Fragment-Based Drug Design of AT13387 - A Novel and Efficacious Inhibitor of Hsp90.

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Introduction

Astex Therapeutics uses a fragment-based screening approach (Pyramid™) which employs a range of biophysical techniques, including X-ray crystallography and NMR (nuclear magnetic resonance) spectroscopy, to discover new leads for drug discovery.

Hsp90 is involved in the folding and maturation of key signalling molecules involved in cell proliferation and survival. Inhibition of Hsp90 can therefore affect multiple signalling pathways involved in tumour malignancy and as such is an attractive target for anti-cancer drug design.

This poster describes the application of Astex's fragment-based screening to Hsp90. This approach has been successfully used to identify multiple (> 10) low affinity, but highly efficient 'fragment hits'. A compound from one of these series (AT13387) with promising xenograft efficacy is currently being evaluated in pre-clinical development.

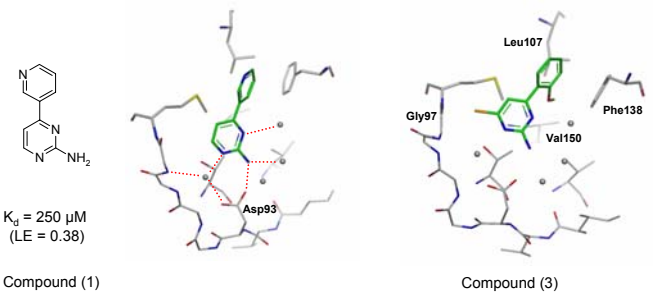
Fragment-based lead discovery

Fragment library design: The screened compound set contained over 1000 diverse, synthetically tractable fragments plus a further 100 fragments identified from virtual screening.

Fragment screening: This was carried out using X-ray crystallography. Soaking apo-crystals of the N-terminal ATPase domain of Hsp90 with targeted fragments allowed the identification of a number of start points for medicinal chemistry. Structure based optimisation of these low affinity hits, with X-ray structural data providing key information about protein-ligand interactions, led to the identification of 4 series with sub μM activity against Hsp90.

Series 1 – Hit to Lead

Figure 1. Structure based Optimisation: The aminopyrimidine (1) was one of a number of hits identified through structural screening. Structural information provided valuable insight into how the fragment binds allowing for rapid optimisation.



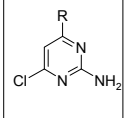
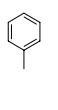
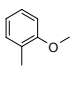
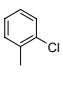
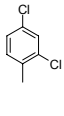
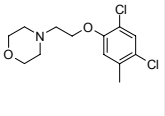
Compound (1) Compound (3)

Structure 1: The aminopyrimidine fragment (1) binds as shown in the ATP binding site via a direct H-bonding interaction between the 2-amino group and Asp93 (H-bonding interactions shown as red dashed lines), plus multiple interactions through a network of conserved water molecules (shown as silver spheres). The 3-pyridyl group is twisted out of plane, which is a disfavoured conformation for this molecule.

Structure 2: Stabilising the twist observed for compound (1) by introducing a 2-substituent gave a 20 fold jump in affinity (compound 3) and also provided a good vector for accessing the small lipophilic pocket (bounded by the residues Val150, Phe138 and Leu107) at the back of the binding site. A chlorine at the 4 position of the pyrimidine ring fills a small lipophilic pocket created by Gly97. This equates to a 700 fold jump in affinity for the addition of 3 heavy atoms.

Table 1. SAR of 2-amino-4-chloropyrimidines

The 2-chloro substituent (4) is a good replacement for the 2-methoxy (3) and introduction of a second chlorine at the 4 position gives a significant increase in binding affinity (5). The solubilising group of compound (6) was primarily used to improve the physical properties of the molecule and has little impact on activity.

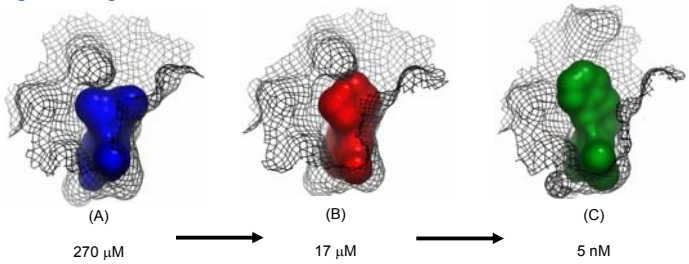
					
Compound	2	3	4	5	6
HSP90, K_d (μM)	9.1	0.35	0.083	0.012	0.005
Ligand Efficiency	0.49	0.55	0.64	0.68	0.45

Ligand efficiency (LE) is the average contribution of each heavy atom to the binding affinity of a ligand against a specified protein. LE = -ΔG/heavy atom count ⇒ LE ~ RTln(IC_{50})/ HAC. Ref. DDT May 2004, 430.

