Identification and Structure-guided Optimisation of Novel Inhibitors of Checkpoint Kinase 1 (Chk1) through Combined Biochemical and Crystallographic Screening

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Abstract

The purpose of this study was to identify novel small inhibitors of Chk1. Confinement and the fact that checkpoint kinases are conserved across species and activated in a wide variety of normal and disease states makes this target of great interest. Inhibition of Chk1 function is known to deregulate cell cycle checkpoints, resulting in cell death. We employed a panel of small molecule inhibitors and a panel of Chk1 inhibitors reported in the literature to screen a series of potential Chk1 inhibitors for their ability to inhibit Chk1. The inhibitors that were identified were then tested for their ability to inhibit Chk1 function in vitro and in vivo. The inhibitors that were identified were then tested for their ability to inhibit Chk1 function in vitro and in vivo. The inhibitors that were identified were then tested for their ability to inhibit Chk1 function in vitro and in vivo. The inhibitors that were identified were then tested for their ability to inhibit Chk1 function in vitro and in vivo.

Methods

Template Library Selection
- Templates are low molecular weight compounds
- Ideal start points for Lead Optimisation
- Selected computationally by Template Descriptors, kinase pharmacophore model & volume constraint
- Templates are screened biochemically and hits are validated by X-ray crystallography
- 361 Templates were screened at 250μM using AlphaScreen™ format

Evaluation of SAR-011797
- SAR-00610 IC50 = 49μM
- SAR-00467 IC50 = 46μM
- SAR-007236 IC50 = 47μM

Optimisation of SAR-011797
- Structure-guided Lead Optimisation

Compound 4 – an example from the optimised lead series:
- Chk1 potency: IC50 = 30nM (DELLFA in-house)
- G2M Abrogation: IC50 = 120nM (MIA in-house)
- Chemistry: MW = 383, C30H26F5N7O9, TP5A = 85A
- Selectivity: Chk-2 IC50 = 84,000 nM (DELLFA in-house)
- CDK1 IC50 = >100,000 nM (DELLFA in-house)
- IC50 = >10,000nM @44/49 kinase tested (Reaction Biology Corp)
- In vivo PK (mouse): Well tolerated at 50 mg/kg (MTD not reached)
- Well distributed to tissue and tumor (e.g. spleen/plasma = 20:1) with sustained levels
- HER2 inhibition: 59% at 10μM (DMSO = 9% – Millipore)
- X-ray structure: 1.9Å (n-house)

Summary

- Template screening identified 13 hits validated by crystallography
- Lead series progressed by iterative synthesis/crystallography
- Lead series example:
  - Potent and selective
  - Good PK properties
  - Biomarker modulation
  - G2 checkpoint abrogation
  - Potentiation of SN-38-induced cytotoxicity

Figure 1: Template descriptors, MA, WH, vdw shape range and generic kinase pharmacophore models used for Template selection

Figure 2: Compound 4 bound to active Chk1 kinase domain

Figure 3: Potentiation of SN-38 cytotoxicity Compound 4 Cytotoxicity IC50 = 11.6μM
SN-38 Cytotoxicity IC50 = 11.6μM
Combined Cytotoxicity IC50 = 0.16μM
Potential = 3.8 fold

Figure 4: Effect on SN-38 induced DNA damage in HT-29 cells
Chemotaxis (4μM) signal diminished
Radial p21 signal at higher concentrations
A decrease in the CDK1-p15 signal is observed

Figure 5: Effect on etoposide induced G2M arrest (2.5μM)
Compound 4 abolished etoposide induced G2M arrest at 2.5μM