A211

Fragment-based Drug Discovery of the Synthetic Small Molecule HSP90 Inhibitor AT13387

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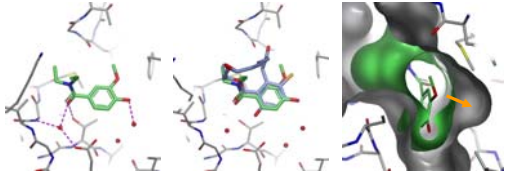
INTRODUCTION

- HSP90 is a molecular chaperone that directs the folding and maturation of its client proteins, many of which are oncogenes regulating tumour cell growth, survival and activation
- Here we describe the identification of the clinical candidate, AT13387, discovered by applying fragment-based drug design to the N-terminal domain of HSP90
- Fragment-based drug discovery is a rapidly growing alternative to high throughput screening in which very small molecules are screened by specialised techniques such as NMR and X-ray
- Only relatively small libraries of fragments are required and observed fragments often possess high potency when normalised to their size (high ligand efficiency)
- Fragment 1, was identified as having a K_d of 790μM for HSP90 and the experimental binding mode indicated a clear pathway to optimise the molecule
- After three iterations of structure-guided medicinal chemistry, compound **4** was identified which is just six heavy atoms larger than fragment **1** but is over 1,000,000 fold more potent
- Lead optimisation led to AT13387 which is currently in Phase I clinical trials for the treatment of cancer
- Early preclinical data on the biological properties of AT13387 are presented here
- The accompanying poster (A217 by Lyons *et al*) gives more preclinical data and discusses the unusually long pharmacodynamics exhibited by AT13387 relative to other HSP90 inhibitors

FRAGMENT-BASED DISCOVERY OF AT13387

Figure 1. NMR screening and X-ray identify fragment 1 binding in the ATP site of N-terminal HSP90

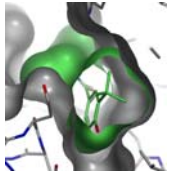


(1)

K_d (ITC) = 790μM
LE = 0.26

- Fragment screening of 1500 fragments identified many hits including fragment **1**, a known respiratory drug
- Despite its poor initial ligand efficiency and potency, the X-ray structure indicated two good design ideas
- Superimposition on the natural product radicicol (top middle) suggested conversion to a resorcinol
- Replacement of the methoxy group with non-planar hydrophobes should provide better fit to the proximal lipophilic pocket (top right)

Figure 2. Experimental binding mode of compound 2 represented as a surface

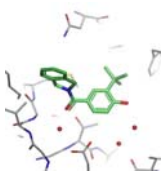


(2)

K_d (ITC) = 8.6μM
LE = 0.38

- Small lipophilic replacements of the methoxy group of fragment **1** were synthesised
- Isopropyl (7μM) and t-butyl (9μM) were the best examples showing approximately 100-fold improvement
- Both analogues give superior hydrophobic contact in the proximal lipophilic pocket as illustrated for compound **2** (above left)

Figure 3. Experimental binding mode of fragment 3

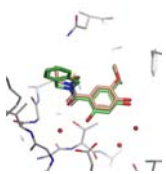


(3)

K_d (ITC) = 0.25μM
LE = 0.41

- Amide replacements were synthesised based on examination of the experimental binding mode
- Tertiary amides were prioritised to preserve the conformational twist observed in fragment **1**
- Isoindoline **3** was one of the most potent offering a 30-fold improvement over compound **2**

Figure 4. Binding mode of lead molecule 4 superimposed on fragment 1

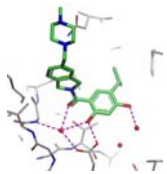


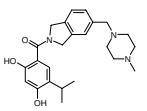
(4)

K_d (ITC) = 0.00054μM
LE = 0.57
cLogP = 3.3
MW = 297 Da
Cell IC_{50} = 0.031μM

- The resorcinol **4** is over 100-fold more potent than the corresponding phenol, and shows good cell activity with a confirmed mechanism of action
- The binding mode was conserved during the fragment optimisation (above left)
- Lead molecule **4** gave weak but encouraging biomarker response