- Aminopyrimidines ~ 50 compounds in 4 months to go from 250 μM to low nM.
- Emphasis on small iterative changes e.g. stabilising conformation, filling small hydrophobic pockets
- Achieved 2 μM cellular activity (HCT116 cell line) with confirmed mode of action.
- Three other series were evaluated in this Hit-to-Lead phase of the project. This includes the one that eventually gave rise to AT13387.
- Work on the pyrimidine series was suspended at the end of Hits-to-Leads due to other more promising lead series.

Series 2 - Fragment-Based Drug Design of AT13387

Figure 2. Fragment Evolution



(A) – Hit identified by fragment screening – unrelated to the pyrimidine hit (compound 1).
(B) – Early hit validation compound.
(C) – Advanced lead molecule, close analogue of AT13387.

AT13387 was developed from this second series of compounds, through the previously described fragment-based screening and structure guided optimisation approach. Figure 2 shows the fragment evolution during Hits-to-Leads for this series of compounds. AT13387 exhibits sub nM affinity for Hsp90 and activity against a wide range of cell lines (Table 2).

Compound Profiling

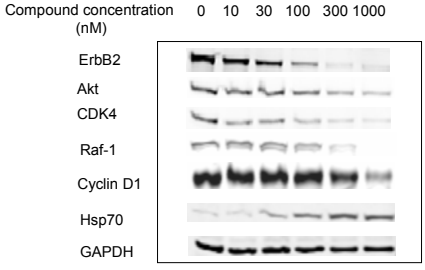
Table 2. Inhibition of Cell Proliferation:

Origin	Cell Line	IC_{50} (nM)
Colon Carcinoma	HCT116	61
	HT-29	80
Lung Carcinoma	A549	22
Breast Carcinoma	MCF-7	64
	MDA-MB-468	26
	BT474	23
Multiple Myeloma	U266	70
	RPMI 8226	70

Origin	Cell Line	IC_{50} (nM)
Pancreatic	PANC1	61
Glioblastoma	U87MG	410
Prostate Carcinoma	DU145	95
	PC3	140
Uterine Sarcoma	MES-SA	90
	MES-SA 5DX	67
Leukaemia	HL60	23
	K562	49

AT13387 shows sub 100nM anti-proliferative activity against a broad range of cell lines, a small selection of which are shown in Table 2.

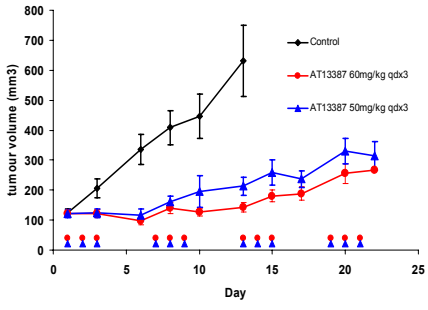
Figure 3. Modulation of Hsp90 Client Proteins *In Vitro*



BT474 cells were treated with AT13387 for 18 hours, followed by Western Blotting

AT13387 inhibits HSP90 client proteins associated with oncogenic pathways in a concentration dependent manner in breast cancer cells (BT474). This is consistent with inhibition of Hsp90.

Figure 4. *In Vivo* Activity of AT13387 in a Colon Cancer Model (HCT116 Xenograft)



AT13387 shows efficacy in a mouse xenograft model (the example shown was via the IP route). PD (pharmacodynamic) profiling shows up-regulation of Hsp70 and Raf-1 degradation.

Summary:

- Structure based screening of Hsp90 using NMR and X-ray crystallography identified multiple fragment hits, 4 of which were pursued in Hits-to-Leads.
- Multiple series progressed in parallel to allow for attrition.
- Structure based optimisation, allowed for rapid identification of lead series with good ATPase and cell activity.
- AT13387 identified as a pre-clinical candidate. It exhibits sub nM affinity for Hsp90 and activity against a wide range of cell lines (Table 2). The compound possesses good aqueous solubility (> 10 mg / ml), is hERG free, shows efficacy in a range of *in vivo* cancer models and has a mode of action consistent with an Hsp90 inhibitor.
  - Salt selection and polymorph screening complete.
  - GMP synthesis complete.
  - Safety evaluation ongoing

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