
2. REVIEW OF LITERATURE

In 1995, David Glover reported the discovery of a new family of mitotic kinases from his study on mutant alleles associated with defective spindle pole organization in *Drosophila melanogaster*. The members of this family of kinases are known as the aurora kinases (AKs).⁴¹

In humans there are three homologous AKs (EC:2.7.11.1), designated as aurora A, aurora B and aurora C kinases.

Aurora A kinase is the mitotic serine/threonine kinase which is involved in the control of cell cycle progression and it belongs to the gene AURKA. Aurora A is associated with the spindle microtubules and centrosome throughout the mitosis and performs a significant role in a range of mitotic events including the centrosome maturation, centrosome duplication, establishment of mitotic spindle, centrosome separation as well as maturation and cytokinesis. Aurora A is mainly required for initial activation of CDK1 at centrosomes to promote timely mitotic entry. Aurora A complex with TPX2, Ajuba, BORA or HEF1 leads to its activation by autophosphorylation on Thr288. After getting activated by phosphorylation at Thr-288; there occurs a change in the conformation of the activation segment. Rate of phosphorylation of aurora A at Thr-288 varies during the cell cycle and is the highest during M phase. The aurora A kinase is highly expressed in testis, colon, ovarian, prostate, neuroblastoma, breast and cervical cancer cell lines and poorly in skeletal muscle, thymus and spleen. Aurora A gene is located on chromosome 20q13.2 which is a well defined hotspot of amplification in tumors. Human aurora A is reported to be amplified in more than 12% of primary breast tumors and more than 50% of primary colorectal cancers. Aurora A also acts as a key regulatory component of the p53 pathway. There is a firm relation observed among aurora A overexpression, p53 function and the observed effect on cellular processes and tumorigenesis. The phosphorylation, stability and transcriptional activity of p53 is controlled by aurora A. The tetraploidisation and centrosome amplification because of over expression of aurora A, which lead to cancer, are directly related to p53 status. These effects mainly occur due to cell cycle arrest in response to DNA damage.^{7,42-44}

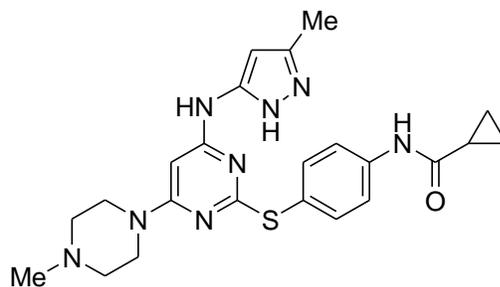
Aurora B complexes with three other proteins, Survivin, Borealin and INCENP to form chromosomal passenger complex (CPC), a complex that acts as a key regulator of mitosis. In human, aurora B gets autophosphorylated within its activation segment on threonine 232. Full

activation of aurora B occurs on autophosphorylation and binding with INCENP. Aurora B gene is located on chromosome 17p13.1. The CPC complex plays vital functions at the centromere in ensuring correct chromosome alignment, segregation and chromosome condensation. Its major role includes promoting chromosome bi-orientation, syntelic chromosome attachments, merotelic chromosome attachments, the spindle assembly checkpoint and cytokinesis. Localization of aurora B to the centromere throughout prometaphase and metaphase needs phosphorylation of the mammalian kinetochore-specific histone-H3 variant centromere protein A (CENP-A). CENP-A associates with the centromere and is necessary for assembly of the kinetochore. Aurora A kinase initiates the phosphorylation of CENP-A at serine 7 to recruit the aurora B to the centromere. Aurora B, once it is recruited then itself can phosphorylate CENP-A at the same residue. Aurora B is expressed during S and G2/M phases and its expression is up-regulated in cancer cells during M phase. It is highly expressed in the thymus. It is also expressed in the lung, testis, spleen, colon, placenta and fetal liver.^{23,45,46}