Figure 5. Lead optimisation yielded the clinical candidate AT13387 whose chemical structure and experimental binding are shown below





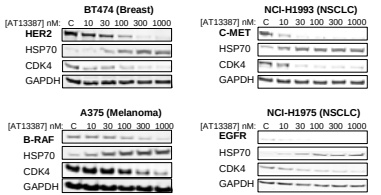
AT13387

K_d (ITC) = 0.00072μM
LE = 0.41
cLogP = 3.5
MW = 410 Da
Cell IC_{50} = 0.048μM

- Initial lead optimisation focussed on improving *in vivo* properties
- Addition of basic groups off the isoindoline was aimed at improving the volume of distribution and led to several compounds with good efficacy and extended pharmacokinetics in tumours
- Some of these compounds possessed activity against hERG (patch clamp) and further SAR showed it was possible to obtain compounds with good efficacy and little or no activity in the hERG assay
- AT13387 was chosen as a clinical candidate after further profiling during a candidate selection phase

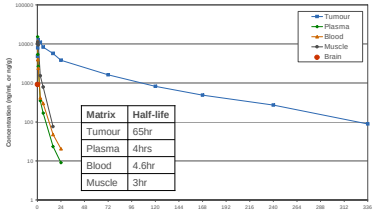
Figure 6. Client protein knock down with AT13387 in breast, melanoma and lung cancer cell lines

Cells were incubated with varying concentrations of AT13387 for 18 hours, lysates harvested and analysed by western blotting.



AT13387 knocks down known oncogenic protein clients of HSP90 (HER2, C-MET, B-RAF and EGFR). In all cases, AT13387 induces HSP70 and knocks down protein client CDK4.

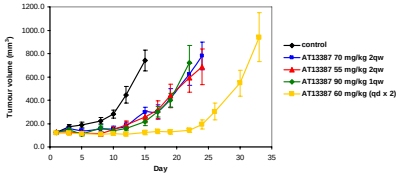
Figure 7. Tumour levels of AT13387 in HCT116 tumour bearing mice



Matrix	Half-life
Tumour	65hr
Plasma	4hrs
Blood	4.6hr
Muscle	3hr

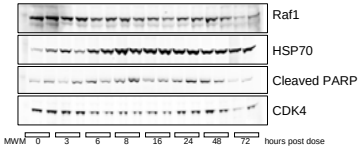
Pharmacokinetic profiling of AT13387 in plasma, blood, brain muscle and tumour after a single 60mg/kg IP dose shows compound retention in only tumour suggesting an opportunity for a greater therapeutic window.

Figure 8. Efficacy of AT13387 in A375 mutant B-raf melanoma xenograft model



AT13387 was dosed weekly or twice weekly by the ip route up to day 21. A treatment group consisted of 8 animals. AT13387 shows good efficacy in this and other models.

Figure 9. Biomarker knock down after dosing of AT13387 in A375 tumour bearing mice



Pharmacodynamic studies show that a single ip dose of 90mg/kg of AT13387 resulted in loss of client proteins Raf1 and CDK4 for 72 hours or more. There was also a concomitant increase in HSP70 and cleaved PARP levels, the latter being indicative of apoptosis.

CONCLUSIONS

- The phenol fragment **1** was identified using fragment screening
- Structure-guided medicinal chemistry was used to identify the highly ligand efficient lead molecule **4**
- Only 6 heavy atoms were added during Hits to Leads yet the potency increased by over 6 orders of magnitude making this one of the most ligand efficient fragment optimisation campaigns so far described
- Only 60 compounds were synthesis in going from fragment **1** to lead **4**
- AT13387 was identified after a lead optimisation campaign centred on increasing the volume of distribution and reducing off-target activities
- AT13387 shows good efficacy and biomarker response in a number of models and exhibits a particularly long half-life in tumours
- The accompanying poster (A217 by Lyons *et al*) gives more details on the favourable biological profile of AT13387
- AT13387 is currently in Phase 1 clinical trials for the treatment of cancer

Presented at the EORTC-NCI-AACR Meeting in Boston on Monday November 16th 2009. This poster can be downloaded from the Astex website at www.astex-therapeutics.com