

Fragment Based Discovery of AT9283; A Multi-targeted Kinase Inhibitor with Potent Aurora Kinase Activity

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INTRODUCTION

- Here we describe the identification of the clinical candidate **5** (AT9283) via structure-based optimisation of a ligand efficient pyrazole-benzimidazole fragment **1**.
- Fragment-based drug discovery is a rapidly growing technique in medicinal chemistry which differs from more traditional approaches such as high throughput screening.¹
- Despite their often low affinity, low molecular weight fragments are efficient binders and can be considered to have high 'ligand efficiency' (LE). LE is the ratio of free binding affinity to molecular size.²

- The optimisation of fragments to low nanomolar inhibitors can be achieved with a limited number of compounds, particularly if good structural data are available.³ In this work X-ray crystallographic structures were generated using a novel soakable form of Aurora A.
- Aurora kinases play a key role in the regulation of mitosis and in recent years have become attractive targets for the treatment of cancer.⁴
- In addition to Aurora A and Aurora B, AT9283 was also found to inhibit a number of other kinases including JAK2 and imatinib-resistant Abl (T315I).
- AT9283 demonstrated *in vivo* efficacy in mouse xenograft models and is currently under evaluation in Phase I/II clinical trials.

FRAGMENT-BASED EVOLUTION OF AT9283

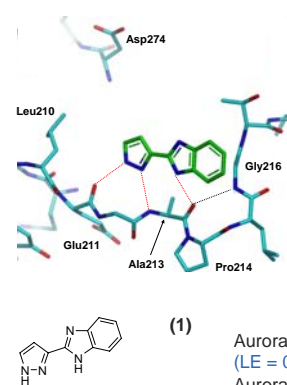
- During the course of our CDK programme it was established that simple pyrazole-benzimidazole had excellent activity and ligand efficiency against Aurora A.⁵ These included fragment **1** and benzamide derivative **2**.
- Compound 1:** Structure of **1** complexed with Aurora A showing the fragment binds deep in the ATP pocket.

- Compound 2:** Overlay of the crystal structures of **2** bound to Aurora A (protein in blue, ligand in green) and bound to CDK2 (protein and ligand in orange). Hydrogen bonds to the kinase hinge region are shown by the red lines.

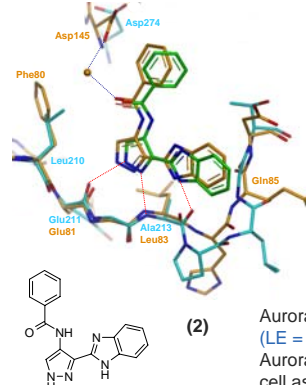
- Compound 3:** Introduction of solubilising group increased cellular activity. However the series suffered from high plasma protein binding (PPB). Analysis of the structure suggested opportunity for replacing the benzamide.

- Compound 4:** Replacing the amide linker with a urea linker significantly reduced PPB. The urea linker adopts a *cis/trans* configuration and places the phenyl group in a twisted conformation adjacent to the benzimidazole.

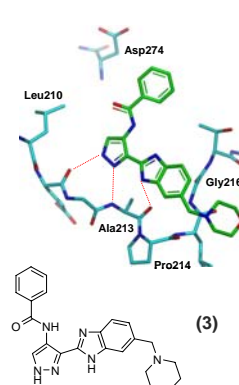
- Compound 5 (AT9283):** Further optimisation of potency and physicochemical properties led to identification of AT9283. Overlay with compound **1** confirms binding mode of original fragment is retained in AT9283. This reflects the high quality interactions between **1** and the protein which are retained throughout the optimisation process.



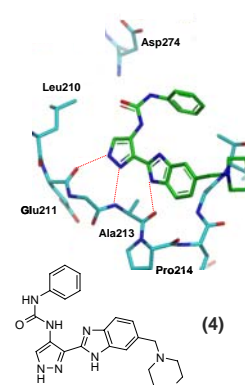
Aurora A = 0.91 μ M
(LE = 0.59)
Aurora B = 6.5 μ M



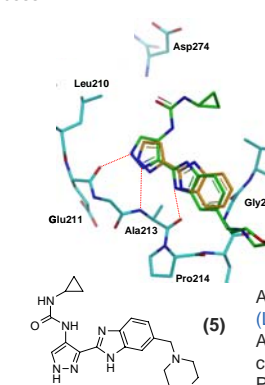
Aurora A = 5 nM
(LE = 0.49)
Aurora B = 180 nM
cell assay¹ = 3 μ M