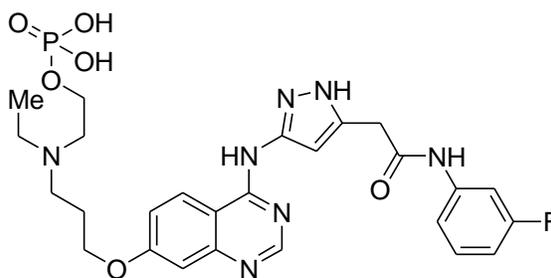
Like aurora B, aurora C is able to bind with the INCENP. This binding results in activation of aurora C. It is not known till now what the exact requirements are for full aurora-C activation in physiology. Aurora C is highly expressed in testis. The expression of aurora C is observed maximum at M phase. Elevated expression levels were observed only in a subset of cancer cell lines such as Hep-G2, Huh-7 and HeLa.^{47,48}

All this information suggests AKs as potential targets for the treatment of cancer suggesting the importance of aurora A followed by aurora B in cancer treatment. Since the identification of AKs as potential targets for the development of cancer chemotherapy, many aurora kinase inhibitors have been discovered, and are currently under development.

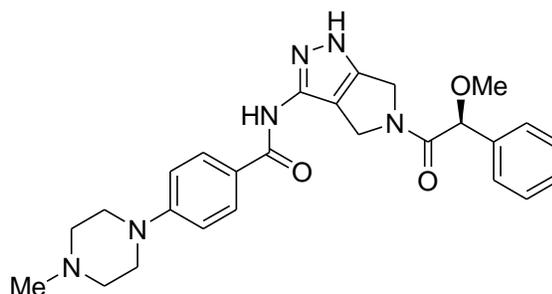
VX-680 (also known as Tozasertib, MK-0457) was the first to enter the clinical trials which was discovered by Vertex Pharmaceuticals, Oxford, UK and subsequently co-developed with Merck. It was designed during the SAR exploitation of a lead amino pyrazole linked to 2-substituted quinazoline. It has been revealed to inhibit aurora A, B, and C with Ki values of 0.7, 18, and 4.6 nM, respectively. In cytotoxicity assays with several tumor cell lines VX-680 inhibited proliferation with IC₅₀ values varying from 15 to 130 nM. Regardless of the promising results in clinical trials, this compound was later withdrawn due to cardiac toxicity observed during open label Phase I/II clinical trials.^{49,50}

**VX-680**

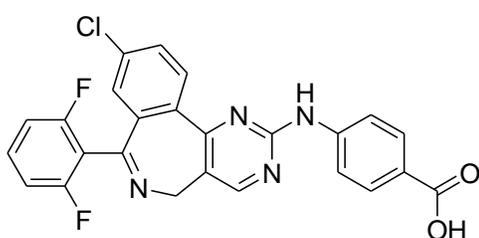
AZD-1152 (Barasertib) is a selective aurora B kinase inhibitor (0.37 nM) developed by AstraZeneca. It recently has passed the Phase I/II and being pursued in Phase II/III clinical trials. It was developed from the lead pyrazole-acetanilide substituted quinazoline. It has shown varying potency levels among different leukemia cells with IC_{50} in the range of 3 to 40 nM.⁵¹

**AZD-1152**

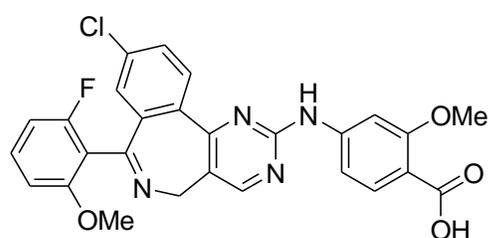
PHA-739358 (Danusertib), developed by Nerviano Medical Sciences, has completed Phase I clinical trials. A recent Phase II clinical trial report in 2013 explained its efficacy and toxicity in patients with metastatic castration-resistant prostate cancer. The most common adverse effects observed here were gastrointestinal disorders in 61.7% cases. This inhibitor is having pharmacophoric features of pyrrolopyrazole scaffold.⁵²

