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Characterization of a highly selective inhibitor of the Aurora kinases

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ABSTRACT

Aurora kinases play an essential role in mitosis and cell cycle regulation. In recent years Aurora kinases have proved popular cancer targets and many inhibitors have been developed. The majority of these clinical candidates are multi-targeted, rendering them inappropriate as tools for studying Aurora kinase mediated signaling. Here we report discovery of a highly selective inhibitor of Aurora kinases A, B and C, with potent cellular activity and minimal off-target activity (PLK4). The X-ray co-crystal structure of Aurora A in complex with compound **2** is reported, and provides insights into the structural determinants of ligand binding and selectivity.

The Aurora kinases are a family of cell-cycle regulated serine/threonine kinases which are primarily active during mitosis.[1] These homologous kinases effect distinct processes via differential expression, localization and interaction partners. Aurora A localizes at the centrosome during interphase, and localizes at mitotic poles and to the spindle throughout mitosis.[2] Aurora A regulates progression of mitosis and promotes centrosome maturation.[1] Aurora B localizes to centromeres during metaphase as part of the chromosomal passenger complex (CPC) and remains associated with the central mitotic spindle during anaphase.[3] The CPC regulates chromosome condensation, via phosphorylation of histone H3, the spindle assembly checkpoint (SAC)[4] and cytokinesis.[5] Unlike Aurora A and B, Aurora C is not expressed in all dividing cells, as its primary role is in male meiosis during spermatogenesis.[2,6]

The Aurora kinases are implicated in a variety of hematological and solid cancers. Aurora A and B are frequently overexpressed in cancer, and have been associated with aneuploidy and poor prognosis.[7,8] Aurora C has also been show to function as an oncogene and overexpression has been reported in thyroid cancer tissues.[6] The Aurora kinases have therefore become attractive drug targets with more than ten Aurora inhibitors undergoing clinical trials.[9] In solid tumors, on-target bone marrow toxicity has precluded the use of Aurora inhibitors in cancer therapy. This is hypothesized to be due to the slower proliferation rate of cells in solid tumors relative to those in the bone marrow, and the requirement for drug exposure through several cell cycles before the maximal cytotoxic effects are realized.[9] Acute hematological tumors, such as Acute Myeloid Leukaemia (AML), have higher proliferation rates and have shown more promising response rates to Aurora kinase inhibitors in the clinic.[10–12]

Many Aurora kinase inhibitors developed to date display polypharmacology, targeting numerous kinases.[9] Whilst this may contribute to clinical efficacy, it does not facilitate detailed study of Aurora kinase mediated signaling and cellular processes. Recently, Carry et al. reported an exquisitely selective pan-Aurora inhibitor,

Abbreviations: PLK4, Polo-like kinase 4; FACS, fluorescence activated cell sorting; DMSO, dimethylsulfoxide; AML, acute myeloid leukemia; T-ALL, T-cell acute lymphoblastic leukemia; ABL1, Abelson Tyrosine-Protein Kinase 1; LRRK2, Leucine Rich Repeat Kinase 2; BRD4, Bromodomain-Containing Protein 4; ERK5, Mitogen-activated protein kinase 7; LC-MS/MS, Liquid Chromatography–Mass Spectrometry/Mass Spectrometry.

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SAR156497. This compound displayed efficacy but had a narrow therapeutic window in colon adenocarcinoma xenograft studies.[13] SAR156497 was used as a control compound in our experiments, is one of the most selective cell permeable pan-Aurora inhibitors described to date.

Here we report discovery of highly selective pan-Aurora kinase inhibitors through phenotypic screening. The co-crystal structure of compound **2** bound in the ATP binding site of Aurora A is described, and provides insight into the possible structural basis for the selectivity of the interaction.

To identify anti-leukemic compounds, a library of pyrimidobenzodiazepinones was tested for anti-proliferative activity in Jurkat cells, a T-cell acute lymphocytic leukemia (T-ALL) cell line. We previously used this phenotypic screening and profiling approach to identify PI3K-δ/γ inhibitors.[14] Compound **1** was identified as a cytotoxic molecule with an IC_{50} of 370 nM. Kinase profiling against a panel of 468 human wild-type and mutant kinases using KINOME*scan*® at 1 μM revealed that compound **1** is a highly selective pan-Aurora inhibitor with exclusively on-target activity detected across the panel at 1 μM compound concentration (Fig. 1). The Aurora A, B and C activity was confirmed using biochemical kinase assays (Fig. 2C).

The Aurora kinases do not contain cysteine residues proximal to their ATP binding sites, therefore we hypothesized that the interaction of compound **1** with Aurora kinases is reversible, and that the acrylamide functionality could be removed without loss of potency. To confirm this we incubated compound **1** at 10-fold excess, with recombinant purified Aurora A protein, and observed no labeling by LC-MS/MS analysis. Compound **2** was synthesized and exhibited similar potency and selectivity to compound **1**. Both compounds have moderate off-target activity on PLK4 (IC_{50} compound **1** = 43 nM, IC_{50} compound **2** = 82 nM) but otherwise display excellent kinome selectivity. The ABL1 Y253 F activity of compound **2** from the kinome screen was not reproduced in a biochemical kinase assay and is likely to be a false positive (IC_{50} > 10,000 nM). Inhibitors based on this scaffold have been reported to bind to LRRK2 and BRD4, proteins not covered in the selectivity panel. Compounds **1** and **2** were tested in biochemical assays against these targets and gratifyingly both molecules were inactive (Supporting Table 1).

