

AT13148, an orally bioavailable AKT kinase inhibitor with potent anti-tumour activity in both *in vitro* and *in vivo* models exhibiting AKT pathway deregulation.

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Introduction

The AKT/PI3K pathway is an important mediator of tumor cell growth and survival. Activation of this pathway is associated with several resistant forms of cancer. This activation can occur by a number of different mechanisms targeting AKT itself or components of the pathway leading to its activation (e.g. PI3K, PTEN). As such, several key steps in this pathway are the focus of intense oncology drug discovery activity and inhibitors of PI3K, PKB and mTOR have moved into development.

AT13148 was recently selected for clinical development and is a novel, small molecule inhibitor of the AGC kinases AKT, p70S6 kinase, ROCKII and PKA. AT13148 potentially inhibits two key enzymes in the AKT/PI3K pathway making it an exciting drug with which to explore the role of this pathway in different cancers in the clinical setting.

Here we describe the work which led to the discovery of AT13148 and the further investigation of the activity of the molecule in pre-clinical models *in vitro* and *in vivo*.

Figure 1. AKT/PI3K pathway

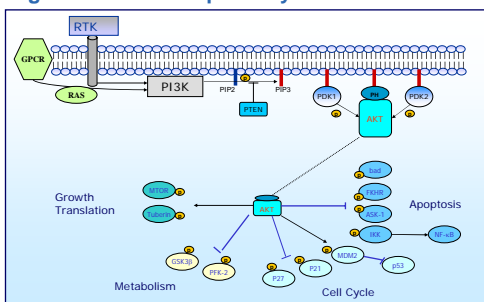
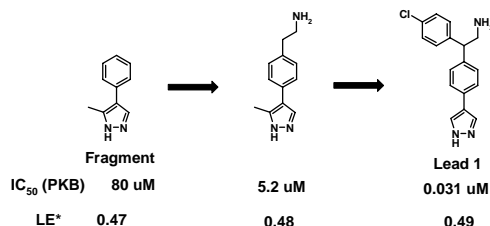


Figure 2. Fragment to Lead Progression

- Fragment Screening
 - Docked ~300,000 available fragments
 - 8 Key fragments validated in Crystallography
 - Inhibitory potencies between 16 μM - 1 mM potency (<250 Da)

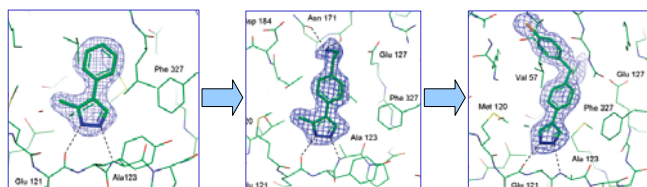


* $\text{LE} = \text{RTIn}(\text{IC}_{50}) / \text{HAC}$ A.L. Hopkins, C.R. Groom et al, Drug Discovery Today, 2004, 9, 430-441

Lead Discovery From Fragment

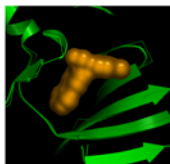
- 14 compounds synthesised to produce nM Lead
- Crystal structures obtained in AKT2 and PKA
- Compounds are low molecular weight with good physical properties and exhibit oral bioavailability

For further detail see:
 J Med Chem. (2007) 50(10):2293-6.
 Identification of inhibitors of protein kinase B using fragment-based lead discovery.
 Saxty G, Woodhead SJ, Berdini V, Davies TG, Verdonk ML, Wyatt PG, Boyle RG, Barford D, Downham R, Garrett MD, Carr RA.



X-ray structures of key inhibitors bound to the ATP site of PKA-PKB chimera.
 The final ligand 2mFo - DFc map (contoured at 1 σ) is shown in blue. Hydrogen bonds are denoted by dashed lines.

Figure 3. AT13148 in AKT2



- AT13148 was identified following further optimisation of this lead series
- Figure shows the crystal structure of the kinase:AT13148 co-complex.