**PHA-739358**

MLN-8237 (Alisertib) reported by Millennium pharmaceuticals is highly selective and potent aurora A kinase inhibitor ($IC_{50} = 1$ nM). This is a predecessor of MLN-8054 reported by the same company which was withdrawn because of off-target toxicities observed during Phase I clinical trial. As per Phase II clinical trial reports published in 2014, MLN-8237 seems clinically active in both B- and T-cell aggressive lymphomas and is now under Phase III testing of clinical trials for relapsed peripheral T-cell lymphoma as a single agent (NCT01482962). MLN-8237 was obtained by optimization of 5*H*-pyrimido[5,4-*d*][2]benzazepine as a lead. Its non-activity towards aurora B was explained by computational model, where it was observed that Glu177 residue in aurora B displayed electrostatic repulsion.⁵³

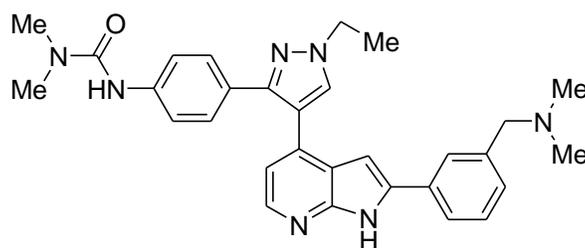


MLN-8054



MLN-8237

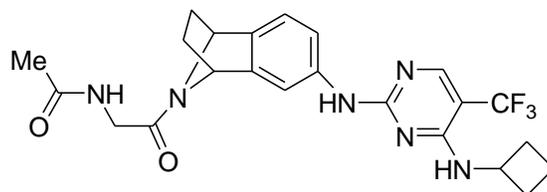
GSK-1070916 reported by GlaxoSmithKline, is an aurora B/C inhibitor against solid tumors as well as hematological malignancies. It has completed Phase I clinical trials. It is reported that GSK-1070916 shows action by inhibiting aurora B-INCENP and aurora C-INCENP complexes with IC_{50} of 5 nM and 6.5 nM respectively. It is a 4-(1*H*-pyrazol-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine analog.⁵⁴



GSK-1070916

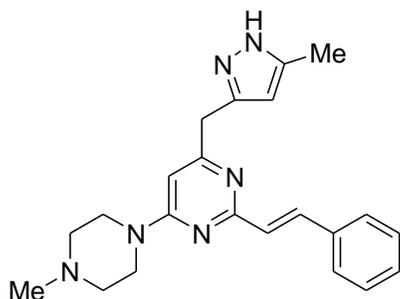
PF-03814735 is an orally bioavailable pan-selective aurora kinase inhibitor developed by Pfizer. It is based on C2 and C4 optimized pyrimidine scaffold. It has shown inhibition of recombinant aurora A and aurora B with IC_{50} values of 5 nM and 0.8 nM respectively. This drug candidate recently completed Phase I clinical trials with safety, PK and PD studies. The major

adverse effects observed were diarrhoea, fatigue, nausea, vomiting and prolonged low-grade neutropenia.⁵⁵

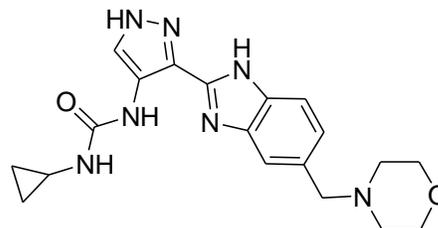


PF-03814735

ENMD-2076 is an orally active vinyl-pyrimidine derivative developed by EntreMed. It is a selective aurora A kinase inhibitor with IC₅₀ value of 14 nM over aurora B kinase (IC₅₀ 290 nM). A Phase II study performed by Matulonis *et al* in 2013 reported the activity and side effect profile of ENMD-2076 in platinum-resistant recurrent epithelial ovarian cancer. The most common adverse effects observed were fatigue, hypertension and diarrhoea. Presently, this drug candidate is undergoing Phase II clinical trials for previously treated advanced and metastatic TNBC (NCT01639248), advanced/metastatic soft tissue sarcoma (NCT01719744) and ovarian clear cell cancers (NCT01914510), as a single agent.⁵⁶