Aurora A = 3.5 nM
(LE = 0.38)
Aurora B = 15 nM
cell assay¹ = 0.1 μ M
PPB (mouse) > 99%



Aurora A = 12 nM
(LE = 0.35)
Aurora B = 54 nM
cell assay¹ = 0.3-1.0 μ M
PPB (mouse) = 95.3%



Aurora A = ~ 3 nM
(LE ~ 0.42)
Aurora B = ~ 3 nM
cell assay¹ = 0.03 μ M
PPB (mouse) = 81.5%

¹Cell assay: Values are given as the minimum concentration of compound required to produce a polyploid morphology in HCT116 cells

Table 1: AT9283 *in vitro* Activity Profile

Protein	IC ₅₀ (nM)
Aurora A	~ 3
Aurora B	~ 3
CDK1/B	1700
CDK2/A	510
JAK2	1.2
JAK3	1.1
Abl (T315I)	4.0
Flt-3	10-30
CYP(3A4, 2D6)	> 10 μ M

Table 2: Physicochemical Properties

MW	381
logD _{7.4}	2.1
Solubility	13 mg/mL (pH5.5) 2 mg/mL (pH7.0)

Figure 1. AT9283 in MV4-11 Xenografts in Nude Mice

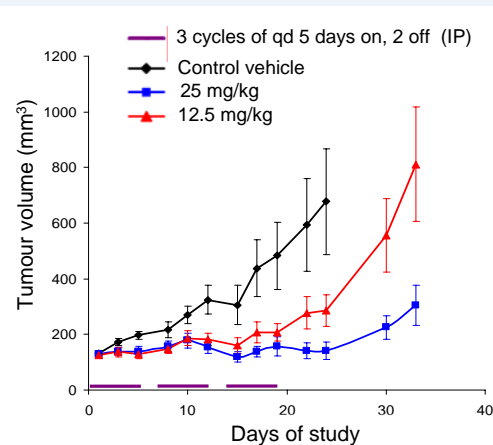


Figure 2. AT9283 in K562 Xenografts in Nude Mice

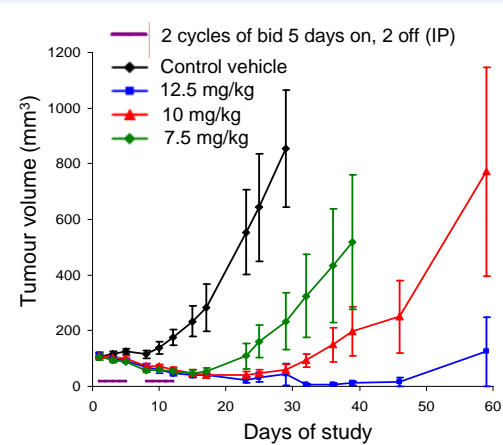


Figure 4. Biomarker Study from K562 Xenografts

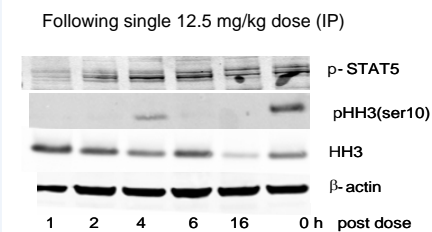


Table 3. PK (mouse)

Cl	114 mL/min/kg
V _{ss}	3.9 L/kg
F _{po}	24 %

Conclusions

- The pyrazole benzimidazole **1** was identified during the course of a CDK programme as a ligand efficient fragment starting point for the discovery of Aurora kinase inhibitors.
- X-ray crystallographic structures of Aurora A were used to drive the optimisation towards potent dual Aurora A / Aurora B inhibitors.
- These compounds inhibited growth and survival of HCT116 cells and produced the polyploid cellular phenotype typically associated with Aurora B kinase inhibition.
- In addition to Aurora A and Aurora B, **5** (AT9283) was also found to inhibit a number of other kinases including JAK2, Flt3 and Abl (T315I) (IC₅₀ = 1 - 30 nM).⁶
- AT9283 shows efficacy in a number of pre-clinical xenograft models.
- AT9283 has shown evidence of benefit in early clinical trials.⁷

References

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