with paclitaxel or bortezomib), R-763/AS-703569 (with gemcitabine), VX-689/MK-5108 (with docetaxel) and BI 811283 (with cytarabine). Alternatively, for this combination therapy with two different drugs, a single drug could be developed to have dual targeting pharmacophoric groups. On these lines, Curis has filed WO2009086012A1 application [89] which claims novel compounds with pharmacophoric features essential for both aurora kinase inhibition and HDAC inhibition. A representative compound 20 was designed to contain the VX-680’s pharmacophoric feature, and also a hydroxamate functional group essential for HDAC inhibition (Figure 1). Such combi-targeting compounds were also claimed by Curis recently for dual EGFR kinase inhibition and HDAC inhibition in PCT application WO2009035718A1.

One important factor to be considered in the future design of new aurora kinase agents is the development of resistance in cancers. It seems that the effectiveness of most aurora kinase inhibitors including VX680, PHA-739538 and AZD1152 are subjected to the expression of the multiple drug efflux pump MDR1 (multiple drug resistance protein) in cancer cells. Therefore, it is important to identify novel compounds that can overcome the MDR1-related resistance and also exhibit improved pharmacological profiles as compared to the existing aurora kinase inhibitors. In this regard, usage of dual acting compounds as those described by Curis in WO2009086012A1 that show activity against multiple targets may be able to overcome drug resistance or at least delay the development of resistance. For example, imatinib–HDAC hybrid compounds were found to overcome imatinib-resistant Abl T315I mutant kinase [99].

**Declaration of interest**

The authors declare no conflict of interest. This work was supported by grants from the National Science Council (NSC99-2323-B-400-006, NSC99-2323-B-400-007, NSC99-2120-M-006-005, NSC98-2119-M-400-001-MY3) and the Department of Health (DOH99-TD-C-111-004, 850 DOH98-TD-G-111-020). The authors also thank the National Health Research Institutes, Taiwan, Republic of China. MS Coumar is an employee of Pondicherry University, India.
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Aurora kinase inhibitor patents and agents in clinical testing: an update (2009 – 10)


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