ENMD-2076

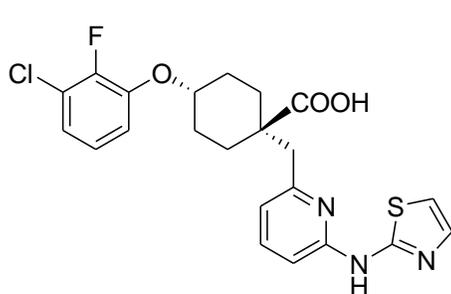


AT-9283

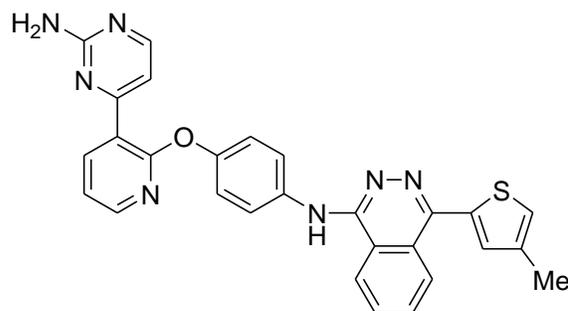
AT-9283 developed by Astex Therapeutics is a multi-targeted kinase inhibitor which inhibits both tyrosine as well as serine/threonine kinases such as aurora A (3 nM) / B (3 nM), JAK2 (1.2 nM), JAK3 (1.1 nM) and Abl (4.0 nM T315I). It is developed by optimization of a pyrazole-benzimidazole lead. This compound was obtained by fragment based drug discovery approach. This drug candidate is now under Phase II clinical trials for refractory multiple myeloma (NCT01145989). This candidate is also in Phase I/II clinical trials in young patients (age under 18 years) for refractory or relapsed acute leukemia (NCT01431664).⁵⁷

VX-689/MK-5108 is a selective aurora A kinase inhibitor ($IC_{50} = 0.064$ nM) developed by Vertex pharmaceuticals. Recently Shan *et al* showed it to be effective for uterine leiomyosarcoma. This drug candidate has completed Phase I clinical trial testing in patients with advanced solid tumors as a single agent and in combination with docetaxel.⁵⁸

AMG-900 developed by Amgen, is a novel pan-aurora kinase inhibitor now undergoing Phase I clinical trials in patients with AML (NCT01380756) and advanced solid tumors (NCT00858377). Amgen obtained patent for this compound in 2011 (WO2011031842A1).⁵⁹

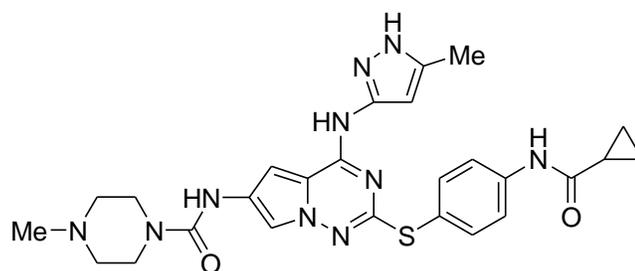


VX-689



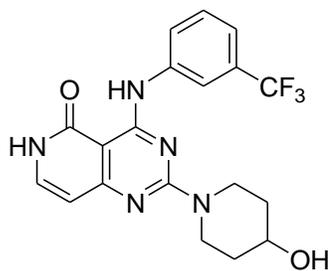
AMG-900

Ambit Biosciences Co. filed a PCT application in 2011 (WO2011088045A1) on pyrrolotriazine series of compounds as aurora kinase inhibitors. The representative structure (**1**) is shown here. It was found that compound (**1**) inhibits all aurora kinases (A, B and C) with K_d values of less than 10 nM.⁶⁰