We have previously described pan-Aurora inhibitors based upon the same chemical scaffold (XMD12-1).[15] However, compounds **1** and **2** have significantly improved kinome selectivity (Fig. 1), making them some of the most selective pan-Aurora kinase inhibitors reported to date.

The cellular activity of compounds **1** and **2** were assessed by their ability to inhibit the phosphorylation of biomarkers of Aurora A and Aurora B by western blot in HCT116 cells. Compounds **1** and **2** were cell permeable and inhibited Aurora A auto-phosphorylation and Aurora B mediated histone H3 S10 phosphorylation at concentrations below 40 nM (Fig. 2A, C, Supporting Fig. 1). These compounds showed improved cellular inhibition of Aurora A auto-phosphorylation and histone H3 S10 phosphorylation compared to the control compound SAR156497.[13]

To investigate the effects of compounds **1** and **2** on the cell cycle, we performed FACS cell cycle analysis in HCT116 cells after 48 h of 500 nM compound treatment (Fig. 2B, Supporting Fig. 2). Treatment with compounds **1**, **2**, or the control pan-Aurora inhibitor

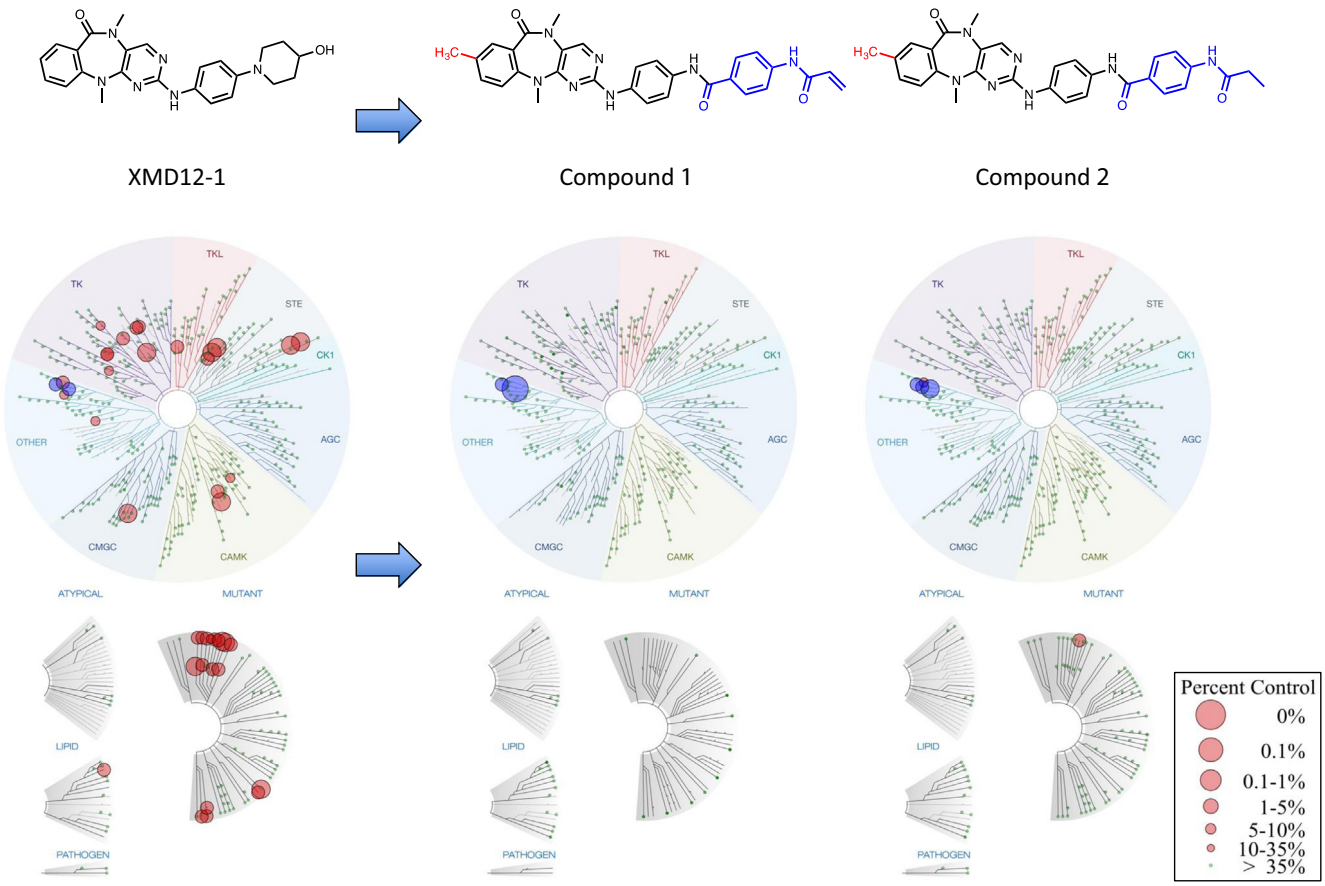

Fig. 1. A) Chemical structure and kinome-wide selectivity plot of compounds **1** and **2** compared to previously reported benzodiazepinone inhibitor XMD12-1. Assay performed by DiscovRX at 1 μM compound concentration. On target activity (Aurora A/B/C) represented by blue circles, off target activity represented by red circles. Chemical modifications to the core (red), and aniline (blue), are highlighted.