Figure 4. Summary of Properties *in vitro*

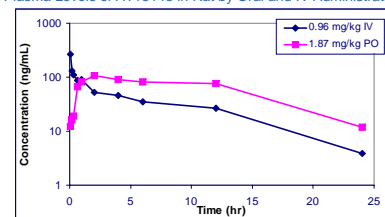
Properties of Lead and Candidate	Lead 1	AT13148
Kinase inhibition (μM)		
AKT2/PKB β	0.031	0.011
P70S6K	0.22	0.012
ROCKII	95% @ 0.1	75% @ 0.01
PKA	0.032	44% @ 0.003
RSK1	40% @ 1.0	0.37
SGK	14% @ 1.0	3.2
Cell based AKT activity (μM)		
U87MG GSK3beta phos. ELISA	n.d.	1.4
PC3 GSK3beta phos. ELISA	3.1	n.d.

Anti-proliferative Activity of AT13148	
Cell Line	IC_{50} (μM)
MES-SA	1.5
MES-SA dx5	1.5
MCF-7	2.1
T47D	1.3
MDA-MB-468	2.9
SKBr3	2.8
HCT116	1.8
HCT116 N7	3.0
A549	2.7
MiaPaCa2	2.6
DU145	38
PC3	4.0
U87MG	4.0

Figure 5. Pharmacokinetics for AT13148

Species	Dose (mg/kg)	route	Cl (mL/min/kg)	T1/2 (hr)	F (%)
Rat	0.96	IV	20	5.9	
	1.87	PO		6.8	100

Plasma Levels of AT13148 in Rat by Oral and IV Administration

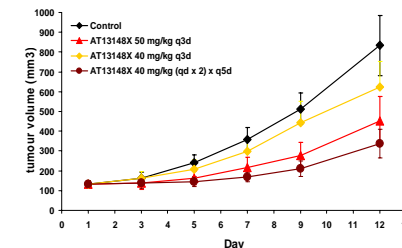


IV formulation - DMA/PBS (40/60)
 PO formulation - Propylene glycol (100%)

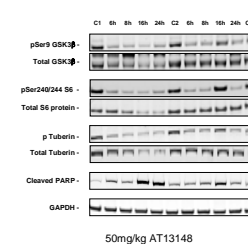
- Oral and intravenous pharmacokinetics of AT13148 were investigated in mice, rats and dogs.
- The compound showed good systemic exposure after oral administration consistent with low plasma clearance in all three species (only rat data shown here)

Figure 6. Activity of AT13148 in PTEN Deficient Mouse Xenograft Model

A: Oral Anti tumour Activity of AT13148 in MES-SA Xenograft Model



B: Biomarker Inhibition in MES-SA Xenografts



Human endometrial tumour cells (MES-SA) were injected sub-cutaneously into the flank of BALB/c nu/nu mice and allowed to grow until they reached approx 100mm³. Groups of 8 mice were then dosed orally with AT13148 as indicated and tumour sizes measured over a 12 day period (A). All doses were well tolerated with <5% body weight loss occurring over the duration of the experiment (data not shown). In a separate experiment tumour-bearing mice were treated with a single dose of 50mg/kg of AT13148 following which tumours were isolated and total protein analysis performed by SDS-PAGE and by western blotting for specific components on the AKT pathway (B).

Conclusion

AT13148 inhibits AKT pathway activation in a range of AKT-dependent tumor cell lines and this inhibition is closely correlated with an anti-proliferative action. The compound shows oral bioavailability and has moderate to low plasma clearance across several species. Studies *in vivo* using mouse xenograft models demonstrate that AT13148 has antitumor effects at 40 - 50mg/kg. The compound is especially effective in an endometrial tumor xenograft line (MES-SA) deficient in PTEN, a negative regulator of the AKT pathway. AT13148 also modulates the activity of components of the AKT pathway in the tumors and induces apoptosis as a downstream consequence of these pathway effects.