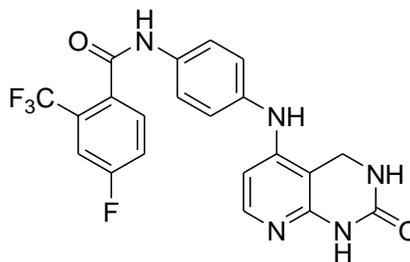


(1)

In 2011, Genosco and Oscotec Inc. obtained a PCT (WO2011053861A1) with the description of pyrido[4,3-d]pyrimidin-5-one derivatives as multiple kinase inhibitors including inhibition of aurora kinases for the treatment of cancer, neurodegenerative and autoimmune diseases. The representative structure (**2**) is shown here. It was observed to be active on various kinases like SYK2, PTK2B, FAK, JAS2, LRRK2 including aurora kinases with 31% inhibition.⁶¹



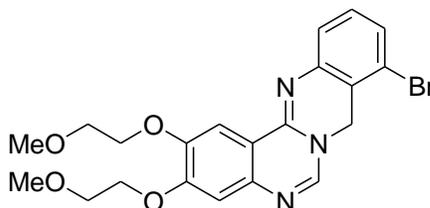
(2)



(3)

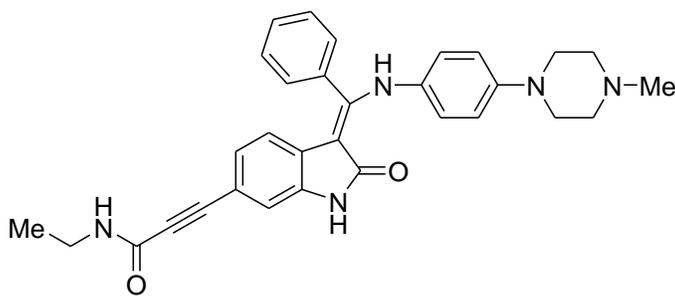
A PCT application on azaheterocyclic compounds as inhibitors of aurora kinase and RON kinase, which is a macrophage stimulating kinase, have been disclosed by Merck GMBH in 2011 (WO2011017142A1). The representative structure (3) is shown here. This compound showed aurora kinase inhibition activity with $IC_{50} < 10$ nM.⁶²

In 2011, Shenzhen Salubris Pharma described polycyclic quinazoline derivatives as aurora kinase and protein tyrosine kinase inhibitors in PCT application (WO2011144059A1). A representative structure (4) is shown here.⁶³

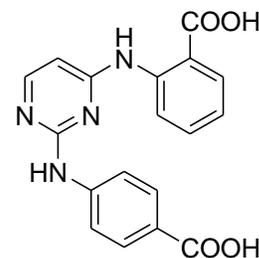


(4)

Boehringer Ingelheim disclosed a PCT application (WO2012095505A1) in 2012 describing the use of indolinone analogs as dual aurora and MEK kinase inhibitors. The representative compound (5) is shown here. This showed aurora B inhibition at 2 nM concentration and MEK1 inhibition with IC_{50} value of 10 nM.⁶⁴



(5)

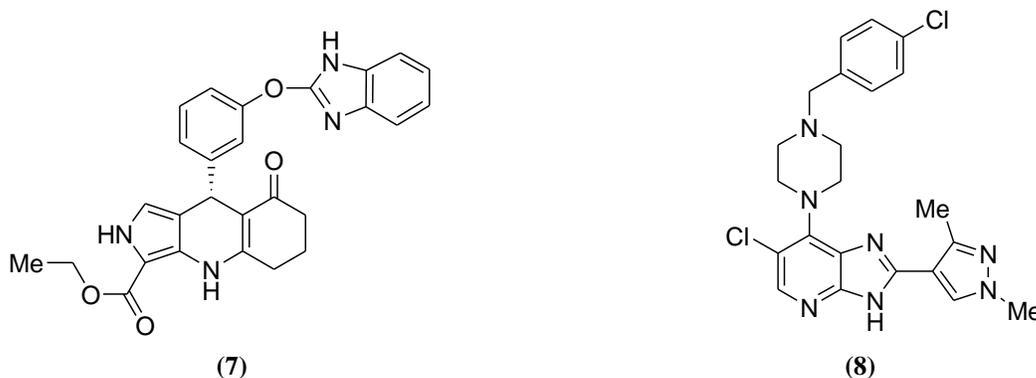


(6)

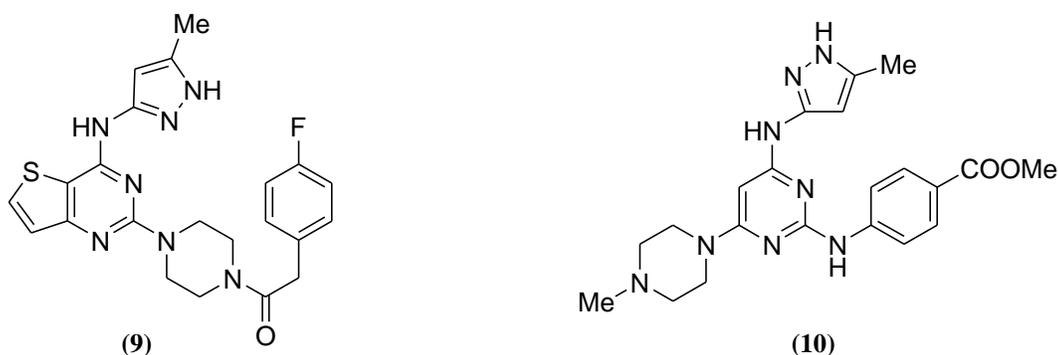
Moffitt Cancer Centre described the role of bisanilinopyrimidines as aurora kinase inhibitors in their patent application (WO2012135641A2) in 2012. The representative compound (6) is shown here. This compound inhibits aurora A with IC_{50} value of 6.1 nM.⁶⁵

Sanofi described compound (7) as aurora A/B kinase inhibitor in their 2012 PCT application (WO2012066486A1). In this application they also disclosed the preparation of sulphate salt of compound 7 and the procedure for chiral resolution. Both the free as well as the salt form of compound 7 showed inhibition of aurora A ($IC_{50} = 2$ nM) and aurora B ($IC_{50} = 1$ nM).⁶⁶

In 2013, Cancer Research Technology Ltd. described the role of imidazopyridines as potent aurora kinase inhibitors in PCT application (WO2013190319A1). The representative structure (8) showed high affinity for aurora A ($K_d = 7.5$ nM) and aurora B ($K_d = 48$ nM). This compound also showed FLT3 inhibition.⁶⁷



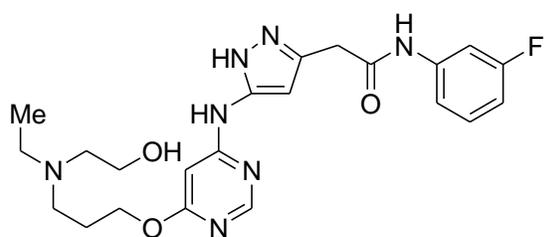
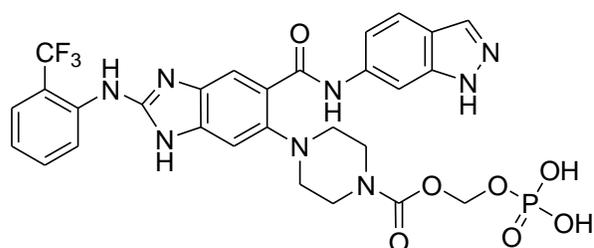
Guangzhou Institute of Biomedicine and Health (Chinese Academy of Sciences), in 2013 described a series of 2,4-disubstituted thieno[3,2-*d*]pyrimidine compounds as aurora kinase inhibitors in a Chinese patent (CN103242341A). The representative structure (9) was found to be potent aurora A ($IC_{50} = 0.69$ nM) and aurora B ($IC_{50} = 84$ nM) kinase inhibitor.⁶⁸



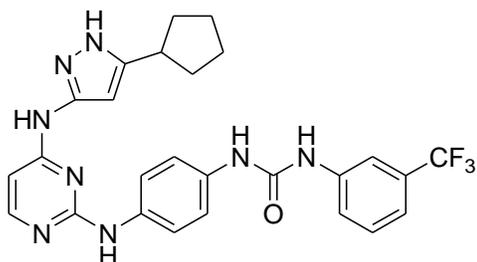
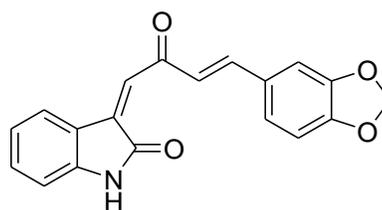
Sun Yar-Sen University in 2013 filed three Chinese patents (CN103059002A; CN103191120A; CN103202843A) discussing the role of pyrimidine derivatives as aurora kinase inhibitors. Compound **(10)** represents the derivatives described in the patents.⁶⁹⁻⁷¹

In 2013, Sunshine Lake Pharma Co. Ltd. described the use of aminopyrimidine derivatives as aurora kinase inhibitors in PCT application (WO2013143466A1). The representative structure **(11)** was reported to be a potent aurora A ($IC_{50} = 4.7$ nM) and aurora B ($IC_{50} = 28$ nM) kinase inhibitor.⁷²

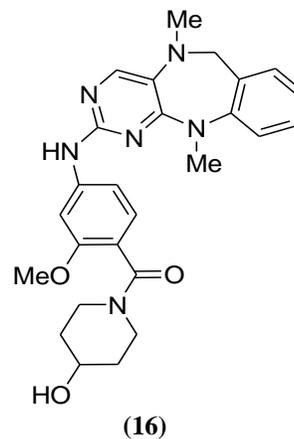
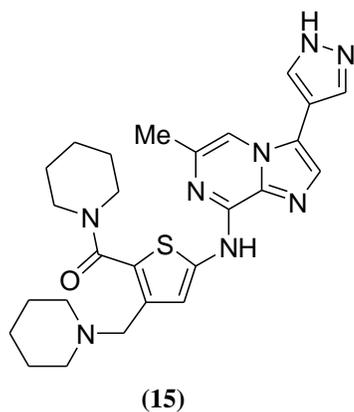
A PCT application (WO2014193696A2) published in 2014 by Transtech Pharma reported the benzimidazole carboxylic acid derivatives as aurora kinase inhibitors. The representative structure **(12)** mentioned here is reported to have inhibitory activity of less than 1 μ M.⁷³

**(11)****(12)**

A PCT application (WO2014190207A1) from The Regents of the University of California in 2014, reported urea derivatives as aurora kinase inhibitors. The representative structure **(13)** is shown here. It inhibits aurora A and is reported to be useful in MYCN-amplified neuroblastoma with IC_{50} value of 45 nM.⁷⁴

**(13)****(14)**

Regents of the University of Minnesota reported isatin derivatives as aurora B kinase inhibitors in their PCT application (WO2014066840A1) published in 2014. The representative compound (**14**) inhibited aurora B kinase with concentration less than 1 μM .⁷⁵



Apart from these reports, many other research organizations have reported various candidates as potential aurora kinase inhibitors. Merck Research Laboratories in three different publications reported imidazo[1,2-*a*]pyrazine derivatives⁷⁶⁻⁷⁸ as aurora kinase inhibitors (**15**; aurora A IC_{50} = 2 nM). The benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6(11*H*)-one derivative⁷⁹ (**16**; aurora A IC_{50} = 2.6 nM) was also reported as aurora kinase inhibitor by another research group.